Iron metabolism disorder and oxidative damage levels in placenta are involved in preeclampsia

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Keywords
oxidative stress, preeclampsia, Iron

Abstract
Introduction
To explore the role of ferritin in placenta, serum and umbilical cord blood of pregnant women and the changes of oxidative stress injury as well as cell apoptosis in placenta in the pathogenesis of preeclampsia (PE).

Material and methods
Sixty pregnant women with severe PE were assigned into early-onset and late-onset PE group. Another 60 cases of normal late pregnant women with similar gestational weeks were divided into early-onset and late-onset control group. Maternal serum and fetal umbilical cord blood ferritin content was determined by automatic biochemical immunoassay system; mRNA expression levels of ferritin and ferritin heavy chain (FTH) were detected by reverse transcription real-time fluorescence quantitative polymerase chain reaction (RT-qPCR); Western Blot was used to detect the relative expression level of ferritin and apoptosis; the contents of total superoxide dismutase (T-SOD) and malondialdehyde (MDA) and glutathione peroxidase (GSH-Px) were detected by colorimetry.

Results
Serum uric acid (UA) and creatinine (Cr) levels of PE groups were significantly higher when compared to the controls. The serum ferritin levels in blood sample and umbilical cord blood sample were significantly higher relative to the controls. However, the mRNA and protein levels of ferritin levels in placenta samples were significantly lower compared with the controls. The placental cleaved caspase-3, Bcl-2 levels were significantly lower than the early onset PE group. The levels of GSH-Px and MDA in placenta were significantly higher.

Conclusions
These results may assist understanding the pathogenesis of PE and provide potential biomarkers for diagnosis of PE.
Iron metabolism disorder and oxidative damage levels in placenta are involved in preeclampsia

Jianying Yan1*, Jie Dong1*, Xiaoqian Lin1, Lichun Chen1, Zhuanji Fang1, Qing Han1, Qinjian Zhang1, Lingling Jiang1, Xia Xu1, Xu Lin2,3

1 Department of Obstetrics, Fujian Provincial Maternity and Child Health Hospital, Affiliated Hospital of Fujian Medical University, Fuzhou 350001, China
2 Key Laboratory of Ministry of Education for Gastrointestinal Cancer, School of Basic Medical Sciences, Fujian Medical University, Fuzhou, 350001, China.
3 Fujian Key Laboratory of Tumor Microbiology, Department of Medical Microbiology, Fujian Medical University, Fuzhou, 350001, China

First author

Jianying Yan, Jie Dong and Xiaoqian Lin are joint first author.

Corresponding author

Jianying Yan, Department of Obstetrics, Fujian Provincial Maternity and Child Health Hospital, Affiliated Hospital of Fujian Medical University, No.18 Daoshan Road, Fuzhou 350001, China. Tel: +86-591-86329265. E-mail: xinyzh2018@126.com

Running title

Iron metabolism disorder and oxidative damage levels

CONFLICT of INTEREST

None

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Abstract

Objective To explore the role of ferritin in placenta, serum and umbilical cord blood of pregnant women and the changes of oxidative stress injury as well as cell apoptosis in placenta in the pathogenesis of preeclampsia (PE). Methods Sixty pregnant women with severe PE were assigned into early-onset and late-onset PE group. Another 60 cases of normal late pregnant women with similar gestational weeks were divided into early-onset and late-onset control group. Maternal serum and fetal umbilical cord blood ferritin content was determined by automatic biochemical immunoassay system; mRNA expression levels of ferritin and ferritin heavy chain (FTH) were detected by reverse transcription real-time fluorescence quantitative polymerase chain reaction (RT-qPCR). Western Blot was used to detect the relative expression level of ferritin and apoptosis; the contents of total superoxide dismutase (T-SOD) and malondialdehyde (MDA) and glutathione peroxidase (GSH-Px) were detected by colorimetry. Result Serum uric acid (UA) and creatinine (Cr) levels of PE groups were significantly higher when compared to the controls. The serum ferritin levels in blood sample and umbilical cord blood sample were significantly higher relative to the controls. However, the mRNA and protein levels of ferritin levels in placenta samples were significantly lower compared with the controls. The placental cleaved caspase-3, Bcl-2 levels were significantly lower than the early onset PE group. The levels of GSH-Px and MDA in placenta were significantly higher. Finally, the neonatal outcomes of PE groups were worse than the early onset PE group. Conclusion Levels of ferritin in blood sample, umbilical cord blood and placenta tissues of PE patients had changed significantly compared with normal pregnant women. Factors related to oxidative stress and cell apoptosis in placenta tissues also altered significantly in PE cases. Factors related to oxidative stress and cell apoptosis in placenta tissues also altered significantly in PE cases. These results may assist understanding the pathogenesis of PE and provide potential biomarkers for diagnosis of PE.

Key words
Iron, preeclampsia, oxidative stress

Introduction
Preeclampsia (PE) and eclampsia are the main causes of perinatal maternal and fetal death [1]. Based on a large-scale systematic review of 40 countries, 129 studies and 39 million patients, the incidence of PE was statistically analyzed. It was found that the overall incidence of PE accounted for 4.6% of the total perinatal pregnant women, and the overall incidence of eclampsia was 1.4% [2]. In addition, the regional differences in its incidence are large. In some developed areas (Eastern Mediterranean), the incidence of PE is only 1.0%, in Africa, the overall incidence is about 5.6% [2], in Brazil, the incidence is 7.5% [3]. Based on 22 monitoring sites in Hebei Province of China, the data of more than 30000 samples showed that the incidence of mild PE and severe PE were 1.38% and 4.02% respectively [4]. The number of perioperative deaths caused by PE accounted for 0.02% of the total pregnant women [5]. In addition, the incidence of stroke, heart failure and diabetes in pregnant women with PE, this is a predictive statement that cannot be known until [6].

According to the guidelines for the diagnosis and treatment of hypertensive disorders during pregnancy (2015 Edition) [7], the diagnostic criteria of PE in China are as follows: after 20 weeks of pregnancy, systolic blood pressure ≥ 140 mmHg and / or diastolic blood pressure ≥ 90 mmHg, accompanied by any of the following items: urinary protein ≥ 0.3g/24h, or urinary protein / creatinine ratio ≥ 0.3, or random urinary protein ≥ (+) (inspection method when urinary protein quantification is not available); no proteinuria but with any of the following organs or systems involved: heart, lung, liver, kidney and spleen, or abnormal changes in the blood system, digestive system, nervous system, placenta fetal involvement.

Previous studies demonstrated that patients with PE usually showed abnormal serum ferritin levels so it was hypothesized that PE may be associated with serum ferritin (SF) so that it may be a potential biomarker for evaluating PE and pregnancy outcome of the patients with PE [8]. Ferritin is a kind of macromolecule containing ferric ion. In the cardiovascular research, serum ferritin is closely related to hypertension, coronary artery disease or cerebrovascular. However, there are few studies investigating the relationship between PE and SF. Soluble transferrin receptor (sTfR) was derived from the proteolysis of cell surface receptors. sTfR, which mainly exist in the form of complexes, came from early red blood cells, it had close relationship with the SF
level[9]. Besides, some studies had revealed that the lipid peroxide significantly increased while antioxidants decreased in pregnant women with PE which may indicated that oxidative stress reactions correlated with development of PE, and oxidative damage levels is referring to that reactive oxygen species (ROS) and reactive nitrogen species (RNS) cause protein oxidative damage value[10]. Furthermore, placental cell apoptosis may play important roles in the procedure of PE and imbalance of cell apoptosis might be one of causes of PE [11]. The cell apoptotic genes including cleaved Caspase-3, Bcl-2, Bax and so on had been reported in previous studies investigating the differentially expressed proteins in PE [12-14]. Therefore, in order to explore the role of SF, and factors involving oxidative stress and cell apoptosis in PE, we had measured and compared the SF levels in maternal blood sample, fetal umbilical cord blood and placenta sample, Caspase-3, Bcl-2, Bax, glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), malondialdehyde (MDA) levels in placenta from pregnant women with early onset PE, late onset PE and normal pregnant women. Furthermore, the neonatal outcomes between the groups were also analyzed. There are study presents a characterization of the profile of biochemical parameters of PE and IUGR, in relation to the occurrence of oxidative stress associated with the expression of VEGF in the muscular layer of the fetal placental vessels and further studies are needed to establish the distinctive role of vasculogenetic factors in the pathophysiology of PE and IUGR, and to determine whether these factors precede or are causally linked to oxidative stress and endothelial dysfunction.

Materials and methods

Subjects

This study has been approved by the ethics committee of Fujian Provincial Maternity and Child Health Hospital, Affiliated Hospital of Fujian Medical University, and the ethical committee approval number is 2019076. Informed consent was obtained from all the included patients. 120 pregnant women with severe PE who received pre-delivery examination, hospitalized and delivered by cesarean section in the obstetric department of Fujian Maternal and Child Health Hospital from December 2017 to December 2018 were included. Among them, 60 were diagnosed with PE with 30 assigned into early-onset PE group (gestational weeks at onset ≤ 34 weeks) and 30 in late-onset PE
group (gestational weeks at onset > 34 weeks) according to their gestational weeks at PE onset. Another 60 cases were normal late pregnant women with similar gestational weeks, and they were assigned into early-onset control group (gestational weeks ≤ 34 weeks) and late-onset control group (gestational weeks > 34 weeks) with 30 subjects in either group. The criteria for the selection of early-onset control group and late-onset control group was as follows:

The most common signs and symptoms of pregnancy included missed period, tender or swollen breasts, nausea with or without vomiting, increased urination and fatigue. While the late-onset was set as the delivery after 37 weeks.

**Diagnosis criteria**

Definition of PE by the International Society for Pregnancy-induced Hypertension Research (ISSHP) in 2014 was used in this study [9]. PE was defined as after 20 weeks of gestation occurrence of newly-developed hypertension combined with proteinuria (> 300 mg) or other maternal organ dysfunction, such as renal insufficiency, liver involvement, neurological or hematological complications, uteroplacental dysfunction or fetal growth restriction.

**Exclusion criteria**

The subjects with any of the following conditions would be excluded: Heart disease, essential hypertension, kidney disease, diabetes mellitus or gestational diabetes mellitus, intrahepatic cholestasis of pregnancy, multiple pregnancies, fetal malformations, parturient pregnant women, having ruptured membranes or signs of clinical infection or non-cesarean section mode of delivery.

**Sample collection**

After fasting overnight, blood samples (3 ml blood) were collected from the peripheral veins of each subject's arm in the morning upon awakening (8 h after “lights-off”). The blood samples were centrifugated (3000g, 15 minutes) and then stored under -80°C. Immediately after delivery, 3 ml cord blood was extracted from the umbilical vein. Placenta tissue was collected from the root of umbilical cord immediately after cesarean
section, and cut into pieces of 1.0 cm * 1.0 cm * 1.0 cm under sterile condition, with blebding, infarction and calcification area avoided. The placenta samples were rinsed with cold saline, wrapped with tin foil, and then put into 1.5 ml cryopreservation tube and stored under -80°C. Since the subjects in the early onset control group were healthy pregnant women, who did not have premature delivery when serum was collected, no placental tissue or umbilical cord blood samples were collected from this group. 

And the SF level of blood samples for all patients in our study was determined by chemiluminescence immunoassay.

qRT-PCR analysis

The placenta tissues were treated with TRIzol reagent to extract the total RNA in the tissues. Revert Aid TW first Strand cDNA Synthesis Kit was used to synthesize the first chain of DNA. QuantiNova SyBr Green PCR Kit was used to perform PCR analysis. Reaction conditions: pre-denaturation at 95°C for 1 minute, denaturation at 95°C for 30 seconds, annealing at 60°C for 30 seconds, and elongation at 72°C for 30 seconds, running 40 cycles. Primers for Ferritin Gene: forward primer 5’-TGTACCTGCAGGCCTCTTACA-3’; reverse primer: 5’-GACGCTTCCAGAGCCACAT-3’; probe primer: 5’-TACCTCTCTCTGGGCTTCTATTTCGACC-3’. Primers for FTH gene: forward primer 5’-AACATGCTGAGAAACTGATGAAGCT-3’; reverse primer 5’-GTCATCACAGTCTGGTTTCTTGATATC-3’; probe primer: 5’-AACCAACGAGGGCGCAATCTTCC-3’. GAPDH was set the internal control and each assay was repeated at least three times. The relative expression of mRNA was calculated by the $2^{-\Delta \Delta Ct}$ and there was no unit in the plotted columns.

Colorimetric Methods to determine T-SOD and MDA levels

First, the placenta tissue was washed 3-5 times with erythrocyte lysate, and then washed with phosphate buffer 3 times. 10% tissue homogenate was prepared by adding saline solution with a weight to volume ratio of 1:9. BCA kit was used to determine protein concentration. The instruction of total superoxide dismutase (T-SOD) and malondialdehyde (MDA) kit were purchased from Beyotime Institute of Biotechnology (Shanghai, China) for tissue homogenate was followed. MDA was measured using a
colorimetric assay kit, according to the reaction of MDA with thiobarbituric acid, in order to produce a red compound. Briefly, the ovary homogenate was centrifuged at 1,600×g/min for 10 min at 4°C, the supernatant was collected, and absorbance was measured at 532 nm using a microplate reader (BioTek, Winooski, VT, United States), according to manufacturer’s instructions. SOD levels were determined by water soluble tetrazolium-8 (WST-8) testing method. The cellular or ovarian homogenate was centrifuged at 1,500×g/min for 5 min at 4°C, the supernatant was collected, and absorbance was measured at 450 nm using a microplate reader (BioTek, Winooski, VT, United States). The above two kits are purchased from Beijing Biyuntian Biotechnology Company. The testing instrument is Ultrospee 2100 Pro ultraviolet/visible spectrophotometer of Amersham Biosciences Company.

**Western Blot Analysis**

First, the placenta tissue was washed 3-5 times with erythrocyte lysate, and then washed 3 times with phosphate buffer. Then the samples were lysate in Radioimmunoprecipitation assay (RIPA) buffer and then centrifuged at 12,000 g (4°C, 10 minutes). Following the manufacturer's instructions, the protein concentration was determined using the BCA protein determination kit (BiYunTian). 40 μg proteins from each tissue were denatured and loaded into each pore, separated by SDS PAGE and transferred to the polyvinylidene fluoride membrane (Millipore, Billerica, MA). The membrane was blocked with 5% skim milk at room temperature for one hour, and incubated with primary antibody in Phosphate Buffer Solution (PBST) (1:1000) overnight. After washing with PBST, the membrane was incubated with second antibody conjugated with horseradish peroxidase at room temperature for 1 hour, and then washed three times. Enhanced chemiluminescence (BeyoECL Plus, 100 ml, BiYunTian) was used to detect the film. Anti-Cleaved Caspase-3 (T), anti-Bax (T), anti-Bcl-2 (T), anti-beta-Actin (70) antibodies were purchased from Cell Signaling Technology Company. Anti-Ferritin (ab75973) and anti-Ferritin Heavy Chain (ab75972) antibodies were purchased from Abcam. Horseradish peroxidase conjugated goat anti-rabbit IgG (A4914) and anti-mouse IgG (A0168) were purchased from Sigma.
TUNEL assay

Frozen sections from placenta samples were prepared. Placental samples, which were collect from basal plate and the decidua was completely removed to avoid the contamination of maternal cells, were fixed overnight with 4% paraformaldehyde. TUNEL immunohistochemical analysis was performed using TUNEL apoptotic assay kit (Roche, Cat. No. 11684795910, US) following the manufacturer's instructions. In short, prepared sections were washed with PBS three times. The slides were incubated in Triton X-100 solution (P0096-100ml, BiYunTian) on ice for 2 minutes. The labeled mixture in TdT enzyme buffer containing biotinylated dUTP was added to the slides, and incubated at 37°C for 60 minutes in a 95% air, 5% CO₂ atmosphere in a humidity controlled incubator. The slides were washed with PBS twice. The area around the sample were dried. According to the tissue size, the sections were added with DAPI (C1006, BiYunTian) and stood for 30 seconds. Then the slides were washed with PBS three times. The presence of green fluorescence in cytoplasm was regarded as positive, while the absence of green fluorescence was negative. Leica inverted microscope was used in 400-fold high-power field of vision. Three non-overlapping fields were selected for each slide. The number of positive cells in 200 cells was counted in each field of vision. The apoptotic rate was calculated as following: apoptotic rate = number of apoptotic positive cells /Total Number of cells *100%.

Statistical analysis

All the above data were processed by SPSS 22.0 software and GraphPad Prism Version 7.0. The continuous variables were shown as means ± SEM. T test or Wilcoxon rank sum test and Kruskal Wallis test were used for comparison between the two groups; Differences between three or more treatment conditions were tested with ANOVA. When comparing the control group with each treatment condition many-to-one testing procedures were used. The test level alpha = 0.05; A p value less than 0.05 was considered statistically significant.

Results

Neonatal characteristics
The characteristics of three groups of newborns were summarized in Table 4. Body weight were 1.80 kg, 2.84 kg and 3.57 kg in early onset PE group, late onset PE group and late onset control group respectively. Body weight of PE groups were significantly lower than that of the control group (both P<0.05). Body length of early onset PE group and late onset PE group were 41.8 cm and 47.8 cm, significantly shorter than 50.6 in control group (P<0.05). In PE groups, 21 (70%) and 8 (26.7%) newborns were admitted to NICU, while only one in control group (P<0.001).

**General information**

There is no difference in respect to the pre-pregnancy age, gestation week between any two groups all (P>0.05). Pre-pregnant and pregnant body weight, gestational week were not significantly different between early onset PE group and early onset control group, or late onset PE group and late onset control group (all P>0.05). Diastolic pressure (DP), systolic pressure (SP) and mean artery pressure (MAP) of the early onset PE group and late onset PE group were significantly higher than those of the corresponding control group (P<0.05). However, DP, SP and MAP were not significantly different between the early onset PE group and late onset PE group (P>0.05) (Table 1, Table 2, figure 1).

**Renal function**

The serum uric acid (UA) and creatinine (Cr) levels of early-onset PE group and late-onset PE group both significantly increased compared with corresponding control group (P<0.05). However, the serum UA and Cr levels between early-onset PE group and late-onset PE group were not significantly different (P>0.05). The 24 hours proteinuria between the early-onset PE group and late-onset PE group were significantly different (P<0.05) (Table 3, figure 2).

**Serum SF**

Serum ferritin level (SF) levels was (60.82±38.14) μg/L and (35.94±21.36) μg/L in early onset PE and late onset PE group respectively and another were (27.22±23.13) μg/L and (21.50±15.83) μg/L in early onset control group and late onset control group respectively as well. SF levels in early onset PE group and late onset PE group were
both significantly up-regulated when compared with corresponding control group (P<0.05). Moreover, the SF levels of early onset PE group was significantly higher than that of late onset PE group (P<0.05) (Figure 3).

**sTfR protein levels**

Soluble transferrin receptor (sTfR) was derived from the proteolysis of cell surface receptors. The sTfR proteins levels were (0.81±0.09) mg/L and (0.85±0.13) mg/L in early onset PE group and late onset PE group respectively and another were (1.00±0.18) mg/L and (1.13±0.28) mg/L in early onset control and late onset control group respectively. The sTfR levels in two PE groups were significantly down-regulated than the corresponding control group (P<0.05). However, the difference between two PE groups or two control groups were both not significant (P>0.05) (Figure 4).

**Levels of SF in fetal umbilical cord blood**
The levels of SF in fetal umbilical cord blood of early onset PE group and late onset PE group were (159.54±73.26) μg/L and (126.84±69.14) μg/L respectively, both significantly higher than late onset control group which showed SF level of 52.53±29.70 μg/L (P<0.05). The levels of SF in fetal umbilical cord blood between early onset PE group and late onset PE group were not significantly different (P>0.05) (figure 5).

**mRNA and protein levels of Ferritin and FTH & protein levels of Cleaved Caspase-3, Bcl-2 and Bax of placenta tissues**

**i. mRNA levels of Ferritin and FTH**
The Ferritin mRNA in placenta tissues of early onset PE group and late onset PE group were 120.38(73.39-308.64) and 172.00(32.51-533.06), significantly lower than late onset control group (283.96(121.09-609.63) (P<0.05). The Ferritin mRNA were not significantly different between the early onset PE and late onset PE group (P>0.05). The FTH mRNA in placenta tissues of early onset PE group, late onset PE group and late onset control group were 218.48(175.77-345.15), 182.43(97.54-322.04) and 234.75(190.66-340.96) respectively. There is no significantly difference between any two groups (P>0.05).
ii. Protein levels of Ferritin and FTH

The protein levels of ferritin were (0.74±0.26), (0.68±0.27), (1.05±0.15) in early onset PE group, late onset PE group and late onset control group respectively. The ferritin protein levels of early onset PE group and late onset PE group were significantly lower than that of late onset control group (both P<0.05). The ferritin protein levels between early onset PE group and late onset PE group were not significantly different (P>0.05).

The protein levels of FTH in placenta tissues were (0.92±0.13), (0.76±0.28) and (1.32±0.49) in early onset PE group, late onset PE group and late onset control group.

The FTH protein levels of early onset PE group and late onset PE group were significantly lower than the late onset control group (P<0.05). However, the FTH protein levels between the early onset PE group and late onset PE group were not significantly different (both P>0.05);

iii. Protein levels of Cleaved Caspase-3, Bcl-2 and Bax in placenta tissues

The cleaved caspase-3 protein levels in placenta tissues of early onset PE group and late onset PE groups were (1.43±0.25) and (1.14±0.25) respectively, significantly higher that of late onset control group (0.64±0.18) (both P<0.05). Moreover, the cleaved caspase-3 protein levels of early onset PE group was significantly higher than that of late onset PE group (P<0.05).

The Bax protein levels in placenta tissue of early onset PE group and late onset PE group were (0.88±0.09) and (1.17±0.37) respectively, significantly higher than that of late onset control group (0.64±0.12) (both P<0.05). Moreover, the Bax protein level of late onset PE group was significantly higher than that of the early onset PE group (P<0.05).

The Bcl-2 protein levels in placenta tissue of early onset PE group and late onset PE group were (1.07±0.15) and (0.89±0.24) respectively, significantly lower than that of the late onset control group (1.25±0.25) (both P<0.05). Moreover, the Bcl-2 protein level of early onset PE group was significantly higher than that of late onset PE group (P<0.05) (figure 6).
Levels of GSH-Px, T-SOD and MDA in placenta tissues

The GSH-Px levels of early onset PE group and late onset PE group were (177.67±65.04) U/mgprot and (201.57±55.97) U/mgprot, significantly higher than that of the late onset control group (95.50±44.98) U/mgprot (both P<0.05); the GSH-Px levels between the early onset PE group and late onset PE group were not significantly different (both P>0.05).

The T-SOD expression levels of early onset PE group and late onset PE group were (1.03±0.24) units and (0.91±0.21) units, significantly lower than that of the late onset control group (1.33±0.48) units (both P<0.05). The T-SOD levels between the early onset PE group and late onset PE group were not significantly different (both P>0.05).

The MDA expression levels of early onset PE group and late onset PE group were (49.93±14.64) μmol/L and (45.66±18.93) μmol/L, significantly higher than that of late onset control group (13.94±7.73) μmol/L (both P<0.05). The MDA levels between the early onset PE group and late onset PE group were not significantly different (both P>0.05) (figure 7).

Cell apoptosis rate in placenta tissue

The cell apoptosis rate in placenta tissue were (14.52%±6.82%) and (16.16%±12.25%) in early onset PE group and late onset PE group respectively, both significantly higher than that of late onset control group (5.51%±7.30%) (both P<0.05). There is no difference in respect to cell apoptosis rate between the early onset PE group and late onset PE group (P>0.05) (figure 8).

Discussion

Our study also demonstrated that ferritin levels in blood sample of PE patients were significantly higher than that of the normal subjects. This result is consistent with the study performed by Su et al [15]. A case-control study in John Radcliffe Hospital [16] compared the serum iron status parameters of 40 PE patients and 40 normal pregnant women. The results showed that the serum iron, ferritin concentration and transferrin saturation of PE patients were significantly higher than those of normal
pregnant women, while the level of unsaturated iron binding capacity and transferrin was significantly lower. **This study suggests that iron overload, the higher iron and ferritin levels in preeclampsia may be the cause of the disease**, and the biochemical reaction may aggravate lipid peroxidation and endothelial cell damage. The ratio of the concentration in maternal serum to that in placental tissue increased during pregnancy for all proteins with the exception of ferritin. It is proposed that the mechanism of secretion of trophoblast specific proteins varies widely.

In vitro culture of PE trophoblasts showed that the expression and activity of ROS producing enzymes increased [18], leading to the imbalance of ROS producing enzymes and antioxidants, promoting the transcription of antiangiogenic factors such as sFlt-1 [19], and inhibiting Wnt/β-catenin signaling pathway that promotes trophoblast invasion [20]. The expression of superoxide dismutase (SOD) and glutathione peroxidase (GSH PX) in placenta of PE patients was lower than that of normal pregnant women [21]. This study found that the content of antioxidant T-SOD in early-onset and late-onset PE group was lower than that in late-onset control group, and the difference was statistically significant; the expression level of MDA in early-onset and late-onset PE group was higher than that in control group, and the difference was statistically significant, indicating that the placental mitochondrial membrane of PE patients was damaged by high-level oxidative stress.

About 27% of ferritin exists in tissues in the form of Fe$^{3+}$ [22]. When the iron content in the body exceeds the storage capacity of cells, the excess iron can be combined with hemosiderin and stored in the reticuloendothelial system [23], while the excess free Fe$^{2+}$ can generate hydroxyl radicals (• oh) through Fenton reaction, leading to oxidative stress [24,25], causing DNA damage and lipid damage. Peroxidation of substance and protein. Fenton reaction in cells may lead to cell death by initiating apoptosis program [26].

There have study presents a characterization of the profile of biochemical parameters of PE and IUGR, in relation to the occurrence of oxidative stress associated with the expression of VEGF in the muscular layer of the fetal placental vessels. In this paper, we have found that the serum ferritin levels in blood sample and umbilical cord blood sample were significantly higher relative to the controls. However, the mRNA and
protein levels of ferritin levels in placenta samples were significantly lower compared with the controls. The placental cleaved caspase-3, Bcl-2 levels were significantly lower than the early onset PE group.

Mitochondria are the main oxygen consuming organs of the body and the place where ROS is produced. It was found that ferritin was also expressed in mitochondria. The most important function of mitochondrial ferritin (FtMt) is to isolate excess iron and respond to oxidative stress. FtMt can chelate iron ions, inhibit the production of reactive oxygen species, and protect cells from iron induced oxidative stress. In addition, intracellular ferritin consists of two different types of 24 subunits: heavy chain (H-ferritin; FH) and light chain (L-ferritin; FTL) [27]. Among them, FH has the activity of iron oxidase, which catalyzes the transformation from ferrous form (Fe$^{2+}$) to ferrous form (Fe$^{3+}$), allowing iron to be incorporated into ferritin shell safely, thus reducing the participation of free iron in the production of ROS. The results showed that the expression of ferritin in the early and late onset PE group was lower than that in the control group at the DNA level, and the difference was statistically significant, but there was no significant difference in the expression of ferritin heavy chain DNA between the three groups. Protein expression: the expression level of ferritin and ferritin heavy chain in early and late onset PE group was lower than that in late onset control group, and the difference was statistically significant. There is no difference in the expression of FTH gene, but the difference in protein expression may be due to the regulation of FTH at the translation level, and FTH protein may have a certain regulatory function on the pathological symptoms of PE. Ferritin gene expression is regulated at the transcription and post transcription levels [28]. Iron is the main posttranscriptional regulator of ferritin. Iron inhibits the interaction between iron regulatory protein (IRP) and iron responsive element (IRE), and the 5'- untranslated region (UTR) of ferritin H and L mRNA promotes recruitment translation mechanism to increase ferritin protein synthesis. In contrast, iron chelators increased IRP-IRE interaction and inhibited ferritin translation [28]. Therefore, ferritin translation is up-regulated by high iron and down regulated by low iron.

It has been reported that a higher degree of apoptosis has been found in PE and placenta with intrauterine growth restriction [29]. The signal pathway of apoptosis
includes exogenous pathway, i.e. death receptor pathway and endogenous pathway, i.e. mitochondrial pathway. The apoptosis of trophoblasts caused by oxidative stress is mainly achieved by endogenous pathway. The permeability of mitochondrial membrane and the release of apoptotic proteins are also regulated by Bcl-2 family proteins. Bcl-2 family can be divided into two categories: one is anti-apoptotic protein (such as BCL XL), the other is pro-apoptotic protein (such as Bax, Bak, mcl-2, etc.).

Due to the imbalance between the pro-apoptotic (Bax, bok and Bak) and pro-survival (Mcl-1, Bcl-2 and bcl-2l1) members of the bcl-2 protein family, the lack of oxygen supply and other conditions can induce apoptosis through the endogenous pathway [30]. Hypoxia has been shown to increase the expression of Bax and decrease the expression of Bcl-2 in human trophoblast cells [31]. Murat can [32] et al. Found that the apoptosis index of villous trophoblasts in PE group increased (P<0.001) by comparing the placental tissues of 32 PE and 31 healthy pregnant women with normal blood pressure. The results of this study also confirmed the difference of expression of cleaved Caspase-3, Bax and other related apoptosis proteins in PE group and control group at the same time. In this study, Western blot showed that the protein levels of cleaved caspase-3 and Bax in placenta of early-onset PE group and late-onset PE group were higher than those of late-onset control group, and the protein levels of cleaved Caspase-3, Bcl-2 and Bax in early-onset and late-onset PE groups were lower than those of late-onset control group (P<0.05). There was statistical significance (P<0.05).

By comparing the content of ferritin and other related proteins between the pre-eclampsia group and the control group, this study hopes to provide some valuable reference information for the further study of the pathogenesis of pre-eclampsia, and provide some theoretical support for the prevention of pre-eclampsia in clinical work.

By the way, this study has some shortcomings. These results did not compare the ferritin levels in early and late onset healthy and preeclamptic pregnant women at the same gestational age. In the foreseeable future, we would pay much more attention on this issue in order to exclude the altered levels in different stages would have a bad effect on the results of placental development.

**Conclusion**
Levels of ferritin in blood sample, umbilical cord blood and placenta tissues of PE patients had changed significantly compared with normal pregnant women. Factors related to oxidative stress and cell apoptosis in placenta tissues also altered significantly in PE cases. These results may assist understanding the pathogenesis of PE and provide potential biomarkers for diagnosis of PE, and a graphical mechanism of the whole study is shown in figure 9.
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Table legends

**Table 1. General information of each groups (x ± s)**

| Group             | Number of cases | Age         | Gestational week | Pre-pregnance BMI | BMI during pregnancy |
|-------------------|-----------------|-------------|------------------|-------------------|---------------------|
| Early onset control | 30              | 30.10±5.17 | 32.20±0.88       | 19.98±1.49        | 25.02±3.06          |
Table 2. Blood pressure comparison between groups (x ± s)

| Group                | Number of cases | SP(mmHg)     | DP(mmHg)     | MAP(mmHg)     |
|----------------------|-----------------|--------------|--------------|--------------|
| Early onset control  | 30              | 114.03±8.98  | 70.57±6.16   | 85.06±5.49   |
| control group        |                 |              |              |              |
| Early onset PE group  | 30              | 147.81±18.40a| 99.67±15.06a | 115.72±15.24 |
|                      |                 |              |              |              |
| Late onset control   | 30              | 117.07±7.56  | 75.20±5.72   | 89.16±5.01   |
| group                |                 |              |              |              |
| Late onset PE group   | 30              | 138.46±17.49b| 91.25±10.86bc| 106.99±12.26 |
|                      |                 |              |              |              |

Note: a represents Compared with early onset control group, \( ^a P<0.05 \); b represents compared with late onset control group, \( ^b P<0.05 \); c represents compared with early onset PE group, \( ^c P<0.05 \).

Table 3 Renal function comparison between groups(x ± s)

| Group                | Number of cases | UA(mmol/L)    | Cr(mmol/L)   | 24h proteinuria (g) |
|----------------------|-----------------|---------------|--------------|---------------------|
| Early onset control  | 30              | 2.99±0.69     | 40.08±6.10   | —                   |
| group                |                 |               |              |                     |
| Early onset PE group  | 30              | 4.59±1.69a    | 57.70±17.27a | 1.67(0.53-11.12)a   |
|                      |                 |               |              |                     |
Table 4. Neonatal characteristics

|                      | Late onset control | Early onset PE group | Late onset PE group |
|----------------------|--------------------|----------------------|--------------------|
| Number of cases      | 30                 | 30                   | 30                 |

Note:  a represents compared with early onset control group,  \( aP<0.05 \);  b represents compared with late onset control group,  \( bP<0.05 \); “—” represents negative result.
|                              |          |          |          |
|------------------------------|----------|----------|----------|
| Body weight (kg)             | 3.57±0.4 | 1.80±0.69* | 2.84±0.47* |
| Body length (cm)             | 50.6±1.5 | 41.8±4.7*  | 47.8±2.2*  |
| Gestational week at birth    | 39.2±0.7 | 33.1±2.7*  | 37.6±1.2*  |
| Number of NICU occupancy     | 1(0.03)  | 21(70.0)*  | 8(26.7)*   |
| Days of NICU occupancy (days)| 0        | 16.8±11.9* | 1.86±1.68* |
| Premature delivery (%)       | 0        | 25(83.3)*  | 0         |
| neonatal respiratory distress syndrome (%) | 0        | 3(0.1)*    | 0         |
| Necrotizing enterocolitis (%) | 0        | 1(0.03)*   | 0         |
| Erythroblastemia (person) (%) | 6(0.2)   | 1(0.03)*   | 1(0.03)*  |
| Bronchopulmonary dysplasia   | 0        | 1(0.03)*   | 0         |

Note: * represents comparison with late onset control group, *P<0.05.
Figure 1. Comparison of blood pressure between groups, * represents $P<0.05$, there was statistical significance.
Figure 2. Comparison of serum Cr and UA between groups, * represents P<0.05, 
There was statistical significance.
Figure 3. Comparison of SF between groups, * represents $P<0.05$, *** represents $P<0.001$, There was statistical significance.

Figure 4. Comparison of sTfR levels between groups, * represents $P<0.05$, ****
represents P<0.0001, There was statistical significance.

Figure 5. Comparison of levels of SF in fetal umbilical cord blood between groups, * represents P<0.05, **** represents P<0.0001, There was statistical significance.
Figure 6. mRNA and protein level of Ferritin and FTH, & protein levels of cleaved caspase-3, Bax, Bcl-2 in placenta tissues of early onset PE group, late onset PE group and late onset control group

A. The standard curve of absolute quantitative RT-q PCR; B and C. the mRNA expression levels of Ferritin and FTH; D. the protein expression level of Ferritin, FTH, cleaved caspase-3, Bax, Bcl-2s, and beta-actin; E. the gray value analysis result of protein bands.
Figure 7. The GSH-Px, T-SOD, MDA levels in placenta tissues of early onset PE group, late onset PE group and late onset control group. A. Comparison of GSH-Px levels; B. comparison of T-SOD levels; C. comparison of MDA levels
Figure 8. TUNEL methods were used to measure the cell apoptosis rate in early onset PE group, late onset PE group and control group. DNA fragmentation (dUTP) (C, F, I) was observed through TUNEL (A, D, G) and DAPI (B, E, H) using double labeling with fluorescent indicator. The apoptosis rate of obtained by dividing the number of TUNEL-positive cells by the total number of DAPI-labeled nuclei (J).
Figure 9 SF can promote progress of PE by upregulating the levels of UA, Cr, T-SOD, MDA, GSH-Px and down-regulating the levels of Bcl-2, CASPASE-3.
