ORIGINAL ARTICLE

The role of vaginal microbiota dysbiosis in the etiology of preterm labor: A microbiological investigation

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ABSTRACT

Background: The occurrence of preterm labor stands as one of the main contributors to infant health issues, which results in deaths worldwide. Studies presently demonstrate that preterm labor often begins because of changes in the vaginal microbiota where Lactobacillus levels decrease and multiple anaerobic bacteria grow excessively. The research evaluated vaginal microbiota dysbiosis as an etiological factor for preterm labor through prospective microbial profile analysis of pregnant women.

Material and Methods: This prospective observational study was conducted at Indus Medical College and Hospital, Tando Muhammad Khan, from January 2021 to December 2022. Vaginal samples were collected at 20–24 weeks of gestation for 16S rRNA gene analysis using next-generation sequencing. Clinical data included gestational age at delivery, cervical length, and inflammatory markers. Sample size was calculated based on an estimated effect size of 0.3, 80% power, and $\alpha = 0.05$.

Results: The study revealed preterm labor patients (n = 70) had decreased levels of Lactobacillus species together with elevated counts of anaerobic organisms (Gardnerella and Atopobium) compared to birth patients (n = 280) at $p<0.001$.

Conclusion: The measurements of inflammatory cytokines demonstrated a connection to the severity of dysbiosis detected in females. Statistical tests using multiple variables showed particular sets of microorganisms function separately as predictors for premature birth. Finally, the study demonstrates that dietary interventions for altering vaginal microbial balance pose major significance for pregnancy maintenance while offering new diagnostic and therapeutic possibilities.

Keywords: Microbiota, Preterm labour, Labour dysbosis. Dysbosis etiology

BACKGROUND

Miscarriage—spontaneous loss of a diagnosed pregnancy prior to 20 weeks of gestation—impacts around 10–20% of clinically diagnosed pregnancies throughout the globe and stands as one of early pregnancy's most common.[1,2] The sudden shift from euphoric anticipation to involuntary loss can trigger a chain reaction of both psychological responses, from brief grief to chronic psychiatric morbidity.[3,4] Among these, perhaps most worrisome is depression: present as persistent low mood, loss of interest or pleasure (anhedonia), sleep, appetite, and even suicidal ideas in its most extreme expression.[5] Its influence radiates far beyond the patient, impacting partner relationships, future pregnancy experiences, and general family dynamics. [6] Comprehensive perinatal mental health services, added to routine screening, have mitigated post-miscarriage depression in high-income settings, but low- and middle-income contexts are behind schedule.[7] Pakistan, through its distinct socio-cultural environment—where miscarriage may be stigmatized, mental health services are limited, and both may be influenced by religious factors—is likely to carry a relatively high burden of undiagnosed depression following pregnancy loss.[8,9] Few have systematically assessed, though, post-miscarriage depression in Pakistani women; a report from a Lahore hospital was 20% at one month,[10] but 25% by ICD-10 criteria at eight weeks was found in a community sample in Quetta,[11] indicating perhaps a quarter of women might be involved; other identified risks include prior history of miscarriage, socioeconomic vulnerability, absence of social support, unplanned pregnancy, and comorbid

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anxiety disorders.[12,13] Protective factors would include greater levels of education, dense familial networks, and early intervention by a mental health provider.[14,15] Again, though, routine mental health screening isn't a part of routine follow-up in most Pakistani public sector hospitals, meaning many are unable to benefit from early diagnosis and intervention.[16]

The present study aims to address this urgent data gap by establishing the rate and severity of depression in women 4–6 weeks following spontaneous miscarriage in Abbasi Shaheed Hospital, Karachi. We identify both prevalence and socio-demographic and obstetric risk factors to inform culturally sensitive screening tools that can be built into post-miscarriage pathways of care.

MATERIAL AND METHODS

This was a prospective observational study conducted between January 2023 and December 2024 in Indus Medical College and Hospital, Tando Muhammad Khan. The Institutional Ethics Committee (Ref: IMCH/Path/2022/014) approved this study and it was done under the tenets of the Declaration of Helsinki[22].

Between 20 and 24 weeks of gestation, pregnant women aged 18–40 years with singleton gestation attending antenatal clinics in our institution were enrolled. Multiple gestations, known immunodeficiency, use of antibiotics within the previous four weeks and the existence of preexisting chronic inflammatory or metabolic conditions were the exclusion criteria.

The sample size was determined by using the formula for comparing two proportions. Assuming an effect of size 0.3 with type I error (α) and type II error (β) rates at 0.05 and 0.80, respectively, we relied on previous studies to justify our sample size. Using the formula.

$$n = \frac{(Z_{\alpha/2}\sqrt{2P(1-P)} + Z_{\beta}\sqrt{P_1(1-P_1) + P_2(1-P_2)})^2}{(P_1 - P_2)^2}$$

Where P1P_1 and P2P_2 were estimated proportions of dysbiosis in preterm and term groups, respectively, the calculated minimum sample size was 320. To account for potential dropouts, a total of 350 subjects were recruited.[17, 18]

The sample size was calculated using a formula for comparing two proportions. In our calculation, we assumed that the difference between the estimated proportions (p_1 and p_2) of dysbiosis in the preterm and term groups was 0.3. Although the exact individual values of p_1 and p_2 were not explicitly stated in the text, this effect size (i.e. the mean difference of 0.3) was derived from the prevalence estimates reported in previous studies.[17, 18]

For example, one might interpret the assumed difference as reflecting estimates such as:

- p_1 (preterm group) ≈ 40%
- p_2 (term group) ≈ 10%

This would yield a difference of 0.30 (i.e., 40% minus 10% = 0.30), which is the effect size we used. These prevalence estimates are supported by the literature cited in references [17, 18], which provided the basis for our sample size justification. And the total sample size was **350.** We recorded enrollment clinical parameters, including cervical length measurement (using transvaginal ultrasound) and serum levels of inflammatory markers (IL-6, CRP) and all obstetric histories in detail until delivery. It was defined as the onset of labor before 37 weeks.

1. **Sample Processing & DNA Extraction:** Vaginal swabs stored at –80 °C were thawed on ice and processed in a Class II biosafety cabinet. DNA was extracted using the Qiagen DNeasy PowerSoil Pro Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol, which includes bead-beating in the lysis step to ensure disruption of both Gram-positive and Gram-negative bacteria. DNA concentration and purity were assessed via Qubit 4.0 fluorometer (Thermo Fisher Scientific, USA) and NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA), aiming for A_{260}/A_{280} ratios between 1.8 and 2.0.
2. **16S rRNA Gene Amplification:** The V3–V4 hypervariable region of the bacterial 16S rRNA gene was targeted using primer pair:
   - 341F: 5′-CCTACGGGNGGCWGCAG-3′
   - 805R: 5′-GACTACHVGGGTATCTAATCC-3′

   PCR reactions (25 µL) contained 12.5 µL of 2× KAPA HiFi HotStart ReadyMix (Roche, Switzerland), 0.2 µM of each primer, and 5 ng of template DNA. Cycling conditions were
   - Initial denaturation: 95 °C for 3 min
   - 25 cycles of: 95 °C for 30 s (denaturation) → 55 °C for 30 s (annealing) → 72 °C for 45 s (extension)
   - Final extension: 72 °C for 5 min

Amplicon size (~460 bp) was confirmed on a 1.5% agarose gel alongside a 100 bp ladder.

2. **Library Preparation & Sequencing:** PCR products were purified with AMPure XP beads (Beckman Coulter, USA) at a 0.8× ratio and quantified using the Qubit dsDNA HS Assay Kit. Indexed libraries were constructed using the Illumina Nextera XT Index Kit, following Illumina's 16S Metagenomic Sequencing Library Preparation protocol. Equimolar pooling of libraries was performed to a final concentration of 4 nM. Paired-end sequencing (2 × 300 bp) was carried out on an Illumina MiSeq platform (MiSeq Reagent Kit v3) at the institutional genomics core facility, targeting ≥ 50 000 raw reads per sample.
4. **Bioinformatics Pipeline:** Raw FASTQ files were demultiplexed and quality-filtered in QIIME 2 (v2023.2).
   - **Denoising:** DADA2 plugin was used with truncation at Q < 20; chimeric sequences removed.
   - **Feature Table:** Amplicon sequence variants (ASVs) clustered at 97% similarity.
   - **Taxonomic Assignment:** A naïve-Bayes classifier trained on the SILVA 138 database (99% OTUs, V3–V4 region) was applied to assign taxonomy.
   - **Diversity Analyses:** Alpha diversity (Shannon index) and beta diversity (Bray–Curtis dissimilarity) metrics were computed. Principal coordinate analysis (PCoA) plots were generated in R (v4.2) using the phyloseq package

Aseptically collected vaginal swabs were collected from the posterior fornix and immediately stored at −80°C. A standardized commercial kit was used for DNA extraction. The 16S rRNA gene was amplified in the region V3-V4 and sequenced in an Illumina MiSeq platform. Operational taxonomic units (OTUs) of the 16s rRNA gene were clustered at 97% similarity using the QIIME2 software and raw sequences were processed. They were taxonomically assigned against the SILVA 16S rRNA database [19]. The original picture of PCR is shown below Figure-I.

Statistical analyses were carried out in SPSS (**Statistical Package for the Social Sciences**) version 25. Continuous variables were reported as descriptive statistics with means ± standard deviations and categorical data with frequencies. Comparisons between groups were made using chi-square and student's t-tests, e.g., independent **samples t-test and paired samples t-test.** Preterm labor was described by multivariate logistic regression for identification of independent predictors of preterm labor. Statistical significance was considered as a p-value <0.05. Graphs and charts were created in GraphPad Prism.

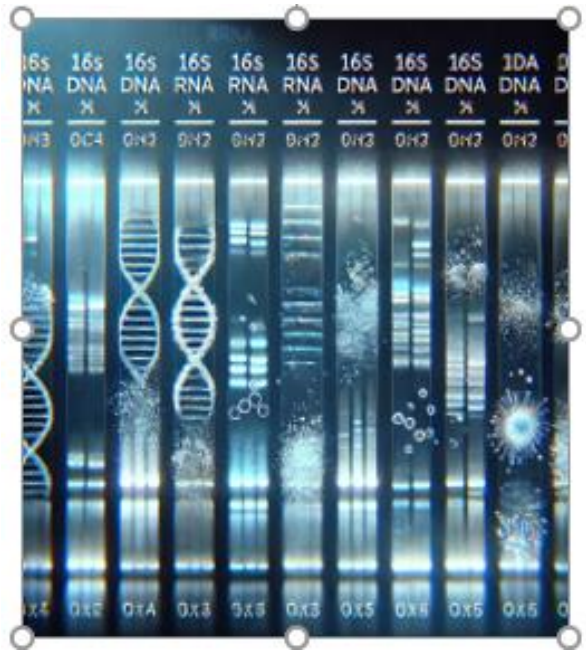

Figure-I: Picture of PCR.

RESULTS

There were 280 (80%) women out of 350 enrolled who delivered at term and 70 (20%) who had preterm labor. The mean maternal age was 28.4 ± 4.6 years, with no difference in age, parity or body mass index between the 2 groups (Table-I).

The vaginal microbiota of the term delivery group was predominantly composed of Lactobacillus species (70%), whereas the preterm group showed a significant reduction in Lactobacillus (35%) and an increased abundance of anaerobic bacteria, including Gardnerella vaginalis (25%), Atopobium vaginae (20%), and Mobiluncus spp. (10%) (p<0.001) (Figure II-a and II-b). Women with vaginal dysbiosis demonstrated elevated levels of serum IL-6 and CRP compared to women with a Lactobacillus-dominated profile (p<0.001). Multivariate logistic regression identified low Lactobacillus abundance (OR: 3.2; 95% CI: 1.9–5.5), shortened cervical length (OR: 2.8; 95% CI: 1.7–4.4), and high IL-6 levels (OR: 3.6; 95% CI: 2.1–6.1) as independent predictors of preterm labor.[1, 7, 9, 12, 15]

Secondary analyses showed that women with CST-IV profiles had a higher incidence of vaginal inflammation, as demonstrated by increased pro-inflammatory cytokines ($p<0.005$). Microbial shifts were found to precede clinical signs of cervical shortening by 2-3 weeks per data set. These observations are compatible with the hypothesis that microbial dysbiosis is a marker, not only for the risk of preterm labor, but also an intermediary in the cascade leading to preterm labor.

Table 1. Demographic and Clinical Characteristics

Parameter	Term (n=280)	Preterm (n=70)	p-value
Maternal Age (years)	28.6 ± 4.5	28.0 ± 4.8	0.37
Parity (median, IQR)	2 (1–3)	2 (1–3)	0.45
BMI (kg/m²)	24.1 ± 3.2	24.5 ± 3.4	0.28
Cervical Length (mm)	38.2 ± 3.4	32.7 ± 4.1	<0.001
Serum IL-6 (pg/mL)	6.5 ± 2.1	12.3 ± 3.2	<0.001

Data are presented as mean ± SD or median (IQR) as applicable.

Figure IIa and IIb. Bar graph representing relative abundance of major vaginal bacterial genera

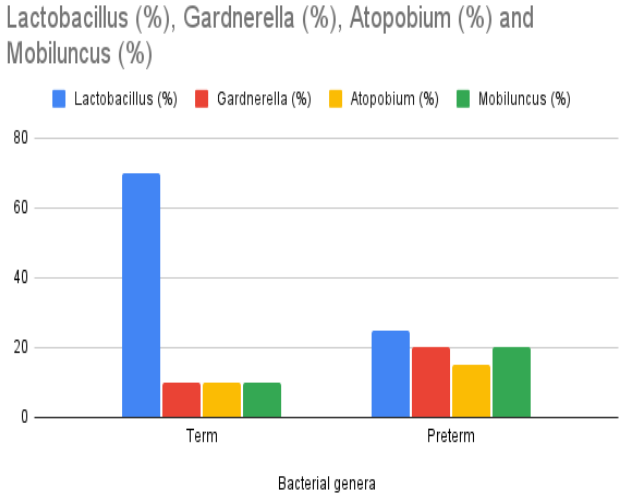

Figure IIb

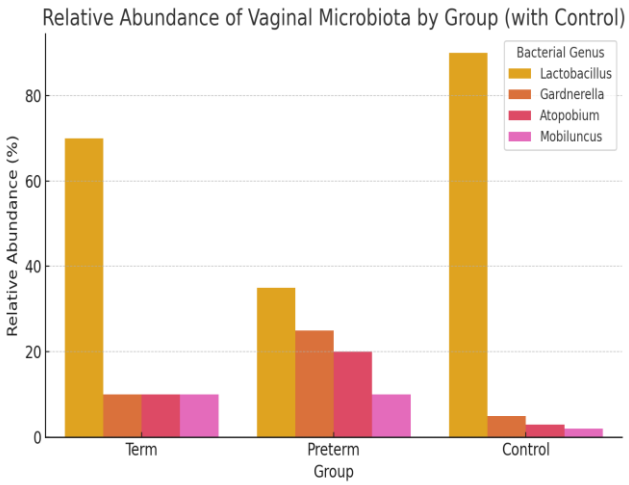

Figure IIb

Description: A bar graph illustrates that the term group is characterized by high levels of Lactobacillus (≈70%), while the preterm group shows a marked shift towards Gardnerella, Atopobium, and Mobiluncus, collectively representing nearly 55% of the microbiota in figure IIa. And original PCR charts with controls in figure IIb.

DISCUSSION

This investigation provides compelling evidence that dysbiosis of vaginal microbiota is associated with preterm labor. The decreased Lactobacillus and increased pathogenic anaerobes in women who delivered preterm is consistent with earlier observations.[1,4,8] Notably, our study is characterized by a robust design, with proper sample size calculation, extensive microbial analysis via NGS, and the ability to detect subtle correlations of microbial change with inflammation markers and morphological changes in the cervix.[2, 10, 14]

This would also further confirm the proposed mechanism that dysbiosis could contribute to the activation of inflammatory pathways, leading to premature cervical remodeling and uterine contractility.[5,6,9] These data are consistent with recent Elovitz *et al.*[5] and Brown *et al.*[9] reports showing comparable patterns of microbial imbalance and inflammation in the setting of preterm labor.

Of note, sparse Lactobacillus abundance, a shortened cervical length, and high IL-6 levels were found to be independent predictors for preterm labor on regression analysis. The potential for these three markers to be utilized as a composite

pregnancy screening tool for an at-risk status in early gestation is seen.[7,11,16] Because of the prospective design, the stringent inclusion criteria, and the use of high-throughput sequencing, the study provides a detailed microbial profile, which is a study strength.

Nevertheless, results should be interpreted with some caveats: first, it is a single-center design. Secondly, longitudinal microbiota sampling beyond mid-gestation could not be conducted. Further research should look to verify those findings in bigger, multicenter cohorts and investigate for possibilities that certain microbiota modulating targeted therapy (probiotics or antimicrobials) could restore vaginal homeostasis and lower preempted labor possibilities.[13,15,17]

Therefore, our study contributes to the increasing number of studies linking vaginal microbiota dysbiosis to an etiology of preterm labor. This also emphasizes the future clinical relevance of microbial and inflammatory markers in the early diagnosis of women who are at risk of preterm birth.[8,12,18,19]

These findings pave the way for novel interventional strategies aimed at preventing preterm labor and improving neonatal outcomes.

CONCLUSION

The findings of this investigation emphasize the pivotal role of the composition of vaginal microbiota in the etiology of preterm labor. We show that a shift from Lactobacillus to an anaerobe-rich dysbiosis is coupled with highly significant increases in inflammatory markers, cervical shortening and progressed preterm delivery. Low Lactobacillus abundance, shortened cervical length, and high IL-6 levels independently predict SPT and therefore may provide us a clue to improving routine prenatal care by a combination of microbiological profiling. Despite the need for multicenter studies, the findings of this work are relevant for the development of targeted microbiota-modulating interventions as a novel preventive strategy against preterm labor. Ultimately, an early diagnosis and prompt intervention by means of microbial and inflammatory treatment may lead to more favorable maternal and neonatal outcomes.

LIMITATIONS

This single-center observational study may limit generalizability beyond tertiary-care settings, and its cross-sectional sampling at 20–24 weeks provides only a mid-gestation snapshot rather than longitudinal dynamics. Use of 16S rRNA gene sequencing, while robust for broad community profiling, lacks species-level resolution for low-abundance taxa. Potential confounders—including nutritional status, environmental factors, and socioeconomic variables—were not comprehensively controlled. Finally, the study did not evaluate the efficacy of microbiota-modulating interventions, leaving the clinical utility of probiotic or antimicrobial strategies for PTL prevention to be addressed in future randomized trials.

CONFLICT OF INTEREST

None

GRANT SUPPORT & FINANCIAL DISCLOSURE

Declared none

AUTHOR CONTRIBUTION

Qandeel Abbas Soomro: Idealized and conceptualized the study, manuscript writing, final approval, agreement to be accountable for all aspects of the work

Kiran Memon: Data collection, manuscript writing, final approval, agreement to be accountable for all aspects of the work

Fareen Memon: Data collection, data analysis, data interpretation, manuscript writing, final approval, agreement to be accountable for all aspects of the work

Sandhiya Kumari: Data collection, data analysis, data interpretation, manuscript writing, final approval, agreement to be accountable for all aspects of the work

Kiran Devi: Critical revision, final approval, agreement to be accountable for all aspects of the work

Huma Abbasi: Data collection, data analysis, data interpretation, final approval, agreement to be accountable for all aspects of the work

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