Original Article

Association between maternal iron status and the risk of pre-eclampsia: a case-control study

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Background and Objectives: This study aimed to assess the associations of maternal iron status and placental iron transport proteins expression with the risk of pre-eclampsia (PE) in Chinese pregnant women. **Methods and Study Design:** A total of 94 subjects with PE and 112 healthy pregnant women were enrolled. Fasting blood samples were collected to detect maternal iron status. The placenta samples were collected at delivery to detect the mRNA and protein expression of divalent metal transporter 1 (DMT1) and ferroportin-1 (FPN1). Logistic analysis was used to explore the associations of maternal iron status with PE risk. The associations of placental iron transport proteins with maternal iron status were explored. **Results:** After adjusting for covariates, dietary total iron, non-heme iron intake and serum hepcidin were negatively associated with PE, with adjusted ORs (95%CIs) were 0.40 (0.17, 0.91), 0.42 (0.18, 0.94) and 0.02 (0.002, 0.13) for the highest versus lowest tertile, respectively. For the highest tertile versus lowest tertile, serum iron (4.08 (1.58, 10.57)) and ferritin (5.61 (2.36, 13.31)) were positively associated with PE. The mRNA expressions and protein levels of DMT1 and FPN1 in placenta were up-regulated in the PE group ($p < 0.05$). The mRNA expressions of DMT1 and FPN1 in placenta showed a negative correlation with the serum hepcidin ($r = -0.71$, $p < 0.001$; $r = -0.49$, $p < 0.05$). **Conclusions:** In conclusion, the maternal iron status were closely associated with PE risk, placental DMT1 and FPN1 were upregulated in PE which may be a promising target for the prevention of PE.

Key Words: pre-eclampsia, iron, hepcidin, divalent metal transporter 1 (DMT-1), ferroportin-1 (FPN1)

INTRODUCTION

Pre-eclampsia (PE), a common pregnancy complication, is defined as the presence of new-onset hypertension and proteinuria or other end-organ damage occuring after 20 weeks gestation.[1] PE affects an estimated 2.2% of pregnancies in China and is the biggest single cause of maternal and child mortality.[2] Serious outcomes caused by PE include abruptio placentae, pulmonary edema, hepatic failure and acute renal failure.[3,4] Currently, there is no effective therapeutic treatment for PE other than termination of the pregnancy.

The pathogenesis of PE has not been fully understood, and it is believed to be associated with endothelial dysfunction,[5] inflammation,[6] genetic factors,[7] and oxidative stress at the maternal-fetal interface.[3] Iron is essential for many fundamental biological processes, including energy metabolism, oxygen transport, DNA synthesis and metabolism, et al.[8] However, excess iron can result in increased oxidative stress by participating in Fenton reactions.[9] It has been revealed that iron homeostasis is disrupted in patients with PE.[10] In addition, recent studies have shown positive associations of serum levels of ferritin and iron with PE.[11,12] In particular, as the key hormone regulating systemic iron metabolism, hepcidin was also detected in PE with inconsistent results. Some studies have found higher serum hepcidin concentrations in PE compared to controls,[13,14] while other studies have shown no difference or even opposite results.[15,16] In addition, dietary iron intake of pregnant women have also been linked with PE,[17,18] which is regarded as a modifiable condition. Most previous studies showed the dietary total iron intake was inversely associated with the development of PE, while the relationship between iron intake from different sources and PE risk was only one study conducted in Iranians.[19] To date, most studies investigating the association of maternal iron status and dietary iron intake with the risk of PE have been conducted in Western countries,[20,21] and evidence from Chinese population remains limited.

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The placenta is the only link between fetus and mother during gestation. Dysregulated iron metabolism in the placenta is also an important factor in the development of PE.[3] Excessive iron accumulation in the placenta can lead to the production of reactive oxygen species (ROS), placental dysfunction and trophoblast injury which are recognized factors invovled in the occurrence of PE.[22] Generally, multiple transporters participate in regulating the absorption and transplacental transport of iron.[23] Divalent metal transporter (DMT1) is a transmembrane divalent metal transfer protein responsible for transferring ferrous iron from endosomes into the cytoplasm of placental syncytiotrophoblast cells.[24] In addition, intracellular iron enters fetal circulation through ferroportin-1 (FPN1) located on the basal membrane of placental syncytiotrophoblasts facing towards the fetus.[25] A recent study showed that the expression of FPN1 protein in placenta was downregulated in PE.[26] Previous study showed that the mRNA of DMT1 in placenta was down-regulated in gestational diabetes mellitus (GDM) patients.[27] The alteration of the expression of placental DMT1 can cause iron metabolism disorder, and then induce the production of ROS which results in endothelial dysfunction in placenta.[28] To date, whether the expression of DMT1 in the placenta is altered in PE has never been examined. In addition, the potential relationships between these placental iron transport proteins and maternal iron status remain unexplored in PE.

In view of the limitations and conflicting outcomes of previous studies, this study aimed to examine the expression of placental iron transport proteins and maternal iron status and evaluate their relationships with PE during late pregnancy.

METHODS

Study population

The subjects of study were recruited from the Affiliated Hospital of Qingdao University (Qingdao, China). PE is new-onset hypertension (systolic blood pressure ≥140 mmHg or diastolic blood pressure of ≥90 mmHg) that develops after 20 weeks of gestation combined with proteinuria (>300mg/day).[1] All subjects were older than 20 years, ≥28 gestational weeks, and had good compliance and communication skills. Exclusion criteria included multiple pregnancies, infectious diseases, immune system diseases, diabetes, heart, liver or renal disease and fetal malformation. Finally, a total of 94 subjects were identified as PE patients and 112 healthy pregnant women with similar age and gestational week hospitalized during the same period (±1 week) were randomly selected as the corresponding controls.

The study was approved by the Ethics Committee of Medical College of Qingdao University (QDU-HEC-2022287). Informed consents were obtained from all participants.

Sample size calculation

Sample size calculation formula for unmatched case-control study was used to estimate sample size. With reference to relevant publications,[29,30] the parameters were determined as follows: P_0 = 0.14, OR = 3.8, α= 0.05, β= 0.10. $P_1=(OR\times P_0)/(1- P_0+ OR\times P_0)$, $\bar{P}=(P_1+P_0)/2$. The high hemoglobin exposure rate (P_0) in the control group was 14%. The odds ratio between the high hemoglobin levels and PE was 3.8. The statistical power (β) was set at 0.10 and the test level was set at 0.05. Plug the above parameters into the formula and confirmed that each group need at least 68 cases. Therefore, the sample size of this study can meet the statistical requirements.

$$n = [z_{1-\alpha/2}\sqrt{2\bar{P}(1-\bar{P})} + Z_{\beta}\sqrt{P_1(1-P_1) + P_0(1-P_0)}]^2/(P_1 - P_0)^2$$

Socio-demographic information survey

Each participant was interviewed using a standardized questionnaire, including age, educational level, gestational weight gain, gravidity, family history of hypertension.

The preconceptional body mass index (BMI) was calculated from self-reported pre-pregnancy body weight and body height. BMI was calculated as a ratio of weight (kg) to height squared (m^2).

Food Frequency Questionnaire (FFQ) survey

Dietary data were collected by a FFQ during face-to-face interviews by an experienced dietitian. The questionnaire was modified on the basis of the questionnaire used in the Chinese nutrition and health surveillance. Participants were asked to report the consumption frequency and the average consumption of food items according to food pictures labeled with standard portion sizes over the past three months. The daily average consumption of each item was calculated by the consumption frequency and the average consumption per time. Finally, the data were imported and computed to obtain the dietary iron intake by using NCCW 12.0 (Qingdao University, China), which is primarily based on China Food Composition 2002 and 2004.

Maternal iron status indicators

Fasting blood samples were collected from the pregnant women before delivery. After centrifuging at 3000 × *g* at 4°C for 5 min, the serum samples were stored at -80°C for further measurement. The concentrations of serum iron were measured using inductively coupled plasma mass spectrometry (ICP-MS). The concentrations of serum ferritin were determined by radioimmunoassay. Serum transferrin levels were measured using an automatic biochemical analyzer (Olympus AU640, Japan). The levels of serum hepcidin were measured using a commercial ELISA kit (Jiancheng Bioengineering Institute, Nanjing, China).

Western blot analysis of placental DMT1 and FPN1

The placenta samples (50 mg) were homogenized in cold RIPA lysis buffer and centrifuged to collect supernatant. The extracted total proteins were qualified by a BCA Protein Quantitation Kit and then loaded onto 12% SDS-PAGE followed by transferring to fluoride (PVDF) membranes. After blocking with skim milk, it was incubated overnight with different primary antibodies (Absin, America): β-actin (1:1000), DMT1 (1:1000), FPN1 (1:1000). Afterwards, the membranes were washed three times with TBST and incubated with a secondary antibody (1:10000) at room temperature for 1 h. The β-actin was utilized as a control. The protein bands were observed by an enhanced chemiluminescence (ECL) localization reagent and quantified by Tanon GIS analysis.

Real-time quantitative PCR analysis of placental DMT1 and FPN1

Trizol reagent and a NanoDrop spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA) were utilized to extract total RNA from the placenta tissues and evaluate the amount and purity of RNA, respectively. Purity and quantity of RNA were assessed and 1μg RNA was reversely transcribed into cDNA. The real-timequantitative PCR was carried out using SYBR Premix Ex Taq fluorescent quantitative PCR (Eppendorf, Framingham, MA, USA). The PCR cycling conditions were as follows: a cycle of 95℃ for 2 min, 40 cycles of 95℃ for 5 s and 60℃ for 10 s.

Statistical analysis

Baseline characteristics were presented as means ± SDs for normally distributed variables, medians (ranges) for non-parametrically distributed variables, and numbers (percentages) for categorical variables. Student's *t*-test or Mann-Whitney *U*-test was used to compare the mean levels of normal distributions or with non-normal distributions. Chi-square test was used to compare the distribution of categorical variables. Dietary and serum indicators were categorized based on tertiles (Tertile 1: <33.3th percentile, Tertile 2: ≥33.3th to 66.6th percentile, Tertile 3: ≥66.6th percentile). Multivariate logistic regression was used to investigate the associations of the dietary iron intake and serum iron indicators with the risk of PE. Odds ratio (OR) and 95% confidence interval (CI) were calculated in Crude Model and Adjusted Model. Crude Model: no covariates were adjusted. Adjusted Model: age, preconceptional BMI, gestational weight gain, gravidity and family history of hypertension were adjusted. Spearman correlation analysis was used to evaluate the relationship between maternal iron status and the expression of placental iron transport proteins. GraphPad Prism 8.0 and Figdarw[3] were used for drawing of figures. All statistical analyses were conducted in SPSS (version 22; IBM Crop., Armonk, NY, USA). A two-tailed *p*-value <0.05 was considered statistically significant. Bootstrap analysis was used to verify the stability of the results.

RESULTS

Characteristics of participants

In this case-control study, 206 participants were included. The characteristics of PE patients (n = 94) and controls (n = 112) were presented in Table 1. Participants in the PE group had higher preconceptional BMI levels ($p < 0.001$) than control group. The proportion of first pregnancy was higher in the PE group. There were no significant differences in age, maternal educational level, family history of hypertension, gestational weight gain, history of abortion and dietary intake of energy, carbohydrate, fat and protein between PE and controls ($p > 0.05$).

Comparison of maternal iron status between two groups

As shown in Figure 1, the dietary intake of total iron and non-heme iron in PE group were significantly lower than those in control group ($p < 0.05$). The PE patients had higher levels of serum iron and ferritin and lower levels of serum transferrin and hepcidin than controls ($p < 0.05$).

Table 1. Baseline characteristics of included participants

Characteristics	Control (n = 112)	PE (n = 94)	t/z/χ^2	*p*
Age, years	28.5 ± 3.76	29.6 ± 4.45	-1.91	0.057
Maternal educational level, n (%)			1.24	0.537
Junior and below	31 (27.7%)	27 (28.7%)		
Senior	26 (23.2%)	16 (17.0%)		
Undergraduate and higher	55 (49.1%)	51 (54.3%)		
Family history of hypertension, n (%)			2.52	0.113
No	109 (97.3%)	87 (92.6%)		
Yes	3 (2.70%)	7 (7.40%)		
Preconceptional BMI, kg/m^2	21.3 (19.5, 24.6)	24.9 (22.6, 27.6)	-5.71	<0.001
Gestational weight gain, kg	15.0 (12.0, 17.3)	16.0 (12.0, 20.0)	-1.26	0.207
Gravidity, n (%)			7.19	0.006
1	10 (8.93%)	21 (22.3%)		
2	38 (33.9%)	33 (35.1%)		
≥3	64 (57.1%)	40 (42.6%)		
History of abortion, n (%)			0.65	0.421
No	63 (67.00%)	69 (61.6%)		
Yes	31 (33.00%)	43 (38.4%)		
Dietary intake				
Energy (kcal/d)	1557 ± 340	1557 ± 316	0.01	0.991
Carbohydrate (g/d)	201 ± 64.5	201 ± 49.9	-0.06	0.950
Fat (g/d)	54.9 (47.8, 65.3)	56.0 (49.2, 65.7)	-0.69	0.488
Protein (g/d)	52.1 (45.0, 66.6)	51.1 (42.4, 62.54)	-1.26	0.209
Serum iron, mg/L	9.11 (7.18, 12.0)	10.7 (9.25, 13.2)	-3.14	0.002
Ferritin, ng/mL	14.3 (10.9, 19.7)	18.6 (11.2, 29.2)	-2.37	0.018
Tansferrin, ng/mL	3.74 (3.42, 4.07)	3.49 (2.89, 3.84)	-2.40	0.016
Hepcidin, ng/mL	41.3 (33.8, 43.6)	26.0 (24.4, 28.3)	-5.70	0.001

PE, pre-eclampsia; BMI, body mass index change

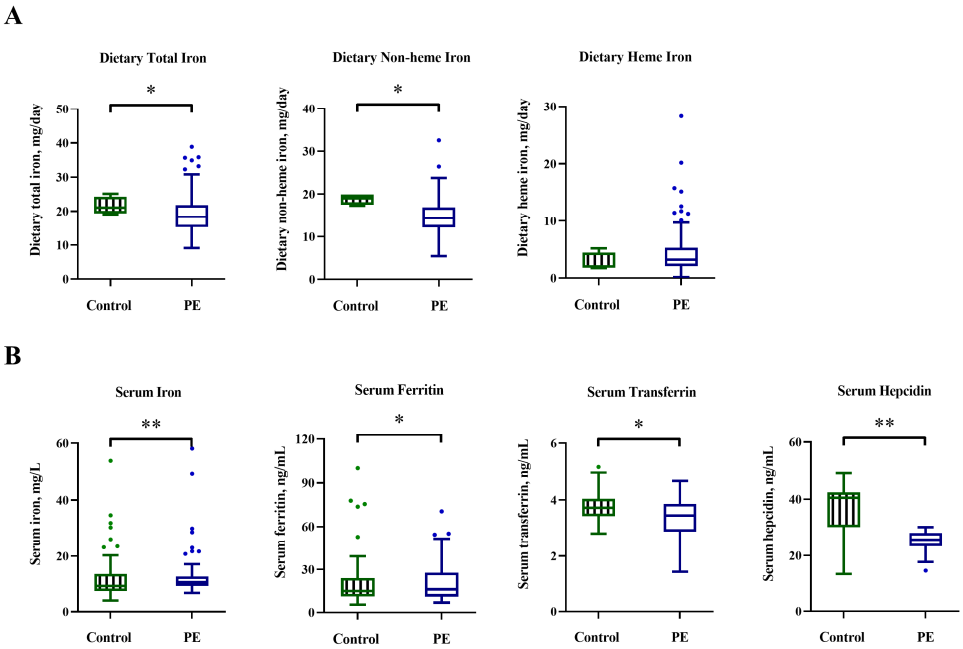

Figure 1. Comparisons of dietary iron intake and maternal iron status between the two groups. (A) Maternal dietary iron intake; (B) Maternal serum iron indicators. Asterisk indicates significant differences between PE and Control group. Data were presented as M (P_{25}, P_{75}). Mann-Whitney *U*-test were used to investigate the difference between control and PE. PE, Pre-eclampsia; * $p < 0.05$.

Table 2. Association between dietary iron intake and risk of PE in pregnant women

	Crude Model OR (95% CI)	Adjusted Model OR (95% CI)
Total iron, mg/day		
< 16.2	1.00 (ref.)	1.00 (ref.)
16.2 to <20.4	0.50 (0.25, 0.99) *	0.39 (0.17, 0.90) *
≥ 20.4	0.45 (0.23, 0.88) *	0.40 (0.17, 0.91) *
Heme iron, mg/day		
< 2.46	1.00 (ref.)	1.00 (ref.)
2.46 to <4.34	1.00 (0.51, 1.98)	0.84 (0.36, 2.00)
≥ 4.34	0.78 (0.39, 1.56)	0.70 (0.29, 1.67)
Non-heme iron, mg/day		
<13.0	1.00 (ref.)	1.00 (ref.)
13.0 to <16.2	0.35 (0.18, 0.71) *	0.41 (0.18, 0.94) *
≥ 16.2	0.40 (0.20, 0.79) *	0.42 (0.18, 0.94) *

Crude Model: no covariates were adjusted.
Adjusted Model: adjusted age and preconceptional BMI, gestational weight gain, gravidity and family history of hypertension. *p <0.05

Table 3. Association between serum iron levels and risk of PE in pregnant women

	Crude Model OR (95% CI)	Adjusted Model OR (95% CI)
Serum iron, mg/L		
< 8.87	1.00 (ref.)	1.00 (ref.)
8.87 to <11.5	5.06 (2.26, 11.3) **	5.22 (2.01, 13.5) **
≥ 11.5	3.64 (1.65, 8.06) **	4.08 (1.58, 10.6) *
Ferritin, ng/mL		
< 15.0	1.00 (ref.)	1.00 (ref.)
15.0 to <20.2	0.56 (0.27, 1.67)	1.54 (0.59, 4.01)
≥ 20.2	2.03 (1.00, 4.12)	5.61 (2.36, 13.3) **
Tansferrin, ng/mL		
< 3.40	1.00 (ref.)	1.00 (ref.)
3.40 to <3.82	0.52 (0.18, 1.50)	0.68 (0.20, 2.32)
≥ 3.82	0.31 (0.10, 0.92) *	0.46 (0.13, 1.65)
Hepcidin, ng/mL		
< 25.9	1.00 (ref.)	1.00 (ref.)
25.9 to <36.3	0.54 (0.15, 2.04)	0.44 (0.09, 2.20)
≥ 36.3	0.04 (0.001, 0.16) **	0.02 (0.002, 0.13) **

Adjusted Model: adjusted age and preconceptional BMI, gestational weight gain, gravidity and family history of hypertension were adjusted.
*p <0.05; **p < 0.01.

Associations of and maternal iron status with PE risk

The associations of maternal iron status with PE risk were presented in Table 2 and Table 3, respectively. After adjusting for age, preconceptional BMI, gestational weight gain, gravidity and family history of hypertension, the participants with highest tertile of dietary total iron, non-heme iron intake and serum hepcidin had a lower risk of PE compared with the lowest tertile, the corresponding ORs (95%CIs) were 0.40 (0.17, 0.91), 0.42 (0.18, 0.94) and 0.02 (0.002, 0.13), respectively. For the highest tertile versus lowest tertile, the concentrations of serum iron (4.08 (1.58, 10.57)) and ferritin (5.61 (2.36, 13.31)) were positively associated with the risk of PE. There was no association between the level of transferrin and PE risk. The ORs and 95%CI of age, pre-pregnancy BMI and gravidity in multivariate logistic regression analysis were shown in Supplementary Table 1.

Comparisions of mRNA expressions and protein levels of placental iron transport proteins between two groups

The mRNA expressions and protein levels of DMT1 and FPN1 in placenta were detected by RT-qPCR and western blot, respectively (Figure 2). Compared with the control group, the mRNA expressions and protein levels of DMT1 and FPN1 were both higher in the PE group ($p < 0.05$).

Correlation analysis of mRNA expressions of placental iron transport proteins and maternal iron status

The correlations between mRNA expressions of DMT1, FPN1 in placenta and maternal iron status were shown in Figure 3. The mRNA expression of FPN1 was positively correlated with the serum concentrations of ferritin (r = 0.33, $p < 0.05$). The mRNA expressions of DMT1 (r = - 0.71, $p < 0.05$) and FPN1 (r = - 0.49, $p < 0.05$) were negatively correlated with serum hepcidin.

DISCUSSION

In present case-control study, we investigated the associations of dietary iron intake and maternal iron status with the risk of PE. We focused on the expressions of the DMT1 and FPN1 in placenta, and explored the correlation between placental iron metabolism and maternal iron status. We found that dietary total iron, non-heme iron intake and serum hepcidin were negatively associated with the risk of PE. Serum iron and ferritin were positively associated with the risk of PE. In addition, the mRNA expressions and protein levels of DMT1 and FPN1 in placenta were up-regulated in PE patients compared to controls. The mRNA expressions of FPN1 and DMT1 were negatively correlated with maternal serum hepcidin, while mRNA expression of FPN1 was positively correlated with maternal serum ferritin.

Previous studies have evaluated the associations between dietary iron intake and the risk of PE.[18,19] Consistent with several previous epidemiological studies, we found dietary total iron intake was inversely associated with the development of PE. Allen demonstrated that insufficient maternal dietary iron intake causes hypoxia, which stimulates the secretion of stress hormones, e.g., norepinephrine and cortisol, which increase the risk of placental oxidative stress.[31] Therefore, ensuring adequate iron intake during pregnancy may reduce the risk of PE effectively. Further, we analyzed the associations between different sources of dietary iron and PE risk, which remains no evidence in the Chinese population. Results showed that dietary non-heme iron intake was inversely associated with the risk of PE, while heme iron intake was not significantly associated with PE risk. Our results were consistent with the results of a study in an Indian,[19] which was the only study to explore the relationship between different sources of dietary iron and PE. The large-scale cohort study in diverse ethnic populations were needed to verify the associations of dietary iron intake with PE.

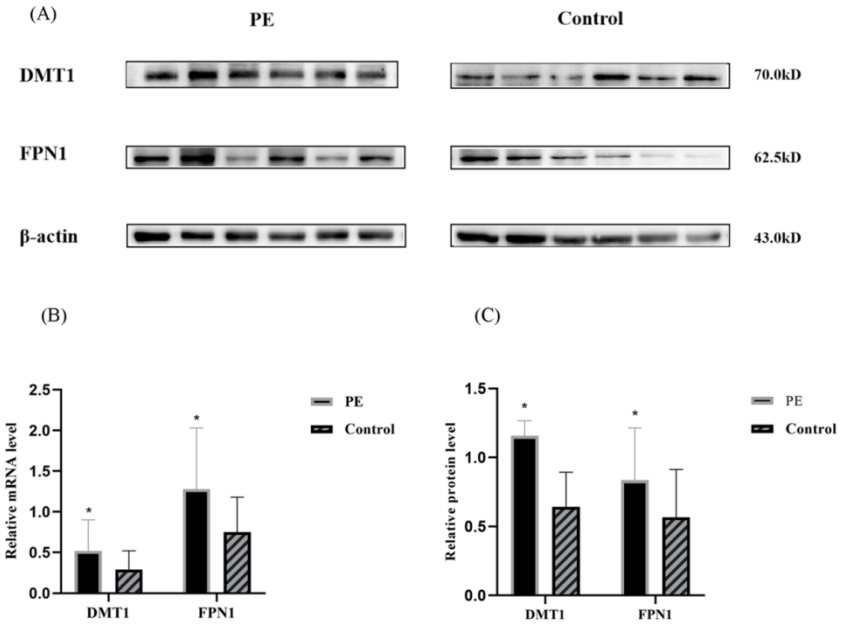

Figure 2. The mRNA expressions and protein levels of DMT1 and FPN1 in the placenta of PE and Control. (A) Western blot assay of proteins in placenta, n = 6 each group; (B) The Relative mRNA expression of DMT1 and FPN1, n = 30 each group; (C) Quantitative analyses of the DMT1 and FPN1 proteins. Data were presented as means ± SD. Student's *t*-test was used to investigate the difference between control and PE. * $p < 0.05$

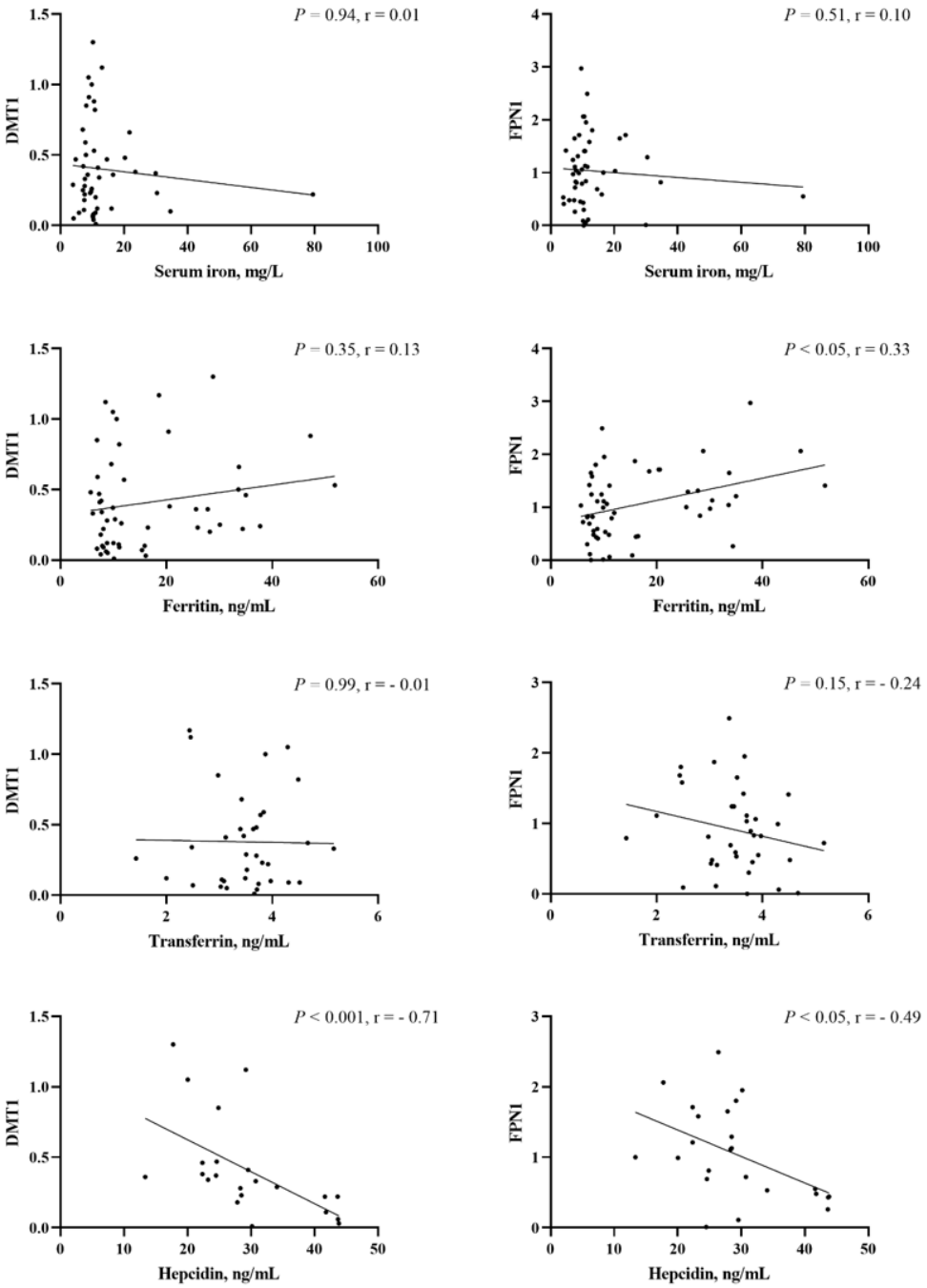

Figure 3. Correlations of placental DMT1 and FPN1 mRNA expressions with maternal serum iron indicators. Spearman correlation analysis was performed on placental DMT1 and FPN1 mRNA expressions and maternal serum iron indicators.

Moreover, the relationship between the maternal iron status and PE risk was analyzed in the study. First, our study showed that higher serum iron was associated with an increased risk of PE, which is consistent with most of previous studies.[20,32-34] Several existing meta-analyses had also confirmed this relationship.[35-37] Although the underlying molecular mechanism of the relationship between iron and PE was unclear, excess iron had been shown to be associated with lipid peroxidation which had been shown to be involved in the pathogenesis of PE. Sedaret al. reported that the levels of iron and lipid peroxides both in plasma and placental tissue of PE patients were significantly elevated.[20] In addition, our results showed that higher serum ferritin was associated with an increased risk of PE. At present, the evidence of this association is limited and controversial,[11,38,39] and it had not been explored in the Chinese pregnant women. As a well-recognized marker of iron storage *in vivo*, serum ferritin plays a key role in the regulation of iron metabolism.[40,41] The increase of iron content in pregnant women can cause the increase of inflammation, oxidative stress and insulin resistance in the body,[42] suggesting that the increase of serum ferritin is related to the higher PE risk. It has been suggested that abnormal increase of serum ferritin level can destroy the structure of vascular endothelial cells, affect their function, and then lead to vasoconstriction, endothelial cell injury and systemic inflammation,[12] which were the promoting factors involved in the development of PE. Therefore, our findings suggested that pregnant women should strengthen the

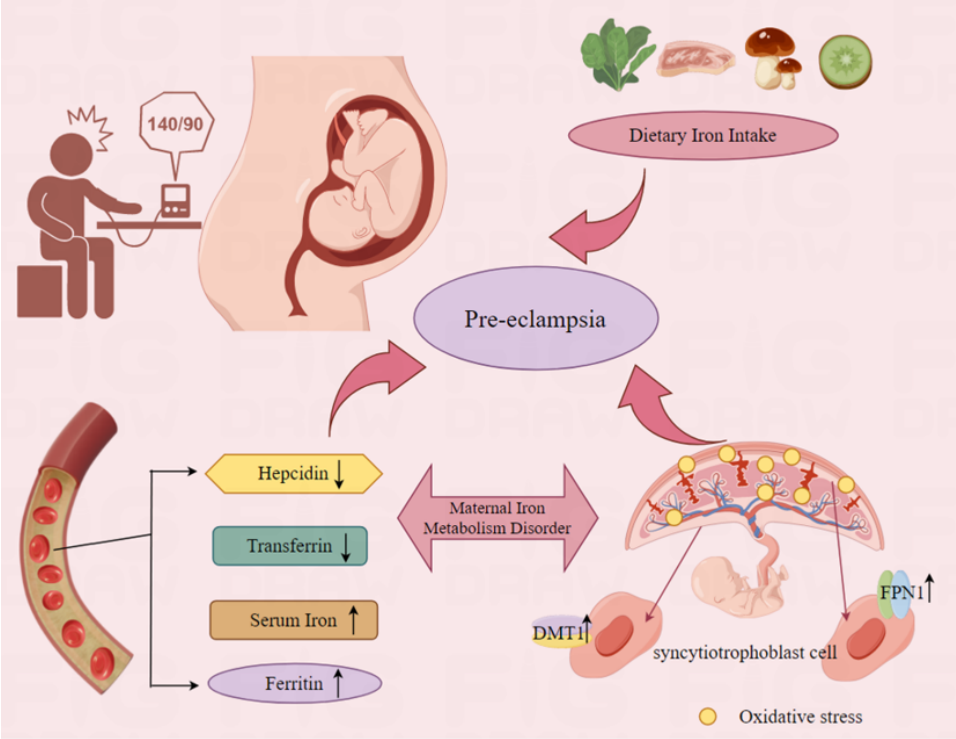

Figure 4. Maternal iron status, placental iron transport and the development of PE

detection of iron status *in vivo* and reasonable iron supplementation is recommended.

Hepcidin had been the focus of many studies on maternal iron regulation in pregnancy and iron transfer to the fetus.[43,44] The association between hepcidin and PE risk has gained much attention with mixed conclusions.[8,16,38,45,46] Consistent with studies of Brunacci and Masoumi,[8,16] our results showed that the serum hepcidin was lower in patients with PE compared with controls. However, Cardaropoli and Ahmed et al. suggested that there was no difference in hepcidin between PE and healthy pregnant women,[10,38] while several other studies reached the opposite conclusion.[10,15] Our present findings seem to conflict with the pronounced inflammatory status of the PE. Brunacci et al. found that hepcidin was lower in the PE group, and at the same time, they found a significant positive correlation between serum hepcidin and CRP concentration and suggested that mechanisms involved in stimulating hormone production in the inflammatory response may be preserved.[16] In addition, previous studies had shown that the disturbance of iron metabolism may result in hypoxia *in vivo*,[47] which may inhibit the expression of hepcidin. Hypoxia inducible factor (HIF) is activated under hypoxia status, which inhibits the promoter region of hepcidin and reduces the expression of hepcidin.[48] The production of erythropoietin (EPO) increased under hypoxia environment, which further down-regulated the expression of hepcidin.[49,50] Lower hepcidin levels observed in PE suggested a hormonal drive to increase maternal iron bioavailability in these pregnancies, a phenomenon that may underlie the iron overload previously described in PE pregnancies.[36] Taken together, our findings suggest that dysregulation of iron homeostasis in PE might be caused by inadequate production of hepcidin, leading to an increased risk of developing iron overload. However, the mechanism of decreased hepcidin levels in PE patients is still unclear and needs further study.

The central role of the placenta in the pathogenesis of PE is undisputed. The imbalance of placental iron homeostasis may lead to the accumulation of lipid peroxidation and lipid peroxides, which may ultimately lead to placental dysfunction and the development of PE.[51,52] As the main iron transport proteins in the placenta, DMT1 and FPN1 play an important role in maintaining the balance of iron intake and release in the placenta. At present, there were few studies focusing on the associations of placental DMT1 and FPN1 with PE development, the present study was the first time to explore it in Chinese population. The results showed that placental protein and mRNA levels of DMT1 and FPN1 were upregulated in PE patients compared to controls, indicating that the uptake and transfer of iron in the placenta of PE patients were increased. This suggests that women with PE transport iron more actively than normal pregnant woman. The upregulation of DMT1 and FPN1 in trophoblasts leads to increased iron uptake, storage, and placental sequestration, contributing to the evoked oxidative stress in placenta.[28] At the same time, the mRNA expressions of DMT1 and FPN1 were shown negatively correlated with maternal serum hepcidin in our study. Previous evidence suggested that the regulation of hepcidin on iron transport proteins in placenta may be similar to that in the intestine, and it is a negative regulator of iron transport.[53] Hepcidin can directly downregulate the expression of DMT1 and FPN1 in the intestine.[54,55] Therefore, the upregulated expression of DMT1 and FPN1 may due to the decrease of maternal serum hepcidin levels. Yang et al. also found a negative correlation between serum hepcidin and placental FPN1 expression.[56] They found that the level of hepcidin was decreased in gestational diabetes patients, while the mRNA expression and

protein level of FPN1 in placenta was increased, suggesting that lower hepcidin level can up-regulate FPN1 expression, thereby increasing the inflow of iron into fetal circulation. Overall, our findings suggested that placental iron transport proteins were pronouncedly associated with the development of PE, DMT1 and FPN1 may be a promising target for the prevention and treatment of PE. However, the systemic and local mechanisms regulating placental iron transport remain poorly understood, the exact mechanism needs to be further explored *in vivo* and *in vitro* in the future.

This study was the first to comprehensively analyze the relationship between dietary iron intake, maternal iron metabolism status, placental iron transport proteins and PE in Chinese pregnant women. In addition, we focused on the expression changes of placental iron transport proteins in PE patients, and tried to explore the possible mechanism related to the associations between iron metabolism and PE development through a series of correlation analyses. Moreover, this study employed a case-control design to provide a theoretical basis for the exploration of the etiology of PE, but causal inference should be cautious. Our study had some limitations. First, PE patients in this present study were not classified according to the severity of the disease. In the future, it may be necessary to further explore the association between the iron status, iron transport protein of placenta and the severity of the disease. Second, our findings rely on self-reported dietary data, which may be subject to recall bias. Third, this study did not detect the indicators of inflammation and oxidative stress in pregnant women. In addition, the sample size is slightly small. Prospective cohort studies with large sample size are needed to confirm the associations in the future.

Conclusion

In conclusion, the dietary iron intake and maternal iron status were closely associated with PE risk, placental DMT1 and FPN1 were upregulated in PE which may be a promising target for the prevention and treatment of PE.

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AUTHOR DISCLOSURES

No competing interests are reported.

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