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Salivary miRNAs in the diagnosis of endometriosis An invited narrative scientific literature review, commissioned by European Board and College of Obstetrics and Gynaecology (EBCOG)

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ABSTRACT

Endometriosis is a common gynaecological condition, the diagnosis of which has been made for many years through invasive procedures, such as laparoscopy. In recent years, non-invasive methods have been proposed, in which the expression of various miRNAs is analyzed in serum or plasma, but none of these methods has been established as routine in daily practice. Recently, miRNA expression has been examined in saliva, which is a simple means of obtaining an unlimited number of samples, the collection of which does not cause inconvenience to the patient. From the relatively small number of studies that have been published so far, a miRNA signature has emerged, which, although validated in the country of production, has not been tested in other countries. In addition, the number of published studies examining individual miRNAs is very small. It should be noted that all published studies have a small sample size, making it difficult to draw clear conclusions. Furthermore, there are limited data on mapping miRNAs across various biological fluids against the molecular structure of endometriosis lesions. Therefore, it remains to be determined whether the findings represent endometriosis per se or reflect the body's general inflammatory or immune response to the disease. This EBCOG invited narrative review analyses existing literature data and their potential clinical applications and raises issues that require further research.

Introduction

Endometriosis is a pathological condition in which functional endometrial like tissue is found outside the uterus, in locations such as the ovaries and/or other pelvic organs, but sometimes also in organs outside the pelvis and peritoneal cavity [1]. It is considered a disease most commonly of reproductive age whose development is directly influenced by ovarian hormones, mainly oestrogen. The prevalence of endometriosis varies widely from 4 to 50% depending on the criteria used and is more common in women with infertility [2]. However, as many as 60% of cases remain undiagnosed [3,4]. It appears that correct diagnosis is delayed on average by 10 years and requires at least 7 visits to a healthcare professional [5–7]. The symptomatology depends on the location of the endometriosis, with dysmenorrhea, pelvic pain and dyspareunia being the main pelvic symptoms [1,8]. Menorrhagia may coexist, but there may also be symptoms from other systems, such as haematuria, intestinal bleeding or even pneumothorax during menstruation [9]. Its aetiology remains largely unknown, with various theories having been proposed over time [10].

Unfortunately, to date, there is no established simple method for diagnosing this condition. The conventional diagnostic methods, i.e. ultrasound and magnetic resonance imaging (MRI), are of significant help, however, the definitive diagnosis is made only by invasive methods, such as laparoscopy/laparotomy combined with biopsy and histological examination of the lesions, the only conclusive diagnostic tool [8,9]. Recently, research has discovered non-invasive techniques, including the expression of various microRNAs (miRNAs) in blood samples [11–13] or in peritoneal fluid samples of women with endometriosis [14]. About 2500–2800 miRNAs have been identified in the human genome, and unique expression profiles of miRNAs have been reported in multiple diseases, including several benign or malignant diseases and degenerative medical disorders [15–17]. These methods have clear advantages, but for endometriosis, despite initial validation, may necessitate a comprehensive evaluation within a broad spectrum of populations before they can be widely embraced as reliable diagnostic tools. More recently, miRNA expression has been studied in saliva samples from women with endometriosis [18]. Saliva is a fluid of the oral cavity that has been tested for several years to measure hormones and other substances in it, but it has not been established as a widely used medium.

Studying the interactions between miRNAs and their target messenger RNA (mRNAs) is essential for understanding the role of miRNAs and how they regulate various biological processes [19]. In terms of diagnostics, the use of miRNAs is promising due to their stability in various biological fluids [20]. While there is optimism about using saliva biomarkers for diagnosis of various conditions [21], the question remains whether the levels of miRNAs detected in saliva are sufficiently abundant and representative of the disease under investigation.

This narrative review aims to evaluate existing literature focusing on

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miRNA expression exclusively in saliva and to assess whether this can be validated widely as a novel non-invasive approach for the diagnosis of endometriosis.

Materials and methods

For the purpose of this study, the electronic databases MEDLINE, EBSCO, Scopus and Cochrane Library were searched to identify the most relevant studies over the past ten years. The following keywords were used in the database: "saliva", "miRNA", "endometriosis". The selection criteria were original studies examining the role of salivary miRNAs in the diagnosis of endometriosis in symptomatic women compared to a control group without relevant symptoms. Studies with miRNAs in blood but not saliva or in other pathological conditions or in animals were excluded.

Results

The search retrieved 19 studies published from January 2022 to June 2026. Of these, 9 studies were excluded as 6 of them were narrative reviews, 1 examined saliva picoRNA, 1 was a survey of test acceptance among German gynaecologic healthcare providers and 1 evaluated the cost-effectiveness in the context of commercial administration of the test. The remaining 10 studies were eligible for full text assessment. Their key elements are listed in Table 1. Of these, 6 studies were produced by the same research group from France (years 2022–2025) and generated a diagnostic miRNA signature, while 4 studies were produced by different groups, i.e. 1 from Austria (year 2025), 1 from Italy (year 2025), 1 from China (year 2026) and 1 from Russia (year 2026), all four evaluating individual miRNAs.

Salivary miRNA diagnostic signature (6 studies)

The first study is described as a prospective ENDOmiRNA study in which extensive sequencing of the human saliva miRNAome was performed in samples obtained from symptomatic women with endometriosis, diagnosed by laparoscopy/histology or magnetic resonance imaging (MRI) and from controls [18]. Next generation sequencing and artificial inteligence (AI) were applied. A 109-miRNA salivary diagnostic signature for endometriosis was generated for the first time with high sensitivity, specificity and area under the curve (AUC).

The same group applied a bioinformatics approach for miRNA sequencing analysis in saliva based on the material of the above discussed study [18,22]. Some 2561 miRNA were differentially expressed in the saliva of women with endometriosis as compared to controls. About 30 (1.17%) miRNAs were up- or downregulated.

The women from the ENDOmiARN study [18] were included in another prospective analysis to develop a saliva-based miRNA signature for diagnosing women with endometriosis at risk of infertility [23]. The feature selection method generated a signature of 34 miRNAs. The overall accuracy of the signature against 10 randomised data sets used for internal validation showed for the most accurate model representing the diagnostic signature sensitivity, specificity and AUC, all 100%.

External validation of the salivary 109-miRNA signature was undertaken in a multicentre prospective ENDOmiRNA saliva test study for the diagnosis of endometriosis [24]. An interim analysis in five centres in France was presented. Reproducibility was confirmed with high accuracy against the validation cohort. The small number of patients, the lack of assessment of the impact of benign conditions in the controls and the lack of analysis on follow-up data were, however, important limitations of this interim analysis, hence more data are awaited.

Further analysis of data from the ENDOmiRNA study [18] has created another study in which an 89 miRNA signature was generated associated with the superficial peritoneal endometriosis phenotype [25]. The overall performance of the Random Forest (RF) model against 9 randomised data sets (for internal validation) regarding sensitivity, specificity and AUC was for the best model, representing the diagnostic signature, 100%.

External validation of the 109-miRNA signature was completed in a subsequent multicentre study in France including 971 patients (including also those in the interim analysis) [26]. The accuracy was defined as the probability of correct classification for both positive and negative results and it was 96.6% (95% CI, 95.2 to 97.6%) with a sensitivity of 97.3% (95% CI, 96.4 to 98.0%), a specificity of 94.1% (95% CI, 91.0 to 96.4%), a positive predictive value of 98.2% (95% CI, 97.3 to 98.9%), a negative predictive value of 91.3% (95% CI, 88.3 to 93.4%), a positive likelihood ratio of 16.6 (95% CI, 10.8 to 26.9), and a negative likelihood ratio of 0.03 (95% CI, 0.02 to 0.04). There has been no validation by researchers outside France.

Salivary individual miRNAs (4 studies)

A study conducted by an Austrian research group has tested miRNA expression in women with endometriosis, confirmed by laparoscopy/histology, compared to controls [27]. Saliva and plasma samples were collected from all women prior to laparoscopy. The miRNA analysis targeted a focused panel of 28 human miRNA, 25 of which had previously been found to be expressed differently in plasma, serum, or blood in women with endometriosis versus controls. All tested miRNAs were expressed in saliva of all patients. According to the authors, the miRNA hsa-mir-135a could be considered a hypothetical biomarker in saliva for the diagnosis of endometriosis.

Recently, another prospective observational study from an Italian group was published with the aim of sequencing miRNAs from saliva samples obtained from women with endometriosis diagnosed either clinically along with ultrasound or histologically and from controls [28]. With principal component analysis (PCA), the control group appeared more clustered and the endometriosis more dispersed indicating more significant variability in miRNA expression. The miRNA hsa-miR-93-5p was downregulated and the hsa-miR-3168 was up-regulated. The study suggests that these two miRNAs could serve as biomarkers for the diagnosis of endometriosis.

More recently, a cross-sectional exploratory sub analysis of material from an ongoing study from China was published in which miRNA expression and prediction of their target genes in serum, saliva and vaginal mucus were tested in women with moderate-severe endometriosis diagnosed during laparoscopy with histological confirmation [29]. This is the first study on vaginal mucus. Two miRNAs in serum, miR-200a-3p and miR-200b-3p, after further validation can be used as non-invasive biomarkers for the diagnosis and monitoring of endometriosis. These two miRNAs, however, were not differentially expressed in either saliva or vaginal mucus. No salivary miRNA was proposed as a potential diagnostic tool.

Finally, a prospective observational pilot study examined the significance of two individual salivary miRNAs, miRNA-451a and miRNA-125b, of which only the first showed increased expression in women with endometriosis [30]. A predictive model combining blood polyunsaturated fatty acids (PUFAs) with these two salivary miRNAs has been developed, which, however, needs further evaluation and validation.

Discussion

General comments

In the diagnosis of endometriosis with miRNAs, blood samples were initially used [11–13]. Recently, studies have been published in which miRNA expression has been performed in saliva samples. Saliva, as a non-invasive procedure, seems to have certain advantages over blood, as even obtaining a blood sample requires a needle puncture. Unlike other biological fluids, saliva is easy to collect and does not require special personnel training or venepuncture, while the collection can be repeated

Table 1
Summary of the main characteristics of studies with salivary miRNAs for the diagnosis of endometriosis.

	Study design	Participants – methods	Type of test	Sensitivity	Specificity	AUC	Limitations
1.Bendifallah et al. 2022 [18]	Prospective observational study (ENDO-miRNA)	200 saliva samples from 200 women (153 endometriosis, 47 controls) Genome-wide miRNA information and a machine-learning algorithm with next-generation sequencing (NGS) Random Forest (RF), a artificial intelligence (AI) algorithm, was used to design the saliva miRNA signature for endometriosis composed of 109 miRNA.	miRNA signature composed of 109 miRNAs (endometriosis)	96.7%	100%	98.3	Not all patients had a laparoscopy. MRI was used instead. Impact of previous hormonal treatment not tested. No external validation.
2.Bendifallah et al., 2022 [22]	Prospective observational study	200 saliva samples (153 endometriosis, 47 controls) (see above No. 1 study). A bioinformatics approach for microRNA sequencing analysis	Some 2561 miRNA were differentially expressed miRNAs in the saliva samples. Of these, 30 (1.17%) were up- or downregulated. Only 3/30 (hsa-miR-34c-5p, hsa-miR-4677-3p, hsa-miR-655-5p) had an AUC > 0.6.	0–≥80%,	0–≥80%,	36.3–≥60%,	No external validation
3.Dabi et al., 2023 [23]	Prospective observational study (ENDO-miRNA or ENDOmiARN study)	200 saliva samples (153 endometriosis, of which 36 infertile and 117 fertile) (No. 1 study) Genome-wide miRNA information and a machine-learning algorithm with next-generation sequencing (NGS) Random Forest (RF), a AI algorithm, was used to design the saliva miRNA signature. The feature selection method was used	A signature of 34 miRNAs linked to endometriosis-associated infertility	100% (for the best model)	100% (for the best model)	100% (for the best model)	Sub-analysis of another study. Small number of infertile patients. Impact of previous hormonal treatment not tested. No external validation.
4.Bendifallah et al., 2023 [24]	Prospective observational study Multicentre external validation of diagnostic accuracy of the 109-miRNA saliva signature – interim analysis (combination of NGS and AI)	200 women 18–43 years old (159 with endometriosis, 41 controls) – validation cohort (five centres in France) Genome-wide miRNA information and a machine-learning algorithm with next-generation sequencing (NGS) Random Forest (RF), a AI algorithm, was used to design the saliva miRNA signature for endometriosis composed of 109 miRNA.	Quantification with no difference in the mean miRNA between saliva sampling and laboratory extraction even if analysis was performed 40 days later miRNA diagnostic signature composed of 109 miRNAs reproducibility was confirmed with no difference in the mean raw read (%) even if samples were analysed 10 days later	96.2%	95.1%	0.96	Small sample size of the interim analysis. Other conditions (eg. myomas) could bias the results. No analysis on follow-up data (recurrences).
5.Bendifallah et al., 2024 [25]	Prospective observational study (ENDOmiRNA study)	200 saliva samples from 153 patients with confirmed endometriosis of whom 16 had superficial peritoneal endometriosis phenotype (SPE) Genome-wide miRNA information and a machine-learning algorithm with next-generation sequencing (NGS) Random Forest (RF), a AI algorithm, was used to design the saliva miRNA signature.	A signature of 89 miRNAs of the SPE phenotype Of the miRNAs of the diagnostic signature, 61 (68.5%) had previously been associated with the pathophysiology of the disease and 28 (31.5%) had not been previously noted. The F1 score (a performance metric used in machine learning) was found to range from 0 to 38.1%.	91.7–100%	91.7–100%	91.7–100%	Analysis of a subpopulation of another study. Small sample size. Some miRNAs are expressed in different phenotypes. Small sample size
6.Bendifallah et al., 2025 [26]	Prospective observational multicentre validation study	Saliva 109-miRNA signature (external validation) 971 women 18–43 years-old (endometriosis prevalence 77%) including also those in the interim analysis (No. 4 study) Genome-wide miRNA information and a machine-learning algorithm with next-generation sequencing (NGS) Random Forest (RF), a AI algorithm, was used	The accuracy was defined as the probability of correct classification for both positive and negative results and it was 96.6% (95% CI, 95.2 to 97.6%)	97.3%	94.1%	NA	Among patients with surgical confirmation, misclassification, underestimation, and overestimation rates were 4.6%, 2.4%, and 2.2% and higher for imaging (27.2%, 15.1%, and 12.2%, respectively). Validation was only done in centres in France and not in other countries

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Table 1 (*continued*)

	Study design	Participants – methods	Type of test	Sensitivity	Specificity	AUC	Limitations
		to design the saliva miRNA signature for endometriosis composed of 109 miRNA.					
7.Perricos et al., 2022 [27]	Prospective observational study from Austria	Saliva and plasma samples were collected prior to laparoscopy from 34 women between 18 and 50 years (17 women with endometriosis and 17 controls) Focused miRNA panel and FireFly microRNA profiling assay	The miRNA analysis targeted a focused panel of 28 human miRNA, 25 of which had previously been found to be expressed differently in plasma, serum, or blood in women with endometriosis versus controls. The miRNAs Hsa-mir-16-5p and hsa-mir-191-5p were detected in all samples, while hsa-mir-145-3p was detected in only 21% of the samples Hsa-mir-135a expressed significantly higher in saliva of women with endometriosis compared to controls and is a putative non-invasive biomarker of endometriosis Similar pattern in plasma samples.	70.6%	88.2%	0.801	Small sample size. Controls with various benign conditions
8.Degano et al., 2025 [28]	Prospective observational study from Italy	16 women with endometriosis diagnosed either clinically along with ultrasound or histologically and treated with dynogest – 12 controls. Saliva samples were obtained from 12 women (4 controls and 8 endometriosis and 4 responders and 4 non-responders) NGS library preparation Bioinformatics analysis	Principal component analysis (PCA). in patients with endometriosis, 5 were upregulated and 8 downregulated Three miRNAs (let7c-5p, mir200a-3p, and mir3168) were downregulated in non-responders compared to responders. The mir93-5p was significantly downregulated and mir3168 was upregulated. These miRNAs could serve as biomarkers for the diagnosis of endometriosis.	NA	NA	NA	Small sample size No validation
9.Lyu et al., 2026 [29]	Prospective cross-sectional exploratory sub analysis of material from an ongoing study	Serum, saliva and vaginal mucus samples were obtained from 20 women (10 with moderate-severe endometriosis and 10 with teratoma – controls) NGS Gene Ontology and KEGG pathway enrichment analyses Serum proteomics by data-independent acquisition LC–MS/MS	No common differentially expressed miRNAs were identified across all body fluids. Expression in vaginal mucus contained higher amounts of detectable miRNA than saliva with the highest amounts in serum. Two miRNAs in serum, miR-200a-3p and miR-200b-3p could be used as potential biomarkers for the diagnosis of endometriosis No salivary miRNA was proposed as a potential diagnostic tool.	NA	NA	0.60–0.64	Small sample size No validation
10. Shansky et al., 2026 [30]	Prospective pilot observational study from Russia	Serum and saliva samples were obtained from 52 women (22 with symptoms and diagnosis of endometriosis by ultrasound and 24 without a diagnosis of endometriosis – controls) Metabolomic analysis and identification of targeted polyunsaturated fatty acids (PUFAs) combined with salivary miR-451a.	Serum levels of PUFAs, i.e. arachidonic acid (AA) and eicosadienoic acid (EDA), but not eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), increased significantly in the endometriosis group. Saliva miR-451a but not miR-125b level was significantly higher in the endometriosis group than in controls. The model OPLS-DA (combined data on PUFAs – AA, EDA, DHA, EPA- and miR-451a and miR-125b showed significant prognostic value in distinguishing test samples between patients with and without endometriosis.	95.8%	96.4%	0.994	Small sample size. No clear diagnosis of endometriosis (only ultrasound). Comorbidities. The selection of these two specific miRNAs and not others is not justified. No validation.

several times in the long term. Although the extent to which saliva volume and properties can be affected by various local factors, such as smoking, oral cavity inflammation, and general oral hygiene has not been established [31], it makes it obvious that standardized procedures for saliva collection and processing should be implemented [32].

Several miRNAs are expressed in saliva, which helps to exploit the potential of creating new methods for diagnosing a range of pathological conditions, including endometriosis. Some miRNAs are overexpressed and others are underexpressed. Regarding the use of salivary biomarkers in clinical diagnostics, there seems to be optimistic prospects [21].

Comparative evaluation of saliva miRNA results

Comparison of research findings from different research groups shows the diversity in the evaluation of results regarding miRNA expression. Concerning the signature 109-miRNA [18], the methodology involved a combination of NGS and AI using Random Forest algorithm. However, the names of the miRNAs in this signature are not mentioned, except four miRNAs (miR-34c-5p, miR-19b-1-5p, miR- 149-5p, and miR-378a-3p), which are the only ones that have been previously reported in the field of endometriosis together with the names of another 27 that have not been reported previously. Of the 109 miRNAs, 84 have been linked to pathophysiological pathways of various benign and malignant diseases. It is obvious that a comparison cannot be made, as the above signature has not been reported by any other research team outside France.

Regarding single miRNA expression, four studies have been published so far. In the first study of the 28 salivary miRNAs tested as a focused panel, only two miRNAs (Hsa-mir-16-5p and hsa-mir-191-5p) were detected in all saliva samples and one (hsa-mir-145-3p) in only 21% of them [27]. For one of the miRNAs, hsa-mir-135a, which was overexpressed in saliva of women with endometriosis compared to controls, the authors concluded that it could be used as a biomarker for the diagnosis of endometriosis [27].

In the second study [28], differential expression of various miRNAs between women with and without endometriosis as well as between women responding and non-responding to dienogest treatment was identified. That study concluded that some miRNAs can be used for the diagnosis of endometriosis and the prediction of responders to progesterone treatment. Importantly, these two studies detected different miRNAs from each other and only very few were in common. Although in the first study [30] miR-135a was considered the most important, in the other study [28] this was the mir-3168. It should be noted that in these two studies the number of women included was remarkably limited, and some participants from the control group had other coexisting gynaecological abnormalities.

The third study in the group of individual miRNAs showed inconsistency between serum, saliva, and vaginal mucus in terms of the differential expression of miRNAs in endometriosis [29]. Two serum miRNAs were proposed as potential diagnostic tools for endometriosis [29], but not any in saliva or vaginal mucus. It should be noted that, similarly to previous studies, this study also included a limited number of patients.

Finally, a fourth study using only two salivary mRNAs along with polyunsaturated fatty acids has developed a predictive model for the diagnosis of endometriosis [30]. Although high sensitivity, specificity, and AUC have been reported, the data are preliminary and need validation of reproducibility and accuracy.

It is evident that comparative data between different biological fluids are currently scarce. This is due to the very limited number of studies using saliva samples. Even the experience using blood samples is not great. Combined serum panels seem to have higher sensitivity, specificity and accuracy than individual miRNAs, which on average is slightly above 0.80 [33,34]. Certainly, more research is needed to determine a suitable non-invasive method of miRNA expression for a more accurate approach to endometriosis diagnosis.

An important observation is that regardless of whether it is a combined panel or individual miRNAs, their expression in saliva does not seem to be affected by hormonal fluctuations of the menstrual cycle, but remains relatively stable [18,29,30].

Endometriosis phenotypes

Endometriosis can have various phenotypes. In two of them, i.e. superficial peritoneal endometriosis [25] and cases with infertility [23], two different signatures have emerged, the 89-miRNA and the 34-miRNA respectively. In the case of peritoneal endometriosis, it is clear that a diagnostic test would be expected to reduce the need for invasive procedures, such as laparoscopy. However, it is important that the signature is exclusive and the miRNAs is not expressed in other phenotypes. Otherwise the diagnostic accuracy is reduced. Also, with the existing data, it is difficult to say to what extent a signature can help in the diagnosis of superficial peritoneal localization in mixed situations, i.e., when foci are also present in other areas. Certainly, the information available so far is interesting, but it should be followed up by more specific signatures. This at present seems quite complex, and becomes even more complicated when the symptomatology is largely common to different phenotypes of the disease. Furthermore, it is possibly difficult to make a positive diagnosis based on a signature alone and when imaging findings are not present. The question that needs to be answered is whether laparoscopic investigation will still be necessary in such cases.

A signature or a single miRNA?

Given that endometriosis is a multifaceted heterogeneous disorder, it would be difficult to argue that its diagnosis can be based on a single miRNA. Theoretically, a single miRNA in saliva may be more vulnerable to alterations due to local factors (infections, smoking, etc.), although there are no relevant data in the literature. Development of a miRNA signature from genome-wide analysis using machine learning may better represent this clinical entity as it has been shown in cases with oral cancer [35]. However, although it can be advantageous, a miRNA signature is a complex data representation that can make interpretation of results and secure validation difficult. This may reduce its reliability, which in some cases can be represented by a single miRNA, which may be easier to understand and standardize [36]. On the other hand, due to the breadth of the clinical picture and the overlap of symptoms in endometriosis, a single miRNA is difficult to be a significant diagnostic factor. In a study using individual miRNAs in blood it has been shown that the sensitivity and specificity ranged from 0.36 to 1.00 and 0.43 to 1.00 respectively, but when several of miRNAs were combined the sensitivity increased to 0.92 and the specificity 0.86 [37]. It is evident from these data that there seem to be advantages and disadvantages to both options.

When attempting to diagnose endometriosis, various parameters may be involved, e.g. a woman with symptoms suggestive of endometriosis does not necessarily have endometriosis. Therefore, to avoid invasive procedures, a diagnostic method must be 100% specific, which is rather unlikely eventuality. It is obvious that to ensure accuracy, a method should be compared with reference standards with histology being one of them. Another parameter is the degree of variation in the amount of RNA in saliva, which could potentially affect the expression of miRNAs, although stability of saliva RNA has been demonstrated [38]. Also, important is the degree of stability of the results between different batches and different laboratories. Finally, it should be noted that, regarding genetic signatures, studies should not only report the AUC but also other essential metrics, such as quality control characteristics, test failure rates, reproducibility and batch measurement errors [39]. It is clear that further research is needed to determine whether signature or individual miRNAs prevail.

Saliva miRNAs vs other biological fluids

The exact way endometriosis-specific miRNAs are transferred to saliva is unclear. From a biological perspective, endometriosis is a disease located primarily in the pelvis, although systemic spread has been reported. One hypothesis is that miRNAs enter saliva from the blood through various cellular mechanisms, such as passive intracellular diffusion and active transport or extracellular ultrafiltration [40]. Local production of miRNAs through apoptosis or cell necrosis is also possible. Circulating miRNAs exit the cell through various means including microvesicles [41–43]. It appears that the transport of miRNAs into the circulation within microvesicles, mainly exosomes, or their association with protein complexes, such as the Argonaute family and high-density lipoproteins (HDL), protect them from degradation by endogenous ribonucleases (RNases), ensuring their stability [44,45]. Alternatively, endometriosis triggers a systemic inflammatory reaction involving the immune system [44,46], which could produce salivary signals even if the lesions are located in distant areas.

There are limited data on the comparison between saliva and blood or other biological fluids regarding the expression of miRNAs. In a recent *meta*-analysis including oesophageal cancer patients, saliva-derived miRNAs have shown higher sensitivity, while blood-derived miRNAs provided higher specificity [47]. The importance of saliva and blood miRNAs as independent biomarkers or as combination tools in cancer diagnosis needs to be further investigated.

In respect to endometriosis, it has been found that a specific endometriosis-related miRNA (mir-143-3p) was overexpressed in saliva and underexpressed in blood [28,37]. This suggests dysregulation of the same miRNA in the same pathological condition and indicates the existence of diversity rather than stability in miRNA expression in endometriosis. When endometriotic tissue was investigated, it was found that the expression of miR-143-3p was significantly upregulated in endometriotic stromal cells compared with normal endometrial stromal cells [48] suggesting that there is no consistency in the expression of some miRNAs across different biological materials. The miR-143-3p was also increased in progesterone non-responders compared to responders and in responders compared to controls [31]. Another miRNA (mir-3168) was found to be upregulated in saliva of endometriosis cases compared to controls and downregulated in progesterone non-responders compared to responders [28]. The same miRNA was found to be significantly downregulated in blood samples in a previous French study, confirming the above inconsistency [49].

A more recent study has investigated miRNA expression in three biological fluids of the same women, i.e. serum, saliva and vaginal mucus [29]. Interestingly, no common differentially expressed miRNAs were identified across the three body fluids [29]. This suggests that the miRNA signature can be distinguished between the three different fluid types reflecting an exclusive biological origin and function. In that study, out of all miRNAs identified, only one (MiR-1304-3p) was differentially expressed between serum and mucus, but it was detected in less than half of the cases. However, it should be noted that vaginal mucus contained a higher amount of detected miRNAs than saliva. According to the conclusions of that study [29], two miRNAs, i.e. miR-200a-3p and miR-200b-3p, subject to further validation, could be used as non-invasive biomarkers in serum for the diagnosis and monitoring of endometriosis. Nevertheless, their relatively low AUC value, 0.60 and 0.64 respectively suggests a moderate discriminatory power of these two miRNAs for this condition.

Over 90% similarity in the expression profile of miRNAs between serum and saliva has been reported [41]. However, in the study by Lyu et al. [29] no common differentially expressed miRNAs were detected between serum and saliva. These differences may be related to disease heterogeneity, but also to the lower abundance of RNA in saliva than in serum. This led Lyu et al. [29] to hypothesize that perhaps salivary miRNAs could serve as complementary biomarkers rather than as standalone diagnostic markers. In any case, that study included a limited cohort of women, and solely those with advanced endometriosis. It is evident that additional studies are warranted to examine whether the same miRNAs may be simultaneously expressed in different biological fluids, as well as in histological samples from the same patients.

A recent *meta*-analysis has investigated the expression of miRNAs in peripheral blood, endometrial tissue, and saliva with the potential to diagnose endometriosis. Although the analysis showed moderate to good diagnostic accuracy (AUC ~ 0.87), only 3 studies examined saliva samples compared to 25 studies using blood samples [34]. This *meta*-analysis highlighted the need for further high-quality studies to develop more effective predictive models.

Regarding other biological fluids, information is limited with one study showing reduced levels of miRNA-451 in the follicular fluid of women with endometriosis undergoing IVF treatment compared to a control group [50].

Validation of salivary miRNA signature

External validation should be predicated on a histologically confirmed diagnosis, as imaging methods are not always accurate. Validation aims to demonstrate the reproducibility of the test, i.e. to show the same performance in different patient groups, a stability of the methodological sample processing, predefined reliability limits, calibration and predefined threshold values, and robustness without the influence of phenotypes or various confounding factors (inflammation, etc.). The 109-miRNA signature [18] derived from extensive sequencing of the human miRNAome is currently the only one in the literature and although validated in a multicentre approach in France [26], there is no validation by research groups outside France. Moreover, the four studies that examined individual miRNAs remain unvalidated.

The strengths of the salivary 190-miRNA signature test in the diagnosis of endometriosis to date are the prospective design of the studies conducted with the inclusion of symptomatic patients, the external validation on a multicentre basis and the discrimination of cases with a peritoneal phenotype but in small numbers. On the other hand, there are weaknesses, mainly the lack of validation by other research groups and laboratories from other countries. Also, the potential impact of oral environmental factors has not been investigated. In addition, the lack of information on the behaviour of the test between batches and populations and the lack of findings in more than one phenotype constitute another group of weaknesses.

Role of salivary miRNAs in the diagnostic toolbox of endometriosis – Degree of acceptance

Although existing studies show an optimistic outlook in diagnosing endometriosis using miRNAs, it is currently rather difficult to clarify to what extent salivary miRNA testing can contribute or what its ultimate role might be. One could hypothesise that salivary miRNAs could be used as the first screening tool in symptomatic women with normal or equivocal imaging followed by the application of another method, e.g. laparoscopy or referral to a specialised centre or earlier initiation of treatment. Where the test will be placed in the diagnostic armamentarium is important to determine with further research, with three possible alternative uses, i.e. as a screening tool for symptomatic patients, as an exclusion tool or as an adjunct to imaging. Regarding the 109 miRNA signature, it appears to be a high-throughput test. However, its specific utility in clinical practice needs to be clearly defined, especially in terms of its contribution to daily practice. For example, it should be demonstrated whether it reduces time to diagnosis and changes the imaging and laparoscopy rates, especially when the diagnosis is obvious on imaging (e.g. endometrioma). Finally, it would be interesting to examine whether in women with chronic pelvic pain the above signature would reduce the number of laparoscopies that are negative for endometriosis.

When using miRNAs as biomarkers for endometriosis, it is essential

to be careful in the selection of the control group, ensuring its homogeneity and the exclusion of women with asymptomatic endometriosis. However, the latter can be ruled out if laparoscopy is performed on these women as they may be included in the control group. The number of participants in each group is also important, as in almost all published studies the sample size is small. In addition, there has been limited analysis regarding various factors, such as age, stage of endometriosis or the coexistence of adenomyosis and fibroids or inflammatory conditions [18]. Furthermore, the presence of other diseases, such as cancer, or hormonal therapy, might have an impact on the reliability of the method. Finally, women under 18 years of age have not been included, which might be of interest for the early diagnosis of the disease.

A recent survey from Germany revealed the attitude of gynaecologists towards the acceptance of saliva miRNA testing in women with endometriosis and whether they would use it in their clinical practice [51]. Interestingly, 135 of the 141 respondents (95.7%) considered the cost of the test to be too high to be affordable, while 119 (84.4%) did not believe that this new test could replace established diagnostic methods (histology, ultrasound, clinical examination). The younger and less experienced generation of professionals had a more favorable view of the usefulness of the test compared to more experienced specialists. This shows that under the current circumstances, German doctors find it difficult to abandon their familiar techniques for a new method with a currently unpredictable perspective. The uncertainty as to whether such a test could be a screening tool for all women or only for those with symptoms may currently be an obstacle to its wider acceptance.

Conclusion

The potential of using saliva to detect miRNA expression for the diagnosis of endometriosis is appealing. However, until more convincing data emerge for healthcare practitioners, thorough research is needed. For example, the extent to which miRNAs detected in saliva and other biological fluids reflect molecular alterations within the endometriotic lesions themselves is unclear. The possibility that the changes observed in these samples reflect systemic inflammatory responses rather than lesion-specific mechanisms cannot be ruled out.

Future prospects

With the existing literature as a starting point for future research, new questions arise regarding the diagnostic potential of salivary miRNAs in endometriosis. One of these is whether miRNAs can be used to screen asymptomatic women for endometriosis, such as those with infertility or a family history of endometriosis or with ultrasound findings suggestive of ovarian endometriomas. Another question is whether a miRNA test could lead to early diagnosis and treatment of endometriosis, thus avoiding potential serious consequences for the woman's reproductive capacity. Furthermore, by using miRNAs, it would be interesting to see whether in women with vague symptoms or imaging findings, invasive procedures, such as laparoscopies can be reduced, thus avoiding the unforeseen risks of surgery. From this perspective, many issues regarding the diagnostic potential of miRNAs in endometriosis are expected to be clarified.

CRediT authorship contribution statement

Ioannis E. Messinis: Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis, Data curation. **Christina Messini:** Writing – review & editing. **George Anifandis:** Writing – review & editing. **Sofia Tsiapakidou:** Writing – review & editing. **Basil Tarlatzis:** Writing – review & editing. **Tahir Mahmood:** Writing – review & editing, Visualization, Validation, Supervision, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Ioannis E. Messinis[a,b,*], Christina Messini[a], George Anifandis[a,c], Sofia Tsiapakidou[b,d,e], Basil Tarlatzis[b,d], Tahir Mahmood[b,f]

[a] *Department of Obstetrics and Gynaecology, Faculty of Medicine, School of Health Sciences, University of Thessaly, 41110 Larissa, Greece*
[b] *European Board and College of Obstetrics and Gynaecology (EBCOG), Leuven, Belgium*
[c] *Department of Embryology, Faculty of Medicine, School of Health Sciences, University of Thessaly, 41110 Larissa, Greece*
[d] *1st Department of Obstetrics and Gynaecology, Papageorgiou General Hospital, Aristotle University of Thessaloniki 56429 Thessaloniki, Greece*
[e] *European Network of Trainees in Obstetrics and Gynaecology (ENTOG), Leuven, Belgium*
[f] *Spire Murrayfield Hospital, Edinburgh, Scotland, UK*

[*] Corresponding author at: Department of Obstetrics and Gynaecology, Faculty of Medicine, School of Health Sciences, University of Thessaly, 41110 Larissa, Greece.
E-mail addresses: messinis@med.uth.gr, messinis.io@gmail.com (I.E. Messinis).