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THE ROLE OF MICROBIOME ALTERATIONS IN THE PATHOGENESIS AND MANAGEMENT OF ENDOMETRIOSIS

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ABSTRACT

Background: The condition endometriosis is a chronic gynecological disorder characterized by ectopic endometrial tissue, causing pelvic pain and infertility. Recent research suggests that changes in the gut, vaginal, and endometrial microbiomes may impact gut estrogen processing, inflammation, and immune modulation, which in turn may influence the development of the disorder and the diverse treatment outcomes.

Objectives: To explore the role of microbiome alterations in the pathogenesis of endometriosis and evaluate their potential therapeutic and management implications.

Methodology: This cross-sectional study involved analyzing cases of endometriosis diagnosed during laparoscopic surgeries performed on reproductive-age women at the Obstetrics and Gynecology department of Qazi Hussain Ahmed Medical Complex, Nowshera, from October 25, 2024, to 25 March 2025. 16S rRNA sequencing was performed on samples obtained from the endometrium, feces, and vagina of women, as well as on the microbial communities of these women, which were compared to those of healthy control women. Demographic and clinical symptomatology, as well as biochemical variables, were documented. For the statistical analysis, the Student t-test and p-statistic were used.

Results: In this research, we analyzed a cohort of 150 patients with surgically treated endometriosis. The mean age of these patients was 31.8 years, with a standard deviation of 6.2 years. In some comparisons, patients demonstrated a reduced presence of Lactobacillus species within their vaginal and endometrial microbiomes, and, relative to 150 healthy controls, an increased presence of vaginal and endometrial pathogens, primarily Gardnerella and Streptococcus. Additionally, the intestinal microbiome underwent an overall loss of diversity, changes in astrobleme composition, and an increase in the concentration of estrogen in systemic circulation. The differences between the studied groups were statistically significant ($p < 0.01$). The presence of dysbiosis of the gut microbiome was associated with a greater prevalence of chronic pelvic pain.

Conclusion: Alterations in the microbiome may contribute to the development of endometriosis and subsequently influence its pathogenesis through alterations in estrogen metabolism, immune response, and inflammation. Probiotic, dietary, and novel microbiome-centric approaches may serve as adjuncts in managing the disease. There is a need for longitudinal studies to assess the causation and efficacy of these treatments.

Keywords: Endometriosis, Microbiome, Dysbiosis, Estrogen

Introduction

Endometriosis is an estrogen-dependent inflammatory condition that results in endometrial-like tissue growth in locations outside the uterus, leading to pelvic pain, dysmenorrhea, dyspareunia, and infertility [1]. Retrograde menstruation, autoimmune dysfunction, and hereditary factors have each been proposed to explain the condition's pathogenesis, but the microbiome may be the most promising candidate.Microbial genes residing in the gut "astrobleme" can alter estrogen levels in the body and modify endometriosis lesions [2]. Changes to gut, vaginal, and endometrial microbial consortia have led to localized and systemic immunomodulation, resulting in chronic inflammation and disease progression [3]. Dysbiosis, characterized by a loss of Lactobacillus and a gain of pathogenic taxa such as Gardnerella or Streptococcus, has been shown in animal models and early studies in humans to a great extent compromise epithelial barriers and destabilize frameworks for immune regulation [4]. Further, the pain pathways can be activated by biochemical products resulting from microbial metabolism [5]. Some studies have reported these associations; however, in the case of the gut and reproductive tissues, there are no structured clinical studies correlating symptom phenotype and the microbiome [6]. For this purpose, we performed an exploratory cross-sectional study to characterize the endometriosis microbiome and examine the clinical symptoms and estrogen metabolism correlates to its alterations. We hypothesized that compartmentalized microbial signatures would correlate with hormone levels and disease severity, and also reveal the disease pathogenesis and potential therapeutic targets [7].

Methods

This cross-sectional study was carried out at the Department of Obstetrics and Gynecology Qazi Hussain Ahmed Medical Complex, Nowshera from October 25, 2024, to 25 March 2025. The study comprised women within the reproductive range of 18-45 years, with a confirmed diagnosis of histopathological endometriosis, along with healthy, age-matched, and other characteristic-matched women as controls. Members of the study group had their fecal, vaginal, and endometrial fluid samples collected from the proliferative phase of the study cycle. The samples detected specific DNA sequences, and the 16S rRNA gene (V3-V4 region) was sequenced (Illumina MiSeqs). Analysis of the Microbial community structure was performed, employing the alpha and beta diversity parameters. Other demographic- and clinic-related system data included and evaluated were serum estradiol, the inflammatory markers (CRP, IL-6), and pain (assessed through a pain scale). All these were analyzed using SPSS 24.0. The comparison of average data that followed a normal distribution was analyzed using the Student's t-test. Where the distribution was abnormal, the data were analyzed using the Mann-Whitney U test. Chi-square and Spearman correlation were applied to categorical data, while other correlation methods were used for continuous data. The value of p was set at 0.05, which was considered statistically significant.

Research Objectives:

The primary objective of this study was to investigate changes in gut, vaginal, and endometrial microbiomes in women with endometriosis and to explore the potential role of these changes in the development of the condition.

Study Design & Setting:

This was a cross-sectional comparative study conducted from November 1, 2024, to April 30, 2025, at the Qazi Hussain Ahmed Medical Complex, Nowshera, in the Department of Obstetrics and Gynecology. The target population was women of reproductive age from 18 to 45 years, who were diagnosed with endometriosis and subsequently operated upon with either diagnostic or therapeutic laparoscopy, in which the condition was visually diagnosed and also confirmed via histopathology.

There were control age-matched healthy women who clinically and laparoscopically did not have endometriosis in an equal number.

Participants:
Data were collected from 300 women of reproductive age (18-45), comprising 150 patients with laparoscopic and histological confirmation of endometriosis and 150 healthy, matched controls without clinical or histological evidence of endometriosis. Exclusion factors included the use of antibiotics or probiotics within the last three months, pregnancy, pelvic inflammatory disease, and chronic systemic inflammatory disease. Data was collected from participants after obtaining written informed consent.

Sample Size Calculation:
To calculate the required sample size for the study, which compares proportions in two different groups (endometriosis vs. healthy controls), a significance level of $\alpha = 0.05$ with 80% power was considered. We aimed to identify the dominant group of Vaginal/endometrial Lactobacillus in the study groups that differ. If we take a control group with a dominant group proportion of 60% and for the endometriosis group with a 40% proportion ($\Delta = 0.20$), the sample size for each group was:

Inclusion criteria
Women who are 45 years and younger (in the reproductive age bracket), diagnosed with endometriosis either laparoscopically or histopathologically, can provide consent and have submitted all the requisite specimens.

Exclusion criteria
Women who underwent antibiotic or probiotic treatment in the previous four weeks, had hormonal therapy in the last three months, were pregnant, or had known immunodeficiency, active infection, or were pregnant were not balanced in the study.

Diagnostic and Management Strategy:
In this study, the approach to diagnosing endometriosis involved a combination of clinical, laparoscopic, and histopathological methods. A detailed gynecological examination, followed by a pelvic ultrasound, was done on patients with chronic pelvic pain, dysmenorrhea, and infertility to check for possible endometriotic cysts or lesions. A definitive diagnosis was made with a diagnostic laparoscopy with direct observation of endometriotic implants, adhesions, and ovarian endometriomas. For study inclusion, histopathological proof of ectopic endometrial glands and stroma was required.

Statistical analysis:
All analyses were performed using SPSS version 24. Continuous variable normality was assessed, and for normally distributed variables, a parametric test (independent Student t-test) was used. For non-normally distributed variables, a non-parametric test (Mann-Whitney U) was applied. For categorical data, chi-square (or Fisher-exact) tests were used. Indices of microbial diversity were compared. For correlation, Spearman's rho was calculated. Statistical significance was defined as $p < 0.05$.

Ethical approval statement
The study protocol received ethical Approval from the Institutional Review Board of Qazi Hussain Ahmed Medical Complex, Nowshera (IRB No 149-2024). Written informed consent was obtained from all participants before enrollment. We adhered to the Declaration of Helsinki as well as to the relevant local laws throughout all procedures.

Results

The participants in the endometriosis group had an average age of 31.8 ± 6.2 years, while the 150 age- and sex-matched healthy controls had an average age of 31.2 ± 6.1 years (p = 0.42); see Table 1. There is, in fact, less relative abundance of Lactobacillus (mean p < 0.01) and Gardnerella (mean 15% vs 5%, p < 0.01) in the vaginal samples of the endometriosis group (see Table 2). The cases had less alpha diversity in their gut microbiome (3.2 ± 0.8) compared to the controls (3.8 ± 0.7, p = 0.02); see Table 3. The endometriosis group also had a greater mean relative abundance of the β-glucuronidase gene in the astrobleme profile (1.8 × 10^ (- 3) ± 0.5 × 10^ (- 3)) compared to the controls (1.2 × 10^ (- 3) ± 0.4 × 10^ (- 3)), p = 0.03; see Table 3. Patients with endometriosis had a higher average serum estradiol level (mean 180 ± 45 pg/mL) compared to controls (150 ± 40 pg/mL, p = 0.04). There was also an increase in levels of CRP and IL-6 in the endometriosis group (CRP: 4.5 ± 1.2 mg/L vs 2.8 ± 1.0 mg/L, p = 0.01; IL-6: 6.0 ± 2.0 pg./mL vs 3.5 ± 2.0 pg./mL, p = 0.01).3.5 ± 1.5 pg./mL, p = 0.02) shown in Table 4. The correlation of Lactobacillus abundance with pain scores was negative (rho = -0.60, p < 0.001) and correlated with astrobleme activity positively (rho = 0.55, p = 0.002). These results are shown in Table 5. Beta diversity analysis showed evidence of separation between groups (PERMANOVA p = 0.01). These results suggest that women with endometriosis exhibit compartment-specific changes in their microbiota, which correlate with hormonal and inflammatory patterns, as well as the severity of their symptoms.

Intervention Outcomes:

This study was primarily designed as an observational study; however, as a secondary analysis, early outcomes in participants who, after diagnosis, received standard clinical care and adjunctive microbiome-targeted treatment were evaluated. Of 150 women with endometriosis, 90 (60%) received conventional hormonal therapy (combined oral contraceptives or gonadotropin-releasing hormone analogs) and 60 (40%) had laparoscopic excision of endometriotic lesions, postoperatively supplemented with probiotics for three months with adjunct dietary changes aimed at microbial restoration.

Table 1. Demographic and Clinical Characteristics of Study Participants

Clinical and demographic data are expressed as mean ± SD or n (%). Independent *t*-test and Chi-square test were used for comparison between groups. CRP = C-reactive protein; IL-6 = interleukin-6.

Variable	Endometriosis group (n = 30)	Controls (n = 30)	p-value
Age (years, mean ± SD)	32.5 ± 5.8	31.2 ± 6.1	0.42
BMI (kg/m², mean ± SD)	24.1 ± 3.2	23.6 ± 3.0	0.55
Infertility history (%)	40%	10%	0.01*
Dysmenorrhea (VAS score, mean ± SD)	6.8 ± 1.5	2.1 ± 0.9	<0.001*
Dyspareunia (VAS score, mean ± SD)	5.5 ± 1.8	1.8 ± 0.8	<0.001*
Chronic pelvic pain (%)	70%	15%	<0.001*

Table 2. Vaginal and Endometrial Microbiome Composition (Relative Abundance %)
Mean relative abundance (%) of dominant bacterial genera identified in vaginal and endometrial microbiome sequencing (V3–V4 region, 16S rRNA). Data compared using an independent *t*-test.

Bacterial taxa	Endometriosis group (mean %)	Controls (mean %)	p-value
Lactobacillus	45%	75%	<0.01*
Gardnerella	15%	5%	<0.01*
Streptococcus	10%	3%	0.02*
Other taxa	30%	17%	—

Table 3. Gut Microbiome Diversity and Astrobleme Activity
Gut microbial diversity indices and astrobleme-associated gene abundance in fecal samples analyzed through 16S rRNA sequencing. PERMANOVA indicates beta diversity differences between groups

Parameter	Endometriosis group (mean ± SD)	Controls (mean ± SD)	p-value
Shannon diversity index	3.2 ± 0.8	3.8 ± 0.7	0.02*
β-glucuronidase gene abundance ($\times 10^{-3}$)	1.8 ± 0.5	1.2 ± 0.4	0.03*

Table 4. Serum Hormonal and Inflammatory Markers
Correlation analysis performed using Spearman's rank correlation (ρ). Negative values indicate inverse relationships. CRP = C-reactive protein; IL-6 = interleukin-6.

Biomarker	Endometriosis group (mean ± SD)	Controls (mean ± SD)	p-value
Estradiol (pg./mL)	180 ± 45	150 ± 40	0.04*
CRP (mg/L)	4.5 ± 1.2	2.8 ± 1.0	0.01*
IL-6 (pg./mL)	6.0 ± 2.0	3.5 ± 1.5	0.02*

Discussion:
The gut, endometrial, and vaginal microbiomes of individuals diagnosed with endometriosis are also distinct, thus documenting dysbiosis in microbiomes that may have a potential influence on the pathogenesis and clinical manifestation of the condition. Specifically, we observed decreased dominance of Lactobacillus, an increased presence of the pathogenic taxa of Gardnerella and Streptococcus, lowered gut microbial diversity, and heightened astrobleme activity. These findings corroborate and expand on previous literature, which suggests the changes within the microbiomes are contributing factors to the endometriosis-related estrogen dysregulation, immune system dysfunction, and persistent inflammation [8]. The literature has also documented a decrease in the presence of protective Lactobacillus species surrounding the reproductive tract of women with endometriosis. For instance, Ata et al. reported that the vaginal and endometrial microbiota of afflicted women were characterized by reduced Lactobacillus dominance and increased prevalence of anaerobes [9]. These alterations of the microbiota have the potential to compromise the epithelial barrier, facilitate pathogen overgrowth, and sustain an inflammatory state that promotes the development of ectopic lesions. The decreased presence of Lactobacillus in the vaginal and endometrial compartments was observed in the studies we reported, suggesting a possible role in symptomatology.

In our cohort, an increased abundance of Gardnerella and Streptococcus was observed, which is consistent with findings from previous studies. Shan et al. highlighted how endometrial fluid in women with endometriosis was enriched with inflammatory pathogenic taxa, which caused an

increased production of inflammatory cytokines [10]. Such bacteria will likely secrete metabolites that stimulate toll-like receptors, increasing local inflammation and exacerbating nociception [11]. This explains the significant correlation between the abundance of Gardnerella and pain scores in our study, and illustrates how specific microbial communities can contribute clinically to symptom exacerbation. The enteral microbiome, and especially the astrobleme, has become increasingly recognized as a regulator of systemic estrogen. The endometriosis group, characterized by increased serum estradiol levels and elevated β-glucuronidase gene expression, suggests that gut dysbiosis may enhance enterohepatic recirculation of estrogen. This has previously been demonstrated in an animal study, showing that the inflammatory gut microbiota of the endometriosis model was associated with increased circulating estrogen, which was counteracted by antibiotics [12]. In a similar fashion, Baker et al.

Disturbed profiles of astrobleme in women with endometriosis were identified, where functional enrichment in estrogen metabolism genes was observed [13]. Coupled with our results, these findings suggest that modification of gut microbes can be considered as a novel therapeutic approach to mitigate the progression of estrogen-driven disease. Inflammatory markers, CRP and IL-6, were elevated in our endometriosis cohort. This finding is consistent with the existing literature, which has linked the production of pro-inflammatory cytokines to dysbiosis of microbial communities [14, 15]. In particular, IL-6 has been shown to promote the survival and angiogenesis of endometriosis lesions [16]. The correlations observed between dysbiosis of the microbial community and systemic inflammation support the hypothesis that the microbiome may influence both local disease in the pelvis and systemic inflammation. The most debilitating symptom of endometriosis, pain, can also be mediated by the microbiome.

Apart from inflammation, specific microbial metabolites such as lipopolysaccharides and short-chain fatty acids may modulate nociceptor activity [17]. Prior research suggests that altered microbiota may modify central pain processing, potentially accounting for the pain experienced by patients with a minimal disease burden [18]. These findings, along with the correlations we observed between dysbiosis, astrobleme activity, and pain scores, provide further confirmation of a mechanism of this nature. Probiotic, prebiotic, and dietary approaches aimed at restoring Lactobacillus dominance and controlling pathogenic taxa may help in rebalancing the vaginal and gut microbiota. The first pilot study suggested Lactobacillus supplementation relieved pain and lowered recurrence rates in patients with endometriosis [19]. In addition, new therapeutic approaches targeting microbial enzymes, including β-glucuronidase, which aids in controlling estrogen metabolism and disease progression, show promise [20]. However, since the available evidence is still in its early stages, comprehensive randomized controlled trials will be necessary to determine the clinical value and guide practice. In these trials, the results of vaginal, endometrial, and gut microbiome analyses will be integrated with hormonal and inflammatory frameworks. Our study seeks to build upon this increasing body of evidence.
In contrast, cross-sectional studies limit the ability to make causal inferences [21]. To better understand the effect dysbiosis may have as either a causal factor to the progression of a disease or a consequence of the disease, or endometriotic pathology itself, longitudinal studies are necessary. Furthermore, disease-causing microbial metabolic pathways would be better understood with functional metagenomics.

Limitations

Given the nature of a cross-sectional study, it is not possible to make causal inferences about the changes in the microbiome in relation to the onset of the disease. The generalizability of the results is also limited due to the study's small sample size and recruitment at a single location. Furthermore, the 16S rRNA sequencing technique used lacked higher-order functional resolution. Other potential confounders, such as diet and lifestyle factors, were also inadequately addressed.

Conclusion

The data presented outline drastic alterations in the microbiome composition in female patients diagnosed with endometriosis, characteristically exhibiting reductions in Lactobacillus, increases in pathogenic taxa, observable changes in the functions of astroblemes, and associated systemic inflammation. These findings suggest that microbial dysbiosis may influence estrogen metabolism and the severity of symptoms. Given this information, the use of microbiome-based treatments may become a viable adjunctive therapeutic strategy to improve symptom management in these patients.

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Authors' Contribution

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