

# Non-invasive diagnosis of endometriosis, a revolutionary step in reproductive endocrinology

## Noninvaziv endometriyozis tanısı, üreme endokrinolojisinde devrimci bir adım

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### ABSTRACT

*Endometriosis is defined as the presence of endometrial glands and stroma out of the uterine cavity. Symptoms of the disease which affects 10-15% of women of reproductive age are chronic pelvic pain, dysmenorrhoea, dyspareunia and infertility. Although definitive diagnosis of endometriosis is made by histopathological examination of surgically removed tissue, noninvasive tests have become important for the diagnosis because of possible difficulties of surgical diagnosis such as patient's fear of invasive procedures, risks of surgical complication or failure to show the endometriotic lesions by surgical inspection. To date, there is not any noninvasive test for the diagnosis of endometriosis that is routinely used in clinical practice. Developments and standardization in noninvasive diagnostic test instruments are getting important in terms of early diagnosis (and thus early treatment) and patient comfort. In this review we are summarizing noninvasive methods which are currently used and discuss a number of candidate methods including specific serum tests, imaging modalities and eutopic endometrial markers for the noninvasive diagnosis of endometriosis have been discussed along with the relevant literature.*

**Keywords:** Endometriosis, noninvasive diagnostic methods, eutopic endometrial markers, serum biomarkers

### ÖZ

*Endometriozis, endometriyal gland ve stromanın uterin kavite dışında bulunmasıdır. Reprodüktif çağıdaki kadınların %10-15'inde görülen hastalığın semptomları arasında, kronik pelvik ağrı, dismenore, dispareni ve infertilite yer almaktadır. Her ne kadar endometriyozisin kesin tanısı cerrahi olarak çıkarılan dokunun histopatolojik incelemesi ile olsa da; invaziv yöntemlere dair hasta korkusu, cerrahi komplikasyon riskleri ya da cerrahi gözlem ile odağın gösterilememesi gibi olası zorluklar nedeniyle non-invaziv testler ile hastalığın tanısını koyma çalışmaları önem kazanmıştır. Günümüzde endometriyozis tanısı için klinik uygulamada rutin olarak kullanılan non-invaziv bir test bulunmamaktadır. Non invaziv tanı test araçlarındaki gelişmeler ve standardizasyon, erken tanı (dolayısı ile erken tedavi) ve hasta konforu açısından önemlidir. Bu derlemede, endometriyozis tanısında kullanılması olası olan non invaziv testler özetlenmiş, spesifik serum belirteçleri, görüntüleme yöntemleri ve ötopik endometrial belirteçleri içeren bir dizi aday tanı testi güncel bilgiler ışığında tartışılmıştır.*

**Anahtar kelimeler:** Endometriyozis, noninvaziv tanı yöntemleri, ötopik endometriyal belirteçler, serum belirteçleri

### INTRODUCTION

Endometriosis is one of the most challenging gynaecological conditions characterized by the presence of the endometrial gland and stroma outside of the uterine cavity<sup>1</sup>. Although the exact prevalence of the disease in general population is unknown, it affects an estimated 176 million women of reproductive age worldwide. In other words, it affects 10-15%

of women in their reproductive years<sup>2</sup>. The condition is mainly characterized by pain including chronic pelvic pain, dysmenorrhea and dyspareunia. Infertility is another hallmark of the disease<sup>3</sup>.

Diagnosis of disease is based on visualisation of the lesions during surgery and histological confirmation of endometriotic tissue outside of the uterus<sup>1,3</sup>. Unfortunately, both laparoscopy and laparotomy are

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invasive procedures and the requirement of these invasive procedures is a deterring factor for patients to make the diagnosis and treatment of disease<sup>4</sup>. Moreover, surgery is associated with rare but significant potential risks for the patients, as expected. Another point is that surgical inspection can not enhance a definitive diagnosis in some endometriosis patients, particularly cases with retroperitoneal or rectovaginal endometriosis. Therefore, ongoing efforts are being made in the relevant literature to develop noninvasive or less invasive diagnostic tools such as imaging techniques and biochemical markers for endometriosis.

Development of noninvasive diagnostic test tools for endometriosis would have a pioneer impact on the management of these cases, diminishes the delay of time of diagnosis and improves the patients' quality of life by enhancing timely treatment of the condition.

However, to date, any noninvasive test for the diagnosis of endometriosis that is routinely utilised in clinical practice is not available<sup>5</sup>. Therefore, there is a need to produce noninvasive diagnostic tests for endometriosis in order to hasten the diagnostic process and optimise management of the cases. Thus, a consensus workshop, declared following the 10<sup>th</sup> World Congress of Endometriosis, proposed that, development of noninvasive diagnostic tests was one of the priorities in endometriosis research<sup>6</sup>.

In this review we summarise the noninvasive methods which are currently evaluated and discuss new insights for the noninvasive diagnosis of endometriosis. In addition to the patient history and pelvic examination, a variety of tests including imaging, serum markers, eutopic endometrium characteristics or peritoneal fluid components have been suggested as valuable diagnostic measures for endometriosis. Although most of these tests are associated with blood or tissue sampling or with intracavity imaging, we will define all tests that do not involve anaesthesia and surgery as 'non-invasive'.

In this chapter, a number of noninvasive diagnostic tests of endometriosis in the relevant literature were evaluated into four main categories: (A) history and clinical examination; (B) imaging modalities; (C) serum biomarkers; (D) eutopic endometrial markers.

### **A-History and clinical examination**

Although endometriosis is mainly characterized by pelvic pain, either menstrual or non-menstrual, other common causes of pelvic pain including adenomyosis, primary dysmenorrhea and leiomyoma may mimic the symptoms of the condition. In addition, women may also be asymptomatic and endometriosis may be diagnosed incidentally with a finding of an ovarian endometrioma on imaging or endometriosis lesions at time of surgery for another indication. In their review, Eskenazi and Warner reported that endometriosis has been found in 4.1 percent of asymptomatic women undergoing laparoscopy for sterilization<sup>7</sup>.

In a retrospective analysis women with endometriosis were more likely to have infertility, dysmenorrhoea, dyspareunia and abdominopelvic pain compared to healthy controls<sup>8</sup>. However, another study demonstrated that these symptoms were not predictive of diagnosis of endometriosis<sup>9</sup>.

Women with endometriosis may also suffer from other syndromes characterized by pain such as irritable bowel syndrome and painful bladder syndrome. Endometriosis, particularly extrapelvic endometriosis may be associated with bladder or bowel symptoms including cyclic hematuria, diarrhea or cyclic hematochezia.

Although taking history of patient is first step in the diagnosis of medical conditions, it is not markedly helpful in the evaluation of women with endometriosis due to the increased prevalence of asymptomatic disease (2% to 50%), wide range of disease-related symptoms and the weak association between the presenting symptoms and severity of the condition<sup>8,10</sup>.

What is the diagnostic value of physical examination

for endometriosis? Some abnormalities on clinical examination may give clues for diagnosis of endometriosis. Local tenderness and nodularity on the pouch of Douglas or on sacro-uterine ligaments indicate deep endometriosis (DE), an enlarged and fixed cystic ovarian mass may suggest an endometrioma, and a fixed uterus or a frozen pelvis may suggest endometriosis-related adhesions. Although some authors have proposed that nodularity of uterosacral ligaments can be better diagnosed during menstrual days, no research have definitively demonstrated this finding<sup>11</sup>. Researches have shown that abnormal clinical findings indicating endometriosis correlate with the presence of endometriotic foci on laparoscopic observation in 70%to 90% of the patients. However, most of the endometriosis-related clinical findings have a wide range of differential diagnosis<sup>12</sup>. Moreover, a normal gynecological examination does not exclude the condition, as more than half of women with a clinically normal pelvic examination have been found to have endometriosis during laparoscopy<sup>9,13</sup>.

Therefore it can be say that history and physical examination solely are not sufficiently sensitive for diagnosis of endometriosis and both can be used as parameters of combined noninvasive diagnostic algorithms.

### **B-Imaging modalities**

Ultrasonography (USG) and magnetic resonance imaging (MRI) are major noninvasive imaging tools to diagnose endometriotic lesions. Ultrasound is cost-effective and readily available, but user-dependent and MRI enhances more accurate and objective data but significantly more expensive.

Endometriosis can be presented in three different forms: a) endometriotic cysts (endometrioma), deep endometriotic lesions and superficial endometriotic implants. Sensitivity and specificity of MRI and USG change for each type of disease.

#### ***Endometriotic ovarian cysts***

The effectiveness and accuracy of transvaginal ultra-

sonography (TVS) for the detection of ovarian endometriotic cysts have been proven in the relevant literature with a considerable number of trials. Detection of diffuse homogenous ground-glass internal echoes, and hyperechogenic foci in cyst wall is the typical sonographic features of endometriomas. In their systematic review, Moore et al.<sup>14</sup> reported that sensitivity and specificity of TVS were 64-89% and 89-100%, respectively. It needs to be added that TVS is more useful in the diagnosis of ovarian endometriomas, which have a largest diameter of 2 cm or more. Sonographic features of endometriomas may be present in haemorrhagic cysts, dermoid cysts, ovarian abscess and epithelial ovarian tumors<sup>14,15</sup>.

MRI has higher diagnostic performance than TVS in detecting endometriomas however this method has not been suggested as primary diagnostic tool due to its high cost. It may be used for the differential diagnosis of endometriomas in case TVS is indeterminate<sup>16</sup>.

As the cost of sonography is less than the more sophisticated imaging techniques such as MRI, currently TVS is the preferred method of diagnosing ovarian endometrioma.

#### ***Deep endometriosis***

Detection of DE is more challenging. Several imaging methods, such as TVS, transrectal ultrasonography (TRU), computerized tomography (CT) and MRI have been used to improve the noninvasive diagnosis of DE. Bazot et al.<sup>17</sup> have reported the potential value of TVS for the diagnosis DE and confirmed in a larger study that TVS effectively detected deep endometriotic lesions of the rectum. Authors have claimed that TVS has a sensitivity, specificity, positive and negative predictive values for the diagnosis of DE as 78.5, 95.2, 95.4 and 77.9%, respectively. Authors also stated that TVS is less useful for vaginal, uterosacral, and rectovaginal septum involvement.

In their systematic review and meta-analysis on diagnostic accuracy of TVS for noninvasive diagnosis of

bowel endometriosis, Hudelist et al. reported a sensitivity of 91% and specificity of 98% for the diagnosis of DE of the rectosigmoid<sup>18</sup>. The TVS is however operator dependent and these high diagnostic rates may not be expected in day-to-day practice, unless the operator has a special expertise with this tool.

Abrao et al.<sup>19</sup> compared the use of bimanual examination, TVS and magnetic resonance imaging (MRI) for the detection of DE of the rectosigmoid in 104 patients, demonstrating higher sensitivity and specificity for TVS when compared with MRI and clinical examination. Saba et al.<sup>20</sup>, on the other hand, reported that TVS and MRI have similar results in the detection of rectosigmoid endometriosis.

Transrectal ultrasonography may also be used to identify rectal endometriosis and the depth of lesions, but it has not been shown to be superior to TVS<sup>21</sup>.

Although comparative studies are scarce and inconsistent, with its low cost and wide availability, TVS seems to be the first-line noninvasive diagnostic approach for DE.

### ***Superficial endometriotic implants***

The superficial implants are typically 2-3 mm in size and generally located under the serosal tissue of the peritoneum, as well as on the surface of pelvic organs. With time, lesions turn to powder burn appearance because of repeated haemorrhage and inflammation with resultant fibrosis and haemosiderin deposition in them. On the contrary to successful diagnosis of endometriotic cysts and DE, both TVS and MRI have a low sensitivity for the diagnosis of superficial endometriotic lesions and pelvic adhesions. Currently, most of the adhesions and superficial implants cannot be identified without surgery. Nonetheless fat saturated MRI increases the detection rate of small haemorrhagic lesions measuring less than 5 mm from 4% at conventional MRI to 50 percent<sup>22</sup>.

In sum, currently TVS is the primary imaging method for the diagnosis of endometriomas and DE.

### **C- Serum biomarkers**

Currently, increased local and systemic inflammation has been accepted as major pathophysiology in the development of endometriosis. Hence, it is possible that women with endometriosis may have different levels of cytokines in their peritoneal fluid or systemic circulation. Therefore, at least in theory, measurement of several serum markers may detect the endometriosis or aggravation of previously diagnosed endometriosis. Serum cytokines, matrix metalloproteinases, adhesion molecules, and markers of angiogenesis have been investigated for this purpose.

Peritoneal markers have also been investigated, but necessity of obtaining peritoneal fluid make this method invasive and will not be discussed in this section.

Since individual serum markers are not specific for the diagnosis of endometriosis, studies are underway to investigate whether the panels of markers is more successful. Amongst more than 100 serum markers suggested in the literature, the most studied biomarkers will be presented in the following section.

### **Ca 125**

The CA- 125 antigen is a large transmembrane glycoprotein derived from both müllerian (fallopian tubal, endometrial, endocervical) and coelomic (pericardium, pleura, peritoneum) epithelia. This biomarker has been used in clinical practice over the last 20 years. However, in a meta-analysis published in 1998, Mol et al.<sup>21</sup> evidenced that the biomarker's performance in diagnosing endometriosis was low. To date, it is accepted that measurement of CA-125 is reasonable to monitor the disease recurrence after treatment rather than the primary diagnosis of the condition<sup>22</sup>.

### **Cytokines**

Cytokines play a role in controlling cell proliferation and adhesion, chemotaxis, immune cell activation

and motility. They are secreted into the extra-cellular environment by leucocytes, macrophages and other inflammatory cells. It has been hypothesized that a change in the function of the immune cells in the peritoneal environment may be an important factor for the development of endometriotic lesions<sup>23,24</sup>.

### **Interleukin 6 (IL-6)**

Numerous investigations have reported a relationship between serum IL-6 levels and endometriosis, however the results are conflicting and a certain cut-off level of IL-6 for the diagnosis of endometriosis has not been standardized. In case of higher IL-6 levels, a sensitivity of 75-90% and specificity of 51-83.3% have been reported for the diagnosis of endometriosis<sup>25-27</sup>. In their prospective cohort study, on the other hand, Somigliana et al.<sup>28</sup> reported that women with and without endometriosis have similar levels of IL-6. Similarly, Seeber et al.<sup>29</sup> have investigated the use of putative serum markers including tumor necrosis factor-alpha, interleukin-6, macrophage chemotactic protein-1, macrophage migration inhibitory factor, interferon-gamma, and CA-125 for the diagnosis of disease, and they reported that only combination of these markers may aid in the detection of endometriosis rather than using them singly.

### **Interleukin 8 (IL-8)**

IL-8, also known as neutrophil chemotactic factor, is a chemokine produced by macrophages and stimulates activation and chemotaxis of neutrophils. Whilst peritoneal fluid or endometrioma fluid levels of IL-8 may be raised, a significant difference in serum IL-8 levels between women with endometriosis and healthy controls was not found<sup>30-32</sup>. In different studies however, serum IL-8 levels were higher in women with early endometriosis and endometriomas than controls<sup>33,34</sup>. To date, there is not enough evidence for the diagnostic value of IL-8 alone in endometriosis.

### **Tumour necrosis factor-alpha (TNF-a)**

TNF-a is produced chiefly by activated macrophages and involved in systemic inflammation with its pro-inflammatory and pro-angiogenic features. Studies have shown inconsistent results on serum TNF-a levels in cases with endometriosis.

Some authors reported lack of any difference between serum levels of TNFa in women with endometriosis and healthy controls<sup>26,32</sup>, others showed elevated levels of serum TNFa in patients with the condition<sup>27,35</sup>. In addition, Cho et al.<sup>36</sup> indicated lack of any difference in serum and urinary levels of TNFa between healthy women and patients with minimal and mild endometriosis, but showed a significant increase in women with advanced stage of endometriosis. On the contrary, one further study demonstrated raised serum TNFa levels in women with early endometriosis and decreasing levels with more advanced disease, whilst the serum level of TNF-a was undetectable in control women<sup>33</sup>.

### **Monocyte chemotactic protein- 1 (MCP-1)**

MCP-1 is a major chemoattractant cytokine that is produced by fibroblasts in response to tissue injury, or inflammation. It is known that peritoneal macrophages are often increased in women with the endometriosis compared with controls. It has been reported that women with endometriosis with early or advanced stages have significantly higher blood MCP-1 levels than control subjects<sup>33,37-39</sup>. Othman et al.<sup>24</sup> investigated MCP-1 as a part of a combination of potential serum biomarkers. They showed that MCP-1 levels were higher in women with endometriosis, IL-6 was a better marker to differentiate between the healthy controls and endometriosis patients; the use of combination of MCP-1 and IL-6 was not able to improve sensitivity or specificity of noninvasive diagnosis of endometriosis.

### **Other cytokines**

IL-1a, IL1b, IL-2, -4, -10, -12, -13, -15, -18, TGF-B, and

RANTES (regulated on activation normal T cell expressed and secreted) are frequently investigated biomarkers for non-invasive diagnosis of endometriosis. Several conflicting results have been reported and no definitive conclusion has been reached<sup>26,27,32,40-43</sup>.

### Antibodies

Endometriosis have been found to be related with defective cell-mediated immunity and activated humoral immunity<sup>5</sup>. Therefore, an extensive ongoing research has been focused on circulating autoantibodies that may be a marker of endometriosis or involved in disease progress. Total immunoglobulin levels, anti-endometrial antibodies, specific markers including antibodies against progesterone-associated endometrial protein, antibodies directed against carbonic anhydrase, anti-laminin-1 antibodies, anti-cardiolipin antibodies were investigated in women with or without endometriosis, however the results have been scarce to reach a definitive conclusion<sup>44-47</sup>.

### D- Eutopic endometrial markers

Biological features of eutopic endometrium of women with endometriosis may differ from the endometrial tissue of healthy controls. Different gene expression profile in eutopic endometrium of patients with endometriosis have been clearly shown in either experimental and human studies which causes these cells to have unique characteristics that facilitate their survival outside the uterine cavity<sup>48-55</sup>. Therefore sampling of endometrial tissue from women with presumptive diagnosis of endometriosis might help to confirm the diagnosis of the condition. Although promising, these endometrial markers are investigational and none of them can be used in clinical practice, currently.

### CONCLUSION

As a widely investigated gynecological condition, definitive noninvasive diagnosis of endometriosis should deserve much more attention than the current status. Development of accurate and noninva-

sive diagnostic tests for women with endometriosis is an inevitable need in reproductive medicine and this target was emphasized at the international consensus workshop at the 10<sup>th</sup> World Congress of Endometriosis in 2008<sup>6</sup>. Easily applicable, widely-available, operator-independent noninvasive diagnostic methods with high sensitivity would help to timely treatment of patients and to better understand the disease pathophysiology. Noninvasive diagnostic tests would also reduce surgery associated risks and high costs. Although various serum markers and imaging modalities have been explored to date, none of them have been applied routinely in clinical practice and surgery is still the mainstay for the diagnosis and classification of the disease.

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