Am J Transl Res 2024;16(9):4662-4670
www.ajtr.org /ISSN:1943-8141/AJTR0158123

Original Article

Disturbance of gut microbiota aggravates the inflammatory response and damages the vascular endothelial function in patients with preeclampsia

Dan Cui[1*], Shujing Zhang[1*], Yefang Zhao[1], Bingjie Wang[2]

[1]Department of Obstetrics, Xingtai People's Hospital, Affiliated Hospital of Hebei Medical University, Xingtai 054001, Hebei, China; [2]Department of Proctology, Xingtai People's Hospital, Affiliated Hospital of Hebei Medical University, Xingtai 054001, Hebei, China. [*]Equal contributors.

Received May 28, 2024; Accepted August 11, 2024; Epub September 15, 2024; Published September 30, 2024

Abstract: Objective: To investigate the influence of intestinal flora imbalance on inflammatory factors in the serum and vascular endothelial functionality in individuals with preeclampsia (PE). Methods: From January 2022 to December 2023, a total of 58 individuals with PE (PE group) and 60 healthy controls (CON group) were included in this study; they were matched for age and pre-pregnancy Body Mass Index (BMI). A comparison was made between the two groups in terms of the general data and the number of unique intestinal flora. Additionally, clinical blood measures, serum inflammatory factors, and vascular endothelial function were also assessed and compared between the groups. Results: Age, gestational age, and pre-pregnancy BMI were similar between the PE and control group. However, diastolic and systolic blood pressure were significantly higher in the PE group. The abundance of Lactobacillus, Bifidobacterium, Enterobacter, and Enterococcus. Interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α) were considerably higher in the PE group compared to the CON group, but Interleukin-4 (IL-4) was noticeably lower, and the amount of White blood cells (WBC), neutrophil count (N) and lymphocyte count (L) in the PE group were significantly higher than those in the CON group. In the PE group, serum vascular endothelin (ET) and soluble endoglin (sEng) were higher than in the CON group, vascular endothelial growth factor (VEGF) and nitric oxide (NO) levels were considerably lower than in the CON group, and the levels of TC, TG, LDL-C and HDL-C were significantly higher in the PE group than in the CON group. The presence of Lactobacillus and Bifidobacterium was inversely associated with levels of TNF-α, IL-6, Interleukin-10 (IL-10), ET, and sEng, and positively associated with levels of IL-4, VEGF, and NO. Nevertheless, there was a positive correlation between the abundance of Enterobacterium and Enterococcus with the levels of TNF-α, IL-6, IL-10, ET, and sEng. Conversely, there was a negative correlation between the abundance of Enterobacterium and Enterococcus and the levels of IL-4, VEGF, and NO. Conclusion: Patients with PE exhibited dysbiosis of intestinal flora, characterized by altered gut microbiota diversity, increased serum pro-inflammatory factors, and impaired vascular endothelial function.

Keywords: Preeclampsia, gut dysbiosis, gut microbiota, inflammatory factors, vascular endothelial function

Introduction

Pre-eclampsia (PE) is a multi-system disorder that occurs during pregnancy and affects 3% to 5% of pregnant women, making it a leading cause of increased maternal and perinatal mortality [1, 2]. PE can cause renal failure, acute pulmonary oedema, stroke, hypertension, and metabolic syndrome in pregnant women [3, 4]. Studies have revealed that oxidative stress, aberrant lipid metabolism, and other factors may cause PE, and damage to endothelial cell activation, but the exact pathogenesis is not clear [5]. PE is associated with an increase in cytokines that promote inflammation and growth factors accompanied by endothelial dysfunction, which ultimately leads to a persistent inflammatory response outcome [6].

Intestinal flora dysbiosis can disrupt intestinal barrier, cause bacterial translocation, and induce systemic inflammation, leading to the onset and progression of various diseases, such as preeclampsia, atherosclerosis, intrahe-

https://doi.org/10.62347/CSJL6508

patic cholestasis in pregnancy, and pregnancy-associated hypothyroidism [7, 8]. Studies have suggested that dysbiosis in PE patients is associated with an increase in intestinal flora that promotes inflammatory responses. This altered flora and its metabolites modulate the body's immune cells and cause an elevation of pro-inflammatory factors [9]. When pro-inflammatory factors outweigh anti-inflammatory factors, it leads to vascular endothelial cell contraction and endothelial dysfunction, exacerbating systemic inflammatory response [10].

Recent studies [11, 12] have shown that PE development is closely related to intestinal flora, and compared to normal pregnant women, those with PE have markedly different gut microbiota. In addition, the main symptoms of PE, such as hypertension, proteinuria and associated inflammation, can also be traced to different gut bacteria separately. This study aims to explore how gut dysbiosis affects gut microbiota and inflammatory markers in PE patients, providing a theoretical basis for the formulation of new strategies for PE management.

Materials and methods

Study population

This retrospective study was approved by the Ethics Committee of Xingtai People's Hospital, Affiliated Hospital of Hebei Medical University. The study involved 118 singleton pregnant women from Xingtai People's Hospital Affiliated with Hebei Medical University between January 2022 and December 2023, including 58 pregnant women who were diagnosed with preeclampsia (PE group) after 20 weeks of pregnancy, and 60 healthy pregnant women with similar age and pre-pregnancy Body Mass Index (BMI) serving as the control group (CON). The study followed the standards for diagnosing and treating hypertensive diseases in pregnancy.

Inclusion criteria: 1) Singleton intrauterine pregnancy; 2) Gestational age between 20-41 weeks; 3) Age between 20-45 years; 4) Absence of psychiatric disorders; 5) Regular antenatal checkups with complete clinical data.

Exclusion criteria: 1) Renal insufficiency or hypertension; 2) History of intestinal surgery prior pregnancy; 3) Comorbid acute or chronic gastrointestinal disorders; 4) Presence of other serious pregnancy complications.

For all participants, data on age, number of deliveries, pre-pregnancy weight, height, BMI, systolic blood pressure (SBP), and diastolic blood pressure (DBP) were recorded.

The level of intestinal flora was detected

Fresh fecal samples (1 g) were collected on the second day after hospital the admission. The samples were dissolved using an anaerobic bacteria diluent and thoroughly mixed using an oscillator. An appropriate amount of normal saline was added to dilute the specimen, and a 10-fold dilution gradient was applied to reach a dilution of 10^(-7). Fifty μL of diluted liquid was inoculated onto a Lactobacillus culture medium, Bifidobacterium culture medium, Escherichia coli culture medium, Bacteroides culture medium, and Enterococcus culture medium for culture. Both the aerobic and anaerobic bacteria were cultivated in an incubator at 37°C for 24 and 48 hours, respectively. After cultivation, the pathogenic bacteria were identified by the professional laboratory personnel of our hospital according to the colony characteristics, morphology and Gram staining results. Additionally, the pathogenic bacteria were identified by mass spectrometer (LH-SH, Bruker, USA) to the genus or species level. The number of target colonies per gram of feces was calculated according to the colony count on the culture medium. The calculation formula: colony number (logN/g) = average colony number × dilution × 500.

Clinical blood index detection

In the morning, 5 mL of venous blood was collected from the elbow of the subjects on an empty stomach and centrifuged at 3000 r/min for 10 min. Total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) were measured by automatic biochemical analyzer (FX8, Toshiba, Japan). Additionally, we took an additional 30 μL of peripheral blood from the finger, which was mixed with EDTA anticoagulant and was used to assess red blood cell (RBC), hematocrit (HCT), white blood cell (WBC), platelets (PLT), neutrophil count (N) and lymphocyte count (L); which were detected by automatic blood cell analyzer (Sysmex5,

Table 1. Baseline characteristics of the PE and CON groups ($\bar{X}$±SD)

	PE (n = 58)	CON (n = 60)	*P* value
Maternal age (years old)	32.53±6.17	32.78±6.70	0.834
Gravidity (N)	1.41±0.59	1.4±0.62	0.902
Pre-pregnancy BMI (Kg/m^2)	22.67±2.10	22.86±2.34	0.648
BP at fecal collection (mmHg)			
Systolic	159.84±8.92	118.68±7.76	<0.001
Diastolic	100.28±7.52	77.92±11.18	<0.001

Note: BMI: body mass index; BP: blood pressure; PE: preeclampsia; CON: healthy controls.

Japan). All samples were tested only after passing quality control.

Serum inflammatory factor assay

On the morning of the 2nd day after hospital admission, fasting venous blood (5 mL) was collected and stored in a blood collection tube without antimicrobial agents. The blood was centrifuged for 20 min (8,000×g). The serum was then transferred to EP tubes for further analysis. The levels of serum tumor necrosis factor alpha (TNF-α) (KE00154, Proteintech, China), interleukin-6 (IL-6) (KE00139, Proteintech, China), interleukin-10 (IL-10) (KE00170, Proteintech, China), and interleukin-4 (IL-4) (KE00016, Proteintech, China) were detected using enzyme-linked immunosorbent assays (ELISA).

Vascular endothelial function test

On the morning of the second day after admission, 5 mL of fasting venous blood was collected into a tube without antimicrobial agents. The blood was centrifuged for 20 minutes at 8,000×g. The resulting serum was then divided into EP tubes for further analysis. The levels of vascular endothelial growth factor (VEGF) (COIBO BIO, China, CB11542-Hu), vascular endothelin (ET) (COIBO BIO, China, CB11084-Hu), nitric oxide (NO) (COIBO BIO, China, CB11614-Hu), and soluble endoglin (sEng) (COIBO BIO, China, CB10919-Hu) levels were detected using enzyme-linked immunosorbent assays (ELISA).

Statistical analysis

SPSS 26.0 was used for all statistical analyses. Data visualization was carried out using GraphPad Prism 8.0. Data were presented as the mean ± standard deviation. An unpaired two-tailed t-test or ANOVA was used to determine the statistical significance of differences between groups. Pearson correlation analysis was used to analyze the correlation of gut microbiota with inflammatory response and vascular endothelial function in preeclampsia patients. A *P* value less than 0.05 was considered to have statistical significance.

Results

General information

There were no significant differences in age, gestational age, and pre-pregnancy BMI between the PE group and the CON group (P>0.05). However, the SBP and DBP were significantly higher in the PE group compared to the CON group (all P<0.05), as shown in **Table 1**.

Comparison of intestinal flora levels

The abundance of intestinal flora in the PE group was as follows: Lactobacillus (5.84±0.50 LogN/g), Bifidobacterium (6.15±1.60 LogN/g), Enterobacter (9.73±2.41 LogN/g), and Enterococcus (9.91±1.46 LogN/g). In the CON, the abundance of Lactobacillus, Bifidobacterium, Enterobacter and Enterococcus was (7.71±1.46) LogN/g, (9.85±1.82) LogN/g, (7.4±0.63) LogN/g and (7.71±0.48) LogN/g, respectively. Notable differences in the abundances of Lactobacillus, Bifidobacterium, Enterobacter, and Enterococcus were observed between the two groups (all P<0.05), as **Figure 1** illustrates.

Comparison of serum inflammatory factors

The results of serum inflammatory factor levels showed that PE had considerably higher levels of IL-6 and TNF-α compared to CON, whereas CON had significantly lower serum levels of IL-4 (all P<0.05). There was no statistically significant difference in IL-10 levels between the CON and PE groups (P>0.05), as **Figure 2** illustrates.

Comparison of blood cell indexes

The results of routine blood test showed that the count of WBC, N and L in the PE group were

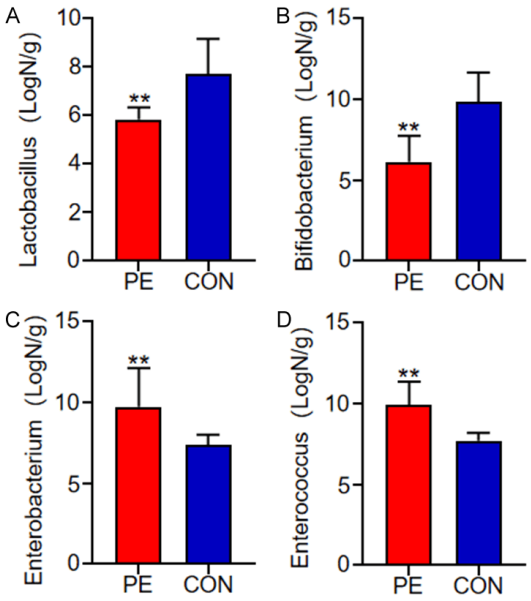

Figure 1. Comparison of the abundance of intestinal flora between the two groups. A. Comparison of the abundance of Lactobacillus. B. Comparison of the abundance of Bifidobacterium. C. Comparison of the abundance of Enterobacterium. D. Comparison of the abundance of Enterococcus. **P<0.01. Note: PE: preeclampsia; CON: healthy controls.

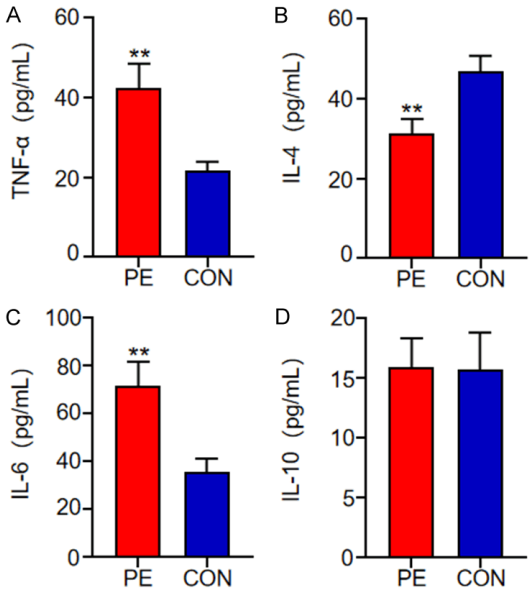

Figure 2. Comparison of serum inflammatory factors between the two groups. A. Comparison of the serum levels of TNF-α. B. Comparison of the serum levels of IL-4. C. Comparison of the serum levels of IL-6. D. Comparison of the serum levels of IL-10. **P<0.01. Note: TNF-α: Tumor necrosis factor alpha; IL-6: interleukin-6; IL-10: interleukin-10; IL-4: interleukin-4; PE: preeclampsia; CON: healthy controls.

significantly higher than those in the CON (all P<0.05). However, there were no statistical differences in PLT, RBC and HCT count between the two groups (all P>0.05), as **Figure 3** illustrates.

Comparison of vascular endothelial function

The results of vascular endothelial function indexes showed that serum ET and sEng levels in the PE group were noticeably higher than those in the CON group (all P<0.05), while serum NO and VEFG levels in the PE group were noticeably lower than those in the CON group (all P<0.05), as **Figure 4** illustrates.

Comparison of serum lipid indexes

The results showed that the levels of TC, TG, LDL-C and HDL-C were significantly higher in the PE group than in the CON group (P<0.05), as **Figure 5** illustrates.

Correlation between intestinal flora disorder and serum inflammatory factors

As shown in **Table 2**, Pearson correlation analysis showed that there was a negative relationship between the abundance of probiotics Lactobacillus, Bifidobacterium and the levels of TNF-α and IL-6. Additionally, a positive correlation was seen between the abundance of these probiotics with the level of IL-4. Conversely, the presence of detrimental bacteria, such as Enterobacter and Enterococcus, was positively correlated with TNF-α and IL-6 levels, while negatively correlated with IL-4 levels.

Correlation between intestinal flora disorder and vascular endothelial function

As shown in **Table 3**, Pearson correlation analysis showed that there was a negative relationship between the abundance of Lactobacillus, Bifidobacterium and the levels of ET and sEng, whereas a positive correlation was seen with the levels of VEGF and NO. On the other hand, the levels of Enterobacter and Enterococcus were positively correlated with ET and sEng, but negatively correlated with VEGF and NO levels.

Discussion

Pre-eclampsia (PE) is a pregnancy-related metabolic syndrome characterized by early onset

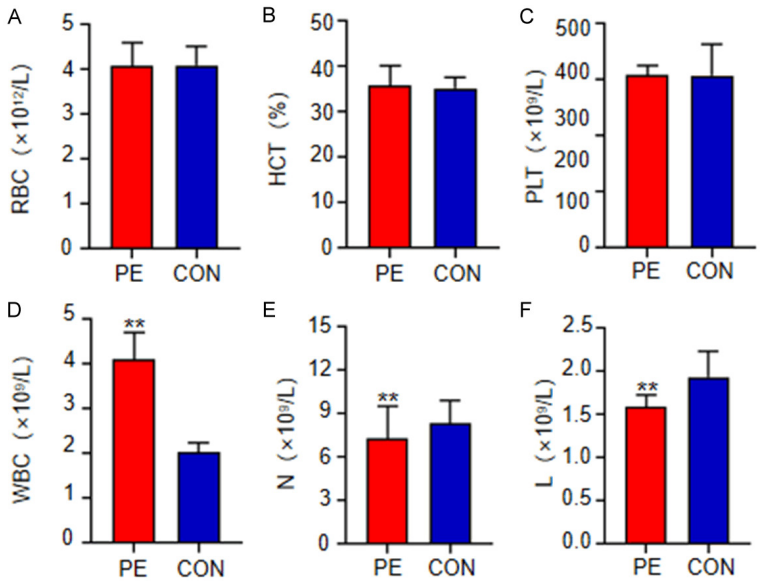

Figure 3. Comparison of blood cell indexes. A. Red blood cell count. B. Hematocrit. C. Platelet count. D. White blood cell count. E. Neutrophil count. F. Lymphocyte count. **P<0.01. Note: RBC: Red Blood Cell; HCT: Hematocrit; WBC: White blood cell; PLT: Platelets; N: neutrophil count; L: lymphocyte count; PE: preeclampsia; CON: healthy controls.

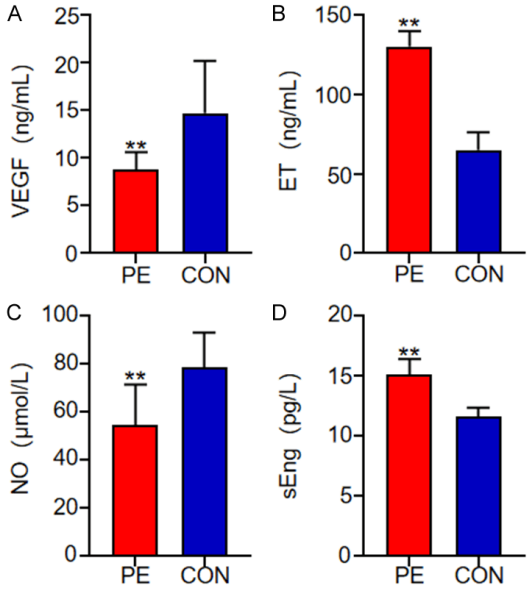

Figure 4. Comparison of vascular endothelial function between the two groups. A. Comparison of the serum levels of VEGF. B. Comparison of the serum levels of ET. C. Comparison of the serum levels of NO. D. Comparison of the serum levels of sEng. **P<0.01. Note: VEGF: Vascular endothelial growth factor; ET: vascular endothelin; NO: Nitric oxide; sEng: soluble endoglin; PE: preeclampsia; CON: healthy controls.

and rapid progression, often leading to preterm delivery and low fetal survival rates [13-15]. It has been found that gut flora may regulate blood pressure through mechanisms such as the sympathetic-gut axis, inflammatory response, and vascular endothelium [16-18]. During pregnancy, metabolic changes in the mother's body adapt to physiological conditions to support fetal growth and development [12, 19]. Additionally, there is a major change in the quantity of the gut flora [20]. When gut flora is balanced, it secretes both inflammatory and anti-inflammatory factors. However, an imbalance leads to a surge in inflammatory factors, which can damage endothelial cells lining blood vessels and affect the stability of the hemostatic system [21]. In this study, the abundance of beneficial bacteria, such as Bifidobacterium and Lactobacillus, was lower in the PE group compared to the CON group, while harmful bacteria such as Enterobacter and Enterococcus, were more prevalent in the PE group. This indicates a disruption in the intestinal flora of preeclampsia patients. These findings are consistent with those of Xie et al., who also reported significant differences in intestinal flora between pregnant women with PE and those without, with these differences correlating with various clinical indicators [22].

Under normal physiological settings, the intestinal flora helps maintain a balance between pro-inflammatory and anti-inflammatory molecules. However, disruptions to the flora disturb the dynamic equilibrium, leading to an increase in inflammatory factors, damage to the vascular endothelial cells, vasospasms, and a consequent rise in blood pressure [23, 24]. In addition, intestinal flora disorders can cause immune dysregulation, damage to the intestinal barrier, bacterial invasion of the placenta, and subsequent placental immune abnormalities, all contributing to preeclampsia [25]. Proinflammatory cytokines (IL-6, TNF-α) play crucial roles in mediating the maternal immune response. In preeclampsia, excessive secretion of IL-6 and TNF-α by maternal immune cells affects the function of endothelial cells by

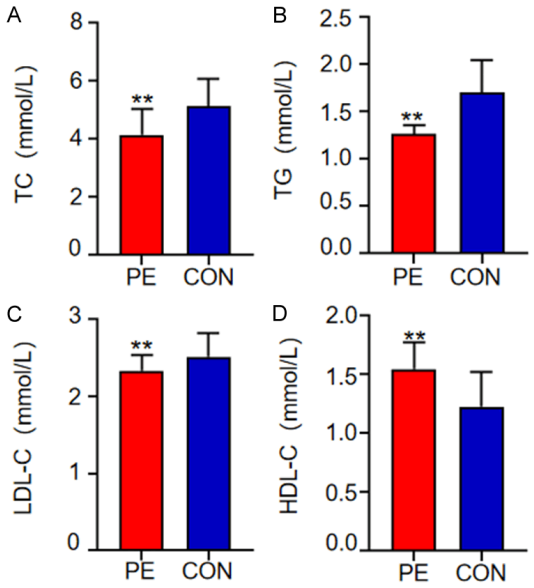

Figure 5. Comparison of serum lipid indexes. A. Total cholesterol. B. Triglyceride. C. Low-density lipoprotein cholesterol. D. High-density lipoprotein cholesterol. **P<0.01. Note: TC: Total cholesterol; TG: Triglyceride; LDL-C: Low-density lipoprotein cholesterol; HDL-C: High-density lipoprotein cholesterol; PE: preeclampsia; CON: healthy controls.

Table 2. Correlation between intestinal flora disorder and serum inflammatory factors

	TNF-α	IL-4	IL-6	IL-10
Lactobacillus	-0.591	0.554	-0.575	-0.093
P value	<0.001	<0.001	<0.001	0.315
Bifidobacterium	-0.713	0.678	-0.654	0.011
P value	<0.001	<0.001	<0.001	0.904
Enterobacterium	0.484	-0.480	0.536	0.02
P value	<0.001	<0.001	<0.001	0.832
Enterococcus	0.643	-0.520	0.612	0.031
P value	<0.001	<0.001	<0.001	0.738

Note: TNF-α: Tumor necrosis factor alpha; IL-6: interleukin-6; IL-10: interleukin-10; IL-4: interleukin-4.

increasing vascular permeability and inducing apoptosis of trophoblast cells [26]. Conversely, anti-inflammatory cytokines (IL-4, IL-10) are essential for the function of T helper cell 2 (Th2) and regulatory T cell (Treg). Alteration in their levels may affect immune and apoptotic pathways, leading to pregnancy-related syndromes [27]. To further investigate whether inflammatory factor expression levels in PE patients are influenced by gut flora dysbiosis, we further examined the serum levels of IL-6, TNF-α, IL-4 and IL-10 in both groups. The findings demonstrated that the PE group had considerably higher serum levels of the pro-inflammatory factors TNF-α and IL-6 than the CON group, and the CON group had substantially reduced serum levels of the anti-inflammatory cytokine IL-4, suggesting that intestinal flora dysbiosis in patients with PE is accompanied by an increased inflammatory response. Moreover, the PE group had significantly higher WBC, N and L compared to the CON group, suggesting that patients with preeclampsia have abnormal immune function and increased inflammatory infiltration. Zhao et al. found intestinal flora disorder and elevated serum proinflammatory factors in PE patients, which aligns with our results [28].

Table 3. Correlation between intestinal flora disorder and vascular endothelial function

Project	VEGF	ET	NO	sEng
Lactobacillus	0.405	-0.585	0.368	-0.609
P value	<0.001	<0.001	<0.001	<0.001
Bifidobacterium	0.457	-0.71	0.476	-0.629
P value	<0.001	<0.001	<0.001	0.904
Enterobacterium	-0.322	0.516	-0.261	0.449
P value	<0.001	<0.001	<0.001	0.832
Enterococcus	-0.293	0.671	-0.508	0.582
P value	<0.001	<0.001	<0.001	0.738

Note: VEGF: Vascular endothelial growth factor; ET: vascular endothelin; NO: Nitric oxide; sEng: soluble endoglin.

The occurrence of preeclampsia in pregnant women leads to increased formation of nitrotyrosine in the placenta, resulting in elevated lipid peroxides, reduced antioxidant enzyme activity, enhanced neutrophil infiltration, and the release of a variety of proteases, collectively culminating in abnormally high levels of inflammatory factors in patients [29, 30]. The inflammatory response aggravates damage to vascular endothelial cells, while toxic substances further impair vascular endothelial contraction and relaxation [31, 32]. Our study found that the serum levels of ET and sEng were significantly higher in the preeclampsia (PE) group compared to the CON group, while serum NO and VEGF levels were lower in the PE group, demonstrating that decreased vascular endothelial function is also associated with gut flora dysbiosis in individuals with PE. Kornacki J et al. showed that endothelial damage may occur

in both early and late stages of PE [33]. This implies a potential mutual causality between intestinal dysbiosis and vascular endothelial injury. In addition, elevated blood lipid level in PE patients, compared to the CON group, can further promote the production and secretion of inflammatory factors, thereby exacerbating vascular endothelial damage and contributing to the progression of preeclampsia. Subsequent analysis revealed that the presence of Lactobacillus and Bifidobacterium was inversely associated with levels of TNF-α, IL-6, IL-10, ET, and sEng, while positively associated with levels of IL-4, VEGF, and NO. Nevertheless, the presence of bacterial Enterobacterium and Enterococcus was positively correlated with the levels of TNF-α, IL-6, IL-10, ET, and sEng, but negatively correlated with IL-4, VEGF, and NO. The imbalance of intestinal flora in PE patients, marked by the increase in harmful bacteria and the decrease of probiotics, may be an important contributing factor to PE. This imbalance leads to the production of endotoxin, which in turn destroys the intestinal barrier and increases the permeability of the intestinal mucosa [34]. Endotoxins entering the bloodstream activate toll-like receptor family members (e.g., TLR4), triggering widespread inflammation [35]. This inflammatory response may exacerbate the damage to vascular endothelial cells and disrupt the process of vascular contraction and relaxation. Therefore, it is speculated that gut microbiota may contribute to the development of preeclampsia by affecting the inflammatory response and vascular endothelial function.

Conclusion

Patients with preeclampsia (PE) exhibit intestinal flora dysbiosis, characterized by altered diversity of intestinal microbiota, increased serum pro-inflammatory factors and impaired vascular endothelial function. A potential strategy for treating and preventing PE could involve managing gut flora balance, regulating inflammatory components, and mitigating endothelial dysfunction.

The innovation of this research lies in its focus on pregnant women as a unique population. It highlights how flora disturbance can affect the maternal immune and metabolic process, resulting in pregnancy complications such as preeclampsia. This study combines the detection of inflammatory and endothelial function levels with assessments of immune-related blood indices and gut flora disturbances to observe immune system changes. Additionally, it examines the relationship between blood lipid changes and preeclampsia, noting that increased lipid peroxidation and inflammatory marker production can exacerbate endothelial damage and promote preeclampsia development. This multi-faceted investigation provides a comprehensive understanding of how intestinal flora imbalance, inflammation, and endothelial function are interconnected in patients with PE.

Despite the insights gained from this study, there are notable limitations. The sample size is relatively small and needs further research with larger cohorts to validate and extend these findings. In addition, while this study confirms significant differences in intestinal flora between PE patients and those with normal pregnancies and demonstrates that intestinal flora imbalance can aggravate the inflammatory reactions and vascular endothelium injury, it does not include causal validation experiments. Further research is needed to explore the potential mechanisms underlying these associations and to establish causality.

Acknowledgements

This work was supported by the 2024 Hebei Province Medical Science Research Project Plan (20240547).

Disclosure of conflict of interest

None.

Address correspondence to: Yefang Zhao, Department of Obstetrics, Xingtai People's Hospital, Affiliated Hospital of Hebei Medical University, No. 16 Hongxing Street, Xiangdu District, Xingtai 054001, Hebei, China. Tel: +86-0319-3286273; E-mail: zhaoyefang315@163.com

References

[1] Dimitriadis E, Rolnik DL, Zhou W, Estrada-Gutierrez G, Koga K, Francisco RPV, Whitehead C, Hyett J, da Silva Costa F, Nicolaides K and Menkhorst E. Pre-eclampsia. Nat Rev Dis Primers 2023; 9: 8.

[2] Arechvo A, Wright A, Syngelaki A, von Dadelszen P, Magee LA, Akolekar R, Wright D and Nicolaides KH. Incidence of pre-eclampsia: ef-

fect of deprivation. Ultrasound Obstet Gynecol 2023; 61: 26-32.
[3] Walter MH, Abele H and Plappert CF. The role of oxytocin and the effect of stress during childbirth: neurobiological basics and implications for mother and child. Front Endocrinol (Lausanne) 2021; 12: 742236.
[4] Ashraf UM, Hall DL, Rawls AZ and Alexander BT. Epigenetic processes during preeclampsia and effects on fetal development and chronic health. Clin Sci (Lond) 2021; 135: 2307-2327.
[5] Qiu D, Wu J, Li M, Wang L, Zhu X and Chen Y. Impaction of factors associated with oxidative stress on the pathogenesis of gestational hypertension and preeclampsia: a Chinese patients based study. Medicine (Baltimore) 2021; 100: e23666.
[6] Qu H and Khalil RA. Vascular mechanisms and molecular targets in hypertensive pregnancy and preeclampsia. Am J Physiol Heart Circ Physiol 2020; 319: H661-H681.
[7] Chen X, Li P, Liu M, Zheng H, He Y, Chen MX, Tang W, Yue X, Huang Y, Zhuang L, Wang Z, Zhong M, Ke G, Hu H, Feng Y, Chen Y, Yu Y, Zhou H and Huang L. Gut dysbiosis induces the development of pre-eclampsia through bacterial translocation. Gut 2020; 69: 513-522.
[8] Ahmadian E, Rahbar Saadat Y, Hosseiniyan Khatibi SM, Nariman-Saleh-Fam Z, Bastami M, Zununi Vahed F, Ardalan M and Zununi Vahed S. Pre-eclampsia: microbiota possibly playing a role. Pharmacol Res 2020; 155: 104692.
[9] Wu J, Zhang D, Zhao M and Zheng X. Gut Microbiota dysbiosis and increased NLRP3 levels in patients with pregnancy-induced hypertension. Curr Microbiol 2023; 80: 168.
[10] Opichka MA, Rappelt MW, Gutterman DD, Grobe JL and McIntosh JJ. Vascular dysfunction in preeclampsia. Cells 2021; 10: 3055.
[11] Deady C, McCarthy FP, Barron A, McCarthy CM, O'Keeffe GW and O'Mahony SM. An altered gut microbiome in pre-eclampsia: cause or consequence. Front Cell Infect Microbiol 2024; 14: 1352267.
[12] Huang L, Liu Z, Wu P, Yue X, Lian Z, He P, Liu Y, Zhou R and Zhao J. *Puerariae lobatae* radix alleviates pre-eclampsia by remodeling gut microbiota and protecting the gut and placental barriers. Nutrients 2022; 14: 5025.
[13] Aditiawarman, Zulhijayanti NA, Ernawati E and Akbar MIA. A retrospective cohort study of hypertension, cardiovascular disease, and metabolic syndrome risk in women with history of preterm and term preeclampsia five years after delivery. Pregnancy Hypertens 2023; 32: 57-63.
[14] Gogoi P, Sinha P, Gupta B, Firmal P and Rajaram S. Neutrophil-to-lymphocyte ratio and platelet indices in pre-eclampsia. Int J Gynaecol Obstet 2019; 144: 16-20.
[15] Lu HQ and Hu R. The role of immunity in the pathogenesis and development of pre-eclampsia. Scand J Immunol 2019; 90: e12756.
[16] Mukherjee I, Dhar R, Singh S, Sharma JB, Nag TC, Mridha AR, Jaiswal P, Biswas S and Karmakar S. Oxidative stress-induced impairment of trophoblast function causes preeclampsia through the unfolded protein response pathway. Sci Rep 2021; 11: 18415.
[17] Robles-Vera I, Toral M and Duarte J. Microbiota and hypertension: role of the sympathetic nervous system and the immune system. Am J Hypertens 2020; 33: 890-901.
[18] Duttaroy AK. Role of gut microbiota and their metabolites on atherosclerosis, hypertension and human blood platelet function: a review. Nutrients 2021; 13: 144.
[19] Miao T, Yu Y, Sun J, Ma A, Yu J, Cui M, Yang L and Wang H. Decrease in abundance of bacteria of the genus Bifidobacterium in gut microbiota may be related to pre-eclampsia progression in women from East China. Food Nutr Res 2021; 65.
[20] Lv LJ, Li SH, Wen JY, Wang GY, Li H, He TW, Lv QB, Xiao MC, Duan HL, Chen MC, Yi ZT, Yan QL and Yin AH. Deep metagenomic characterization of gut microbial community and function in preeclampsia. Front Cell Infect Microbiol 2022; 12: 933523.
[21] Wang J, Chen WD and Wang YD. The relationship between gut microbiota and inflammatory diseases: the role of macrophages. Front Microbiol 2020; 11: 1065.
[22] Xie F, Zhang H, Peng M and Jiang T. Clinical study on the difference in intestinal microecology between patients with preeclampsia and pregnant women at different stages of pregnancy. Acta Biochim Pol 2024; 71: 12020.
[23] Al Bander Z, Nitert MD, Mousa A and Naderpoor N. The gut microbiota and inflammation: an overview. Int J Environ Res Public Health 2020; 17: 7618.
[24] Di Vincenzo F, Del Gaudio A, Petito V, Lopetuso LR and Scaldaferri F. Gut microbiota, intestinal permeability, and systemic inflammation: a narrative review. Intern Emerg Med 2024; 19: 275-293.
[25] Chen Y, Cui W, Li X and Yang H. Interaction between commensal bacteria, immune response and the intestinal barrier in inflammatory bowel disease. Front Immunol 2021; 12: 761981.
[26] Zhou B, Yuan Y, Zhang S, Guo C, Li X, Li G, Xiong W and Zeng Z. Intestinal flora and disease mutually shape the regional immune system in the intestinal tract. Front Immunol 2020; 11: 575.

[27] Mora Palazuelos CE, Aguilar Medina EM, Avendano Felix MM, Payan RR, Cortez MB, Lara Avila L, Herrera Salazar A, Murillo Llanes J, Sanchez-Schmitz G and Romero Quintana JG. IL-6, IL-10 and TNF-alpha gene polymorphisms in preeclampsia: a case-control study in a Mexican population. Ginekol Pol 2024; 95: 108-113.

[28] Zhao Y, Wang B, Zhao X, Cui D, Hou S and Zhang H. The effect of gut microbiota dysbiosis on patients with preeclampsia. Front Cell Infect Microbiol 2023; 12: 1022857.

[29] Sutton EF, Gemmel M and Powers RW. Nitric oxide signaling in pregnancy and preeclampsia. Nitric Oxide 2020; 95: 55-62.

[30] Chiarello DI, Abad C, Rojas D, Toledo F, Vazquez CM, Mate A, Sobrevia L and Marin R. Oxidative stress: normal pregnancy versus preeclampsia. Biochim Biophys Acta Mol Basis Dis 2020; 1866: 165354.

[31] Theofilis P, Sagris M, Oikonomou E, Antonopoulos AS, Siasos G, Tsioufis C and Tousoulis D. Inflammatory mechanisms contributing to endothelial dysfunction. Biomedicines 2021; 9: 781.

[32] Shao Y, Saredy J, Yang WY, Sun Y, Lu Y, Saaoud F, Drummer C 4th, Johnson C, Xu K, Jiang X, Wang H and Yang X. Vascular endothelial cells and innate immunity. Arterioscler Thromb Vasc Biol 2020; 40: e138-e152.

[33] Kornacki J, Wirstlein P and Wender-Ozegowska E. Markers of endothelial injury and dysfunction in early- and late-onset preeclampsia. Life (Basel) 2020; 10: 239.

[34] Sun Q, Zhang S, Liu X, Huo Y, Su B and Li X. Effects of a probiotic intervention on Escherichia coli and high-fat diet-induced intestinal microbiota imbalance. Appl Microbiol Biotechnol 2020; 104: 1243-1257.

[35] Ciesielska A, Matyjek M and Kwiatkowska K. TLR4 and CD14 trafficking and its influence on LPS-induced pro-inflammatory signaling. Cell Mol Life Sci 2021; 78: 1233-1261.