

Original Research

Levels and Significance of Arachidonic Acid Metabolites in Hypertensive Disorders of Pregnancy: A Prospective Cohort Study

Xueyan Cao^{1,†}, Jiaqi Yang^{1,†}, Yu Zhu¹, Xiaoqing Huo¹, Junze Bao², Lihong Shi^{3,*}, Min Su^{1,*}

¹Department of Obstetrics and Gynecology, Affiliated Hospital of Nantong University, Medical School of Nantong University, 226001 Nantong, Jiangsu, China

²Medical School of Nantong University, 226001 Nantong, Jiangsu, China

³Department of Obstetrics and Gynecology, the Third People's Hospital of Tongzhou, 226001 Nantong, Jiangsu, China

*Correspondence: 425777671@qq.com (Lihong Shi); sumin_nt@126.com (Min Su)

†These authors contributed equally.

Academic Editor: Michael H. Dahan

Submitted: 2 July 2023 Revised: 5 September 2023 Accepted: 27 September 2023 Published: 13 December 2023

Abstract

Background: This project aimed to investigate the role of two arachidonic acid metabolites (14,15-epoxyeicosatrienoic acid (14,15-EET) and 15-hydroxyeicosatetraenoic acid (15-HETE)) as a precursor of hypertensive disorders in pregnancy by comparing their serum levels between third-trimester hypertensive and normal pregnant women. The relationship between their differential levels and pregnancy outcomes was investigated to clarify the role of arachidonic acid metabolites in the occurrence and development of hypertensive disorders during pregnancy. **Methods:** This is a prospective cohort study and a total of 88 patients were included in the study. 17 of them were recognized as the gestational hypertension and 25 of them were considered to be the preeclampsia. 24 women were diagnosed with severe preeclampsia. The control group consisted of 22 healthy patients pregnancy course, with no disease at the present and in the history. For this purpose, the serum levels of 14,15-EET and 15-HETE of gestational hypertension, preeclampsia (PE) and normal pregnant women group were detected by enzyme-linked immunosorbent assay (ELISA) during the third trimester of pregnancy. It was followed by detecting placental cytochrome P450 2J2 (CYP2J2) and 15-lipoxygenase-2 (15-LOX-2) expression and localization using immunohistochemistry, their corresponding proteins employing western blotting. All outcomes of maternal pregnancy were then statistically analyzed. **Results:** The analysis indicated that the levels of 14,15-EET, and 15-HETE were significantly higher ($p < 0.05$) in gestational hypertension compared to control, where the same differences were observed when severe preeclampsia and preeclampsia groups were compared, which were altogether higher than gestational hypertension group patients. Similarly, the 14,15-EET and 15-HETE levels were significantly ($p < 0.05$) higher in the adverse pregnancy outcome than in the normal pregnancy outcome group in severe preeclampsia. The immunohistochemical analysis results revealed that the positive staining for CYP2J2 and 15-LOX-2 expression in the placenta of the preeclampsia group was significant ($p < 0.05$) compared to the normal pregnant control and gestational hypertension groups, with significantly higher ($p < 0.05$) relative CYP2J2 and 15-LOX-2 protein levels in preeclampsia compared to normal control pregnancy group. **Conclusions:** The study concluded that the 14,15-EET and 15-HETE might be involved in gestational hypertension pathophysiology and preeclampsia, associated with disease severity and adverse pregnancy outcomes. Moreover, CYP2J2 and 15-LOX-2 signaling expression in the placenta may be related to arachidonic acid metabolites generated in preeclampsia.

Keywords: 14,15-EET; 15-HETE; arachidonic acid metabolites; gestational hypertension; preeclampsia

1. Introduction

China has currently completely and successively liberalized the second and third-child policy, which translated into an increase in the number of high-risk pregnancies, particularly a higher number of patients with hypertensive disorders pregnancy (HDP), which not only seriously threatens maternal and infant health but is also the primary cause of maternal and perinatal death. The HDP has a higher recurrence rate and pregnancy termination is currently the only viable cure. Therefore, an in-depth understanding of gestational hypertension and preeclampsia (PE) pathogenesis is envisaged to offer the possibility of early prediction and intervention, an important subject that needs to be explored in the field.

Recent study has shown that arachidonic acid (AA) metabolites are antihypertensive and protect vascular endothelial function [1]. Since various vascular dysfunction diseases are caused by metabolic abnormalities [2–4]. The AA and eicosanoids are metabolized by sets of enzymes in three pathways, including cyclooxygenase (COX) [5], lipoxygenase (LOX) [6], and cytochrome oxidase P450 (CYP450) [7]. The 15-hydroxyeicosatetraenoic acid (15-HETE) is produced via the AA-LOX pathway [8], while epoxyeicosatrienoic acids (EETs) and 20-hydroxyeicosatetraenoic acid (20-HETE) are CYP450-derived [9]. Current studies indicated that EETs and 20-HETE are essential in regulating local vascular tone and developing hypertension [10,11]. A report published re-



Table 1. The basic information of each group.

| | Control | Gestational hypertension | PE | Severe PE | <i>p</i> |
|--------------------------|---------------|--------------------------|---------------|---------------|----------|
| Age (years) | 28.55 ± 2.36 | 28.35 ± 2.50 | 27.48 ± 2.63 | 29.29 ± 2.56 | >0.05 |
| Week of delivery | 38.91 ± 1.19 | 38.24 ± 1.03 | 35.44 ± 1.19 | 34.29 ± 1.04 | <0.01 |
| BMI (kg/m ²) | 23.78 ± 3.09 | 22.90 ± 2.33 | 22.50 ± 2.11 | 23.37 ± 2.39 | >0.05 |
| SP (mmHg) | 122.27 ± 8.64 | 148.35 ± 4.43 | 148.40 ± 3.96 | 168.13 ± 6.64 | <0.01 |
| DP (mmHg) | 76.14 ± 6.27 | 100.76 ± 4.91 | 100.80 ± 4.84 | 120.71 ± 5.99 | <0.01 |
| PRO (g/24 h) | - | 0.22 ± 0.04 | 3.85 ± 0.41 | 4.15 ± 0.59 | <0.01 |

Values are the mean ± standard deviation, BMI, body mass index; SP, systolic pressure; DP, diastolic pressure; PRO, urine protein; PE, preeclampsia.

cently used mass spectrometry for analyzing dihydroxyeicosatrienoic acids (DHETs), an EETs metabolite, in the PE patient's urine sample and found significantly lower levels compared to normal pregnant women [12], suggesting that the metabolism of EETs may be associated with renal dysfunction and hypertension in PE patients. It has already been confirmed that 20-HETE plays a vital role in hypertension pathogenesis, and some PE patients were found to have an increased 20-HETE serum level [10]. Similarly, 15-HETE is also highly expressed in the serum of patients with pulmonary hypertension [13]. However, there are few reports on the levels of 15-HETE and 14,15-epoxyeicosatrienoic acid (14,15-EET) in the peripheral blood of gestational hypertension and PE patients, with scarce investigations on the LOX and CYP, which are responsible for the synthesis of AA metabolites in gestational hypertension and PE patients.

Lipoxygenases (LOXs) are a family of dioxygenases that catalyze the peroxidation of polyunsaturated fatty. The fatty acid hydroperoxides formed by the different LOX isoenzymes involved in many relevant cell processes and human pathologies. One of the most important LOX enzymes is 15-LOX. Human 15-LOX-2 metabolizes AA exclusively to 15-HETE [14]. Cytochrome P450 comprises a superfamily of heme-thiolate proteins named for the spectral absorbance peak of their carbon-monoxide-bound species at 450 nm. Although 12 human CYP genes have been reported to possess epoxygenase activity, the most important appear to be the CYP2J and CYP2C families. CYP2J2 remains a strong candidate for a vascular protective lipid metabolising epoxygenase. CYP2J2 protein metabolises arachidonic acid to all four cis-EETs and is highly enantio-selective for 14,15-EET [15]. Our study is to investigate the expression of CYP2J2 and 15-LOX-2 proteins in placental tissue, also serum 14,15-EET and 15-HETE levels in the pregnant women of gestational hypertension and preeclampsia.

2. Materials and Methods

2.1 Grouping of Study Subjects

A prospective cohort study was established to collect peripheral blood, and as a result that a total of 88 normotensive pregnant women and pregnant women with gestational

hypertension and preeclampsia between 34–40 weeks of gestational age who were undergoing routine obstetric examination and inpatient delivery at Affiliated Hospital of Nantong University from January 2018 to January 2022 were selected. All patients were grouped into experimental groups, i.e., gestational hypertension (*n* = 17), PE (*n* = 25), severe PE (*n* = 24), and control group, i.e., normal healthy pregnant women (*n* = 22) in the same gestational period. There was no significant difference in the age and body mass index (BMI) of all groups (*p* > 0.05). In contrast, significant differences (*p* < 0.05) were observed for gestational age, blood pressure, and urinary proteins. The patients were included in the study per the diagnostic HDP guidelines in American College of Obstetrics and Gynecology (ACOG) Practice Bulletin [9]. Inclusion criteria: (1) aged 18–35 years; (2) single pregnancy and natural conception; (3) no hypertensive disease in the past; (4) no previous obstetric complications and internal and surgical complications, no recent infection, fever, etc., no previous history of serious cardiovascular disease, diabetes, kidney disease, no infectious disease, no autoimmune disease, no bad habits such as smoking and drinking, no family history of diabetes, hypertension, coronary heart disease and other diseases. Exclusion criteria: (1) initial antenatal examination greater than 20 weeks; (2) there are other obstetric complications or medical and surgical diseases; (3) abortion or termination of pregnancy for reasons other than preeclampsia; (4) loss of follow-up. The hospital's Ethics Committee approved this study (Ethics number: 2019-L123), and all patients signed informed consent. All puerpera were able to be followed up for three months after delivery during the study period, where after three months, blood pressure levels returned to normal in all patient groups, essentially the same as before pregnancy, with no need for further medication. The basic information of each group is shown in Table 1.

2.2 Serum Collection and ELISA

All study participants were put on fasting for ≥8 hours, followed by withdrawal of venous blood samples by trained nursing personnel, followed by centrifuging the samples at 3000 rpm for 10 minutes to separate serum from other cellular components and stored at –80 °C refrigera-

tor till further use. The serum samples were analyzed for 14,15-EET and 15-HETE levels using enzyme-linked immunosorbent assay (ELISA) in the same batch. ELISA kits were purchased from the Jiangsu Meimian Industry Co., Ltd. (Yancheng, Jiangsu, China), and assays were performed per the manufacturer's instructions. Each sample was analyzed twice, and the results were averaged.

2.3 Pregnancy Outcomes

The adverse pregnancy outcomes in this study included perinatal death (intrauterine death or induced labor and neonatal death), neonates intensive care unit (NICU) stay after birth, very low birth weight, 5 min Apgar score <7 after delivery, intrauterine growth restriction, neonatal respiratory distress syndrome, placental abruption, oligohydramnios, postpartum hemorrhage, hemolysis, elevated liver enzymes, and low platelets (HELLP) syndrome, and heart failure.

2.4 Immunochemistry Analysis of CYP2J2 and 15-LOX-2 in Placental Tissue

The placental tissues were sliced with the help of microtome, fixed in paraformaldehyde (Servicebio, Wuhan, Hubei, China), and rinsed with 1% tween-phosphate buffered saline solution (Servicebio, Wuhan, Hubei, China) twice for 5 minutes. The slides were then immersed in 3% hydrogen peroxide (Servicebio, Wuhan, Hubei, China) for 20 minutes to quench endogenous peroxidase activity. The specimens were pre-blocked for 30 minutes with bovine serum albumin (BSA, Servicebio, Wuhan, Hubei, China). Subsequently, the slides were incubated with rabbit monoclonal antibody CYP2J2 and 15-LOX-2 (Invitrogen, Carlsbad, CA, USA, 1:200 dilution) for 1 hour at room temperature. After washing thrice with phosphate buffered saline (PBS) (Servicebio, Wuhan, Hubei, China), the slides were incubated in biotin-conjugated goat antirabbit immunoglobulin (IgG, EnVision, Dako, Ely, UK, 1:500 dilution) for 1 hour at room temperature. Negative controls were prepared by replacing the primary antibody with tris-buffered saline (Servicebio, Wuhan, Hubei, China). The positive controls were performed on the samples of known cases. Respective sections were photographed at 200× magnification and analyzed using Image J software (version 1.8.0, National Institutes of Health, Bethesda, MD, USA).

2.5 Analysis of CYP2J2 and 15-LOX-2 in Placental Tissue by Western Blot

Patient-derived tissue samples were lysed by radio immunoprecipitation assay (RIPA) buffer (Servicebio, Wuhan, Hubei, China). Protein concentrations were determined by bicinchoninic acid (BCA) Protein Assay Kit (Beyotime Biotechnology, Shanghai, China). Protein extracted from above samples was loaded in sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE, Servicebio, Wuhan, Hubei, China), resolved by electrophoresis, and transferred to polyvinylidene fluoride (PVDF) mem-

branes (Millipore, Billerica, MA, USA). Nonspecific protein bands on these membranes were blocked by BSA for 30 minutes, followed by incubation of rabbit monoclonal antibody CYP2J2 and 15-LOX-2 (Invitrogen, Carlsbad, CA, USA) at 1:1000 dilution at 4 °C overnight. Following incubation with goat anti-rabbit at 1:20,000 dilution for 2 hours at room temperature, the bands of specific proteins on the membranes were developed using Odyssey (LICOR, Lincoln, NE, USA). The level of proteins was quantified using the ImageJ software (National Institutes of Health, Bethesda, MD, USA). The expression of β -actin was used as control.

2.6 Statistical Processing

All data were statistically analyzed using GraphPad Prism (version 8.00, GraphPad Software, Inc., San Diego, CA, USA). The intra-group measurement data were expressed as mean \pm standard deviation ($M \pm S$). Student's *t*-test was used to analyze the differences between the two groups, while multiple group comparisons were analyzed using one-way analysis of variance (ANOVA). A *p*-value < 0.05 indicate a statistically significant difference.

3. Results

3.1 Serum Levels of 14,15-EET and 15-HETE in Pregnant Women of Each Group

The ELISA results of all samples for serum levels of 14,15-EET and 15-HETE are shown in Fig. 1. Results indicated that the levels of 14,15-EET (Fig. 1A) and 15-HETE (Fig. 1B) were significantly higher ($p < 0.05$) with elevated blood pressure and disease severity among different groups (control, gestational hypertension, PE, and severe PE groups).

3.2 Analysis of Serum 14,15-EET and 15-HETE Levels and Pregnancy Outcomes during PE

Based on pregnancy outcomes, the severe PE group was further divided into adverse pregnancy ($n = 14$) and normal pregnancy outcome groups ($n = 10$). The association between serum 14,15-EET, 15-HETE levels and adverse pregnancy outcomes in the pregnant women was analyzed, and results showed that the 14,15-EET and 15-HETE levels in the adverse pregnancy were significantly higher ($p < 0.05$) than normal pregnancy outcome group, as shown in Table 2.

3.3 CYP2J2 and 15-LOX-2 Expression in Placental Tissue Samples by Immunohistochemistry

The immunohistochemical analysis results of placental tissue specimens revealed that CYP2J2 and 15-LOX-2 were localized and expressed in the cytoplasm of trophoblast cells in the placenta. The positive staining for CYP2J2 (Fig. 2B) and 15-LOX-2 (Fig. 2E) in the severe PE group was significantly higher than the normal pregnant control group (Fig. 2A,D), as shown in Fig. 2C,F.

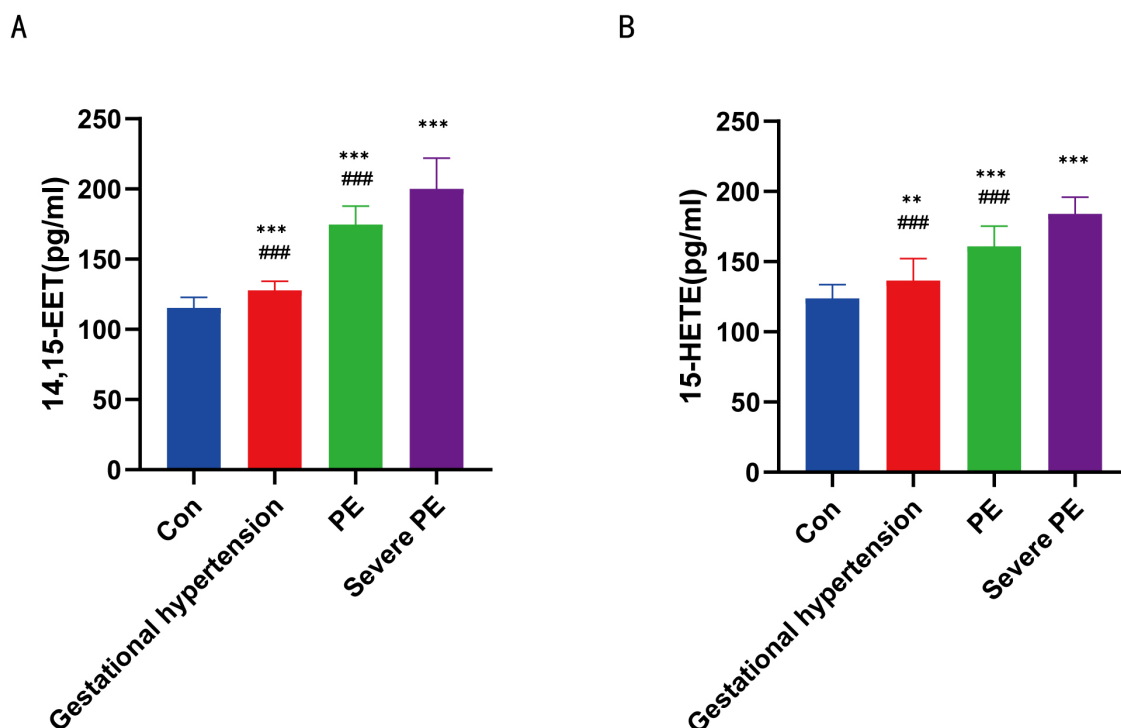


Fig. 1. Levels of serum 14,15-EET (A), and 15-HETE (B) in control, gestational hypertension, PE, and severe PE groups. ** $p < 0.01$, *** $p < 0.001$, compared with the control group; ### $p < 0.001$, compared with the severe PE (ANOVA). 14,15-EET, 14,15-epoxyeicosatrienoic acid; 15-HETE, 15-hydroxyeicosatetraenoic acid; con, control; PE, preeclampsia.

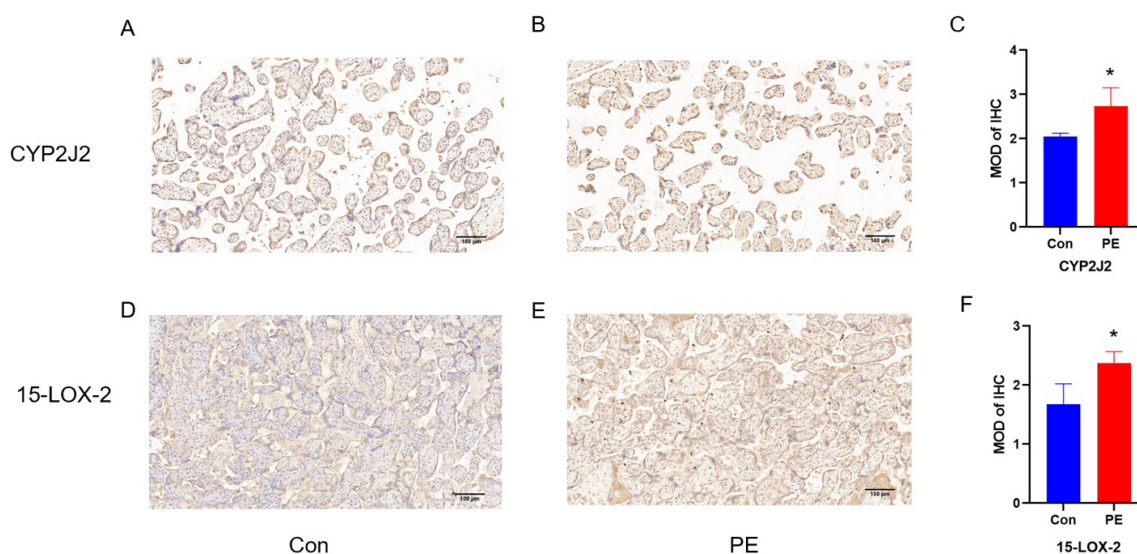


Fig. 2. CYP2J2 and 15-LOX-2 expression in placental tissue samples by immunohistochemistry. Expression of CYP2J2 (A,B) and 15-LOX-2 (D,E) in placental tissues of control and PE group detected by immunohistochemistry (200 \times). Analysis of CYP2J2 (C) and 15-LOX-2 (F) protein relative expression in PE tissues compared to the control tissues using *T*-test, * $p < 0.05$. MOD, mean optical density; IHC, immunohistochemistry; CYP2J2, cytochrome P450 2J2; 15-LOX-2, 15-lipoxygenase-2.

3.4 CYP2J2 and 15-LOX-2 Expression in Placental Tissue Samples by Western Blot

The western blot analysis results revealed that the protein levels of CYP2J2 (Fig. 3A) and 15-LOX-2 (Fig. 3C) in the PE placental group were significantly higher than the normal pregnant control group, as shown in Fig. 3B,D.

4. Discussion

The pathogenesis of HDP is complex, and characterized by extensive endothelial dysfunction and placental changes. Different factors have been found to contribute to HDP development, including inflammatory [16], immuno-

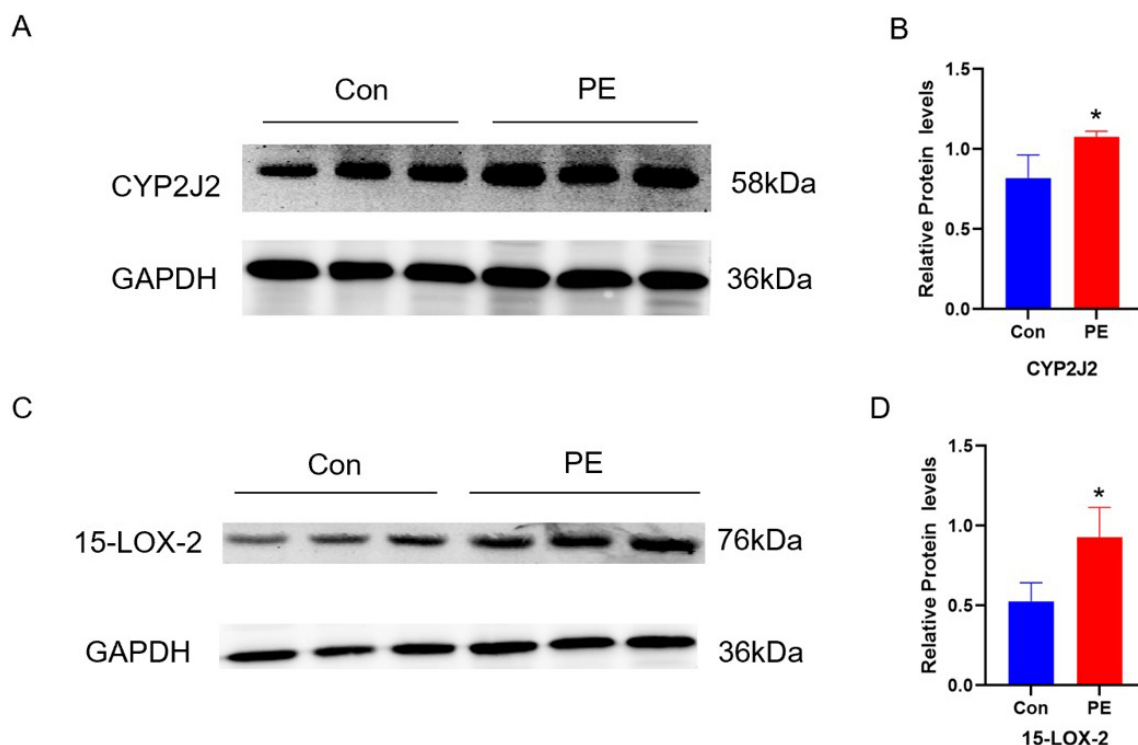


Fig. 3. Expression of CYP2J2 and 15-LOX-2 in placental tissue samples by western blot. Analysis of relative protein levels of CYP2J2 (A,B) and 15-LOX-2 (C,D) in PE placental tissues compared with the control tissues using *T*-test, $*p < 0.05$. GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

Table 2. Analysis of serum 14,15-EET, 15-HETE and pregnancy outcome during severe PE.

| | 14,15-EET (pg/mL) | 15-HETE (pg/mL) |
|---------------------------------|--------------------|--------------------|
| Adverse pregnancy outcome group | 201.92 \pm 25.73 | 191.22 \pm 10.01 |
| Normal pregnancy outcome group | 121.07 \pm 5.35 | 132.26 \pm 4.71 |
| T-value | 9.727 | 17.23 |
| <i>p</i> -value | <0.0001 | <0.0001 |

logical [17], and nutritional [18], however, no clear conclusion has yet been drawn till date, but generally, it is now accepted that HDP pathogenesis is closely related to uterine spiral artery recasting disorders [19] and cardiovascular endothelial dysfunction. Owing to rapid and uncontrollable HDP progression, the accuracy of predicting the HDP occurrence risk only through risk factors assessment could be low. Work has been in progress for predicting HDP occurrence by analyzing different biochemical indicator levels in maternal blood from the early onset before 20 weeks of gestation, as well as to delay disease development via timely intervention, further prolonging the gestational weeks, improving maternal and infant survival and prognosis, and ultimately achieving HDP cure. For example, Diniz *et al.* [20] used the ratio of soluble fms-like tyrosine kinase 1 (sFlt-1) to placental growth factor (PlGF) for predicting PE occurrence and prognosis, while a study also reported the effects of vitamin D supplementation on PE [21]. However, there are still no clear, well-accepted, early, rapid, economical and effective biochemical indicators for HDP monitoring and clinical disease severity assessment.

Endothelial cells are the core participants in the recasting process of uterine spiral arteries, while AA metabolites are critical regulatory factors for the proliferation and apoptosis of vascular endothelial cells. Their metabolic abnormalities are associated with dysfunctional vascular diseases. EETs are produced by CYP450 from AA, which exists in four different regioisomers forms, including 14,15-EET, distributed in trophoblasts, placenta, fetal membranes, and decidua. Similarly, 15-HETE is a major AA metabolite formed via the LOX-COX pathway. The study used liquid chromatography coupled with mass spectrometry to compare placental EET and HETE levels in normal pregnant and PE patients, where 5-, 12- and 15-HETE contents in PE patients were significantly higher than in normal pregnant women, meanwhile PE patient's placental EET contents were positively correlated with HETEs [22], suggesting that EETs and HETEs may play a particular role in the cellular regulatory mechanism of the placental vasculature. Our results showed that the serum 14,15-EET and 15-HETE levels were related to disease severity and adverse pregnancy outcome in the hypertensive disorders pregnancy.

Previous study has reported that EETs mainly function as vasodilators and could promote angiogenesis [23]. The plasma EETs tend to decrease in non-pregnant patients with secondary hypertension, such as renal hypertension, but increase during pregnancy. The study has shown that HDP patients secrete large amounts of 14,15-EET in their urine, suggesting that 14,15-EET might have a regulatory effect on HDP [24]. EETs are also produced in the placenta, trophoblast cells, amniotic membrane, chorion, decidua, and myometrium of the pregnant uterus, and increased EETs levels in the embryo-placenta are more significant than those in plasma [25]. EETs contribute to the physiological response to normal pregnancy and the pathophysiology of pregnancy-induced hypertension [26]. Moreover, a study showed that the expression of cytochrome P450-2J2 (CYP2J2) in the placenta and decidua of HDP patients was higher than that in normal pregnant women, which could promote the synthesis and secretion of EETs in the placenta, helping to dilate spasmodic and narrowed placental vessels, in order to improve maternal-fetal circulation [27]. Our results indicated that the CYP2J2 protein was specifically localized in the placental trophoblasts and was upregulated in PE, in agreement with previous study. Enhanced EET level in circulation reflects incremental CYP2J2-mediated EET biosynthesis in the maternal-fetal interface in PE, indicating CYP2J2 might be regarded as a novel potential candidate for the disturbed uteroplacental vascular remodeling, leading to pregnancy-induced hypertension.

The related functions of HETEs on uterine spiral artery endothelial cells and smooth muscle cells may cause dysfunction of trophoblast invasion, resulting in the inadequate recasting of the uterine spiral arteries, aggravating placental ischemia and hypoxia, and forming a vicious circle [28]. Hypoxia induces the simultaneous synthesis of 15-HETE in the maternal serum and placenta in HDP patients, also significantly increasing of 15-HETE in the serum of hypertensive patients. Previous study has shown that hypoxia induces the secretion of 15-HETE from trophoblast cells in the cytotrophoblast and umbilical artery rings [29]. Those increased 15-HETE levels promote constriction of the umbilical artery and placental vessels, resulting in placental ischaemia and hypoxia, thereby affecting the recasting of the uterine spiral arteries and accelerating the progression of HDP. However, the underlying mechanism by which 15-HETE affects vascular regulation in human vascular endothelial cells is still poorly understood. The study has shown that extracellular regulated protein kinases (ERK) are necessary for the vascular regulation of human umbilical cord endothelial cells by 15-HETE, which may be a potential novel target for treating of vascular regulation-related diseases [30].

The immunostaining results showed an increment of 15-LOX-2 expression in the placenta, leading to an increased synthesis of AA metabolites in PE. 15-HETE produced under hypoxic conditions is derived mainly from 15-LOX-2 [31]. LOX are non-heme iron enzymes that can

oxygenate unsaturated fatty acids such as AA to produce bioactive metabolites. The LOX family of enzymes is characterized by members including 5-, 8-, 12- and 15-LOX. The 15-LOX-1 and -2 have been identified as two subtypes of 15-LOX, where the latter has a limited tissue distribution, with mRNA detected in the prostate, lung, skin, and cornea, but not in numerous other tissues. The primary metabolites of AA produced by the LOX family are 5-, 8-, 12-, and 15-HETE. Increased free AA could produce leukotrienes, HETEs, and other bioactive substances produced by 15-LOX [32], contributing to pathophysiological processes such as oxidative stress and inflammation. Hypoxia-inducible factor 1 α (HIF-1 α)/15-LOX/15-HETE axis activation induced abnormal endothelial cell migration process, vasoconstriction, microangiopathy and increased blood pressure [24].

The results of this study suggested that the levels of 14,15-EET and 15-HETE in the serum showed an increasing trend with the progression of HDP, especially in patients with severe PE. Their abnormal levels in HDP patients are suggestive that 14,15-EET and 15-HETE are closely related to the progression of HDP. In the severe PE group, 14,15-EET and 15-HETE levels were associated with adverse pregnancy outcomes, where their levels in adverse pregnancy outcome group were significantly higher than the normal pregnancy outcome group. It has been reported that higher 15-HETE in PE is closely related to EETs, where the EETs concentration in the umbilical cord blood of newborns was 3-5 times higher than maternal circulation. The blood gas analysis in the fetal umbilical cord suggested that partial pressure of carbon dioxide (pCO₂) was positively correlated with the level of placental plasma EETs [33], suggesting 14,15-EET and 15-HETE levels are related to neonatal hypoxia and acidosis. Consequently, the effects of 14,15-EET and 15-HETE on the maternal-fetal interface might be involved in the occurrence and development of adverse pregnancy outcomes in HDP.

5. Conclusions

This study suggested that 14,15-EET and 15-HETE are involved in the occurrence and progression of HDP. Detection of serum 14,15-EET and 15-HETE levels in HDP patients might predict the severity of the disease. Our findings shed light on the involvement of 14,15-EET and 15-HETE in PE by CYP2J2 and 15-LOX signaling.

Abbreviations

14,15-EET, 14,15-epoxyeicosatrienoic acid; 15-HETE, 15-hydroxyeicosatetraenoic acid; PE, preeclampsia; ELISA, enzyme-linked immunosorbent assay; CYP2J2, cytochrome P450 2J2; 15-LOX-2, 15-Lipoxygenase-2; HDP, hypertensive disorders of pregnancy; AA, arachidonic acid; COX, cyclooxygenase; LOX, lipoxygenase; CYP, cytochrome P450; EETs, epoxyeicosatrienoic acids; 20-HETE, 20-hydroxyeicosatetraenoic acid; DHETs,

dihydroxyeicosatrienoic acids; BMI, body mass index; SP, systolic pressure; DP, diastolic pressure; PRO, urine protein; NICU, new-born intensive care unit; HELLP, haemolysis, elevated liver enzymes, and low platelets; BSA, bovine serum albumin; sFlt-1, soluble fms-like tyrosine kinase 1; PlGF, placental growth factor; ERK, extracellular regulated protein kinases.

Availability of Data and Materials

The datasets supporting the conclusions of this study are included in the article, further inquiries can be directed to the corresponding author.

Author Contributions

MS and LS conceived and designed the study. XC and JY were responsible for the materials. XC and YZ drafted the manuscript. YZ reorganized and compiled the materials. XH, JB contributed to acquisition, analysis, and interpretation of data. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The studies involving human participants were reviewed and approved by the ethical committee of Affiliated Hospital of Nantong University (2019-L123). The patients/participants provided their written informed consent to participate in this study.

Acknowledgment

We would like to thank all tutors for their guidance in scientific research and all participants for their support and encouragement in research data collection.

Funding

This work was supported by the Maternal and Child Health Technology Project of Jiangsu Province (grant F201905), Nantong Science and Technology Bureau Plan Project (grant JC2021045) and Nantong Health Commission General Project (grant MS2023018).

Conflict of Interest

The authors declare no conflict of interest.

References

- [1] Gawrys O, Husková Z, Baranowska I, Walkowska A, Sadowski J, Kikerlová S, *et al.* Combined treatment with epoxyeicosatrienoic acid analog and 20-hydroxyeicosatetraenoic acid antagonist provides substantial hypotensive effect in spontaneously hypertensive rats. *Journal of Hypertension*. 2020; 38: 1802–1810.
- [2] Mavangira V, Brown J, Gandy JC, Sordillo LM. 20-hydroxyeicosatetraenoic acid alters endothelial cell barrier integrity independent of oxidative stress and cell death. *Prostaglandins & other Lipid Mediators*. 2020; 149: 106425.
- [3] Dakarapu R, Errabelli R, Manthathi VL, Michael Adebesin A, Barma DK, Barma D, *et al.* 19-Hydroxyeicosatetraenoic acid analogs: Antagonism of 20-hydroxyeicosatetraenoic acid-induced vascular sensitization and hypertension. *Bioorganic & Medicinal Chemistry Letters*. 2019; 29: 126616.
- [4] Sporková A, Husková Z, Škaroupková P, Rami Reddy N, Falck JR, Sadowski J, *et al.* Vasodilatory responses of renal interlobular arteries to epoxyeicosatrienoic acids analog are not enhanced in Ren-2 transgenic hypertensive rats: evidence against a role of direct vascular effects of epoxyeicosatrienoic acids in progression of experimental heart failure. *Physiological Research*. 2017; 66: 29–39.
- [5] Zhang D, Chang X, Bai J, Chen ZJ, Li WP, Zhang C. The Study of Cyclooxygenase 2 in Human Decidua of Preeclampsia. *Biology of Reproduction*. 2016; 95: 56.
- [6] Herse F, LaMarca BB, Hubel CA, Laivuori H, Huppertz B, Verloren S, *et al.* OS066. Intrauterine CYP2J2 expression and circulating epoxyeicosatrienoic acid levels in preeclampsia. *Pregnancy Hypertension*. 2012; 2: 212–213.
- [7] Johnson RD, Polakoski KL, Huang X, Sadovsky Y, Nelson DM. The release of 15-hydroxyeicosatetraenoic acid by human placental trophoblast is increased in preeclampsia. *American Journal of Obstetrics and Gynecology*. 1998; 178: 54–58.
- [8] Zhu D, Medhora M, Campbell WB, Spitzbarth N, Baker JE, Jacobs ER. Chronic hypoxia activates lung 15-lipoxygenase, which catalyzes production of 15-HETE and enhances constriction in neonatal rabbit pulmonary arteries. *Circulation Research*. 2003; 92: 992–1000.
- [9] Shi Z, He Z, Wang DW. CYP450 Epoxygenase Metabolites, Epoxyeicosatrienoic Acids, as Novel Anti-Inflammatory Mediators. *Molecules (Basel, Switzerland)*. 2022; 27: 3873.
- [10] Roman RJ, Fan F. 20-HETE: Hypertension and Beyond. *Hypertension (Dallas, Tex.: 1979)*. 2018; 72: 12–18.
- [11] Garcia V, Gilani A, Shkolnik B, Pandey V, Zhang FF, Dakarapu R, *et al.* 20-HETE Signals Through G-Protein-Coupled Receptor GPR75 (G_q) to Affect Vascular Function and Trigger Hypertension. *Circulation Research*. 2017; 120: 1776–1788.
- [12] Zhang C, Booz GW, Yu Q, He X, Wang S, Fan F. Conflicting roles of 20-HETE in hypertension and renal end organ damage. *European Journal of Pharmacology*. 2018; 833: 190–200.
- [13] Ruffenach G, O'Connor E, Vaillancourt M, Hong J, Cao N, Sarji S, *et al.* Oral 15-Hydroxyeicosatetraenoic Acid Induces Pulmonary Hypertension in Mice by Triggering T Cell-Dependent Endothelial Cell Apoptosis. *Hypertension (Dallas, Tex.: 1979)*. 2020; 76: 985–996.
- [14] Suardiá R, Jambriña PG, Masgrau L, González-Lafont À, Rosta E, Lluch JM. Understanding the Mechanism of the Hydrogen Abstraction from Arachidonic Acid Catalyzed by the Human Enzyme 15-Lipoxygenase-2. A Quantum Mechanics/Molecular Mechanics Free Energy Simulation. *Journal of Chemical Theory and Computation*. 2016; 12: 2079–2090.
- [15] Askari A, Thomson SJ, Edin ML, Zeldin DC, Bishop-Bailey D. Roles of the epoxygenase CYP2J2 in the endothelium. *Prostaglandins & other Lipid Mediators*. 2013; 107: 56–63.
- [16] Croke L. Gestational Hypertension and Preeclampsia: A Practice Bulletin from ACOG. *American Family Physician*. 2019; 100: 649–650.
- [17] Bartoloni E, Alunno A, Valentini V, Luccioli F, Valentini E, La Paglia G, *et al.* Role of Inflammatory Diseases in Hypertension. *High Blood Pressure & Cardiovascular Prevention: the Official Journal of the Italian Society of Hypertension*. 2017; 24: 353–361.
- [18] Valencia-Ortega J, Saucedo R, Peña-Cano MI, Hernández-Valencia M, Cruz-Durán JG. Immune tolerance at the maternal-placental interface in healthy pregnancy and pre-eclampsia. *The*

Journal of Obstetrics and Gynaecology Research. 2020; 46: 1067–1076.

- [19] Godhamgaonkar AA, Sundrani DP, Joshi SR. Role of maternal nutrition and oxidative stress in placental telomere attrition in women with preeclampsia. *Hypertension in Pregnancy*. 2021; 40: 63–74.
- [20] Diniz ALD, Paes MMBM, Diniz AD. Analyzing Preeclampsia as the Tip of the Iceberg Represented by Women with Long-Term Cardiovascular Disease, Atherosclerosis, and Inflammation. *Current Atherosclerosis Reports*. 2020; 22: 13.
- [21] Müller A, Horvat V, Vulin M, Mandić S, Šerić V, Vidosavljević D. The soluble fms-like tyrosin kinase-1 (sFLT-1) to placental growth factor (PIGF) ratio as a possible indicator for the severity of preeclampsia - single institution experience. *Medicinski Glasnik: Official Publication of the Medical Association of Zenica-Doboj Canton, Bosnia and Herzegovina*. 2019; 16: 53–59.
- [22] Pearson T, Zhang J, Arya P, Warren AY, Ortori C, Fakis A, *et al.* Measurement of vasoactive metabolites (hydroxyecosatrienoic and epoxyecosatrienoic acids) in uterine tissues of normal and compromised human pregnancy. *Journal of Hypertension*. 2010; 28: 2429–2437.
- [23] Rand AA, Barnych B, Morisseau C, Cajka T, Lee KSS, Panigrahy D, *et al.* Cyclooxygenase-derived proangiogenic metabolites of epoxyecosatrienoic acids. *Proceedings of the National Academy of Sciences of the United States of America*. 2017; 114: 4370–4375.
- [24] Szczuko M, Kikut J, Komorniak N, Bilicki J, Celewicz Z, Ziętek M. The Role of Arachidonic and Linoleic Acid Derivatives in Pathological Pregnancies and the Human Reproduction Process. *International Journal of Molecular Sciences*. 2020; 21: 9628.
- [25] Boldt C, Röschel T, Himmerkus N, Plain A, Bleich M, Labes R, *et al.* Vasopressin lowers renal epoxyecosatrienoic acid levels by activating soluble epoxide hydrolase. *American Journal of Physiology. Renal Physiology*. 2016; 311: F1198–F1210.
- [26] Sari I, Pinarbasi H, Pinarbasi E, Yildiz C. Association between the soluble epoxide hydrolase gene and preeclampsia. *Hypertension in Pregnancy*. 2017; 36: 315–325.
- [27] Dalle Vedove F, Fava C, Jiang H, Zanconato G, Quilley J, Brunelli M, *et al.* Increased epoxyecosatrienoic acids and reduced soluble epoxide hydrolase expression in the preeclamptic placenta. *Journal of Hypertension*. 2016; 34: 1364–1370.
- [28] Dalle Vedove F, Fava C, Jiang H, Zanconato G, Quilley J, Brunelli M, *et al.* 8C. 05: Epoxyecosatrienoic acids are increased in placentas of preeclamptic pregnancies. *Journal of Hypertension*. 2015; 33: e111.
- [29] Plenty NL, Faulkner JL, Cotton J, Spencer SK, Wallace K, LaMarca B, *et al.* Arachidonic acid metabolites of CYP4A and CYP4F are altered in women with preeclampsia. *Prostaglandins & other Lipid Mediators*. 2018; 136: 15–22.
- [30] Long A, Ma S, Li Q, Lin N, Zhan X, Lu S, *et al.* Association between the maternal serum levels of 19 eicosanoids and preeclampsia. *International Journal of Gynaecology and Obstetrics: the Official Organ of the International Federation of Gynaecology and Obstetrics*. 2016; 133: 291–296.
- [31] Wang S, Cao W, Xing H, Chen YL, Li Q, Shen T, *et al.* Activation of ERK pathway is required for 15-HETE-induced angiogenesis in human umbilical vascular endothelial cells. *Journal of Receptor and Signal Transduction Research*. 2016; 36: 225–232.
- [32] Gao Y, Cao X, Zhang X, Wang Y, Huang H, Meng Y, *et al.* Brozapine Inhibits 15-LOX-2 Metabolism Pathway After Transient Focal Cerebral Ischemia in Rats and OGD/R-Induced Hypoxia Injury in PC12 Cells. *Frontiers in Pharmacology*. 2020; 11: 99.
- [33] Jiang H, McGiff JC, Fava C, Amen G, Nesta E, Zanconato G, *et al.* Maternal and fetal epoxyecosatrienoic acids in normotensive and preeclamptic pregnancies. *American Journal of Hypertension*. 2013; 26: 271–278.