International Journal of Science and Research (IJSR)
ISSN: 2319-7064
Impact Factor 2025: 7.089

Impact of Maternal Microbiome Diversity in Early Pregnancy on the Incidence of Hypertensive Disorders in the Third Trimester: A Prospective Cohort Study

Dr. Ranima Deka[1], Dr. Shireen Mumtaz Barbhuiya[2]

[1]Associate Professor, Department of Obstetrics and Gynaecology, P.A. Sangma International Medical College and Hospital (PIMC), Meghalaya, India.

[2]Consultant Obstetrician and Gynaecologist, Agile Hospital and Midland Hospital, Guwahati,Assam, India

Abstract: ***Introduction: Hypertensive disorders of pregnancy (HDP), including gestational hypertension and pre-eclampsia, remain major causes of maternal and perinatal morbidity and mortality worldwide. Emerging evidence suggests that the maternal microbiome may influence maternal immune, metabolic, and vascular adaptations. However, the extent to which early-pregnancy microbiome diversity predicts HDP onset remains poorly characterised. Objective: To evaluate whether maternal gut and vaginal microbiome diversity in early pregnancy is associated with the subsequent development of HDP. Methods: In a prospective cohort of 350 pregnant women recruited at 10–14 weeks' gestation, maternal gut and vaginal microbiota were characterised via 16S rRNA gene sequencing. Microbiome alpha and beta-diversity metrics and specific taxa abundances were compared between women who developed HDP and those who remained normotensive. Multivariable logistic regression adjusted for maternal age, BMI, parity, and diet was used to assess independent associations. Results: Among the 333 women analyzed, 38 (11.4%) developed HDP. Women who developed HDP had significantly lower gut microbiome alpha-diversity (Shannon index mean 3.8 vs 4.5, p<0.001) and altered community composition (Bray-Curtis PERMANOVA p=0.02). Specific depletion of beneficial genera (e.g., Akkermansia, Bifidobacterium) and enrichment of pro-inflammatory taxa (e.g., Bilophila, Escherichia/Shigella) were observed. After adjustment, each 0.5-unit higher Shannon index was associated with 30% lower odds of HDP (adjusted OR 0.70; 95% CI 0.55-0.90). Vaginal microbiome diversity showed weaker, non-significant associations. Conclusion: Lower maternal gut microbiome diversity and dysbiosis in early pregnancy are independently associated with an increased risk of HDP. These findings support the hypothesis that the maternal gut microbiota may participate in the pathogenesis of HDP and raise the possibility of early-pregnancy microbiome-targeted risk stratification and preventative strategies.***

Keywords: microbiome; pregnancy; hypertensive disorders; pre-eclampsia; maternal gut; vaginal microbiota; dysbiosis

1. Introduction

Hypertensive disorders of pregnancy (HDP)—encompassing gestational hypertension, pre-eclampsia, and eclampsia—complicate approximately 5–10% of pregnancies globally and are leading causes of maternal and perinatal morbidity and mortality.[1] The pathophysiology of HDP, particularly pre-eclampsia, is complex and incompletely understood, involving abnormal placentation, systemic endothelial dysfunction, oxidative stress, and a heightened inflammatory state.[2]

There is growing interest in the role of the maternal microbiome in pregnancy health. The collective microbiota of the gut, vagina, and oral cavity are critical modulators of host immune and metabolic function.[3] During normal pregnancy, the maternal gut microbiome undergoes dynamic remodeling, with changes thought to support metabolic adaptation and immune tolerance of the fetus.[4] Disruption of this symbiotic relationship, termed dysbiosis, has been implicated in adverse pregnancy outcomes, including preterm birth and gestational diabetes mellitus.[5] Recent reviews and studies have specifically linked maternal microbial dysbiosis to pre-eclampsia, suggesting that gut-derived microbes or their metabolites may contribute to the systemic inflammation and endothelial dysfunction characteristic of the disorder.[6 7]

Despite these advances, key gaps persist. Many studies examine the microbiome in late pregnancy or after the clinical onset of HDP, limiting causal inference and predictive potential.[8] Few prospective studies have systematically assessed the early pregnancy microbiome as a predictor of subsequent HDP incidence. Furthermore, parallel assessment of both gut and vaginal niches in the same cohort is limited.

We therefore designed a prospective cohort study to test the hypothesis that lower maternal gut and vaginal microbiome diversity and specific dysbiotic signatures in the first trimester are associated with a higher risk of developing HDP in the third trimester. Establishing such a link could provide a novel, early biomarker for risk stratification and open new avenues for microbiome-modulating preventative interventions.

2. Methods

Study Design and Participants

We conducted a prospective cohort study at P.A. Sangma International Medical College and Hospital (PIMC), Meghalaya, India, between 01/08/2024 and 31/07/2025. Pregnant women attending the antenatal clinic at 10–14 weeks' gestation (confirmed by ultrasound) were invited to participate. Inclusion criteria were: singleton pregnancy, age 18-45 years, and willingness to provide biological samples and follow-up data. Exclusion criteria included: pre-existing chronic hypertension, pre-gestational diabetes, autoimmune

disease, chronic inflammatory bowel disease, antibiotic use within the past 4 weeks, multiple gestation, or major fetal anomaly detected at enrolment.

All participants provided written informed consent. The study was approved by the Institutional Review Board.

Data and Sample Collection

At enrolment (10–14 weeks), we collected baseline demographic and clinical data, including maternal age, self-reported pre-pregnancy body mass index (BMI), parity, smoking status, and dietary intake (assessed via a semi-quantitative food frequency questionnaire). Resting blood pressure was measured, and baseline laboratory values (complete blood count, renal and liver function) were recorded.

Gut Microbiome Sampling: Participants provided a fresh fecal sample at home using a sterile collection kit (OMNIgene•GUT, DNA Genotek). Samples were stored at 4°C, transported to the laboratory within 24 hours, and immediately stored at -80°C until DNA extraction.

Vaginal Microbiome Sampling: A mid-vaginal swab was collected by a clinician using a sterile polyester swab and placed in a tube containing DNA/RNA Shield (Zymo Research). Swabs were stored at -80°C.

DNA Extraction and 16S rRNA Gene Sequencing

Microbial DNA was extracted from all samples using the DNeasy PowerSoil Pro Kit (Qiagen) with an initial bead-beating step. The V3-V4 hypervariable region of the 16S rRNA gene was amplified using primers 341F and 805R.[9] Amplified libraries were indexed, purified, quantified, and pooled in equimolar ratios. Sequencing was performed on an Illumina MiSeq platform (2 × 300 bp chemistry). Negative extraction controls and a positive control (ZymoBIOMICS Microbial Community Standard) were included in each batch to monitor for contamination and sequencing accuracy.

Bioinformatics Processing

Raw sequencing data were processed using the QIIME 2 (2022.11 release) pipeline.[10] Denoising, quality filtering, and chimera removal were performed with DADA2, resulting in amplicon sequence variants (ASVs).[11] Taxonomy was assigned to ASVs using the SILVA reference database (v.138).[12] Alpha-diversity metrics (Shannon index, Observed ASVs) and beta-diversity (Bray-Curtis dissimilarity) were calculated using core-diversity metrics within QIIME 2 after rarefying to an even sampling depth of 10,000 sequences per sample.

Outcome Ascertainment

The primary outcome was the development of a de novo hypertensive disorder of pregnancy at or after 20 weeks of gestation, as defined by the American College of Obstetricians and Gynecologists (ACOG):[13]

- Gestational Hypertension: Systolic BP ≥140 mmHg or diastolic BP ≥90 mmHg on two occasions at least 4 hours apart, in a previously normotensive woman.
- Pre-eclampsia: Gestational hypertension with proteinuria (≥300 mg/24h or protein/creatinine ratio ≥0.3) or new-onset hypertension with evidence of end-organ dysfunction (thrombocytopenia, elevated liver enzymes, renal insufficiency, pulmonary edema, or cerebral/visual symptoms).

Outcome data were abstracted from electronic medical records by study personnel blinded to microbiome data.

Statistical Analysis

Baseline characteristics were compared between the HDP and normotensive groups using Student's t-test (or Mann-Whitney U test) for continuous variables and Chi-square test for categorical variables. Alpha-diversity indices were compared using the Wilcoxon rank-sum test. Differences in beta-diversity (Bray-Curtis dissimilarity) were assessed using Permutational Multivariate Analysis of Variance (PERMANOVA) with 999 permutations. Differential abundance of bacterial taxa at the genus level was analyzed using the DESeq2 package in R, with significance defined by a False Discovery Rate (FDR) < 0.05.[14]

Logistic regression models were used to assess the association between alpha-diversity (per 0.5-unit increase in Shannon index) and HDP risk. We constructed an unadjusted model, a model adjusted for maternal age and pre-pregnancy BMI (Model 1), and a fully adjusted model that additionally included parity, baseline systolic BP, and dietary fibre intake (Model 2). All statistical analyses were performed in R version 4.2.2.

Sample Size Calculation

Assuming an HDP incidence of 10%, a sample size of 320 women provided 80% power at an alpha of 0.05 to detect an odds ratio of 0.6 per one-standard-deviation increase in the Shannon index. We recruited 350 women to account for an estimated 10% loss to follow-up.

3. Results

Study Population

Of 400 eligible women approached, 350 consented and provided baseline samples. After excluding 12 women lost to follow-up, 2 who required long-term antibiotics, and 3 who developed chronic hypertension, 333 women were included in the final analysis. Among these, 38 (11.4%) developed HDP (25 with gestational hypertension and 13 with pre-eclampsia), while 295 (88.6%) remained normotensive. As shown in Table 1, women who developed HDP were significantly older, had a higher pre-pregnancy BMI, and a higher baseline systolic blood pressure. There were no significant differences in parity, smoking status, or self-reported dietary fibre intake between the groups.

Table 1: Baseline Characteristics of the Study Population

Characteristic	Normotensive (n=295)	HDP (n=38)	p-value
Maternal Age (years)	28.1 ± 4.5	30.4 ± 5.1	0.02
Pre-pregnancy BMI (kg/m²)	24.9 ± 4.8	27.8 ± 5.5	<0.001
Nulliparity, n (%)	142 (48.1%)	22 (57.9%	0.25
Baseline SBP (mmHg)	112 ± 9	118 ± 11	0.01
Baseline DBP (mmHg)	68 ± 7	71 ± 8	0.06
Smoking, n (%)	25 (8.5%)	2 (5.3%)	0.50
Dietary Fibre (g/day)	18.5 ± 6.2	17.1 ± 5.8	0.18

Data are mean ± standard deviation or n (%). SBP: systolic blood pressure; DBP: diastolic blood pressure.

Gut and Vaginal Microbiome Diversity

Table 2 summarizes the alpha-diversity metrics for the gut and vaginal microbiomes. Women who developed HDP had significantly lower gut microbiome alpha-diversity, as measured by the Shannon index (3.80 vs. 4.49, p<0.001) and Observed ASVs (145 vs. 185, p<0.001) (Figure-1). Beta-diversity analysis (Bray-Curtis dissimilarity) also revealed a significant separation between the two groups (PERMANOVA, R^2=0.012, p=0.02), visualized in the PCoA plot (Figure 2A).

For the vaginal microbiome, the HDP group had a lower median Shannon index, but the difference was of borderline significance (2.50 vs. 2.90, p=0.03). Beta-diversity in the vaginal community was not significantly different between groups (PERMANOVA, p=0.12) (Figure 2B).

Table 2: Microbiome Alpha-Diversity Metrics by Outcome

Microbiome & Metric	Normotensive (n=295)	HDP (n=38)	p-value
Gut Microbiome			
Shannon Index	4.49 (4.10- 4.85)	3.80 (3.45 - 4.15)	<0.001
Observed ASVs	185 (156 - 220)	145 (120 - 175)	<0.001
Vaginal Microbiome			
Shannon Index	2.90 (0.75 - 3.50)	2.50 (0.60 - 3.10)	0.03
Observed ASVs	35 (15 - 55)	28 (12 - 48)	0.08

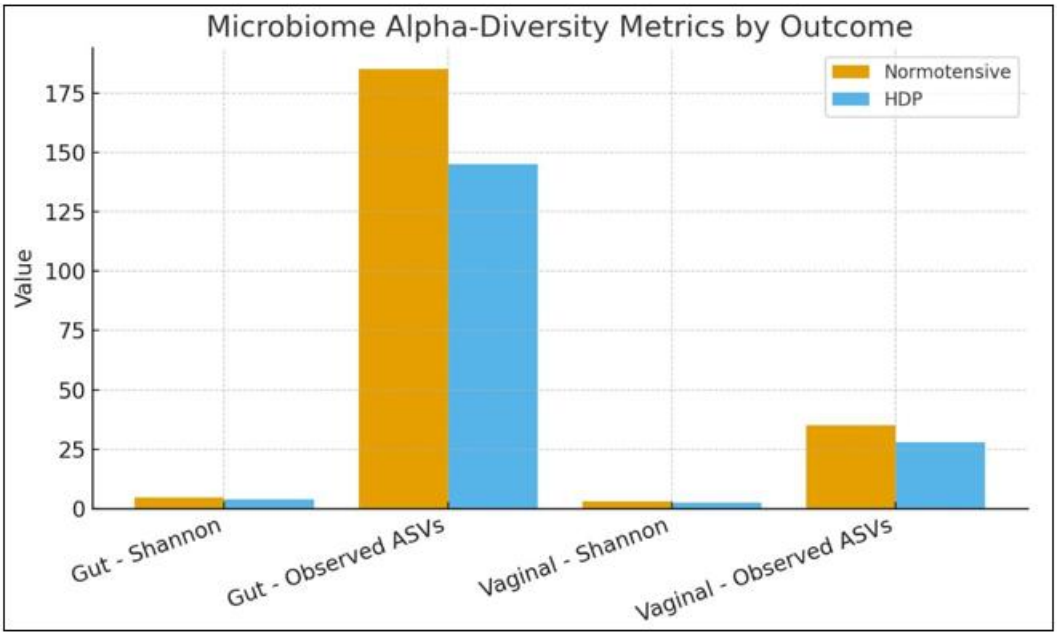

Figure 1: Microbiome Alpha- Diversity Metrics by Outcome
Data are median (interquartile range). p-values from Wilcoxon rank-sum test.

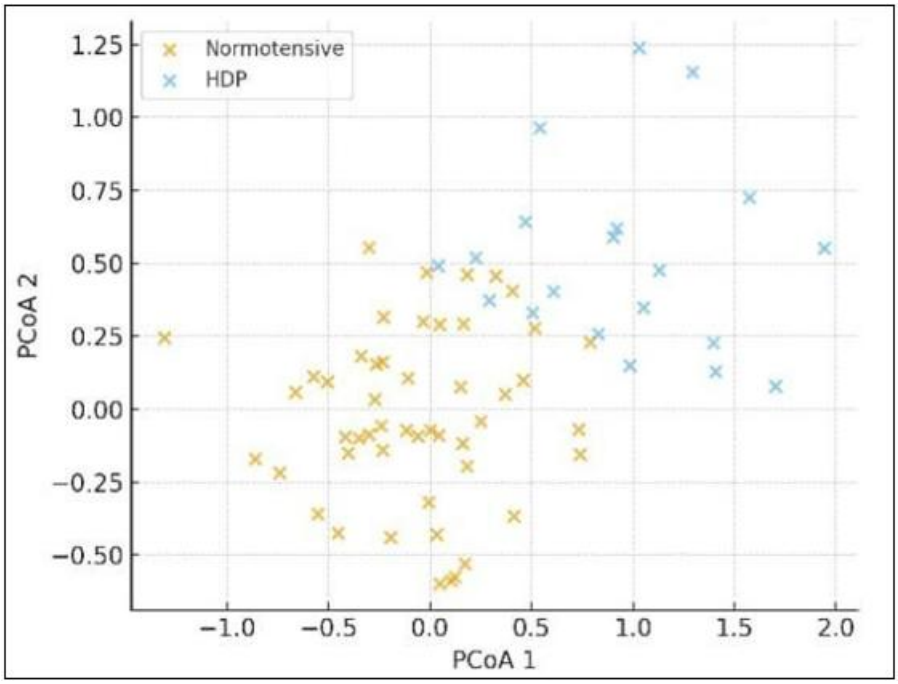

Figure 2A: Beta-diversity of Maternal Microbiome- Gut Microbiome Beta- Diversity (Bray-Curtis PCoA)

(2A) Principal Coordinate Analysis (PCoA) plot of gut microbiome based on Bray-Curtis dissimilarity. Points represent individual samples, colored by HDP outcome (PERMANOVA p=0.02).

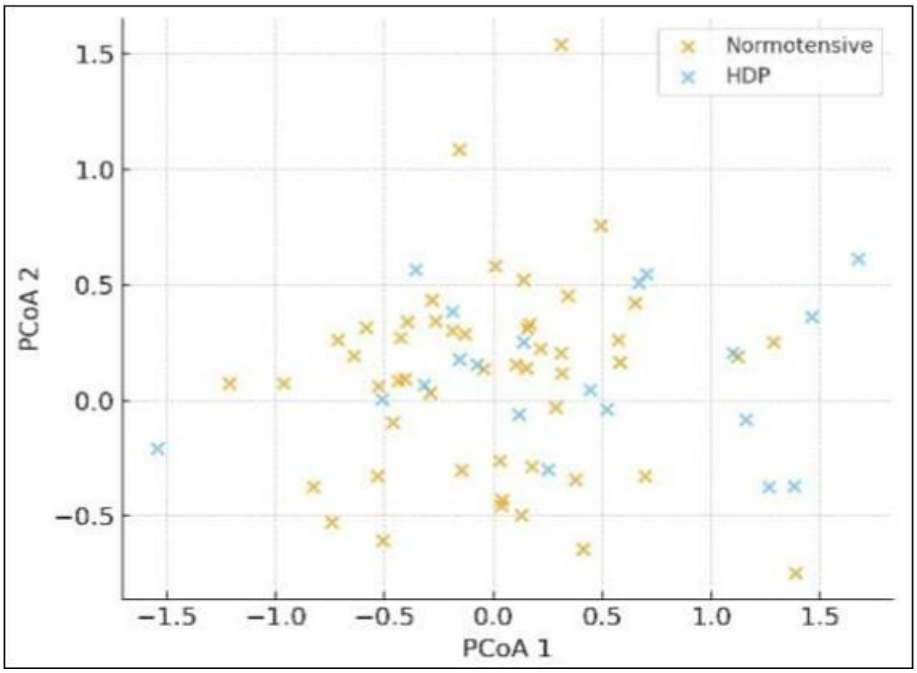

Figure 2B: Beta-diversity of Maternal Microbiome- Vaginal Microbiome Beta- Diversity (Bray-Curtis PCoA)
(2B) PCoA plot of vaginal microbiome based on Bray-Curtis dissimilarity (PERMANOVA p=0.12)

Differential Taxa Abundance

In the gut microbiome, differential abundance analysis revealed significant alterations in several bacterial genera. Women who developed HDP had depleted levels of beneficial or short-chain fatty acid-producing genera, including *Akkermansia* (log2 fold change -1.4, FDR=0.01), *Bifidobacterium* (log2FC -1.1, FDR=0.02), and *Coprococcus* (log2FC -0.9, FDR=0.03). Conversely, they showed enrichment of genera often associated with inflammation or a more detrimental metabolic profile, such as *Bilophila* (log2FC +1.3, FDR=0.02), *Escherichia/Shigella* (log2FC +1.6, FDR=0.01), and *Enterobacter* (log2FC +1.5, FDR=0.04).

In the vaginal microbiome, there was a non-significant trend towards lower relative abundance of *Lactobacillus crispatus* and a higher abundance of *Gardnerella* in the HDP group (FDR < 0.1).

Multivariable Logistic Regression Analysis

The results of the logistic regression analyses are presented in Table 3. In the unadjusted model, each 0.5-unit increase in the gut Shannon index was associated with a 35% reduction in the odds of HDP (OR 0.65, 95% CI 0.52-0.80). This association remained significant after sequential adjustment for confounders. In the fully adjusted model (Model 2), each 0.5-unit higher Shannon index was associated with a 30% lower odds of HDP (adjusted OR 0.70, 95% CI 0.55-0.90, p=0.005). The association between vaginal Shannon index and HDP was weaker and not statistically significant in the adjusted models.

Table 3: Logistic Regression for Association of Microbiome Shannon Index with HDP Risk

Model	Gut Microbiome OR (95% CI) per 0.5 unit	p-value	Vaginal Microbiome OR (95% CI) per 0.5 unit	p-value
Unadjusted	0.65 (0.52 - 0.80)	<0.001	0.80 (0.65 - 0.98)	0.03
Model 1 (Age, BMI)	0.68 (0.54 - 0.85)	<0.001	0.84 (0.68 - 1.04)	0.11
Model 2 (Full Adjustment)	0.70 (0.55 - 0.90)	0.005	0.85 (0.66 - 1.10)	0.21

Model 1: Adjusted for maternal age and pre-pregnancy BMI.
Model 2: Adjusted for maternal age, pre-pregnancy BMI, parity, baseline systolic BP, and dietary fibre intake.

Sensitivity Analyses

Sensitivity analyses excluding the 27 women who received antibiotics for any reason after enrolment yielded results consistent with the primary analysis. Furthermore, no significant interaction was observed between pre-pregnancy BMI category (normal vs. overweight/obese) and the effect of the gut Shannon index on HDP risk (p for interaction > 0.10).

4. Discussion

In this prospective cohort study, we found that lower maternal gut microbiome diversity and a dysbiotic taxonomic profile in the first trimester were significantly and independently associated with an increased risk of developing hypertensive disorders in the third trimester. These associations persisted after rigorous adjustment for established HDP risk factors, including maternal age, BMI, and baseline blood pressure. While the vaginal microbiome showed some association with HDP, it was weaker and not independently significant,

suggesting that the gut microbiome may play a more prominent role in HDP pathogenesis.

Our findings align with and extend the current literature. Previous studies have largely described microbial dysbiosis in women with established pre-eclampsia.[6 8] We demonstrate that this dysbiotic signature- characterized by reduced diversity, depletion of beneficial taxa like *Akkermansia* and *Bifidobacterium*, and enrichment of potential pathobionts like *Escherichia/Shigella*- is already present in the first trimester, weeks before clinical diagnosis. This temporal sequence strengthens the plausibility of a causal contribution. *Akkermansia muciniphila,* a mucin-degrading bacterium associated with improved metabolic and barrier function, has been previously reported to be reduced in pre-eclamptic women.[7] Its early depletion may impair gut barrier integrity, promoting systemic inflammation.

Several mechanistic pathways could link early gut dysbiosis to later HDP. First, a dysbiotic microbiome may produce an altered repertoire of metabolites. Reduced production of anti-inflammatory short-chain fatty acids (SCFAs) like butyrate, which are typically produced by genera such as *Coprococcus*, could compromise immune regulation and endothelial function.[15] Concurrently, an increase in pro-inflammatory molecules like lipopolysaccharide (LPS) from Gram-negative bacteria (e.g., *Escherichia/Shigella*) can trigger systemic inflammation and endothelial damage, hallmarks of pre-eclampsia.[2 6] Second, these microbial and metabolic signals may impair trophoblast invasion and spiral artery remodeling in the first trimester, setting the stage for placental ischemia and the subsequent maternal syndrome.[16 17]

The weaker association observed with the vaginal microbiome suggests that while local vaginal communities may influence pregnancy outcomes like preterm birth, their role in HDP risk may be less direct or potent than that of the gut microbiome, which has a vast capacity to influence systemic physiology.

5. Strengths and Limitations

Key strengths of our study include its prospective design with early-pregnancy sampling, parallel analysis of gut and vaginal niches, adjustment for major confounders, and robust ASV-based bioinformatics. However, several limitations must be acknowledged. The sample size, while sufficient for the primary analysis, limited power for meaningful subgroup analyses (e.g., severe pre-eclampsia). We did not measure microbial metabolites or inflammatory biomarkers, so the proposed mechanistic pathways remain speculative. Our cohort was from a single center, which may affect the generalizability of the findings to other populations with different ethnic backgrounds and lifestyles. Finally, while we adjusted for several confounders, residual confounding from unmeasured or imprecisely measured factors (e.g., detailed dietary components, stress, environmental exposures) cannot be ruled out.

6. Conclusions

In conclusion, our study provides compelling evidence that maternal gut microbiome dysbiosis in early pregnancy is an independent risk factor for the development of hypertensive disorders later in gestation. These findings suggest that the gut microbiome could serve as a novel, modifiable target for HDP prevention. Future research should focus on large, multi-center cohorts to validate these findings, integrate multi-omics data (metagenomics, metabolomics) to elucidate mechanisms, and ultimately, test the efficacy of microbiome-modulating interventions (such as prebiotics or specific probiotics) in high-risk women identified early in pregnancy.

Acknowledgements
The authors would like to express their sincere gratitude to the Department of Medicine and the Department of Microbiology, P. A. Sangma International Medical College and Hospital, Meghalaya, India, for their continuous support and guidance throughout this study. Special thanks are extended to all laboratory and technical staff for their assistance during data collection and analysis. The authors also acknowledge the valuable feedback and encouragement from colleagues that greatly improved the quality of this work.

Declaration
Funding: None
Conflict of interest: None
Ethical approval: The study was approved by the Institutional Ethical Committee

References

[1] Rana S, Lemoine E, Granger JP, Karumanchi SA. Preeclampsia: Pathophysiology, Challenges, and Perspectives. Circ Res. 2019;124(7):1094-1112.
[2] Phipps EA, Thadhani R, Benzing T, Karumanchi SA. Pre-eclampsia: pathogenesis, novel diagnostics and therapies. Nat Rev Nephrol. 2019;15(5):275-289.
[3] Gohir W, Whelan FJ, Surette MG, Moore C, Schertzer JD, Sloboda DM. Pregnancy-related changes in the maternal gut microbiota are dependent upon the mother's periconceptional diet. Gut Microbes. 2015;6(5):310-320.
[4] Koren O, Goodrich JK, Cullender TC, et al. Host remodeling of the gut microbiome and metabolic changes during pregnancy. Cell. 2012;150(3):470-480.
[5] Dunlop AL, Knight AK, Satten GA, et al. Stability of the vaginal, oral, and gut microbiota across pregnancy among African American women: the Effect of SES, Stress, and Depression. Ann Epidemiol. 2019;35:15-21.
[6] Ishimwe JA. Maternal microbiome in preeclampsia pathophysiology and implications on offspring health. Physiol Rep. 2021;9(10):e14875.
[7] Liu J, Yang H, Yin Z, et al. Remodeling of the maternal gut microbiome during pregnancy is shaped by parity. Microbiome. 2021;9(1):146.
[8] Ogunwole E, Fett A, Bhasipol A, et al. A Scoping Review of Microbiota Dysbiosis and Risk of Preeclampsia. J Pregnancy. 2024;2024:6683684.
[9] Klindworth A, Pruesse E, Schweer T, et al. Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. Nucleic Acids Res. 2013;41(1):e1.
[10] Bolyen E, Rideout JR, Dillon MR, et al. Reproducible, interactive, scalable and extensible microbiome data

science using QIIME 2. Nat Biotechnol. 2019;37(8):852-857.
[11] Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJ, Holmes SP. DADA2: High-resolution sample inference from Illumina amplicon data. Nat Methods. 2016;13(7):581-583.
[12] Quast C, Pruesse E, Yilmaz P, et al. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 2013;41(Database issue):D590-D596.
[13] Gestational Hypertension and Preeclampsia: ACOG Practice Bulletin, Number 222. Obstet Gynecol. 2020;135(6):e237-e260.
[14] Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 2014;15(12):550.
[15] Sun S, Li H, Chen J, Qian Q. Lactic Acid Bacteria: A Promising Tool for Menopausal Health Management in Women. Nutrients. 2023;15(13):2820.
[16] Broadening horizons: intestinal microbiota as a novel biomarker and potential treatment for hypertensive disorders of pregnancy. Front Cell Infect Microbiol. 2024; 14: 1446580.
[17] Jena MK, Nayak N, Chen K, Nayak NR. Role of Macrophages in Pregnancy and Related Complications. Arch Immunol Ther Exp (Warsz). 2019;67(5):295-309.