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The Relationship Between of Expression Mirna Let-7a-1 and Mirna Let-7f-1 with Expression of Genes IL6, IL8, and Vascular Endothelial Growth Factor A (VEGF A) in Endometrioum Endometriosis Patients

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ABSTRACT: Endometriosis is a chronic inflammatory, oestrogen-dependent disease in which local inflammation and angiogenesis, mediated in part by interleukin (IL)-6, IL-8, and vascular endothelial growth factor A (VEGF-A), drive lesion growth, yet the contribution of the let-7 family of microRNAs to this process remains poorly defined. This cross-sectional study evaluated endometrial expression of miRNA let-7a-1 and let-7f-1 in relation to disease severity and to IL-6, IL-8, and VEGF-A expression. Sixty-five women undergoing clinically indicated hysteroscopy, laparoscopy, or operative laparotomy at three hospitals in Makassar and Maros, Indonesia (January–June 2023) were enrolled by consecutive sampling: 32 with histologically confirmed endometriosis (7 mild, 12 moderate, 13 severe, ASRM classification) and 33 disease-free controls. Endometrial tissue miRNA and mRNA levels were quantified by reverse-transcription quantitative PCR. Groups did not differ in education level ($p = 0.563$) or nutritional status ($p = 1.000$) but differed in age ($p < 0.001$). Let-7a-1 expression did not differ significantly across severity categories ($p = 0.265$) or between cases and controls ($p = 0.068$). In contrast, let-7f-1 expression differed significantly across severity categories ($p = 0.023$) and was

markedly lower in all endometriosis subgroups than in controls (overall $p < 0.001$; mild vs control $p = 0.018$, moderate vs control $p < 0.001$, severe vs control $p < 0.001$). Neither miRNA correlated with IL-6, IL-8, or VEGF-A expression within any endometriosis severity subgroup, but in controls let-7f-1 correlated positively with IL-8 ($r = 0.478$, $p = 0.033$) and with VEGF-A ($r = 0.467$, $p = 0.050$). These findings indicate that let-7f-1, rather than let-7a-1, is consistently downregulated in endometriotic endometrium and warrants further evaluation as a candidate biomarker, while its coupling to inflammatory–angiogenic signalling appears to be disrupted specifically in disease tissue.

1. INTRODUCTION

Endometriosis is a chronic, oestrogen-dependent inflammatory disease defined by the presence of endometrium-like glands and stroma outside the uterine cavity, most commonly within the peritoneal cavity and pelvis (Monnaka et al., 2021). The ectopic tissue remains hormonally responsive and is sustained by a local milieu of chronic inflammation and pathological angiogenesis, which together promote lesion implantation, survival, and progression (Ahn et al., 2015). Clinically, the disease manifests as dysmenorrhoea, dyspareunia, dyschezia, dysuria, and infertility, and imposes a substantial burden on reproductive-age women; a small proportion of lesions may also undergo malignant transformation (Gaia-Oltean et al., 2021). Diagnosis is frequently delayed because no sufficiently accurate non-invasive test exists, and laparoscopy with histological confirmation remains the diagnostic gold standard, an invasive standard that current clinical guidelines encourage researchers to move beyond (Kaspote et al., 2024; Becker et al., 2022). Among the inflammatory and angiogenic mediators implicated in this process, interleukin (IL)-6, IL-8, and vascular endothelial growth factor A (VEGF-A) have each been linked to macrophage activation, extracellular-matrix remodelling, and neovascularisation within endometriotic lesions (Kai et al., 2023; Hosseini et al., 2023).

Beyond these effector cytokines, increasing attention has focused on the post-transcriptional layer of gene regulation that governs their expression. Non-coding RNAs, and microRNAs (miRNAs) in particular, act as post-transcriptional regulators that bind the 3'-untranslated regions of target messenger RNAs and thereby fine-tune inflammatory and angiogenic gene networks (Ghafouri-Fard et al., 2020). Dysregulated miRNA expression has repeatedly been observed in eutopic and ectopic endometrium from women with endometriosis, supporting a mechanistic role for these small RNAs in disease pathogenesis and raising interest in their use as diagnostic biomarkers (Azam et al., 2022; Moustafa et al., 2020).

Among miRNA families implicated in inflammatory and angiogenic signalling, the let-7 family is of particular interest. Let-7 members participate in a regulatory loop with nuclear factor kappa B (NF- κ B), Lin28, and IL-6, in which inflammatory signalling suppresses let-7 biogenesis while let-7 itself restrains IL-6 and downstream oncogenic signalling (Kaspote et al., 2024). Two subtypes, let-7a-1 and let-7f-1, are biologically plausible candidates in endometriosis: let-7a shares this IL-6-linked regulatory circuit, while let-7f additionally regulates aromatase expression and endothelial angiogenic behaviour, including vascular sprouting and ischaemia-induced neovascularisation (Dhahri et al., 2017). Reduced let-7f expression has been associated with activation of the TGF- β /ALK5 pathway and impaired vascular responses, suggesting a mechanistic link to the aberrant angiogenesis characteristic of endometriotic lesions.

Despite this biological rationale, direct evidence linking let-7a-1 and let-7f-1 expression to IL-6, IL-8, and VEGF-A expression in human endometriotic tissue, and to endometriosis severity specifically, remains limited. Existing studies have largely examined let-7 family members in circulating biofluids or in other gynaecological and oncological contexts, so whether the pattern of let-7a-1 and let-7f-1 expression varies systematically with disease severity, and whether either miRNA is coordinately expressed with local inflammatory and angiogenic markers in endometrial tissue, has not been clearly established.

We therefore conducted a cross-sectional study to characterise endometrial let-7a-1 and let-7f-1 expression in women with endometriosis of varying severity and in disease-free controls, and to examine their association with IL-6, IL-8, and VEGF-A expression. The primary objective was to determine whether let-7a-1 and let-7f-1 expression differs according to endometriosis severity and between endometriosis and control tissue, and whether either miRNA is associated with IL-6, IL-8, or VEGF-A expression within each study group.

2. MATERIALS AND METHODS

2.1 Study design and setting

This was a cross-sectional study conducted between January and June 2023 at three hospitals in South Sulawesi, Indonesia: Wahidin Sudirohusodo Hospital (Makassar), Primaya Hospital Makassar, and La Palaloi Regional Hospital (Maros).

2.2 Participants and eligibility criteria

Women of reproductive age presenting to the gynaecology outpatient clinics of the participating hospitals and undergoing clinically indicated hysteroscopy, laparoscopy, or operative laparotomy were enrolled by consecutive sampling. The endometriosis group comprised women with a diagnosis of endometriosis who were not pregnant, were not receiving hormonal therapy, and provided written informed consent. The control group comprised women without a diagnosis of endometriosis who had an independent indication for hysteroscopy (infertility work-up), were of reproductive age, were not pregnant, were not receiving hormonal therapy, and provided written informed consent. Women with a malignancy or with pelvic infection/pelvic inflammatory disease were excluded, as were samples in which RNA degradation or insufficient RNA yield precluded molecular analysis. Endometriosis severity was classified according to the revised American Society for Reproductive Medicine (ASRM) staging system on the basis of laparoscopic findings.

The minimum sample size was calculated for a correlational design (two-sided $\alpha = 0.05$ corresponding to a one-tailed $Z\alpha = 1.64$, power 90% so $Z\beta = 1.28$, and a minimum meaningful correlation coefficient $r = 0.50$), giving a minimum requirement of 31 participants. A total of 66 participants were initially recruited; one sample was excluded because it did not meet the criteria for miRNA extraction, leaving 65 participants for analysis (32 endometriosis cases: 7 mild, 12 moderate, 13 severe; 33 controls).

2.3 Sample collection

Endometrial tissue was obtained at the time of hysteroscopy, laparoscopy, or operative laparotomy according to clinical indication, immediately stabilised, and stored at -80°C until processing.

2.4 RNA extraction

Total RNA, including the miRNA fraction, was extracted from 20–30 mg of tissue using the miRNeasy Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol with β -mercaptoethanol-supplemented RLT buffer (600 μL per sample). Tissue was disrupted and homogenised using an ultrasonic disruptor for 20–40 seconds. Lysates were processed through QIAamp spin columns with sequential RW1 and RPE buffer washes, and RNA was eluted in 30–50 μL RNase-free water. RNA purity and concentration were assessed by spectrophotometry (NanoPhotometer P-Class, Implen).

2.5 cDNA synthesis and miRNA quantification

Complementary DNA (cDNA) for miRNA quantification was synthesised using the TaqMan MicroRNA Reverse Transcription Kit (catalogue no. 4366596; Thermo Fisher Scientific, Waltham, MA, USA) in a 15- μL reaction containing 100 mM dNTPs (0.15 μL), MultiScribe Reverse Transcriptase (1 μL), 10 $\times$ RT buffer (1.50 μL), RNase inhibitor (0.19 μL), nuclease-free water (4.16 μL), primer (3 μL), and RNA template (5 μL). Thermal cycling conditions were 16 $^{\circ}\text{C}$ for 30 min, 42 $^{\circ}\text{C}$ for 30 min, 85 $^{\circ}\text{C}$ for 5 min, and hold at approximately 4 $^{\circ}\text{C}$.

Expression of let-7a-1 and let-7f-1 was quantified by quantitative real-time PCR (qPCR) using TaqMan Fast Advanced Master Mix and TaqMan Advanced miRNA Assays in a 10- μL reaction (5 μL master mix, 2 μL nuclease-free water, 0.50 μL assay, 2.50 μL cDNA). Cycling conditions were 95 $^{\circ}\text{C}$ for 20 s (polymerase activation, 1 cycle), followed by 40 cycles of 95 $^{\circ}\text{C}$ for 3 s (denaturation) and 60 $^{\circ}\text{C}$ for 30 s (annealing/extension).

2.6 Quantification of IL-6, IL-8, and VEGF-A expression

IL-6, IL-8, and VEGF-A messenger RNA expression was quantified separately from the same tissue samples using a complementary DNA-based RT-qPCR workflow with the ReverTra Ace qPCR RT Master Mix with gDNA Remover (Toyobo, Osaka, Japan), following the manufacturer's protocol; RNA template concentration was standardised to 25 ng/ μL in a final reaction volume of 6 μL prior to reverse transcription. miRNA and mRNA quantification were performed using distinct, assay-specific chemistries and are reported separately.

2.7 Statistical analysis

Data were analysed using IBM SPSS Statistics version 25 (IBM Corp., Armonk, NY, USA). Normality of continuous variables was assessed with the Kolmogorov–Smirnov test. Because miRNA expression values were non-normally distributed, group comparisons were performed using the Kruskal–Wallis test (>2 groups) or the Mann–Whitney U test (2 groups), and post hoc pairwise comparisons after a significant Kruskal–Wallis result were performed using the Mann–Whitney U test. Associations between miRNA expression and IL-6, IL-8, and VEGF-A expression were assessed using Spearman's rank correlation coefficient (r) for non-normally distributed data and Pearson's correlation for normally distributed data. Categorical variables were compared using the chi-square test. All tests were two-sided, and $p < 0.05$ was considered statistically significant; exact p -values are reported where available.

2.8 Ethical considerations

The study was conducted in accordance with the Declaration of Helsinki and was approved by the appropriate institutional research ethics committee. Written informed consent was obtained from all participants prior to enrolment, and participant data were handled confidentially throughout the study.

3. RESULTS AND DISCUSSION

3.1 Participant characteristics

Of 66 women initially recruited, one sample was excluded because it did not meet the criteria for miRNA extraction, leaving 65 participants for analysis: 32 (49.2%) with endometriosis and 33 (50.8%) controls (Figure 1). Cases were older than controls (median 38.5 vs 30.0 years; $p < 0.001$) (Table 1). Education level did not differ significantly between groups ($p = 0.563$), nor did nutritional status, classified as normal or overweight body mass index ($p = 1.000$). Among endometriosis cases, the largest subgroup had severe disease (13/32, 40.6%), followed by moderate (12/32, 37.5%) and mild disease (7/32, 21.9%) by revised ASRM classification.

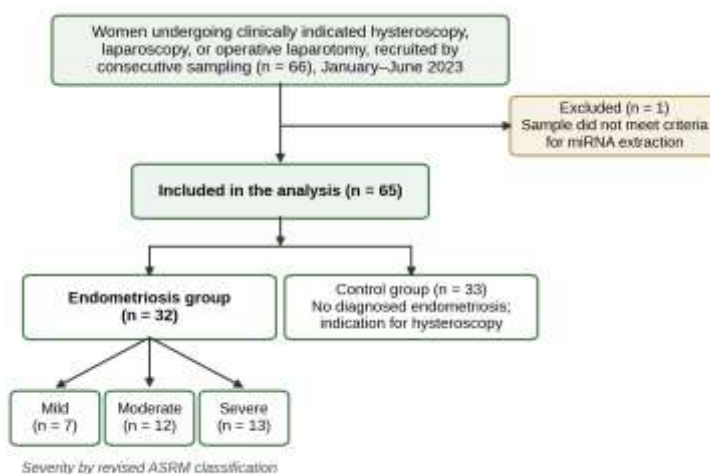


Figure 1. Participant flow diagram.

Table 1. Clinical and demographic characteristics of the study participants.

Characteristic	Endometriosis (n = 32)	Control (n = 33)	p-value
Age, years, median (min–max)	38.50 (24.00–45.00)	30.00 (20.00–42.00)	<0.001*
Education level, n (%)			0.563†
Junior high school	1 (3.1)	3 (9.1)	
Senior high school	15 (46.9)	16 (48.5)	
Bachelor's degree	16 (50.0)	14 (42.4)	

Nutritional status, n (%)			1.000†
Normal	16 (50.0)	17 (51.5)	
Overweight	16 (50.0)	16 (48.5)	

*Mann–Whitney U test. †Chi-square test. Endometriosis severity (ASRM classification): mild, n = 7; moderate, n = 12; severe, n = 13.

3.2 miRNA let-7a-1 and let-7f-1 expression according to endometriosis severity

Among endometriosis cases, let-7a-1 expression did not differ significantly across severity categories (median 0.72, 1.54, and 0.44 in mild, moderate, and severe disease, respectively; Kruskal–Wallis $p = 0.265$) (Table 2, Figure 2A). In contrast, let-7f-1 expression differed significantly across severity categories (median 0.29, 0.10, and 0.02 in mild, moderate, and severe disease, respectively; $p = 0.023$), with the lowest expression in severe disease (Table 2, Figure 2B).

Table 2. miRNA let-7a-1 and let-7f-1 expression according to endometriosis severity.

miRNA, median relative expression	Mild (n = 7)	Moderate (n = 12)	Severe (n = 13)
<i>let-7a-1</i>	0.72	1.54	0.44
<i>let-7f-1</i>	0.29	0.10	0.02
Kruskal–Wallis p-value		0.265 (let-7a-1) / 0.023 (let-7f-1) across mild/moderate/severe	

3.3 Comparison of miRNA expression between endometriosis and control groups

When cases were compared with controls, let-7a-1 expression was numerically highest in the control group (median 3.28) and lowest in severe disease (median 0.44), but the overall difference across the four groups did not reach statistical significance ($p = 0.068$) (Table 3, Figure 2A). Let-7f-1 expression showed a markedly different pattern: median expression in controls (3.15) was more than two orders of magnitude higher than in severe disease (0.02), and the overall difference across groups was highly significant ($p < 0.001$) (Table 3, Figure 2B). Post hoc pairwise comparisons confirmed that let-7f-1 expression was significantly lower in every endometriosis severity subgroup than in controls (mild vs control $p = 0.018$; moderate vs control $p < 0.001$; severe vs control $p < 0.001$), and that the difference between mild and severe disease also reached significance, whereas let-7a-1 differed significantly only between severe disease and controls ($p = 0.012$) (Table 4).

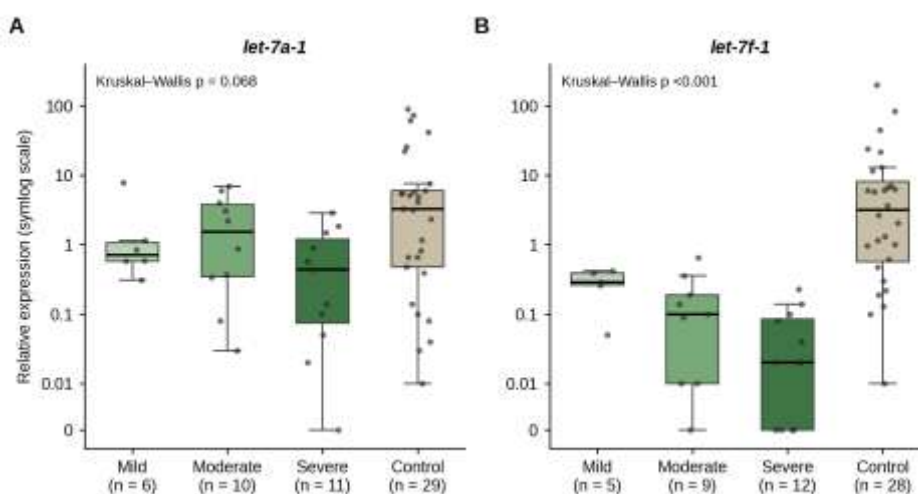


Figure 2. let-7a-1 (A) and let-7f-1 (B) expression across mild, moderate, and severe endometriosis and controls. Boxes show median and interquartile range; points are individual samples plotted on a symmetric log scale. p-values are from the Kruskal–Wallis test.

Table 3. Comparison of miRNA expression between endometriosis severity subgroups and controls.

miRNA, median	Mild	Moderate	Severe	Control
<i>let-7a-1</i>	0.72	1.54	0.44	3.28
<i>let-7f-1</i>	0.29	0.10	0.02	3.15

Overall Kruskal–Wallis $p = 0.068$ (*let-7a-1*) and $p < 0.001$ (*let-7f-1*) across the four groups.

Table 4. Post hoc pairwise comparisons (Mann–Whitney U) of miRNA expression by severity subgroup, p-values.

Comparison	Mild	Moderate	Severe	Control
<i>let-7a-1</i> vs Mild	–	0.914	0.228	0.381
<i>let-7a-1</i> vs Moderate/Severe		–	0.139	0.260
<i>let-7f-1</i> vs Mild	–	0.162	0.007	0.018
<i>let-7f-1</i> vs Moderate/Severe		–	0.217	<0.001

Values are exact p-values from Mann–Whitney U pairwise comparisons following a significant Kruskal–Wallis test; the severe-vs-control comparison for *let-7a-1* was significant ($p = 0.012$) despite the non-significant omnibus test in Table 3.

3.4 Association of miRNA expression with nutritional status

Let-7a-1 and *let-7f-1* expression did not differ significantly between women with normal and overweight nutritional status (*let-7a-1*: median 0.66 vs 1.86, $p = 0.424$; *let-7f-1*: median 0.29 vs 0.30, $p = 0.883$) (Table 5).

Table 5. miRNA expression according to nutritional status.

miRNA, median (min–max)	Normal BMI	Overweight	p-value
<i>let-7a-1</i>	0.66 (0.00–90.16)	1.86 (0.04–73.33)	0.424
<i>let-7f-1</i>	0.29 (0.00–199.88)	0.30 (0.00–84.35)	0.883

Mann–Whitney U test.

3.5 Correlations between miRNAs and IL-6, IL-8, and VEGF-A

Within each endometriosis severity subgroup, correlation analyses between *let-7a-1* or *let-7f-1* expression and IL-6, IL-8, or VEGF-A expression were performed separately. No statistically significant correlation was observed between either miRNA and IL-6, IL-8, or VEGF-A expression in the mild, moderate, or severe subgroups (all $p > 0.05$). In the moderate subgroup, body mass index correlated positively with *let-7a-1* expression ($r = 0.782$, $p = 0.008$), a strong association not observed in the mild or severe subgroups; no other anthropometric correlation reached significance within any severity subgroup. In the control group, however, *let-7f-1* expression correlated positively with both IL-8 expression (Spearman $r = 0.478$, $p = 0.033$) and VEGF-A expression (Spearman $r = 0.467$, $p = 0.050$) (Table 6, Figure 3). *Let-7a-1* expression did not correlate significantly with IL-6, IL-8, or VEGF-A in the control group.

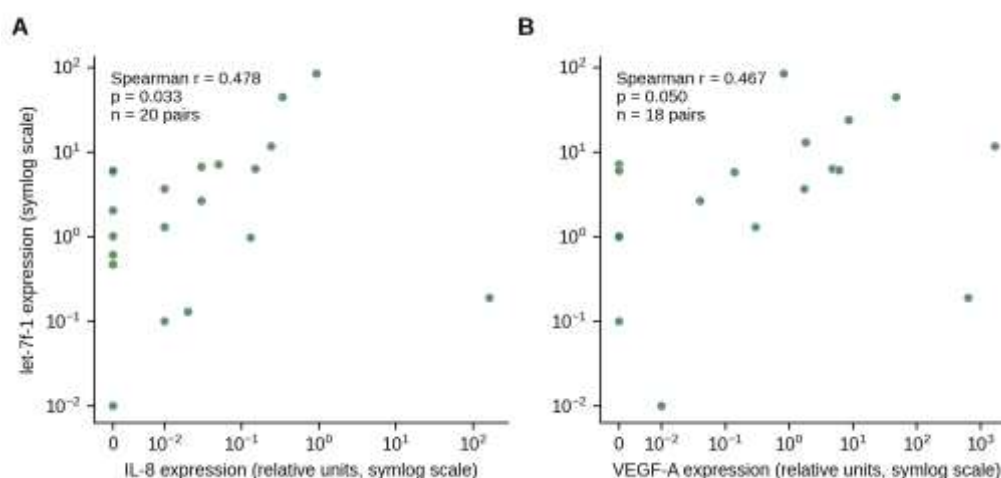


Figure 3. Correlation between let-7f-1 expression and IL-8 (A) and VEGF-A (B) expression in the control group (Spearman correlation; axes on symmetric log scale).

Table 6. Correlations between miRNA expression and IL-6, IL-8, and VEGF-A expression, by study group.

Group	Association	r	p-value
Mild	let-7a-1/let-7f-1 vs IL-6, IL-8, VEGF-A	n.s.	>0.05
Moderate	let-7a-1/let-7f-1 vs IL-6, IL-8, VEGF-A	n.s.	>0.05
Moderate	BMI vs let-7a-1	0.782	0.008
Severe	let-7a-1/let-7f-1 vs IL-6, IL-8, VEGF-A	n.s.	>0.05
Control	let-7f-1 vs IL-8	0.478	0.033
Control	let-7f-1 vs VEGF-A	0.467	0.050

Spearman correlation coefficients (r); n.s. = not statistically significant (all $p > 0.05$). BMI = body mass index.

3.6 Biological interpretation and implications

The principal finding of this study is a clear dissociation between the two let-7 family members examined: let-7f-1, but not let-7a-1, was consistently and significantly lower in endometriotic endometrium than in disease-free tissue, with a stepwise decline from mild to severe disease. This pattern is consistent with reports that let-7f regulates angiogenic and vascular remodelling processes relevant to lesion growth, including its role as a negative regulator of TGF- β /ALK5 signalling during ischaemia-induced neovascularisation (Dhahri et al., 2017), and with evidence that let-7 family members are embedded in a feedback loop with NF- κ B, Lin28, and IL-6 that is characteristically dysregulated in chronic inflammatory states (Kaspute et al., 2024).

Let-7a-1, in contrast, showed only a non-significant downward trend with increasing severity and differed significantly from controls only in severe disease. This is compatible with let-7a and let-7f sharing some regulatory targets, such as IL-6-related signalling, while differing in others: let-7f additionally suppresses aromatase expression and thereby local oestrogen generation (Dhahri et al., 2017), a pathway not shared by let-7a-1, which may explain why let-7f-1 tracked disease severity more consistently in this cohort. Because both miRNAs converge on overlapping but non-identical target networks, a uniform decline was not necessarily expected, and the observed divergence itself is informative about which let-7 subtype is more tightly coupled to endometriosis pathophysiology.

Within endometriosis tissue, neither let-7a-1 nor let-7f-1 correlated significantly with IL-6, IL-8, or VEGF-A expression in any severity subgroup, despite the biological plausibility of such coupling. Several non-exclusive explanations are consistent with this observation. First, cytokine and growth-factor expression in endometriotic lesions is governed by a dense network of transcription factors, epigenetic modifications, and multiple co-regulatory miRNAs, so that the influence of any single miRNA subtype on transcript-level cytokine expression may be diluted or masked (Banerjee et al., 2023). Second, the chronic hypoxic and fibrotic microenvironment that characterises more advanced lesions may engage HIF-1 α -driven transcriptional programmes that operate largely independently of let-7-mediated regulation, decoupling miRNA expression from cytokine transcript abundance in diseased tissue (Mitranovici et al., 2024). Third, the relatively small size of each severity subgroup limits statistical power to detect small-to-moderate correlations within strata.

The positive correlation between let-7f-1 and both IL-8 and VEGF-A observed specifically in the control group, but not in endometriosis tissue at any severity, suggests that this physiological coupling between let-7f-1 and inflammatory-angiogenic signalling may be disrupted once ectopic implantation and chronic inflammation are established. In healthy endometrium, let-7f-1 may participate in homeostatic regulation of IL-8- and VEGF-A-driven vascular remodelling that occurs across the normal menstrual cycle, whereas in endometriotic tissue this relationship appears uncoupled, potentially reflecting the persistent, dysregulated inflammatory and angiogenic drive described in endometriosis pathophysiology models (Ahn et al., 2015; Kai et al., 2023).

More broadly, these observations are consistent with reviews describing endometriosis as a disease of disordered eutopic and ectopic endometrial signalling in which multiple non-coding RNAs, rather than any single miRNA, jointly shape the inflammatory-angiogenic phenotype of lesions (Ochoa Bernal & Fazleabas, 2024; Letafati et al., 2022).

With respect to clinical and demographic covariates, endometriosis cases were older than controls, consistent with the tendency for endometriosis to be diagnosed after a period of unrecognised or unexplained pelvic pain (Kaspute et al., 2024), whereas education level and nutritional status did not differ between groups, suggesting that these socio-demographic and metabolic factors did not confound the case-control comparison of miRNA expression in this cohort. The absence of an association between nutritional status and let-7a-1 or let-7f-1 expression overall is consistent with a predominantly tissue-intrinsic, rather than systemic metabolic, regulation of these miRNAs in the endometrium; the isolated positive correlation between body mass index and let-7a-1 observed in the moderate-severity subgroup did not extend to mild or severe disease and should be interpreted cautiously given the subgroup size.

These results add to a broader literature nominating circulating and tissue miRNAs as candidate non-invasive biomarkers for endometriosis, including panels unrelated to the let-7 family (Chico-Sordo & García-Velasco, 2024; Nothnick, 2022), and reinforce the view that progesterone- and angiogenesis-linked miRNA pathways, rather than a single universal marker, are likely to underlie future diagnostic strategies (Duval et al., 2024; Steinbuch et al., 2024).

Taken together, these findings support let-7f-1 as a candidate biomarker that distinguishes endometriotic from healthy endometrium and tracks with disease severity, while indicating that let-7a-1 plays a more limited role in this cohort. Neither miRNA can currently be considered a validated diagnostic or prognostic biomarker: the cross-sectional design precludes causal inference, and no receiver-operating-characteristic validation, external replication cohort, or longitudinal follow-up was performed. Because IL-6, IL-8, and VEGF-A were not significantly correlated with either miRNA within diseased tissue, functional studies are needed to establish whether let-7f-1 acts causally upstream of angiogenic signalling in endometriosis or is instead a marker of a shared but independently regulated pathological process. From a precision-medicine perspective, a validated let-7f-1 signature could eventually complement clinical and imaging criteria in risk-stratifying women with suspected endometriosis, but this application remains exploratory at present.

3.7 Limitations

This study has several limitations. The cross-sectional design precludes causal inference and does not allow the temporal sequence of miRNA and cytokine changes to be established. The sample size, while meeting the pre-specified minimum for correlation analysis, was relatively modest once stratified by endometriosis severity, which limited the power to detect small-to-moderate within-subgroup correlations. Participants were recruited from three hospitals within the Makassar–Maros area of South Sulawesi, so generalisability to other populations should be confirmed in multicentre studies with broader geographic representation. No external validation cohort or receiver-operating-characteristic analysis was performed, and no longitudinal

follow-up was available to assess whether miRNA expression changes with disease progression or treatment. Finally, the associations reported here reflect statistical correlation rather than established causal or functional mechanisms, and mechanistic or functional validation studies are needed.

4. CONCLUSION

In this cross-sectional study of 65 women, endometrial let-7f-1 expression was significantly lower in endometriosis tissue than in disease-free controls and declined progressively with increasing disease severity, whereas let-7a-1 expression did not differ significantly by severity and differed from controls only in severe disease. Within endometriosis tissue, neither miRNA was significantly associated with IL-6, IL-8, or VEGF-A expression at any severity level, while in control tissue let-7f-1 correlated positively with both IL-8 and VEGF-A expression, suggesting that this coupling is specifically disrupted in diseased endometrium. These findings are associative rather than causal, but they identify let-7f-1 as a candidate biomarker of endometriosis that warrants further validation in larger, longitudinal, and externally replicated cohorts before any diagnostic or prognostic application can be considered.

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Author Contributions

Wahyuni Saddang: Conceptualization, Methodology, Investigation, Data Curation, Formal Analysis, Writing Original Draft, Writing Review & Editing; Nusratuddin Abdullah: Conceptualization, Supervision, Writing Review & Editing; Firdaus Hamid: Supervision, Critical Review, Writing Review & Editing; Rusdiana Bte Ladju: Supervision, Critical Review, Writing Review & Editing; Irfan Idris: Supervision, Critical Review, Writing Review & Editing; Sitti Wahyuni: Critical Review, Writing Review & Editing; Sriwijaya: Critical Review, Writing Review & Editing; Mila Maidarti: Critical Review, Writing Review & Editing.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethics Statement

The study was conducted in accordance with the Declaration of Helsinki and was approved by the appropriate institutional research ethics committee. Written informed consent was obtained from all participants prior to enrolment, and participant data were handled confidentially throughout the study.

Data Availability Statement

Data supporting the findings of this study are available from the corresponding author upon reasonable request, subject to applicable ethical and institutional restrictions.

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Figure Legends

Figure 1. Participant flow diagram, from initial recruitment (n = 66) through exclusion and allocation to the endometriosis (n = 32, subdivided by ASRM severity) and control (n = 33) groups.

Figure 2. Box-and-scatter plots of (A) let-7a-1 and (B) let-7f-1 relative expression across mild, moderate, and severe endometriosis and controls, on a symmetric log scale; horizontal lines are group medians. p-values are from the Kruskal–Wallis test.

Figure 3. Spearman correlation of let-7f-1 expression with (A) IL-8 and (B) VEGF-A expression in the control group, on a symmetric log scale.