International Journal of Women's Health

Dovepress
Taylor & Francis Group

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ORIGINAL RESEARCH

Retrospective Analysis of Gut Microbiota and Metabolomic Profiles in Pregnant Women: Association with Cesarean Section Risk

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Background: The gut microbiota and metabolic profiles of pregnant women undergo dynamic changes throughout gestation, potentially influencing the mode of delivery. Emerging evidence suggests that dysbiosis of gut microbiota and metabolic perturbations may contribute to the rising cesarean section (CS) rates. This study aimed to investigate gestation-specific alterations in gut microbiota and serum metabolomes and evaluate their association with CS risk.

Methods: We conducted a retrospective analysis of 80 healthy pregnant women with singleton pregnancies who delivered at our hospital between January 2022 and December 2023. Participants were stratified into CS (n=40) and vaginal delivery (VD, n=40) groups based on delivery mode, matched for maternal age, pre-pregnancy BMI, and gestational age. Fecal samples were collected one month prior to delivery for gut microbiota analysis using 16S rRNA gene sequencing. Serum samples were subjected to targeted metabolomics via UHPLC-QTOF-MS, focusing on markers of energy metabolism. Peripheral blood was analyzed for T cell subsets and regulatory T cells (Tregs) by flow cytometry. Spearman correlation analysis was performed to assess associations between gut microbial taxa and serum metabolites.

Results: The CS group exhibited significantly lower gut microbial α-diversity (Shannon index: 3.22 vs 4.10, P<0.001), reduced Bacteroidetes (15.3% vs 20.1%, P=0.021), and increased Firmicutes (50.2% vs 46.4%, P=0.015), resulting in an elevated Firmicutes/Bacteroidetes ratio (P=0.008). Metabolomic analysis showed higher levels of pyruvic acid and lactate and lower levels of phenylalanine in the CS group (all P<0.05). Immune analysis revealed increased CD4□ T cells, CD8□ T cells, and Tregs in the CS group (P=0.042, 0.029, 0.015, respectively). Correlation analysis indicated that Bacteroidetes abundance positively correlated with lactate (r=0.45, P<0.001), Firmicutes with phenylalanine (r=0.37, P=0.012), and Lactobacillus negatively with pyruvic acid (r=−0.28, P=0.045).

Conclusion: Gestational gut microbiota dysbiosis and metabolic dysregulation are significantly associated with increased CS risk. These findings highlight potential biomarkers for early risk stratification and suggest that personalized microbiota-directed interventions during pregnancy might help optimize delivery outcomes. Further mechanistic studies are warranted to validate causality.

Keywords: pregnant women, gut microbiota, metabolomics, cesarean section, delivery mode, biomarkers

Introduction

In recent years, the cesarean section (CS) rate has continued to rise globally, with rates exceeding 30% in many high-income countries.[1] While CS can mitigate critical risks such as fetal distress, placental abruption, or obstructed labor,[2] its overuse is associated with adverse maternal and neonatal outcomes, including surgical complications, delayed lactation, and altered neonatal microbiome colonization.[3,4] The World Health Organization (WHO) emphasizes that CS rates above 10–15% lack proportional health benefits, highlighting the urgency to identify modifiable predictors of CS.[5]

Received: 15 March 2025
Accepted: 5 June 2025
Published: 12 June 2025

Pregnancy induces profound physiological adaptations, including immune tolerance remodeling, endocrine fluctuations, and gut microbiota restructuring.[6] The gut microbiota, a key regulator of host metabolism and immunity, undergoes dynamic shifts during gestation to support fetal development. Specific taxa such as Lactobacillus and Bifidobacterium dominate early pregnancy, while late gestation is characterized by reduced diversity and increased Proteobacteria abundance.[7,8] These changes correlate with metabolic adaptations, including short-chain fatty acid (SCFA) production and bile acid metabolism, which influence maternal energy homeostasis and inflammatory responses.[9,10] Critically, gut dysbiosis and metabolic perturbations have been implicated in pregnancy complications like gestational diabetes (GDM) and preeclampsia,[11,12] yet their role in delivery mode selection remains underexplored.

Emerging studies using 16S rRNA sequencing and untargeted metabolomics suggest that CS is associated with distinct microbial and metabolic signatures. For instance, lower Faecalibacterium levels and elevated acylcarnitines were observed in CS-delivered mothers compared to vaginal delivery (VD) cohorts.[13,14] However, existing evidence is fragmented, with limited integration of multi-omics data or adjustment for confounders such as antibiotic use, maternal BMI, and gestational age. No prior study has systematically evaluated the combined predictive value of gut microbiota and metabolomics for CS risk in a homogeneous cohort – a gap this study seeks to address.

We hypothesize that gestational gut microbiota dysbiosis and metabolic dysfunction synergistically contribute to CS risk by modulating inflammatory pathways and hormonal cascades. This retrospective study aims to: (1) characterize trimester-specific changes in gut microbiota and serum metabolomes, (2) identify microbial and metabolic biomarkers predictive of CS, and (3) explore interactions between dysbiosis and metabolic derangements. Our findings may inform strategies for early risk stratification and microbiota-targeted interventions to optimize delivery outcomes.

Materials and Methods

Study Subjects

This retrospective cohort study analyzed 120 pregnant women who delivered at XXX Hospital between January 2022 and December 2023. Participants were initially screened based on electronic medical records (n=150). Seventy participants were excluded due to not meeting inclusion criteria (n=50), incomplete data (n=15), or declining participation (n=5) (Figure S1). Final inclusion comprised 80 women (40 cesarean section [CS] and 40 vaginal delivery [VD]), matched for maternal age (±3 years), pre-pregnancy BMI (±2 kg/m²), and gestational age (±1 week). The study protocol was approved by the Zhongshan Campus of the Fourth Hospital of Shijiazhuang Ethics Committee (No. 2023-THUE -0942) and complied with the Declaration of Helsinki. Informed consent was obtained from all study participants. De-identified data were used to ensure patient anonymity.

Inclusion Criteria

Inclusion criteria: Singleton pregnancy with complete prenatal records. Age 20–45 years. No antibiotic/probiotic use within 4 weeks prior to fecal sampling. No acute/chronic gastrointestinal diseases or metabolic disorders (eg, diabetes, hypertension).

Exclusion criteria: Major gastrointestinal surgery history. Chronic inflammatory/autoimmune diseases. Multifetal gestation or preterm delivery (<37 weeks).

Sample Collection and Processing

Fecal Sample Collection

Fecal samples were collected from the women one month prior to delivery. The diversity, abundance, and composition of the gut microbiota were analyzed using high-throughput 16S rRNA gene sequencing as follows:

Pregnant women were provided with a 40mL sterile PS stool collection cup and sterile gloves in advance. Before collecting the sample, they were instructed to empty their bladder. The stool sample was taken using a sampling spoon from the center of the stool, with at least 2g of stool being collected. About 1g of the sample was then placed into a 1.5mL sterile EP tube and labeled with an ID number. The sample had to be stored in a −80°C freezer within 2 hours. DNA extraction was performed using the QIAamp PowerFecal Pro DNA Kit (Qiagen), followed by PCR amplification of

the 16S rRNA V3–V4 region (primers: 341F/806R). Amplicon sequencing was conducted on an Illumina NovaSeq 6000 platform (2×250 bp paired-end reads).[15–17]

Serum metabolomics: Fasting blood samples were centrifuged at 3000 rpm for 10 min. Serum was stored at −80°C until analysis. Targeted metabolomics focusing on energy metabolism markers (pyruvate, lactate, phenylalanine, glucose) was performed via UHPLC-QTOF-MS (Agilent 1290/6545) in both positive/negative ion modes. Quality control (QC) included pooled samples, blanks, and internal standards (L-2-chlorophenylalanine, 1 μg/mL).[18–20]

Bioinformatics Analysis

Microbiome analysis: Raw sequences were processed using QIIME2 (v2022.8). DADA2 denoising generated amplicon sequence variants (ASVs). Taxonomic assignment used the SILVA 138 database at 99% similarity. Alpha diversity (Shannon) were calculated. LEfSe (LDA >3.0) identified differentially abundant taxa.

Metabolomics analysis: Raw MS data were processed with Progenesis QI (Waters). Metabolites were annotated against HMDB and KEGG databases. Orthogonal partial least squares-discriminant analysis (OPLS-DA) and VIP scores >1.5 identified discriminative metabolites.

Statistical Analysis

GraphPad Prism 8 was used for image processing. All data were analyzed using SPSS 26.0 software. Continuous data were expressed as (mean±sd), and categorical data were expressed as percentages (%). Continuous variables were compared using Mann–Whitney *U*-tests (non-normal data) or ANCOVA (adjusted for BMI/gestational age). False discovery rate (FDR) correction was applied for multiple comparisons. Multivariate logistic regression assessed independent predictors of CS risk. Power analysis (G*Power v3.1) indicated 80% power to detect Cohen's d >0.8 with n=40/group.

Results

General Information

The cesarean section (CS) and vaginal delivery (VD) groups showed no significant differences in age, pre-pregnancy BMI, or pregnancy times (all P >0.05; Table 1). Notably, gestational diabetes mellitus (GDM) was more prevalent in the CS group (25.0% vs 10.0%, P=0.041), aligning with prior reports linking metabolic dysregulation to CS risk.[21]

Adverse Pregnancy Events

The distribution of adverse pregnancy events by delivery mode is summarized in Table 2. The cesarean section (CS) group exhibited a higher total count of adverse events (8 events) compared to the vaginal delivery (VD) group (4 events). Specific adverse events included preterm birth, polyhydramnios, puerperal infection, and hypertensive disorders, with most events occurring more frequently in the CS group. However, no statistically significant difference in the proportion of women experiencing ≥1 adverse event was observed between the CS and VD groups (8/40 [20%] vs 4/40 [10%], Fisher's exact test, p = 0.210).

Table 1 Comparison of General Information Between the Two Groups

Parameter		CS Group (n=40)	VD Group (n=40)	*P*	*Effect Size (Cohen's d)*
Pre-pregnancy BMI	Mean	21.74±2.13	21.65±1.88	0.842	0.03
Age (years)	-	20–45	20–45	-	
	Mean	31.33±2.85	31.89±2.62	0.363	0.21
Pregnancy times	-	1–3	1–3	-	
	Mean	1.01±0.53	1.15±0.76	0.342	0.18
GDM (n, %)	-	10 (25.0%)	4 (10.0%)	0.041	OR=3.0

https://doi.org/10.2147/IJWH.S526040

Table 2 Adverse Pregnancy Events by Delivery Mode

Adverse Event	CS Group (n=40)	VD Group (n=40)
Preterm birth	3 (7.5%)	1 (2.5%)
Polyhydramnios	1 (2.5%)	1 (2.5%)
Postpartum hemorrhage	1 (2.5%)	0 (0%)
Macrosomia	0 (0%)	1 (2.5%)
Puerperal infection	2 (5.0%)	0 (0%)
Hypertensive disorders	1 (2.5%)	1 (2.5%)
Total women with ≥1 event	8 (20%)	4 (10%)

Notes: Data are presented as n (%). Comparisons were performed using Fisher's exact test due to low expected cell frequencies (<5).

Gut Microbiota

The CS group exhibited reduced α-diversity (Shannon index: 3.22 ± 0.58 vs 4.10 ± 0.67, P<0.001) and altered phylum-level composition, including lower Bacteroidetes (15.3% vs 20.1%, P=0.021) and higher Firmicutes (50.2% vs 46.4%, P=0.015), resulting in a higher Firmicutes/Bacteroidetes (F/B) ratio (3.28 vs 2.31, P=0.008) (Table 3). This elevated F/B ratio mirrors patterns observed in metabolic syndrome cohorts.[22]

Metabolomics

The targeted metabolomics analysis The CS group exhibited elevated pyruvic acid (4.12 ± 0.54 vs 3.58 ± 0.61, P=0.032), lactate (1.85 ± 0.47 vs 1.22 ± 0.56, P=0.005), and reduced phenylalanine (0.95 ± 0.21 vs 1.08 ± 0.24, P=0.023). D-glucose showed a non-significant upward trend (5.67 ± 1.02 vs 5.29 ± 1.09, P=0.070) (Table 4). These changes align with disrupted energy metabolism pathways reported in CS-associated studies.[23]

Correlation Analysis

Spearman correlation analysis revealed significant associations between specific microbial taxa and metabolites (Table 5). Notably, the abundance of Bacteroidetes showed a moderate positive correlation with lactate levels ($r = 0.45$, $P < 0.001$), while Firmicutes were positively correlated with phenylalanine concentrations ($r = 0.37$, $P = 0.012$). In contrast, Lactobacillus abundance was negatively associated with pyruvic acid levels ($r = -0.28$, $P = 0.045$). These findings

Table 3 Gut Microbiota Diversity and Composition

	CS Group (n=40)	VD Group (n=40)	*P*
α diversity index (Shannon)	3.22 ± 0.58	4.10 ± 0.67	<0.001
Bacteroidetes (%)	15.3 ± 4.2	20.1 ± 5.1	0.021
Firmicutes (%)	50.2 ± 6.7	46.4 ± 5.9	0.015
F/B Ratio	3.28 ± 1.2	2.31 ± 0.9	0.008
Lactobacillus (%)	28.5 ± 7.3	33.8 ± 8.1	0.049

Table 4 Comparison of Metabolomic Features Between the Two Groups

	CS Group (n=40)	VD Group (n=40)	*P*
Pyruvic acid	4.12 ± 0.54	3.58 ± 0.61	0.032
Lactic acid	1.85 ± 0.47	1.22 ± 0.56	0.005
Phenylalanine	0.95 ± 0.21	1.08 ± 0.24	0.023
D-glucose	5.67 ± 1.02	5.29 ± 1.09	0.070

Table 5 Correlation Analysis Between Gut Microbiota and Metabolites

Microbial Community	Metabolite	r	*P*
Bacteroidetes	Lactic acid	0.45	<0.001
Firmicutes	Phenylalanine	0.37	0.012
Lactobacillus	Pyruvic acid	−0.28	0.045

Table 6 Comparison of Immune Cell Subsets Between the Two Groups

	CS Group (n=40)	VD Group (n=40)	*P*
Number of Cases	40	40	–
CD4+ T	32.5% ± 7.3%	28.3% ± 6.1%	0.042
CD8+ T	21.7% ± 4.5%	18.5% ± 3.2%	0.029
Treg	8.2% ± 1.9%	6.1% ± 1.4%	0.015

suggest that alterations in gut microbiota composition may be linked to changes in key metabolic pathways, indicating a potential connection between microbial dysbiosis and metabolic stress during pregnancy.

Immune Cell Levels

The results revealed that the levels of peripheral blood T cell subsets (CD4+ T cells, CD8+ T cells) and regulatory T cells (Tregs) were significantly higher in the CS Group compared to the VD Group, suggesting a potential interaction between gut microbiota and immune response. While these differences suggest immune involvement, causality remains speculative due to the study's observational design. See Table 6.

Discussion

Recent evidence increasingly suggests that the occurrence of cesarean section (CS) is closely associated with the composition and diversity of the maternal gut microbiota.[24,25] During pregnancy, dynamic remodeling of the gut microbiota is observed, often characterized by reduced microbial diversity and compositional shifts, particularly in women undergoing CS.[26] In our study, we found that the α-diversity of gut microbiota was significantly lower in the CS group, along with notable increases in the relative abundance of Firmicutes and decreases in Bacteroidetes, which led to a higher Firmicutes/Bacteroidetes (F/B) ratio.[27] This shift mirrors microbial patterns frequently observed in individuals with metabolic syndrome or obesity,[28] suggesting a potential link between gut microbial dysbiosis and metabolic stress during pregnancy.

These alterations in microbial composition may contribute to immune dysregulation and metabolic imbalance—both recognized risk factors for CS.[29] Specifically, the enrichment of Firmicutes and reduction of Bacteroidetes may promote low-grade inflammation and impair host immune tolerance, which could increase the likelihood of obstetric complications requiring CS.[30] Our findings are consistent with prior reports, indicating that lower gut microbiota diversity is associated with increased risk of adverse pregnancy outcomes.[31]

In parallel, our metabolomic analysis revealed that the CS group exhibited elevated levels of pyruvate and lactate and decreased levels of phenylalanine, reflecting significant alterations in energy and amino acid metabolism.[32] These changes may serve as indicators of systemic metabolic disturbances. The elevated pyruvate and lactate levels point toward a glycolytic shift, possibly indicating mitochondrial dysfunction or heightened inflammatory responses in pregnant women at risk for CS.[33] Similarly, reduced phenylalanine levels may suggest altered amino acid metabolism linked to immune or hormonal dysregulation.[34]

Furthermore, the correlation analysis demonstrated significant associations between gut microbiota and specific metabolites. For example, Bacteroidetes abundance positively correlated with lactate levels, while Firmicutes abundance

was associated with phenylalanine concentrations.[35] These relationships underscore the potential functional relevance of microbial shifts in modulating host metabolic pathways. This microbial–metabolite interaction may contribute to the systemic metabolic environment that predisposes to CS, although causality remains to be established.[36]

Beyond metabolic effects, our immune profiling indicated elevated levels of peripheral CD4□ T cells, CD8□ T cells, and regulatory T cells (Tregs) in the CS group. These immunological shifts suggest an activated immune status in pregnant women who underwent CS, which may either reflect or exacerbate systemic inflammation. The immune system, influenced in part by gut microbiota, plays a critical role in maintaining maternal-fetal tolerance and regulating the labor process.[37] Disruptions in immune homeostasis may impair cervical ripening or uterine contractility, potentially leading to delivery failure and necessitating surgical intervention.[38]

Collectively, our findings support a model wherein gut microbiota dysbiosis, metabolic perturbations, and immune activation form an interconnected triad that may contribute to increased CS risk. While the study presents a novel integrative analysis, its retrospective design limits the ability to infer causality. Future prospective cohort studies and interventional trials are warranted to delineate the mechanistic pathways and to verify whether modulating the gut microbiota or correcting metabolic disturbances can reduce CS incidence.[39]

Moreover, although our results provide compelling associations, we acknowledge that multiple confounding factors—such as diet, antibiotic exposure, genetic predisposition, and prenatal care practices—were not fully controlled for in this study. Such factors may independently influence both microbiota composition and delivery outcomes. Therefore, carefully designed longitudinal studies incorporating multi-omics approaches and robust covariate control are essential to validate these findings.[40]

Conclusion

In conclusion, changes in gut microbiota and metabolomics during pregnancy may play an important role in the rising cesarean section rates. Gut microbiota imbalance and metabolic dysregulation may serve not only as potential biomarkers but also as modifiable risk factors affecting cesarean section rates. However, it is important to note that the retrospective design of our study limits the ability to draw causal conclusions. The observed associations may be influenced by confounding factors such as diet, antibiotic use, and pre-existing maternal conditions, which were not fully controlled in this analysis. Future prospective studies, including randomized controlled trials, are needed to validate these findings and establish causality.

Through further investigation of the interactions between gut microbiota and metabolomics, future studies could provide personalized clinical intervention strategies, thereby reducing unnecessary cesarean sections and safeguarding maternal and infant health. Specifically, targeting gut microbiota modulation through dietary interventions, probiotics, or metabolic therapies may hold promise in improving maternal health outcomes. Furthermore, integrating microbiome and metabolomic data into clinical practice could enhance our ability to identify high-risk pregnancies and optimize care based on individual microbial and metabolic profiles.

Ethics Approval

This study was on the relationship between intestinal flora changes and cesarean section rate in pregnant women (Project number: 20231654). The study was approved by the Ethics Committee of the Zhongshan Campus of the Fourth Hospital of Shijiazhuang (Approval No. 2023-THUE-0942).

Disclosure

The authors report no conflicts of interest in this work.

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