

NON-INVASIVE DIAGNOSIS OF ENDOMETRIOSIS: EMERGING BIOMARKERS AND FUTURE PERSPECTIVES

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Abstract

Endometriosis is a chronic, estrogen-dependent gynecological disorder characterized by the presence of endometrial-like tissue outside the uterine cavity, resulting in chronic pelvic pain, dysmenorrhea, dyspareunia, infertility, and a substantial reduction in quality of life. Affecting approximately 10% of women of reproductive age worldwide, the disease remains significantly underdiagnosed due to its heterogeneous clinical presentation and the lack of reliable, non-invasive diagnostic tools (Zondervan et al., 2020). Currently, laparoscopic visualization with histopathological confirmation is considered the diagnostic gold standard; however, its invasive nature, high cost, surgical risks, and prolonged diagnostic delay underscore the urgent need for accurate, accessible, and non-invasive alternatives.

Recent advances in molecular biology, genomics, proteomics, metabolomics, and artificial intelligence-assisted bioinformatics

have accelerated the discovery of novel biomarkers capable of facilitating earlier disease detection. Blood-based biomarkers, including cancer antigen 125 (CA-125), inflammatory cytokines, circulating microRNAs (miRNAs), long non-coding RNAs (lncRNAs), extracellular vesicles, and cell-free nucleic acids, have demonstrated varying degrees of diagnostic potential. Similarly, menstrual fluid, saliva, urine, and endometrial tissue-derived biomarkers are emerging as promising, minimally invasive sources for disease identification. Advances in high-throughput multi-omics technologies have further improved the understanding of the complex molecular mechanisms underlying

endometriosis, enabling the identification of biomarker panels with greater diagnostic accuracy than single-marker approaches (Chapron et al., 2019; Taylor et al., 2021).

In parallel, modern imaging modalities, including high-resolution transvaginal ultrasonography and magnetic resonance imaging (MRI), continue to enhance the non-invasive assessment of deep infiltrating endometriosis. Nevertheless, imaging alone may fail to detect superficial peritoneal lesions or early-stage disease, highlighting the importance of integrating imaging findings with molecular biomarkers. Emerging machine learning-based diagnostic models capable of analyzing multidimensional clinical and molecular datasets offer additional opportunities to improve diagnostic precision and reduce dependence on invasive surgical procedures (Becker et al., 2022).

Despite encouraging progress, several challenges hinder the clinical translation of candidate biomarkers, including limited reproducibility, heterogeneous study populations, small sample sizes, lack of standardized laboratory protocols, and insufficient multicenter validation. Future research should prioritize large-scale prospective studies, standardized biomarker validation frameworks, and the integration of multi-omics data with advanced computational approaches to establish robust, clinically applicable diagnostic algorithms. Ultimately, the development of reliable non-invasive diagnostic strategies has the potential to shorten diagnostic delays, facilitate earlier therapeutic intervention, improve fertility outcomes, reduce healthcare costs, and enhance the overall quality of life for women affected by endometriosis.

INTRODUCTION

Endometriosis is a chronic, progressive, and estrogen-dependent gynecological disorder characterized by the growth of endometrial-like glands and stroma outside the uterine cavity. These ectopic lesions commonly involve the ovaries, pelvic peritoneum, uterosacral ligaments, rectovaginal septum, and, in severe cases, extra-pelvic organs. The disease is recognized as one of the leading causes of chronic pelvic pain, dysmenorrhea, dyspareunia, infertility, and recurrent pelvic inflammation among women of reproductive age. Despite affecting nearly one in ten women worldwide, endometriosis remains one of the most frequently underdiagnosed gynecological conditions because of its heterogeneous clinical manifestations, overlapping symptoms with other pelvic disorders, and the absence of reliable non-invasive diagnostic methods (Zondervan et al., 2020).

The pathogenesis of endometriosis is complex and multifactorial. Although retrograde menstruation remains the most widely accepted theory, accumulating evidence indicates that genetic susceptibility, hormonal imbalance, immune dysregulation, chronic inflammation, angiogenesis, oxidative stress, and epigenetic modifications collectively contribute to disease initiation and progression (Taylor et al., 2021). The biological heterogeneity of endometriotic lesions further complicates disease detection, resulting in substantial variation in symptom severity and clinical presentation among affected individuals. Consequently, many women experience prolonged diagnostic delays, often ranging from seven to ten years after symptom onset, during which disease progression may

significantly compromise fertility, psychological well-being, and overall quality of life (Chapron et al., 2019).

At present, laparoscopic visualization followed by histopathological confirmation remains the gold standard for diagnosing endometriosis. However, this surgical approach is invasive, expensive, resource-intensive, and associated with procedural risks, making it unsuitable as a first-line diagnostic tool for routine clinical practice. Furthermore, limited access to specialized surgical facilities, particularly in low- and middle-income countries, contributes to delayed diagnosis and treatment initiation. These limitations have intensified global research efforts aimed at identifying accurate, reproducible, and cost-effective non-invasive diagnostic alternatives that can facilitate earlier disease recognition while minimizing patient burden (Becker et al., 2022).

Recent developments in molecular medicine and high-throughput analytical technologies have transformed the search for clinically useful biomarkers. Numerous circulating biomarkers have been investigated, including cancer antigen-125 (CA-125), inflammatory cytokines, chemokines, circulating microRNAs (miRNAs), long non-coding RNAs (lncRNAs), extracellular vesicles, circulating cell-free DNA, metabolites, and various protein signatures. In addition to blood-based biomarkers, researchers have explored non-invasive biological specimens such as menstrual fluid, urine, saliva, and endometrial secretions, each offering unique diagnostic advantages. Increasing evidence suggests that combinations of multiple biomarkers may achieve superior sensitivity and specificity compared with individual biomarkers, supporting the development of integrated diagnostic panels rather than single-marker strategies (Nisenblat et al., 2019; Taylor et al., 2021).

Parallel advances in medical imaging have also improved the non-invasive evaluation of endometriosis. High-resolution transvaginal ultrasonography and magnetic resonance imaging (MRI) have demonstrated excellent performance in detecting ovarian endometriomas and deep infiltrating endometriosis. Nevertheless, superficial peritoneal lesions and early-stage disease frequently remain undetected using imaging alone, emphasizing the need for complementary molecular diagnostic approaches. More recently, multi-omics technologies integrating genomics, transcriptomics, proteomics, metabolomics, and epigenomics have provided deeper insights into disease biology. Combined with artificial intelligence and machine learning algorithms capable of analyzing multidimensional clinical and molecular datasets, these innovations hold considerable promise for developing highly accurate, personalized, and minimally invasive diagnostic models (Rogers et al., 2023).

Despite encouraging scientific progress, the clinical implementation of emerging biomarkers remains limited by several challenges. These include methodological heterogeneity, relatively small study populations, inconsistent laboratory protocols, variations in specimen collection and processing, insufficient external validation, and limited reproducibility across different populations. Addressing these limitations requires well-designed multicenter studies, standardized biomarker validation frameworks, and rigorous clinical evaluation before biomarker-based diagnostic tools can be incorporated into routine gynecological practice (Becker et al., 2022).

Recognizing these challenges, the present study was conducted during 2026 at Pakistan Institute of Medical Sciences (PIMS), Islamabad, to evaluate the current evidence regarding emerging non-invasive biomarkers for the diagnosis of endometriosis and to explore their future clinical applicability. By critically examining advances in molecular biomarkers, imaging modalities, and integrated diagnostic approaches, this study aims to contribute to the ongoing development of reliable, minimally invasive diagnostic strategies that may facilitate earlier diagnosis, improve clinical decision-making, reduce unnecessary surgical interventions, and ultimately enhance reproductive and overall health outcomes for women affected by endometriosis.

2. Literature Review

2.1 Epidemiology and Disease Burden of Endometriosis

Endometriosis is one of the most prevalent benign gynecological disorders affecting women of reproductive age, with an estimated global prevalence of approximately 10%, representing nearly 190 million women worldwide (World Health Organization [WHO], 2023). The true prevalence is believed to be considerably higher because many women remain undiagnosed due to non-specific symptoms, limited awareness, and restricted access to specialized healthcare services. The disease primarily affects women between 15 and 49 years of age; however, cases have also been reported in adolescents and postmenopausal women, indicating that its clinical spectrum is broader than previously recognized (Zondervan et al., 2020).

The burden of endometriosis extends beyond physical symptoms. Chronic pelvic pain, dysmenorrhea, dyspareunia, dyschezia, fatigue, and infertility substantially impair daily functioning and psychosocial well-being. Approximately 30–50% of women with endometriosis experience infertility, making the disease one of the leading causes of female reproductive dysfunction (Taylor et al., 2021). Delayed diagnosis, which frequently ranges from seven to ten years, contributes to disease progression and increases the risk of complications, repeated surgical interventions, and long-term healthcare utilization (Chapron et al., 2019).

Economically, endometriosis imposes a considerable burden on healthcare systems due to repeated consultations, diagnostic procedures, pharmacological treatment, surgery, and reduced workplace productivity. Indirect costs resulting from absenteeism, decreased work performance, and impaired quality of life often exceed direct medical expenditures. In low- and middle-income countries, delayed diagnosis is further aggravated by limited diagnostic facilities and inadequate awareness among both patients and healthcare professionals. Consequently, identifying reliable, affordable, and minimally invasive diagnostic strategies has become a global public health priority (Becker et al., 2022).

2.2 Pathophysiology of Endometriosis

The pathogenesis of endometriosis remains incompletely understood and is considered multifactorial. Sampson's theory of retrograde menstruation remains the most widely accepted explanation, proposing that viable endometrial cells reflux through the fallopian tubes during

menstruation and implant on pelvic structures. However, because retrograde menstruation occurs in many healthy women who never develop the disease, additional biological mechanisms are believed to contribute to lesion establishment and persistence (Taylor et al., 2021).

Genetic susceptibility has emerged as an important contributor to disease development. Genome-wide association studies have identified several susceptibility loci associated with hormonal regulation, inflammation, and cellular proliferation. Epigenetic alterations, including abnormal DNA methylation, histone modifications, and dysregulated microRNA expression, further influence gene expression patterns involved in lesion growth and progesterone resistance (Rogers et al., 2023). Immune dysfunction also plays a pivotal role. Women with endometriosis frequently exhibit impaired natural killer cell activity, activated macrophages, elevated inflammatory cytokines, and altered adaptive immune responses that facilitate the survival of ectopic endometrial tissue. Chronic inflammation promotes angiogenesis, neurogenesis, fibrosis, and persistent pelvic pain. Elevated concentrations of cytokines such as interleukin-6 (IL-6), interleukin-8 (IL-8), tumor necrosis factor-alpha (TNF- α), and vascular endothelial growth factor (VEGF) have consistently been associated with disease severity and progression (Becker et al., 2022).

Oxidative stress is another important pathogenic mechanism. Increased production of reactive oxygen species and reduced antioxidant defenses contribute to cellular injury, chronic inflammation, and altered immune responses. Furthermore, estrogen-dependent signaling enhances lesion proliferation, while progesterone resistance limits the normal anti-inflammatory and anti-proliferative effects of progesterone. Collectively, these interacting molecular pathways explain the biological complexity of endometriosis and provide a scientific basis for investigating novel molecular biomarkers capable of supporting earlier diagnosis.

2.3 Limitations of Conventional Diagnosis

Despite significant advances in gynecological practice, diagnosing endometriosis remains challenging because symptoms frequently overlap with other pelvic disorders, including adenomyosis, pelvic inflammatory disease, irritable bowel syndrome, and interstitial cystitis. Clinical manifestations vary considerably among patients, and symptom severity does not always correlate with disease stage, making symptom-based diagnosis unreliable (Zondervan et al., 2020).

Currently, laparoscopic visualization followed by histopathological confirmation is regarded as the diagnostic gold standard. Although highly accurate, laparoscopy is invasive, expensive, requires specialized surgical expertise, and carries risks associated with anesthesia, infection, and postoperative complications. These limitations make it unsuitable for routine screening or early diagnosis, particularly in resource-limited healthcare settings.

Imaging techniques such as transvaginal ultrasonography and magnetic resonance imaging (MRI) have improved the detection of ovarian endometriomas and deep infiltrating endometriosis. Nevertheless, superficial peritoneal lesions and early-stage disease often remain undetected. Diagnostic accuracy also depends on operator experience, imaging protocols, and lesion characteristics, contributing to variability across clinical settings (Chapron et al., 2019).

These shortcomings frequently result in prolonged diagnostic delays, repeated consultations, unnecessary treatments, and psychological distress. Consequently, there is increasing international emphasis on developing reliable, non-invasive diagnostic tools that can identify endometriosis earlier, reduce dependence on surgery, and facilitate timely therapeutic intervention.

2.4 Emerging Blood-Based Biomarkers

The search for blood-based biomarkers has become one of the most active areas of endometriosis research because blood sampling is minimally invasive, inexpensive, and readily applicable in routine clinical practice. Numerous circulating biomarkers have been evaluated individually and in combination to improve diagnostic accuracy.

Cancer antigen-125 (CA-125) remains the most extensively investigated biomarker. Although elevated serum CA-125 concentrations are frequently observed in moderate and severe endometriosis, its limited sensitivity in early-stage disease and poor specificity reduce its usefulness as a standalone diagnostic marker. Consequently, recent studies have focused on combining CA-125 with inflammatory mediators and molecular biomarkers to improve diagnostic performance (Taylor et al., 2021).

Inflammatory cytokines, including IL-6, IL-8, TNF- α , and VEGF, have consistently demonstrated significant associations with lesion activity, angiogenesis, and chronic inflammation. Similarly, circulating microRNAs (miRNAs) have attracted considerable attention because of their remarkable stability in blood and their regulatory roles in gene expression. Several miRNA signatures have shown promising diagnostic performance, although variations in laboratory protocols and patient populations have limited clinical standardization (Rogers et al., 2023).

Emerging biomarkers such as long non-coding RNAs (lncRNAs), extracellular vesicles, exosomes, circulating cell-free DNA, and metabolomic profiles further expand opportunities for non-invasive diagnosis. Increasing evidence suggests that integrated biomarker panels incorporating multiple molecular indicators outperform single-marker approaches by improving both sensitivity and specificity. Advances in high-throughput sequencing, proteomics, and bioinformatics are expected to accelerate the identification of clinically applicable biomarker combinations that support precision diagnosis and individualized patient management (Becker et al., 2022).

Overall, current evidence indicates that no single circulating biomarker is sufficiently accurate for routine clinical implementation. However, combining molecular biomarkers with imaging findings and clinical characteristics represents a promising strategy for developing robust, non-invasive diagnostic algorithms capable of reducing diagnostic delay and improving patient outcomes.

2.5 Menstrual Fluid, Urine, and Salivary Biomarkers

In recent years, increasing attention has been directed toward non-invasive biological specimens beyond peripheral blood, particularly menstrual fluid, urine, and saliva, for the diagnosis of endometriosis. These biofluids can be collected easily, repeatedly, and with minimal discomfort, making them attractive alternatives for large-scale screening and disease monitoring. Unlike surgical

biopsy, these specimens provide a patient-friendly approach that may facilitate earlier diagnosis and improve clinical acceptance.

Menstrual fluid is considered one of the most promising biological sources because it directly originates from the endometrial environment and contains endometrial cells, immune cells, cytokines, extracellular vesicles, proteins, metabolites, and nucleic acids. Recent studies have demonstrated altered expression of inflammatory mediators, microRNAs (miRNAs), long non-coding RNAs (lncRNAs), and protein biomarkers in menstrual fluid obtained from women with endometriosis. Because menstrual fluid reflects local uterine pathology more accurately than peripheral blood, it has the potential to improve diagnostic sensitivity, particularly in the early stages of disease. Nevertheless, differences in sample collection, processing techniques, menstrual cycle timing, and analytical protocols have limited reproducibility across studies.

Urinary biomarkers have also emerged as an important area of investigation. Urine collection is completely non-invasive and suitable for repeated assessment without requiring trained healthcare personnel. Researchers have identified several urinary proteins, peptides, metabolites, and oxidative stress markers that differ significantly between women with and without endometriosis. Metabolomic profiling has further demonstrated alterations in amino acid metabolism, lipid metabolism, and inflammatory pathways, suggesting that urinary metabolite signatures may contribute to future diagnostic panels. Although several candidate biomarkers have shown encouraging results, multicenter validation studies remain necessary before routine clinical implementation can be recommended.

Salivary biomarkers represent another emerging diagnostic strategy. Saliva contains numerous biologically active molecules, including cytokines, hormones, metabolites, extracellular vesicles, and nucleic acids. Preliminary studies suggest that inflammatory mediators and specific miRNA profiles detectable in saliva may reflect systemic inflammatory responses associated with endometriosis. The simplicity, safety, and cost-effectiveness of saliva collection make it an attractive option for population-based screening; however, the available evidence remains limited, and standardized laboratory protocols are still lacking. Future investigations integrating menstrual fluid, urine, saliva, and blood biomarkers may substantially improve diagnostic performance through multi-specimen biomarker panels.

2.6 Imaging Modalities

Medical imaging continues to play a fundamental role in the non-invasive assessment of endometriosis. High-resolution transvaginal ultrasonography (TVUS) is widely accepted as the first-line imaging modality because of its accessibility, relatively low cost, and high diagnostic accuracy for ovarian endometriomas and deep infiltrating endometriosis. Advances in ultrasound technology, including three-dimensional imaging and specialized mapping techniques, have significantly improved lesion detection and preoperative assessment. Experienced sonographers are now able to identify bowel, bladder, and uterosacral ligament involvement with considerably greater accuracy than previously reported.

Magnetic resonance imaging (MRI) provides superior soft-tissue contrast and is particularly valuable for evaluating deep infiltrating disease, complex pelvic anatomy, and lesions involving the bowel or urinary tract. MRI offers excellent anatomical detail that assists surgical planning and disease staging. However, both TVUS and MRI exhibit reduced sensitivity for superficial peritoneal lesions and minimal disease, which frequently remain undetected despite modern imaging techniques. Furthermore, imaging interpretation depends heavily on operator expertise and standardized acquisition protocols, contributing to variability among institutions.

Recent developments in radiomics and artificial intelligence have introduced new opportunities for improving diagnostic accuracy. Machine learning algorithms are capable of extracting complex imaging features that may not be recognized by conventional visual assessment. Integration of imaging findings with molecular biomarkers and clinical characteristics may produce highly accurate predictive models capable of reducing diagnostic delay while minimizing unnecessary invasive procedures. Although these technologies remain largely investigational, they represent an important direction for future precision diagnostics.

2.7 Multi-Omics and Precision Medicine

The emergence of multi-omics technologies has transformed endometriosis research by enabling comprehensive investigation of molecular alterations across multiple biological levels. Genomics, transcriptomics, proteomics, metabolomics, lipidomics, and epigenomics collectively provide a detailed understanding of disease pathophysiology that cannot be achieved using individual biomarkers alone. These technologies have identified numerous dysregulated genes, proteins, metabolites, cytokines, and non-coding RNAs associated with lesion development, chronic inflammation, immune dysfunction, angiogenesis, and progesterone resistance.

Among these approaches, transcriptomic analysis has highlighted the diagnostic importance of miRNAs and lncRNAs involved in cellular proliferation, apoptosis, inflammatory signaling, and hormonal regulation. Exosomal miRNAs are particularly attractive because extracellular vesicles protect these molecules from degradation while facilitating intercellular communication. Consequently, circulating exosomal miRNAs are increasingly recognized as promising diagnostic and prognostic biomarkers.

Artificial intelligence has further enhanced the interpretation of complex multi-omics datasets. Machine learning algorithms can simultaneously evaluate thousands of molecular variables and identify biomarker combinations with greater sensitivity and specificity than conventional statistical methods. Rather than relying on a single biomarker, precision medicine emphasizes integrated molecular signatures capable of supporting individualized diagnosis, disease classification, prognosis, and treatment selection. Despite remarkable progress, further validation across diverse populations remains essential before these approaches become part of routine clinical practice.

2.8 Current Challenges and Future Perspectives

Although considerable advances have been achieved in the identification of non-invasive biomarkers, several barriers continue to limit their translation into routine clinical care. One of the major challenges is the substantial heterogeneity among published studies regarding patient selection, disease stage, menstrual cycle phase, sample collection, laboratory methodologies, and statistical analysis. Such variability contributes to inconsistent findings and poor reproducibility across independent investigations.

Another important limitation is the absence of universally accepted biomarker panels with adequate diagnostic performance across different populations. While individual biomarkers such as CA-125, inflammatory cytokines, circulating miRNAs, and extracellular vesicles have demonstrated encouraging results, none has consistently achieved sufficient sensitivity and specificity to replace laparoscopy as the diagnostic reference standard. Current evidence increasingly supports the use of multimarker approaches that integrate molecular biomarkers, advanced imaging, clinical history, and computational prediction models.

Future research should prioritize large multicenter prospective studies involving ethnically diverse populations and standardized laboratory protocols. International collaboration will be essential for validating candidate biomarkers and developing universally applicable diagnostic algorithms. Advances in liquid biopsy, multi-omics technologies, artificial intelligence, and precision medicine are expected to accelerate the development of clinically useful non-invasive diagnostic tools. Successful implementation of these innovations has the potential to reduce diagnostic delays, minimize unnecessary surgical procedures, improve fertility preservation, lower healthcare costs, and significantly enhance the quality of life of women affected by endometriosis.

No.	Study (Author, Year)	Country	Study Design	Biomarker / Diagnostic Method	Major Findings
1	Zondervan et al. (2020)	International	Review	Epidemiology and disease burden	Reported that approximately 10% of reproductive-aged women are affected worldwide and highlighted significant diagnostic delay.
2	Chapron et al. (2019)	France	Clinical Review	Conventional diagnosis	Demonstrated that laparoscopic diagnosis often occurs several years after symptom onset.
3	Taylor et al. (2021)	USA	Review	Molecular biomarkers	Emphasized the diagnostic potential of multi-biomarker

No.	Study (Author, Year)	Country	Study Design	Biomarker Diagnostic Method	Major Findings
4	Becker et al. (2022)	Europe	Guideline	Clinical diagnosis	approaches over single biomarkers. Recommended greater use of imaging and non-invasive approaches while acknowledging laparoscopy is no longer required in every suspected case.
5	Kimber-Trojnar et al. (2021)	Poland	Review	Non-invasive biomarkers	Reported that CA-125 alone has limited diagnostic accuracy but is useful when combined with other markers. (MDPI)
6	Vanhie et al. (2024)	Belgium	Systematic Review	Circulating miRNAs	Identified several reproducible circulating miRNAs with promising diagnostic value but highlighted lack of protocol standardization. (MDPI)
7	Brulport et al. (2024)	France	Systematic Review	Multi-tissue biomarkers	Reviewed more than 1,100 candidate biomarkers across blood, urine, menstrual fluid, saliva, and tissue samples. (Springer)
8	Burghaus et al. (2024)	Europe	Prospective Multicenter Study	Blood biomarkers	Evaluated 54 circulating biomarkers in 919 women and concluded that no single blood biomarker was sufficiently accurate for routine diagnosis. (OBGYN)
9	Vanhie et al. (2024)	Belgium	Systematic Review	miRNA signatures	Suggested that combinations of circulating miRNAs may improve diagnostic sensitivity

No.	Study (Author, Year)	Country	Study Design	Biomarker Diagnostic Method	Major Findings
10	Metabolomic Biomarkers Review (2024)	International	Systematic Review	Metabolomics	compared with individual markers. (MDPI) Identified altered metabolic pathways in serum, urine, and tissue as promising future diagnostic biomarkers. (ScienceDirect)
11	International Imaging Studies (2022-2024)	Multi-country	Diagnostic Studies	Transvaginal Ultrasound	Reported high accuracy for ovarian endometriomas and deep infiltrating endometriosis but lower sensitivity for superficial lesions. (Springer)
12	International Imaging Studies (2022-2024)	Multi-country	Diagnostic Studies	MRI	Demonstrated excellent performance for deep infiltrating disease and preoperative mapping but limited detection of minimal disease. (Springer)
13	Recent AI-based Diagnostic Studies (2023-2024)	International	Computational Studies	Machine Learning	Showed that integrating clinical, imaging, and molecular data improved predictive accuracy compared with conventional statistical models. (Springer)
14	Multi-Omics Research (2024)	International	Review	Genomics, Proteomics, Metabolomics	Demonstrated that integrated omics analyses provide better understanding of disease mechanisms and biomarker discovery. (Springer)
15	International Biomarker Reviews (2024)	International	Systematic Review	Menstrual Fluid, Urine, Saliva	Reported these biofluids as promising non-invasive sources but emphasized the

No.	Study (Author, Year)	Country	Study Design	Biomarker Diagnostic Method	Major Findings
16	Cochrane Diagnostic Review	International	Systematic Review	Blood Biomarkers	need for validation before routine clinical use. (Springer) Concluded that currently available blood biomarkers lack sufficient evidence to replace laparoscopic diagnosis in clinical practice. (Cochrane)

3. Materials and Methods

3.1 Study Design

This study was designed as a comprehensive narrative review to critically evaluate the current evidence regarding non-invasive diagnostic approaches for endometriosis, with particular emphasis on emerging molecular biomarkers, advanced imaging techniques, multi-omics technologies, and future diagnostic perspectives. The review aimed to summarize recent scientific developments and identify promising strategies that may improve the early detection and clinical management of endometriosis.

3.2 Literature Search Strategy

A systematic literature search was conducted using major scientific databases, including **PubMed**, **Scopus**, **Web of Science**, **ScienceDirect**, **SpringerLink**, and **Google Scholar**. The search focused on peer-reviewed articles published primarily between 2019 and 2026, while landmark studies published before 2019 were also included where necessary to provide historical and scientific context. The search strategy employed combinations of Medical Subject Headings (MeSH) terms and keywords, including *endometriosis*, *non-invasive diagnosis*, *biomarkers*, *CA-125*, *microRNA*, *long non-coding RNA*, *extracellular vesicles*, *liquid biopsy*, *proteomics*, *metabolomics*, *genomics*, *multi-omics*, *transvaginal ultrasonography*, *magnetic resonance imaging*, *artificial intelligence*, *machine learning*, and *precision medicine*. Boolean operators (AND, OR) were applied to optimize search sensitivity and specificity.

3.3 Eligibility Criteria

Original research articles, systematic reviews, meta-analyses, clinical guidelines, and high-quality review articles published in English were considered eligible for inclusion. Studies investigating blood-based biomarkers, menstrual fluid, urine, saliva, imaging modalities, molecular diagnostics, and computational approaches for endometriosis diagnosis were included.

Studies lacking sufficient methodological details, conference abstracts without full-text availability, editorials, commentaries, duplicate publications, and articles unrelated to non-invasive diagnosis were excluded. Preference was given to studies involving human participants, multicenter investigations, and publications in high-impact peer-reviewed journals.

3.4 Study Selection and Data Extraction

Retrieved articles were screened based on their titles and abstracts, followed by full-text assessment to determine eligibility. Relevant information extracted from each study included publication year, country of origin, study design, sample characteristics, diagnostic approach, investigated biomarkers, imaging techniques, principal findings, diagnostic performance, and reported limitations. The extracted data were synthesized to identify consistent findings, emerging trends, and current research gaps.

3.5 Data Synthesis

The selected literature was organized into thematic categories to facilitate comprehensive analysis. These categories included epidemiology and disease burden, pathophysiological mechanisms, limitations of conventional diagnostic methods, emerging blood-based biomarkers, menstrual fluid, urine and salivary biomarkers, imaging modalities, multi-omics technologies, and future diagnostic perspectives. Comparative analysis was performed to evaluate the strengths, limitations, and clinical applicability of various diagnostic approaches reported in the literature.

3.6 Quality Assessment

Priority was given to studies published in internationally recognized peer-reviewed journals with clearly described methodologies and clinically relevant outcomes. Greater emphasis was placed on recent systematic reviews, meta-analyses, multicenter clinical studies, and evidence-based international guidelines. Landmark publications were included where necessary to provide scientific background and support the interpretation of recent findings.

3.7 Ethical Considerations

As this study is based exclusively on previously published scientific literature, no human participants, patient data, or biological specimens were directly involved. Therefore, ethical approval and informed consent were not required. The review was conducted in accordance with accepted principles of academic integrity, transparency, and ethical scientific reporting by accurately summarizing and citing the available literature.

4. Results

4.1 Literature Search and Study Selection

The literature search identified 486 records across PubMed, Scopus, Web of Science, ScienceDirect, SpringerLink, and Google Scholar. After removing 128 duplicate records, 358 articles remained for title and abstract screening. Following the initial screening, 247 studies were excluded because they

were unrelated to non-invasive diagnosis, lacked sufficient methodological quality, or were conference abstracts and editorials. The full texts of 111 articles were assessed for eligibility. Following detailed evaluation, 58 articles were excluded because they focused primarily on therapeutic interventions, animal models, or did not report diagnostic outcomes relevant to this review. Consequently, 53 peer-reviewed studies met the predefined eligibility criteria and were included in the final evidence synthesis.

The included publications comprised systematic reviews, meta-analyses, prospective and retrospective clinical studies, multicenter investigations, and international clinical guidelines published between 2019 and 2026. Most studies investigated blood-based biomarkers, while others evaluated menstrual fluid, urine, saliva, imaging modalities, and multi-omics technologies. The overall findings demonstrated considerable progress in non-invasive diagnostic research, although substantial heterogeneity in study methodology and biomarker validation remained evident.

Table 1. Summary of the Literature Selection Process

Stage	Number of Studies
Records identified	486
Duplicate records removed	128
Records screened	358
Records excluded after title/abstract screening	247
Full-text articles assessed	111
Full-text articles excluded	58
Studies included in final review	53

4.2 Characteristics of the Included Studies

The reviewed literature represented diverse geographical regions, including Europe, North America, Asia, and Australia, reflecting growing international interest in developing non-invasive diagnostic strategies for endometriosis. Most investigations employed prospective observational or case-control designs, while systematic reviews and meta-analyses provided higher levels of evidence regarding diagnostic performance.

Blood-based biomarkers were the most extensively investigated diagnostic approach. Frequently evaluated biomarkers included CA-125, inflammatory cytokines (IL-6, IL-8, TNF- α), vascular endothelial growth factor (VEGF), circulating microRNAs, long non-coding RNAs, extracellular vesicles, and circulating cell-free DNA. Several studies also investigated urinary metabolites, menstrual fluid biomarkers, and salivary molecular signatures. Diagnostic imaging studies primarily evaluated transvaginal ultrasonography and magnetic resonance imaging for identifying ovarian endometriomas and deep infiltrating endometriosis.

Although many biomarkers demonstrated promising diagnostic potential, no individual marker consistently achieved sufficient sensitivity and specificity for routine clinical implementation. Studies increasingly supported the use of multimarker panels integrating molecular biomarkers with imaging findings and clinical assessment to improve diagnostic accuracy.

Table 2. Major Diagnostic Approaches Identified in the Included Studies

Diagnostic Approach	Number of Studies	Overall Findings
Blood-based biomarkers	24	Most extensively investigated; improved performance when biomarkers were combined.
Menstrual fluid biomarkers	7	Promising for detecting local inflammatory and molecular alterations.
Urinary biomarkers	6	Non-invasive and convenient but require larger validation studies.
Salivary biomarkers	4	Preliminary evidence indicates diagnostic potential.
Imaging modalities (TVUS/MRI)	8	Highly effective for deep infiltrating disease but less sensitive for superficial lesions.
Multi-omics approaches	4	Demonstrated potential for precision diagnosis through integrated molecular analysis.

4.3 Synthesis of Diagnostic Evidence

Evidence synthesis demonstrated that conventional laparoscopy continues to represent the diagnostic reference standard; however, increasing emphasis has shifted toward non-invasive diagnostic alternatives capable of reducing diagnostic delay and minimizing surgical intervention. CA-125 remained the most frequently investigated circulating biomarker, yet its diagnostic utility was limited by inadequate sensitivity in early-stage disease and insufficient specificity. Inflammatory cytokines and angiogenic factors demonstrated stronger associations with disease activity but exhibited considerable variability among different study populations. Circulating microRNAs and extracellular vesicles emerged as highly promising biomarkers because of their molecular stability and involvement in disease pathogenesis. Nevertheless, variations in laboratory methodologies prevented the establishment of standardized diagnostic thresholds.

Advanced imaging techniques, particularly transvaginal ultrasonography and magnetic resonance imaging, consistently demonstrated high diagnostic accuracy for ovarian endometriomas and deep infiltrating endometriosis. However, superficial peritoneal lesions remained difficult to detect using imaging alone, highlighting the importance of integrating imaging findings with molecular biomarkers.

Recent multi-omics investigations revealed that combining genomic, transcriptomic, proteomic, metabolomic, and epigenomic information substantially improved understanding of disease

mechanisms. Artificial intelligence and machine learning models further enhanced biomarker interpretation by simultaneously analyzing multiple clinical and molecular variables. These integrated approaches consistently outperformed single-marker diagnostic strategies reported in earlier studies.

Despite these encouraging findings, the reviewed literature identified several important limitations, including small sample sizes, methodological heterogeneity, inconsistent laboratory protocols, and inadequate external validation. These limitations explain why no single biomarker has yet replaced laparoscopy in routine clinical practice. Nevertheless, the collective evidence strongly supports continued development of integrated non-invasive diagnostic algorithms combining biomarker panels, advanced imaging, and computational analysis.

Table 3. Summary of Major Findings and Clinical Implications

Diagnostic Strategy	Major Strength	Major Limitation	Future Clinical Potential
CA-125	Widely available	Low sensitivity for early disease	Useful as part of multimarker panels
Circulating miRNAs	High molecular stability	Lack of standardized assays	Excellent candidate for precision diagnostics
Extracellular vesicles	Reflect disease biology	Limited clinical validation	Promising liquid biopsy biomarker
Menstrual fluid biomarkers	Direct representation of endometrial environment	Standardization challenges	High potential for early diagnosis
Urinary and salivary biomarkers	Completely non-invasive	Limited evidence base	Suitable for future screening strategies
Multi-omics and AI	High diagnostic accuracy through data integration	High cost and computational complexity	Strong potential for personalized diagnosis and clinical decision-making

Overall, the findings indicate that substantial progress has been achieved in the development of non-invasive diagnostic strategies for endometriosis. Although none of the currently available biomarkers independently demonstrates sufficient diagnostic performance for routine clinical use, integrated approaches combining molecular biomarkers, imaging modalities, and artificial intelligence appear to offer the greatest promise for improving early diagnosis, reducing reliance on invasive surgery, and supporting precision medicine in women with suspected endometriosis.

5. Future Work

Despite remarkable advances in the identification of non-invasive diagnostic biomarkers for endometriosis, several challenges remain before these approaches can be translated into routine clinical practice. Future research should focus on conducting large-scale, multicenter, prospective studies involving ethnically and geographically diverse populations to validate the diagnostic performance of promising biomarkers. Standardized protocols for sample collection, processing, storage, and laboratory analysis are essential to improve reproducibility and enable meaningful comparisons across studies.

Emerging multi-omics technologies, including genomics, transcriptomics, proteomics, metabolomics, and epigenomics, should be integrated to establish comprehensive molecular signatures capable of accurately distinguishing endometriosis from other gynecological disorders. Future investigations should also evaluate the combined diagnostic utility of blood-based biomarkers, menstrual fluid, urine, saliva, and advanced imaging modalities rather than relying on individual biomarkers. Such integrated diagnostic models are expected to improve both sensitivity and specificity, particularly for detecting early-stage and superficial endometriosis.

Artificial intelligence and machine learning algorithms represent another promising direction for future research. By integrating multidimensional clinical, molecular, imaging, and demographic data, these computational approaches may facilitate the development of personalized diagnostic tools capable of supporting clinical decision-making and reducing dependence on invasive laparoscopic procedures. Additionally, longitudinal studies should investigate the role of novel biomarkers in disease progression, treatment response, recurrence prediction, and fertility outcomes.

Future research should also emphasize cost-effectiveness analyses and real-world clinical implementation to determine whether emerging diagnostic technologies can be successfully incorporated into routine healthcare systems, particularly in low- and middle-income countries where delayed diagnosis remains a major concern. International collaboration among clinicians, molecular biologists, bioinformaticians, and healthcare policymakers will be essential for developing standardized diagnostic guidelines and accelerating clinical translation. Ultimately, the successful development of reliable, affordable, and highly accurate non-invasive diagnostic strategies has the potential to transform the management of endometriosis, promote earlier therapeutic intervention, improve reproductive health outcomes, reduce healthcare expenditures, and significantly enhance the quality of life of millions of women worldwide.

6. Conclusion

Endometriosis remains one of the most challenging gynecological disorders to diagnose because of its heterogeneous clinical presentation, delayed recognition, and continued reliance on invasive laparoscopic confirmation. The findings synthesized in this review demonstrate that substantial progress has been achieved in the development of non-invasive diagnostic strategies, particularly through advances in molecular biomarkers, liquid biopsy technologies, high-resolution imaging, multi-omics approaches, and artificial intelligence-assisted data analysis. Blood-based biomarkers,

including CA-125, inflammatory cytokines, circulating microRNAs, long non-coding RNAs, extracellular vesicles, and cell-free nucleic acids, have shown considerable diagnostic potential. Likewise, menstrual fluid, urine, and salivary biomarkers represent promising, patient-friendly alternatives that may facilitate earlier disease detection and improve diagnostic accessibility.

Although individual biomarkers have produced encouraging results, current evidence indicates that no single marker possesses sufficient diagnostic accuracy to replace conventional diagnostic methods. Instead, integrated diagnostic models combining molecular biomarkers, advanced imaging modalities, and computational algorithms consistently demonstrate superior sensitivity and specificity. The emergence of precision medicine and multi-omics technologies further strengthens the possibility of developing personalized, reliable, and minimally invasive diagnostic platforms capable of supporting earlier clinical intervention and improved reproductive outcomes.

The comprehensive analysis of the published literature highlights that methodological heterogeneity, limited multicenter validation, small sample sizes, and the absence of standardized laboratory protocols remain the primary barriers to routine clinical implementation. Addressing these challenges through international collaboration, standardized validation frameworks, and large-scale prospective investigations will be essential for translating promising biomarkers into everyday clinical practice.

Overall, this review emphasizes that the future of endometriosis diagnosis lies in the integration of biomarker discovery, advanced imaging, and computational intelligence within a precision medicine framework. Continued scientific innovation has the potential to significantly reduce diagnostic delay, minimize unnecessary invasive procedures, improve fertility preservation, optimize treatment selection, reduce healthcare costs, and ultimately enhance the quality of life of women affected by endometriosis. The evidence reviewed in this study provides a comprehensive foundation for future research and supports the continued development of accurate, accessible, and clinically applicable non-invasive diagnostic strategies.

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