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Differential Expression of EMT-Related Transcription Factors and Mitochondrial Dynamics Genes across Endometriosis Stages

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Abstract

Background: Endometriosis is a chronic, debilitating gynecological disorder characterized by the ectopic presence of endometrial-like tissue, often leading to pain and infertility. However, the role of mitochondrial dynamics, mitophagy, and mitochondrial-related complexes in the development of endometriosis—and their relationship with epithelial-mesenchymal transition (EMT)—remains unclear. **Objective:** This study aims to investigate the role of mitochondrial oxidative phosphorylation (OXPHOS) dysfunction and EMT in the pathogenesis and progression of endometriosis, and to explore how these molecular alterations correlate with disease severity. **Materials and Methods:** A total of 180 women with surgically confirmed endometriosis, categorized into minimal-mild (Stage I-II) and moderate-severe (Stage III-IV) groups, along with 180 healthy controls, were included. Clinical, hormonal, and demographic data were recorded. Endometrial tissue samples were analyzed using quantitative real-time PCR, flow cytometry, and phase-contrast microscopy. Gene expression profiling focused on OXPHOS components, mitochondrial dynamics (fission/fusion), mitophagy-related genes, hypoxia markers, EMT transcription factors, and mesenchymal cell surface markers. In vitro cultures of endometrial stromal cells were evaluated morphologically and molecularly to assess EMT progression. **Results:** Quantitative PCR and in vitro analyses re-

vealed that advanced endometriosis is associated with significant downregulation of OXPHOS genes (ND1, ND6, CYTB, CO2, CO3, ATP6) and mitochondrial dynamics genes (DRP1, OPA1, MFN1/2, LCLAT1), along with decreased mitophagy markers (PINK1, PARKIN) and elevated hypoxia marker HIF-1*α*. EMT activation was confirmed by morphological transitions in cultured stromal cells, reduced E-cadherin, increased N-cadherin, and elevated expression of mesenchymal and EMT-related markers (CD73, CD90, CD105, TWIST, SNAIL, SLUG). These molecular alterations were most pronounced in advanced (Stage III-IV) disease, minimally evident in Stage I-II, and not significantly different from controls. **Conclusion:** Our findings demonstrate that mitochondrial dysfunction and EMT activation are interrelated processes contributing to the severity and persistence of endometriosis. The distinct alterations in gene expression associated with mitochondrial bioenergetics and cellular plasticity in advanced disease highlight their potential as biomarkers for diagnosis, disease staging, and as targets for novel therapeutic interventions.

Keywords

Endometriosis, Mitochondrial Oxidative Phosphorylation, Epithelial to Mesenchymal Cell Transitioning

1. Introduction

Endometriosis is a chronic, estrogen-dependent inflammatory condition characterized by the presence of endometrial-like tissue outside the uterine cavity [1]. It affects approximately 10% - 15% of women of reproductive age and is frequently associated with pelvic pain, dysmenorrhea, and infertility [2]. Despite its prevalence and significant impact on quality of life, the pathogenesis of endometriosis remains incompletely understood [3].

Endometriosis is a multifactorial disorder influenced by immunological, molecular, and environmental factors that contribute to its development [4]. Current theories, including retrograde menstruation, coelomic metaplasia, and stem cell implantation, fail to fully explain the cellular and molecular dynamics underlying lesion establishment and progression. Increasing evidence suggests that a complex interplay of immune dysregulation, oxidative stress, and hormonal imbalances contributes to the pathophysiological landscape of the disease [5] [6].

Mitochondria, the powerhouses of the cell, play a central role not only in energy production via oxidative phosphorylation (OXPHOS) but also in regulating redox homeostasis, apoptosis, and cellular metabolism [7]. Recent studies suggest that mitochondrial dysfunction is a critical contributor to the pathophysiology of endometriosis. Impairments in OXPHOS compromise ATP generation, leading to bioenergetic stress and excess reactive oxygen species (ROS), which can exacerbate inflammation and tissue injury [8]. Disruptions in mitochondrial dynamics, including altered fission and fusion processes and impaired mitophagy, may fur-

ther aggravate mitochondrial damage and cellular stress [9]. These dysfunctions could not only facilitate the survival of ectopic endometrial cells but also promote their resistance to apoptosis and metabolic reprogramming [10] [11].

Pathogenic mutations in mitochondrial DNA (mtDNA), particularly those affecting genes encoding subunits of the mitochondrial respiratory chain complexes, may further exacerbate mitochondrial dysfunction. Such mutations can disrupt OXPHOS efficiency and elevate ROS production, potentially contributing to oncogenic processes. The functional impact of these mutations can be assessed by examining the expression of OXPHOS-related genes in endometrial tissues [12]. Moreover, dysregulated mitochondrial quality control mechanisms, including mitophagy, may facilitate disease progression through sustained oxidative and metabolic stress [13]. Despite these insights, the role of OXPHOS gene expression in the pathophysiology of endometriosis, particularly among Indian patients, remains inadequately characterized.

Epithelial-mesenchymal transition (EMT) is a dynamic process in which epithelial cells transform into motile and invasive mesenchymal cells such as enhanced motility and invasiveness. This transition enables individual epithelial cells to migrate, invade surrounding tissues, and facilitate metastasis through intravasation and extravasation [14] [15].

Molecularly, EMT is driven by transcription factors such as TWIST, SNAIL, and SLUG, and is characterized by a cadherin switch—the downregulation of epithelial markers like E-cadherin and the upregulation of mesenchymal markers including N-cadherin, CD73, CD90, and CD105 [16] [17]. These mesenchymal traits enhance cellular plasticity, invasiveness, and resistance to apoptosis, hallmarks of more aggressive or treatment-resistant forms of the disease.

2. Materials and Methods

2.1. Study Design

The study protocol was approved by the Institutional Ethics Committee with ethics registration number ECR/1609/Inst/TG/2021, and written informed consent was obtained from all participants prior to their enrollment in the study.

2.2. Study Population

A total of 300 women presenting with symptoms suggestive of endometriosis were initially recruited from 1st March 2023 to 31st March 2025 in the Department of Obstetrics and Gynecology, MHRT Hospital & Research Centre, Hyderabad, India as per the inclusion and exclusion criteria. Of these, 240 underwent diagnostic laparoscopy. Endometriosis was confirmed in 180 cases based on surgical and histopathological criteria. These patients were stratified into two groups: Stage I-II (minimal to mild disease, n = 90) and Stage III-IV (moderate to severe disease, n = 90) according to the revised American Society for Reproductive Medicine (rASRM) classification. Additionally, 180 healthy women without clinical or laparoscopic evidence of endometriosis were included as controls. Demographic data,

reproductive history, body mass index (BMI), and infertility duration were recorded. Serum hormone levels including FSH, LH, AMH, and estradiol were measured using standard immunoassay techniques.

2.3. Tissue Collection and Cell Culture

Cell viability of isolated cells from endometriotic tissue biopsies was evaluated using the Trypan Blue Exclusion Assay and Fluorescein Diacetate (FDA) staining. Endometrial biopsy samples were obtained from all participants during the proliferative phase of the menstrual cycle. Single-cell suspensions were prepared from the tissue samples, and cell viability was assessed using FDA staining, while total and viable cell counts were performed using a hemocytometer. Viable cells were subsequently cultured under appropriate conditions to evaluate adherence and morphological characteristics over a five-day period. Phase-contrast microscopy was employed to monitor morphological changes associated with epithelial-mesenchymal transition (EMT).

2.4. Collection of Peripheral Blood Samples

Peripheral blood samples (4 mL) were collected from all study participants, including individuals with endometriosis and healthy controls, into EDTA-coated tubes. Samples were anonymized using coded identifiers and stored at 4˚C until further processing for RNA extraction.

2.5. RNA Isolation and Quantitative Real-Time PCR

Total RNA was extracted from endometrial tissues and cultured cells using a commercial RNA isolation kit. Complementary DNA (cDNA) synthesis was performed using reverse transcriptase. Five nanograms of complementary DNA (cDNA) were used for gene expression analysis. Conventional PCR was employed for the detection of selected gene transcripts, while quantitative real-time PCR (RT-qPCR) was performed to quantify transcript levels relative to endogenous reference genes. Quantitative real-time PCR (RT-qPCR) was used to evaluate the expression of genes associated with mitochondrial oxidative phosphorylation (ND1, ND6, CO2, CO3, CYTB, ATP6, ATP8), mitochondrial dynamics (DRP1, OPA1, MFN1, MFN2, LCLAT1), mitophagy (PINK1, PARKIN), hypoxia (HIF-1α), mesenchymal markers (CD73, CD90, CD105), EMT transcription factors (TWIST, SNAIL, SLUG), and cadherins (E-cadherin, N-cadherin). Gene expression levels were quantified relative to those in control subjects, using the endogenous housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as an internal reference for normalization. Relative gene expression was calculated using the 2^−ΔΔCt method, and fold-change values were determined with the aid of StepOne™ Software (Applied Biosystems).

2.6. Flow Cytometry

Mesenchymal stem/stromal cell surface markers CD73, CD90, and CD105 were

analyzed using flow cytometry on day 5 of in vitro culture. Cells were stained with fluorochrome-conjugated antibodies and analyzed using a flow cytometer to determine the expression profiles.

2.7. Statistical Analysis

Data were analyzed using appropriate statistical software. Group comparisons were performed using one-way ANOVA followed by post hoc tests. A p-value of less than 0.05 was considered statistically significant. Results were expressed as mean ± standard error of the mean (SEM) or percentages where applicable.

3. Results

A total of 300 subjects were initially enrolled in the study. Out of these, 240 patients underwent laparoscopic examination. Endometriosis was confirmed in 180 of the laparoscopically evaluated patients. Among the confirmed cases, 90 patients were classified with Stage I-II endometriosis, representing minimal to mild disease, while the remaining 90 patients were diagnosed with Stage III-IV endometriosis, corresponding to moderate to severe disease severity (Table 1).

Table 1. Summary of subjects with surgically confirmed endometriosis.

No of subjects	No of patients undergoing laparoscopy	No of the patients confirmed diagnosis of Endometriosis
300	240	180 Stage I-II (Minimal to mild): 90 Stage III-IV (Moderate to severe): 90

The study included 180 women diagnosed with surgically confirmed endometriosis, evenly divided into minimal to mild (Stage I-II) and moderate to severe (Stage III-IV) groups, alongside 180 healthy control subjects. The mean age was notably lower in the minimal to mild group (26 ± 0.8 years) compared to the moderate to severe group (33 ± 0.4 years) and controls (35.3 ± 0.7 years). Body mass index (BMI) values were similar between the endometriosis groups (23.2 ± 0.7 and 23.5 ± 0.4, respectively), but markedly lower than in controls (35.3 ± 0.4).

Hormonal profiles showed minor variations, with follicle-stimulating hormone (FSH) levels slightly higher in the moderate to severe group (7.13 ± 0.4 mIU/mL) compared to controls (7.02 ± 0.2 mIU/mL) and the mild group (6.13 ± 0.5 mIU/mL). Anti-Müllerian hormone (AMH) levels were somewhat reduced in both endometriosis groups relative to controls. Luteinizing hormone (LH) and estradiol concentrations were comparable across all groups.

Regarding reproductive and demographic factors, the majority of participants in all groups were married. A higher proportion of women with endometriosis were nulliparous (72 and 77 in mild and severe groups, respectively) compared to

controls (13). Duration of infertility was longer in women with endometriosis, averaging 7 ± 1.8 years in the minimal to mild group and 9 ± 2.5 years in the moderate to severe group, compared to 5 ± 1.1 years in controls. Primary infertility was more prevalent than secondary infertility among women with endometriosis (Table 2).

Table 2. Demographic, hormonal, and clinical outcome measures in individuals with surgically confirmed endometriosis.

Baseline parameter	Surgical endometriosis (n = 180)		Controls (n = 180)
	Minimal to mild (n = 90)	Moderate to severe (n = 90)	
Age (mean ± SEM)	26 ± 0.8	33 ± 0.4	35.3 ± 0.7
BMI in kg/m² (median [IQR])	23.2 ± 0.7	23.5 ± 0.4	35.3 ± 0.4
FSH (mean ± SEM)	6.13 ± 0.5	7.13 ± 0.4	7.02 ± 0.2
AMH (ng/mL)	1.82 ± 3.02	1.78 ± 2.02	2.78 ± 3.02
LH (mIU/mL)	4.18± 1.11	4.56± 2.58	3.12 ± 1.72
Estradiol (pg/mL)	42.16 ±3.18	44.73 ± 5.92	40.26 ±1.62
Marital status (n)			
Single	12	16	13
Married	74	71	165
Divorced	4	3	2
Parity (n)			
0	72	77	13
1	18	13	87
Duration of infertility (years)	7 ± 1.8	9 ± 2.5	5 ± 1.1
Infertility type (n)			
Primary	65	72	NA
Secondary	25	18	NA
Total number of subjects	90	90	180

3.1. In Vitro Culture of Cells Isolated from Different Forms of Endometrial Tissue Biopsies

Single cells were isolated from endometrial tissue biopsy samples and assessed for viability using fluorescein diacetate (FDA) staining, which selectively labels live cells with intact membranes. Following confirmation of viability, the cells were cultured under appropriate in vitro conditions to support adhesion and proliferation. Over time, the adherent cells exhibited morphological transitions from a spherical to a spindle-shaped phenotype, indicative of epithelial-mesenchymal transition (EMT). These morphological changes provided the basis for subsequent investigations into EMT at both the cellular and molecular levels (Figure 1).

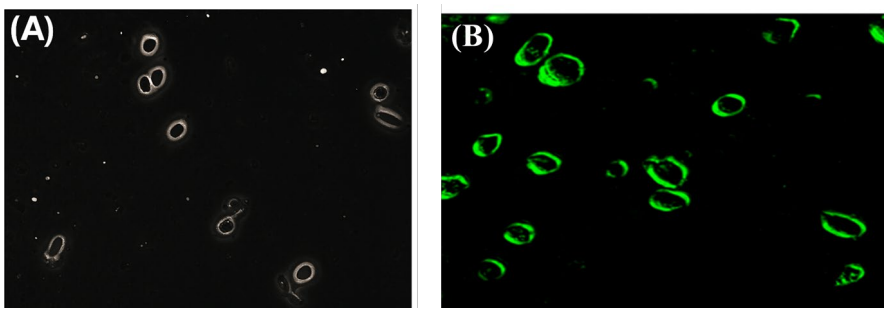

Figure 1. Assessment of membrane integrity. Panel (A) shows unstained cells, while Panel (B) shows cells stained with fluorescein diacetate (FDA), indicating viable cells with intact membranes. Images were captured at 40× magnification. Scale bar = 20 μm.

3.2. Identification of EMT-Related Morphological Alterations in Endometrial Biopsies

Morphological evaluation was performed using phase-contrast microscopy from day 0 to day 5 of in vitro culture. Initially, cells from both mild and severe endometriosis samples exhibited predominantly spherical, epithelial-like morphology. As the culture progressed, a gradual transition toward an elongated, spindle-shaped phenotype—characteristic of mesenchymal cells—was observed, indicating epithelial-mesenchymal transition (EMT). Notably, by day 5, cultures derived from severe endometriosis biopsies showed a visibly higher population of mesenchymal-like cells compared to those from mild cases. These observations suggest a more pronounced EMT progression in cells derived from severe forms of endometriosis. Scale bars = 100 μm (Figure 2).

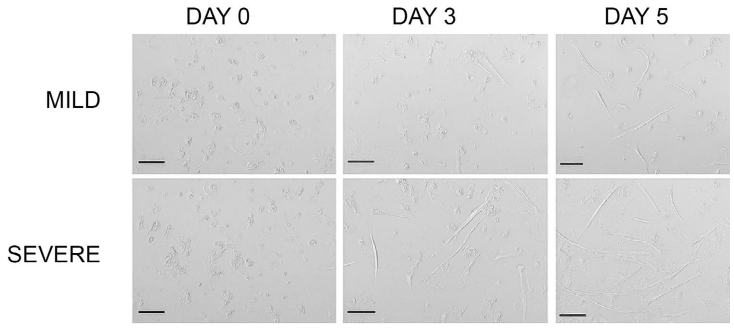

Figure 2. Morphological characterization of cells isolated from mild and severe endometrial tissue biopsies. Representative images show differences in cellular morphology between the two conditions. Images were captured at 10× magnification. Scale bar = 20 μm.

3.3. Quantification of OXPHOS Gene Expression in Endometrial Biopsies

Quantitative PCR analysis (Figure 3) revealed a marked downregulation of genes associated with oxidative phosphorylation (OXPHOS) in endometrial biopsies from women with severe endometriosis compared to both healthy controls and those with mild disease ($p < 0.0001$). In contrast, the expression levels of these genes showed no statistically significant differences between the control and mild

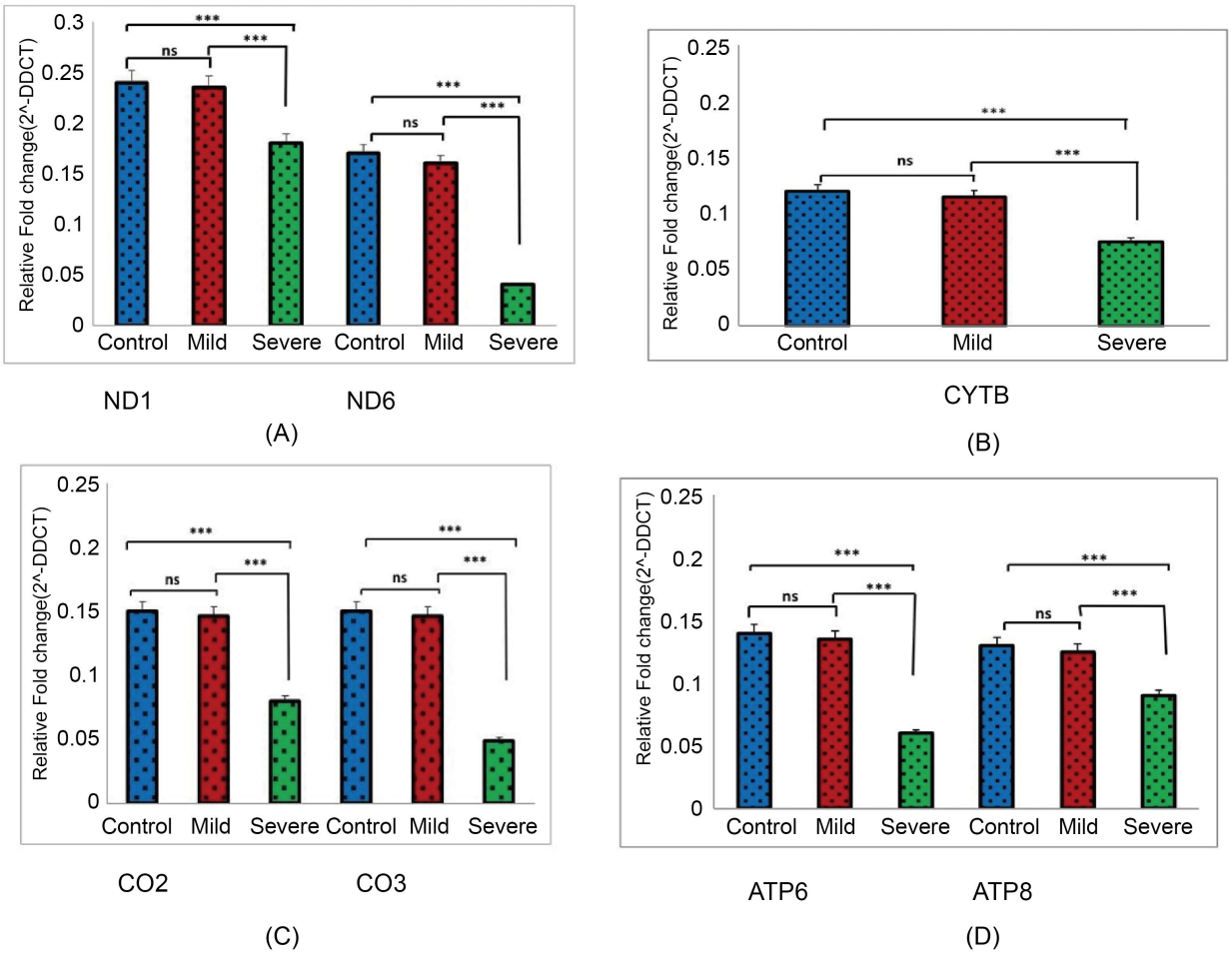

Figure 3. Relative expression of OXPHOS genes in endometrial biopsies. Gene expression levels were assessed in samples from healthy controls, women with mild endometriosis, and women with severe endometriosis. (A) Complex I (ND1 and ND6); (B) Complex III (CYT-B); (C) Complex IV (CO2 and CO3); (D) Complex V (ATP6 and ATP8). Note: (***$p < 0.0001$) represents significance, ns*-non significant.

endometriosis groups, suggesting that substantial alterations in mitochondrial gene expression occur predominantly in advanced stages of the condition.

The genes analyzed are central to mitochondrial bioenergetics. ND1 and ND6, components of Complex I, initiate the electron transport process. CYTB, part of Complex III, facilitates electron transfer from ubiquinol to cytochrome. CO2 and CO3, subunits of Complex IV, are involved in the terminal reduction of oxygen to water. Finally, ATP6 and ATP8, constituents of Complex V (ATP synthase), are directly responsible for ATP generation.

The consistent downregulation of these genes in severe endometriosis points to widespread mitochondrial impairment. This dysfunction likely compromises ATP production, leading to energy deficits in endometrial cells and potentially contributing to elevated oxidative stress. Such disturbances in mitochondrial homeostasis may play a critical role in disease pathophysiology, exacerbating tissue damage and promoting disease progression.

The absence of significant changes in OXPHOS gene expression between control and mild cases suggests that mitochondrial dysfunction may be a hallmark of more advanced disease stages. This pattern of expression could serve as a molecular indicator of disease severity and offers insight into potential therapeutic targets aimed at improving mitochondrial function and cellular energy metabolism in endometriosis (**Figure 3**).

3.4. Analysis of Mitochondrial Gene Expression in Fission and Fusion Genes

Mitochondrial dysfunction appears to intensify with the progression of endometriosis. In this study, the expression levels of key genes involved in mitochondrial dynamics and energy homeostasis—DRP1, OPA1, MFN1, MFN2, and LCLAT1—were evaluated. These genes play critical roles in regulating mitochondrial fission, fusion, and lipid remodeling. A significant downregulation, particularly in severe stages of the disease, suggests impaired mitochondrial integrity and function.

In the mild stage of endometriosis, the expression profiles of these genes remain relatively stable and comparable to those of healthy controls, indicating that mitochondrial function is largely preserved. However, as the disease advances, a consistent and significant reduction in gene expression is observed. This decline likely disrupts mitochondrial morphology and bioenergetics, leading to reduced ATP synthesis, elevated reactive oxygen species (ROS) production, and increased oxidative stress—all of which contribute to cellular dysfunction and tissue damage.

The observed correlation between endometriosis severity and decreased expression of mitochondrial-related genes supports the notion that mitochondrial bioenergetic failure is a pivotal factor in disease pathophysiology. These findings not only provide insight into the molecular mechanisms underlying endometriosis progression but also highlight the potential of mitochondrial dynamics-associated genes as biomarkers for disease severity and as therapeutic targets for restoring mitochondrial function and tissue homeostasis (**Figure 4**).

3.5. Relative Expression of Mitophagy Genes PINK1 *and* PARKIN in Endometrial Tissues

The expression of mitophagy-related genes PINK1 and PARKIN was evaluated in endometrial tissues from control, mild, and severe endometriosis groups. While no significant differences were observed between control and mild cases, a marked downregulation of both genes was evident in severe endometriosis ($p < 0.0001$).

PINK1 and PARKIN are essential for mitochondrial quality control, initiating the removal of damaged mitochondria via mitophagy. Their reduced expression in severe disease suggests impaired mitophagic activity, potentially leading to mitochondrial dysfunction, oxidative stress, and inflammation—key features of advanced endometriosis. These findings highlight PINK1 and PARKIN as potential biomarkers of disease severity and therapeutic targets to restore mitochondrial homeostasis (**Figure 5**).

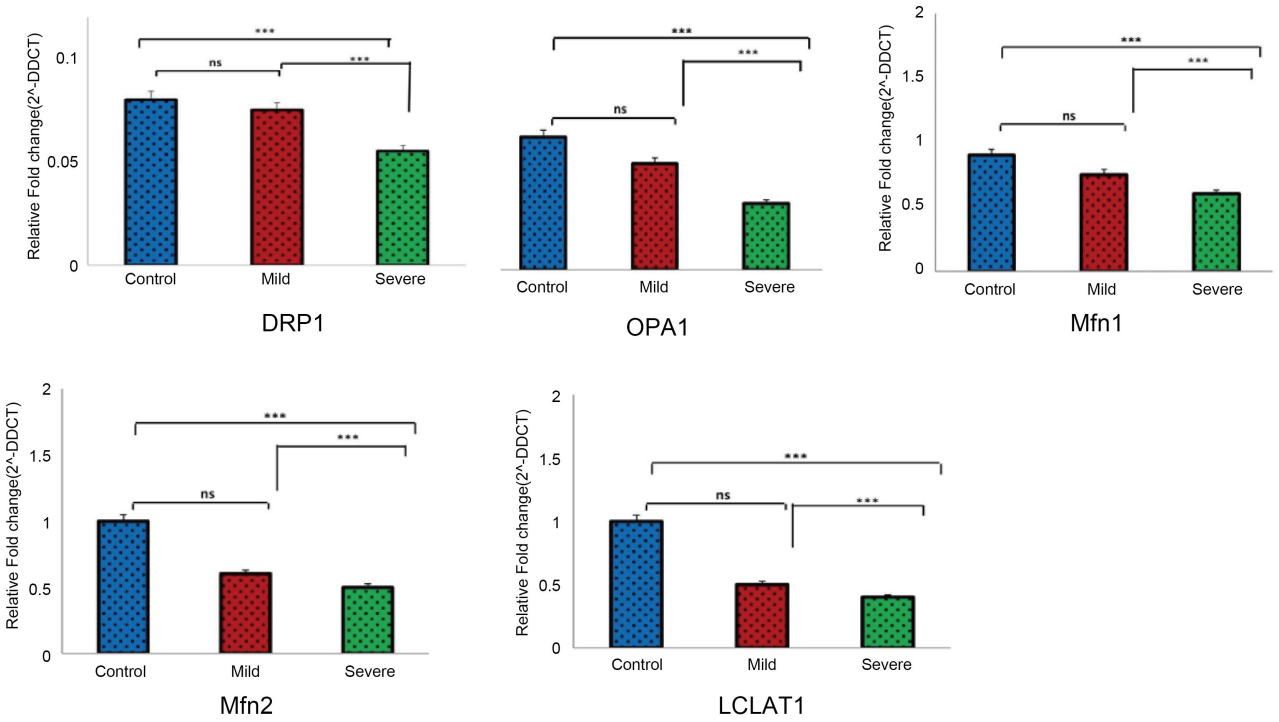

Figure 4. Relative expression of the *fission and fusion* gene in endometrial biopsies. Gene expression was quantified in endometrial tissues from healthy controls, and from women with mild and severe endometriosis. Note: (***p < 0.0001) represents significance, ns*-non significant.

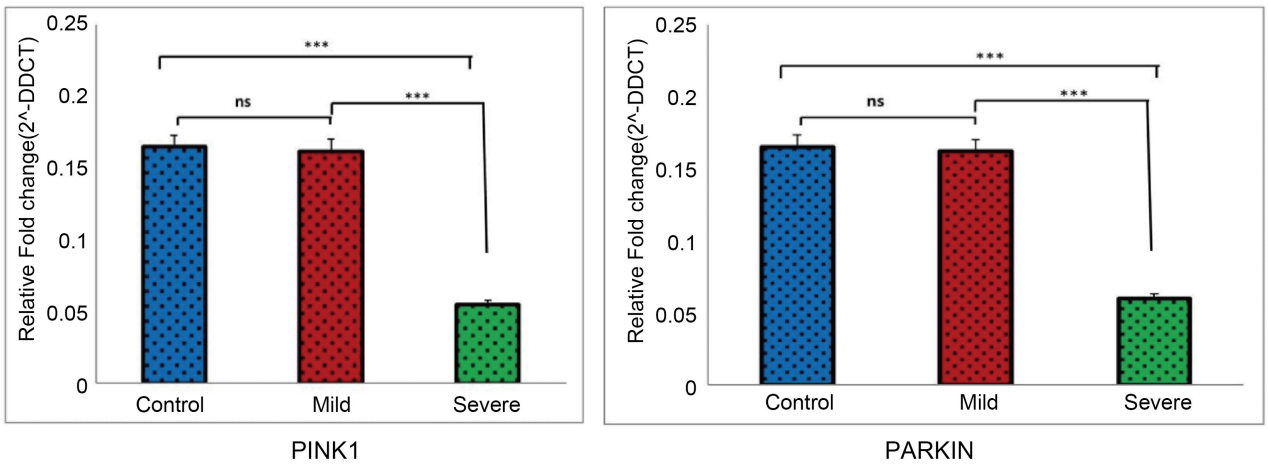

Figure 5. Relative expression of mitophagy-related genes in endometrial biopsies. Expression levels were measured in samples from healthy controls, and from women with mild and severe endometriosis. (A) PINK1; (B) PARKIN. Note: (***p < 0.0001) represents significance, ns*-non significant.

3.6. Elevated Expression of HIF-1*α* in Severe Endometriosis

The expression of HIF-1α was evaluated in endometrial biopsies across control, mild, and severe endometriosis groups. No significant difference was observed between control and mild groups, suggesting minimal hypoxic signaling in early stages. However, a significant increase in HIF-1α expression was detected in severe endometriosis ($p < 0.0001$), indicating activation of hypoxia-associated path-

ways in advanced disease.

HIF-1*α*, a key transcription factor in cellular response to low oxygen, promotes genes involved in angiogenesis, inflammation, and metabolic adaptation. Its upregulation in severe cases suggests a hypoxic microenvironment contributing to disease progression, including abnormal vascularization and fibrosis. These findings underscore the relevance of HIF-1α as a potential biomarker of disease severity and a therapeutic target in advanced endometriosis (Figure 6).

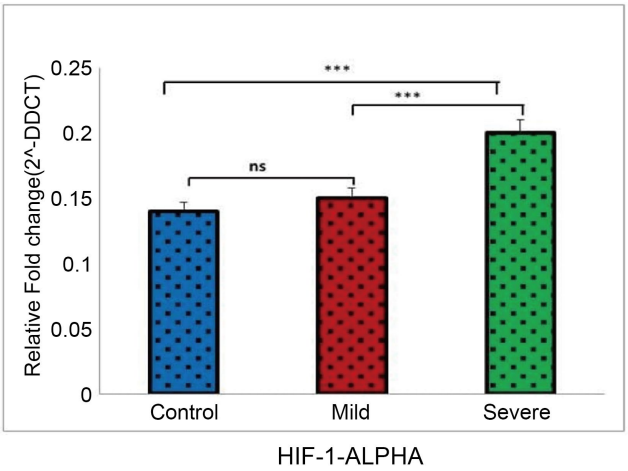

Figure 6. Relative expression of *HIF1α* in endometrial biopsies. *HIF1α* gene expression was quantified in endometrial tissues from healthy controls, and from women with mild and severe endometriosis. Note: (***p < 0.0001) represents significance, ns*-non significant.

3.7. Expression of Mesenchymal Markers CD73, CD90, and CD105 in Endometrial Stromal Cells

CD73, CD90, and CD105 are established markers of mesenchymal stem/stromal cells (MSCs). Flow cytometry revealed a marked increase in their expression in stromal cells from severe endometriosis compared to mild cases, indicating a higher proportion of MSC-like cells in advanced disease.

This enrichment suggests a phenotypic shift toward a mesenchymal state, potentially driven by epithelial-to-mesenchymal transition (EMT), contributing to increased cellular plasticity, fibrosis, and invasiveness. The elevated expression of these markers reflects enhanced regenerative and invasive capabilities, supporting lesion persistence and progression.

Overall, the data highlight a significant mesenchymal transformation in severe endometriosis, underscoring the role of MSC-like stromal cells in disease pathogenesis and their potential as therapeutic targets (Figure 7).

3.8. Upregulation of CD73, CD90, and CD105 Indicates EMT Progression in Severe Endometriosis

Quantitative RT-PCR analysis was conducted on day 5 of in vitro cultures to assess the expression of key mesenchymal markers—CD73, CD90, and CD105—across

control, mild, and severe endometriosis samples. As shown in Figure 8, expression levels of all three markers were markedly elevated in stromal cells from severe endometriosis cases compared to both mild forms and healthy controls ($p < 0.0001$). A modest but statistically significant increase was also observed in mild cases relative to controls ($p < 0.01$).

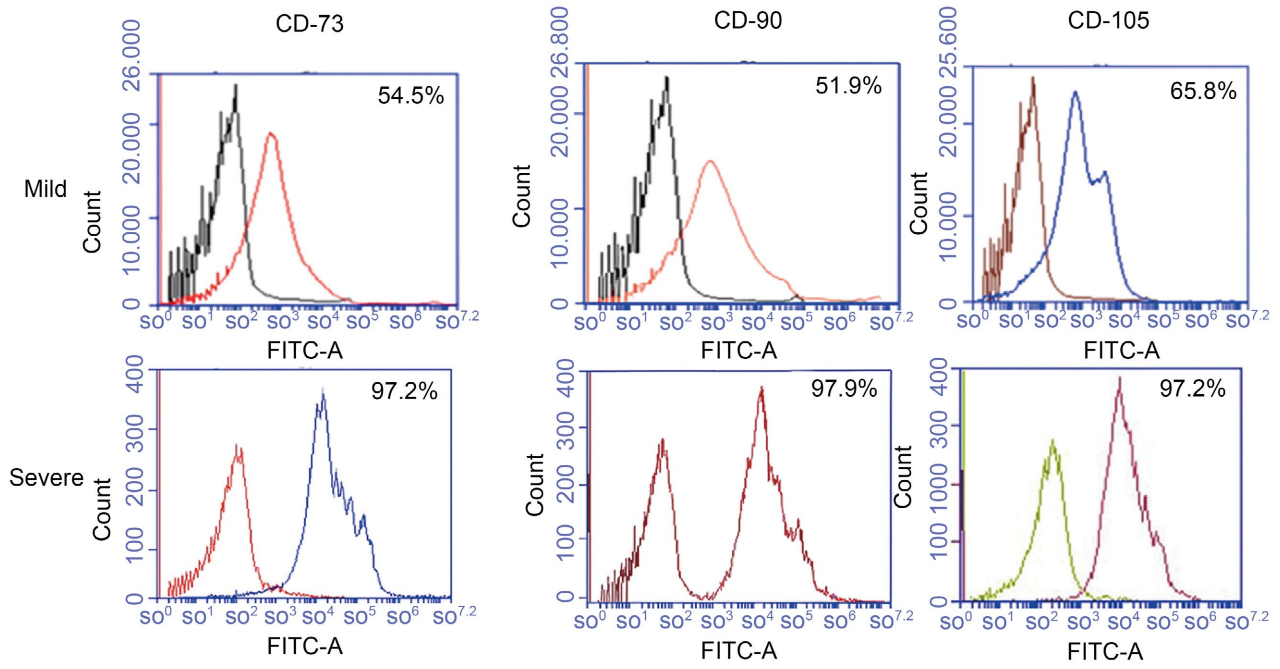

Figure 7. Immunophenotypic characterization of mesenchymal stem cells (MSCs) isolated from endometrial biopsies. Flow cytometric analysis of MSCs derived from women with mild and severe endometriosis, performed on day 5 of in vitro culture.

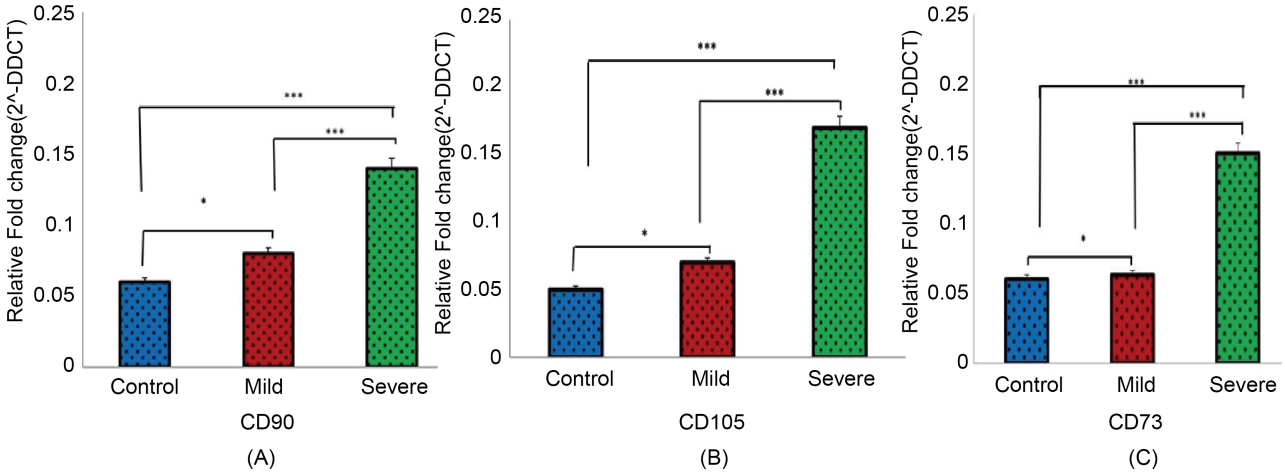

Figure 8. Expression of mesenchymal stem cell-specific genes in cultured endometrial MSCs. Relative mRNA levels of CD90 (A), CD105 (B), and CD73 (C) were assessed by real-time quantitative PCR (RT-qPCR) on day 5 of in vitro culture. MSCs were isolated from endometrial biopsies of healthy controls, and women with mild and severe endometriosis. Note: (***$p < 0.0001$) represents significance, ns*-non significant.

This progressive upregulation of mesenchymal markers indicates a phenotypic

shift toward a mesenchymal state, suggesting a heightened epithelial-to-mesenchymal transition (EMT) in severe endometriotic conditions. These findings reinforce the morphological observations and support the notion of enhanced EMT activity as a hallmark of disease severity in endometriosis (Figure 8).

3.9. Reduced E-Cadherin and Elevated N-Cadherin Facilitate EMT

Quantitative real-time PCR analysis demonstrated a marked downregulation of E-cadherin and a significant upregulation of N-cadherin in endometrial samples from individuals with severe endometriosis when compared to both mild and control groups (***$p < 0.0001$). No statistically significant difference in expression was detected between the control and mild groups for either gene. This pattern reflects a cadherin switch, a key feature of the epithelial-mesenchymal transition (EMT), and underscores the enhanced activation of EMT in more advanced stages of endometriosis, implicating its contribution to disease progression (Figure 9).

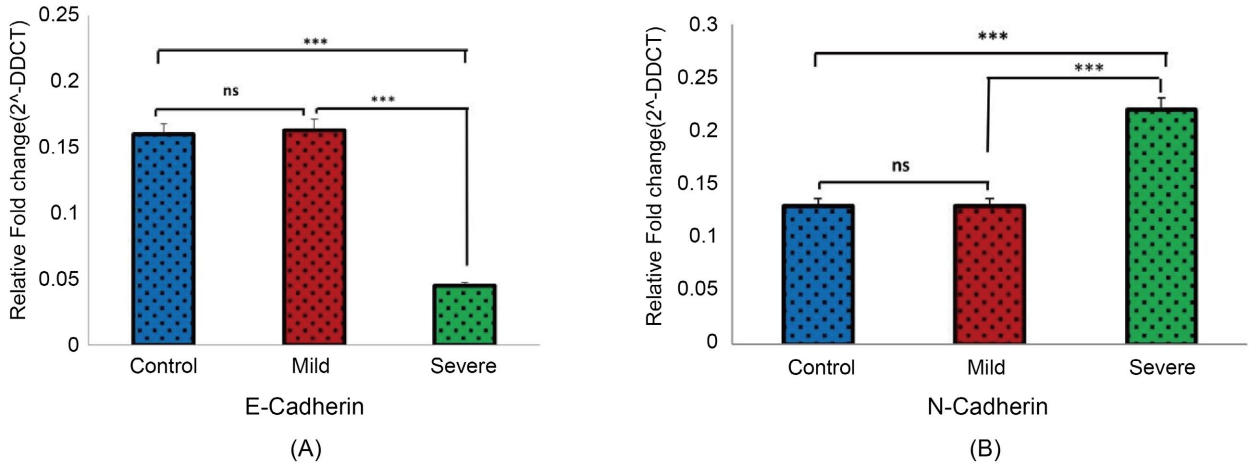

Figure 9. Expression analysis of epithelial-mesenchymal transition (EMT) markers in endometrial biopsies. Relative mRNA levels of *E-cadherin* (A) and *N-cadherin* (B) were quantified in endometrial tissues from healthy controls, and from women with mild and severe endometriosis to assess EMT progression. Note: (***$p < 0.0001$) represents significance, ns*-non significant.

3.10. Stage-Dependent Upregulation of TWIST, SNAIL, and SLUG Highlights EMT Activation in Severe Endometriosis

TWIST, SNAIL, and SLUG are critical transcription factors that suppress E-cadherin expression and drive the epithelial-mesenchymal transition (EMT). Quantitative real-time PCR analysis showed a significant increase in the expression of these EMT-inducing factors in severe endometrial samples compared to both mild and control groups (***$p < 0.0001$). In contrast, no significant difference in expression was observed between the mild and control groups.

These results indicate a disease severity-dependent elevation in TWIST, SNAIL, and SLUG levels, suggesting their enhanced role in the molecular events underlying EMT activation during advanced endometriosis. When considered alongside the reduced E-cadherin expression, this pattern strongly supports the contribution of EMT-related transcriptional regulation to the progression and invasive-

ness of severe endometrial lesions (Figure 10).

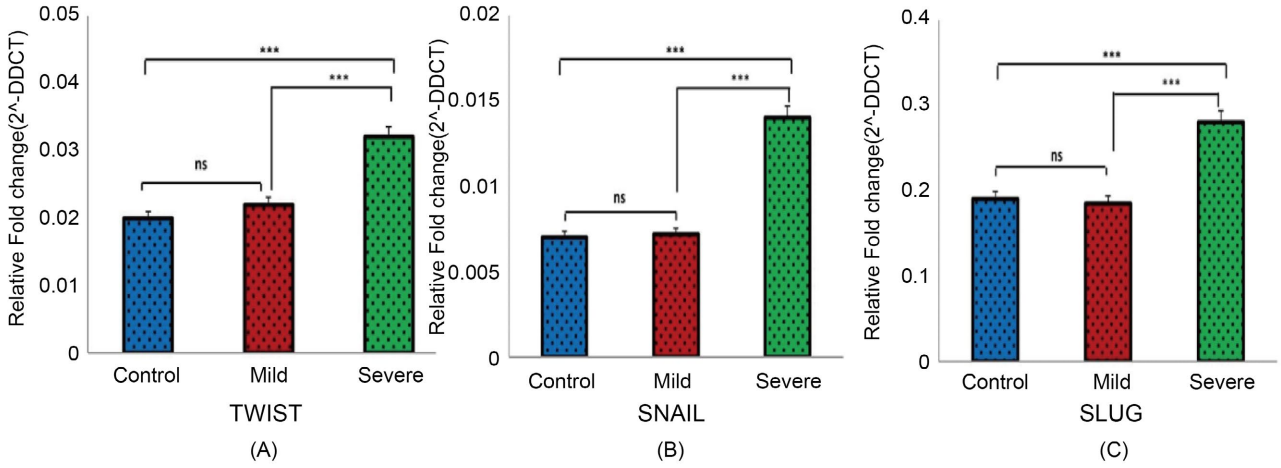

Figure 10. Relative expression of EMT-associated transcription factors in endometrial biopsies. Bar graphs show mRNA levels of TWIST (A), SNAIL (B), and SLUG (C) in tissues from healthy controls, women with mild endometriosis, and women with severe endometriosis. Note: (***$p < 0.0001$) represents significance, ns*-non significant.

4. Discussion

The present study highlights the critical role of mitochondrial dysfunction and epithelial-to-mesenchymal transition (EMT) in the progression of endometriosis. By evaluating molecular and cellular markers in control, mild, and severe disease groups, we provide evidence that impaired mitochondrial bioenergetics and increased mesenchymal characteristics are key features of advanced pathology.

Importantly, the control group consisted of 180 healthy women without clinical symptoms suggestive of endometriosis, recruited from the same catchment population as patients. All control participants underwent thorough gynecological evaluation and pelvic ultrasonography to exclude pelvic pathology. Women with any suspicion of endometriosis or other gynecological disorders were excluded. As part of ethical and methodological rigor, controls also had no prior surgical diagnosis of endometriosis, no infertility history linked to pelvic pathology, and normal hormonal profiles. This ensured that the comparison group truly represented endometriosis-free individuals, thereby strengthening the validity of observed molecular differences.

Quantitative analysis of oxidative phosphorylation (OXPHOS) genes revealed marked mitochondrial respiratory impairment in severe endometriosis [18], with significant downregulation of ND1, ND6, CYTB, CO2, CO3, ATP6, and ATP8. This disruption indicates reduced electron transport chain activity and ATP synthesis, changes largely absent in mild cases, suggesting mitochondrial dysfunction predominantly arises in late-stage disease and may serve as a biomarker of progression [19] [20]. Reduced ATP levels likely disturb cellular homeostasis, promoting chronic inflammation and abnormal cell survival.

Similarly, genes regulating mitochondrial dynamics—DRP1, OPA1, MFN1,

MFN2, and LCLAT1—were markedly decreased in advanced disease, indicating disrupted fission, fusion, and lipid remodeling. These impairments threaten mitochondrial integrity and exacerbate metabolic stress [21]-[24]. The parallel downregulation of mitophagy regulators PINK1 and PARKIN further suggests defective clearance of damaged mitochondria, potentially leading to excessive ROS accumulation and persistent inflammation, consistent with previous reports.

The observed upregulation of HIF-1α in severe cases indicates a hypoxic microenvironment within endometriotic lesions, which may drive angiogenesis, fibrosis, and metabolic reprogramming—hallmarks of invasive disease.

In vitro, stromal cells from patients displayed progressive EMT, evident both morphologically and at the molecular level. Severe disease was characterized by elevated mesenchymal stem cell markers (CD73, CD90, CD105) and EMT transcription factors (TWIST, SNAIL, SLUG), coupled with a cadherin switch—decreased E-cadherin and increased N-cadherin —indicative of enhanced invasiveness, loss of cell-cell adhesion, and increased migratory potential [25]-[28].

Collectively, these findings support a central hypothesis: advanced endometriosis involves a synergistic interplay between mitochondrial impairment and EMT activation. Mitochondrial stress may trigger EMT programs, while mesenchymal transition can further compromise mitochondrial efficiency, creating a feedback loop that sustains lesion persistence and promotes tissue remodeling.

In conclusion, mitochondrial bioenergetic failure and EMT emerge as interrelated drivers of severe endometriosis. The identified molecular signatures show promise as diagnostic markers and therapeutic targets. Strategies aimed at restoring mitochondrial function or inhibiting EMT could offer novel approaches for managing progressive, treatment-resistant disease.

5. Conclusion

Our study underscores the central role of mitochondrial dysfunction and EMT in the pathophysiology of advanced endometriosis. These processes are tightly interlinked, and their dysregulation may drive the transition from early-stage to severe disease. The identified molecular markers could serve as valuable tools for diagnosis, prognosis, and targeted intervention, opening new avenues for personalized treatment approaches in endometriosis management.

Consent to Publication

Consent to publication was obtained from all those included in the study.

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Authors' Contributions

RR designed the study; RR and WT conducted the laboratory work; AAK, WT, TNA, AGM, and VAA wrote the manuscript. AAK and WT performed the statis-

tical analysis. All authors reviewed and approved the final manuscript.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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