Background: Although preeclampsia causes maternal and infantile morbidity and mortality, its pathophysiology is unclear. We aimed to study the correlation between CXC chemokine receptor (CXCR)4 and CXCR7 protein expression levels in the placentas of women with preeclampsia.

Material/Methods: The study included 42 women who delivered in Wenzhou People’s Hospital China from September 2019 to March 2020. There were 3 groups: 13 patients with gestational hypertension, 12 patients with preeclampsia, and 17 patients with normal pregnancy (control). We measured placental CXCR4 and CXCR7 levels with ELISA. We compared differences between groups with t test and ANOVA, and Pearson’s correlation was used to test correlations between CXCR4 and CXCR7 protein expression levels and lag time of preeclampsia.

Results: The preeclampsia and gestational hypertension groups showed statistically higher levels of CXCR4 than did the control group (54.43±10.31, 51.53±9.62 vs 42.81±10.06 ng/g, respectively), with no difference between the preeclampsia and gestational hypertension groups. There were no significant differences in CXCR7 levels between the preeclampsia, gestational hypertension, and control groups. Among patients with preeclampsia, the CXCR4 level was significantly higher in the severe preeclampsia group (systolic blood pressure ≥160 and/or diastolic blood pressure ≥90 mmHg) than in the mild hypertension group. CXCR4 and CXCR7 levels were higher in early-onset preeclampsia (<34 weeks) than in late-onset preeclampsia. CXCR4 and CXCR7 levels were not correlated with the lag time of preeclampsia.

Conclusions: CXCR4 and CXCR7 protein may play roles in the pathophysiology of preeclampsia.

Keywords: ACKR3 protein, human • CXCR4 protein, human • Preeclampsia Eclampsia 2

Full-text PDF: https://www.medscimonit.com/abstract/index/idArt/931192
Background

Preeclampsia is a multi-system condition in pregnant women, with an incidence of approximately 5% to 7% [1, 2]. Preeclampsia is a major cause of perinatal maternal and perinatal mortality. Previous studies report that preeclampsia is among the top 3 causes of maternal death in developing countries [3, 4]. Its clinical manifestations include hypertension and proteinuria, and it can cause fetal growth restriction and stillbirth, thus severely affecting the health of mothers and fetuses. Preeclampsia is characterized by an abrupt onset and in most women it occurs without any warning symptoms [5]. A lag time exists between the discovery of high blood pressure and a preeclampsia diagnosis. Currently, the pathogenesis of preeclampsia is not fully known. Several studies are currently exploring the correlation between CXC motif chemokine ligand (CXCL)12, CXC chemokine receptor (CXCR)4, and CXCR7, and the pathogenesis of preeclampsia. Studies report that dysregulation of the CXCR4/CXCR7 signaling pathway causes pregnancy-related diseases, such as preeclampsia, through complex pathways [6, 7]. CXCR4 is expressed in chorionic villous tissue throughout pregnancy, with high expression levels observed in early trimester chorionic tissue [8]. Studies report that changes in CXCR4 expression are inextricably linked to the pathogenesis of hypertensive disorders that occur during pregnancy, with significant clinical and pathological changes observed during mid to late pregnancy. This study aimed to explore the expression levels of CXCR4 and CXCR7 in the placental tissues of women with normal pregnancy and those with preeclampsia. Further, the correlation between the CXCR4/CXCR7 signaling pathway and preeclampsia was analyzed, showing that the CXCR4/CXCR7 signaling pathway is implicated in the occurrence and development of preeclampsia.

Material and Methods

Clinical Data

A total of 25 pregnant women who presented with hypertension during pregnancy and were hospitalized in Wenzhou People’s Hospital from September 2019 to March 2020 were included in this study, following the guidelines for hypertensive disorders in pregnancy [9]. Participants were grouped into a gestational hypertension group (n=13) and preeclampsia group (n=12). In addition, 17 women with normal pregnancies who gave birth at the same time were included as normal controls. Prior to the study, a power analysis was conducted (power=98.6%), which showed that the sample size was sufficient. All women included in the study had singleton pregnancies and had no history of organic diseases, kidney diseases, chronic hypertension, diabetes, systemic lupus erythematosus, and blood-related diseases. Patients who were using certain medications during pregnancy and patients with severe infections during pregnancy were excluded. This study was reviewed and approved by the ethics committee of our hospital (ID 2018-18). Written informed consent was obtained from all participants.

Methods

A piece of full-thickness placenta tissue was obtained from the center of the maternal surface of the placenta and at the 3, 6, 9, and 12 o’clock positions within 15 min after the placenta was delivered. Each piece was about 2×2×1 cm in size. Placenta edges, hemorrhagic foci, necrosis, and calcification foci were avoided during sampling. Samples were rinsed with sterile saline repeatedly, and blood and water on the tissue were repeatedly dried with sterile filter paper. All samples were placed in a sterile cryotube and stored in a refrigerator at -20°C for analysis of the levels of CXCR4 and CXCR7 proteins.

Experimental Materials

CXCR4 and CXCR7 enzyme-linked immunoassay (ELISA) kits were purchased from Wuhan Chundu Biological Co., Ltd.

Observation Index

The ELISA method was used to determine the expression levels of CXCR4 and CXCR7 proteins in the placenta samples from each group. The placenta samples were cut into sections and weighed. Samples were placed into phosphate buffer (pH 7.4), frozen, and stored in liquid nitrogen for later use. The test was carried out by professional researchers following the manufacturer’s instructions. The optical density (OD) value of each well was measured at a wavelength of 450 nm. A standard curve was generated using the concentration of the standard substance as the abscissa and the OD value as the ordinate. The corresponding concentration from the standard curve was then determined based on the OD value of the sample, and then multiplied by the dilution factor. Alternatively, the concentration of the standard substance was used to calculate the linear regression equation of the standard curve with the OD value. The OD value of the sample was substituted into the equation to calculate the sample concentration. The value was multiplied by the dilution factor to obtain the actual concentration of the sample.

Statistical Analysis

Statistical analysis was performed using SPSS version 24.0. All data were expressed as mean±standard deviation. The LSD t test was used to compare differences between the 2 groups. One-way ANOVA was used to compared differences between the 3 groups. The correlation between CXCR4 and CXCR7 protein expression levels and lag time of preeclampsia was determined by Pearson’s correlation analysis. P<0.05 indicated a statistically significant difference.
**Results**

**General Features of the 3 Groups**

Analysis of maternal age, maternal weight, gravidity, parity, and gestational age of the 3 groups of pregnant women showed no significant differences ($P>0.05$, **Table 1**).

**Determination of CXCR4 and CXCR7 Protein Expression Levels in Placenta Samples from the 3 Groups**

CXCR4 protein expression levels in the placenta samples from the 3 groups were significantly different ($F=5.540$, $P<0.05$). However, CXCR7 protein expression levels in placenta tissues from the 3 groups were not significantly different ($F=0.374$, $P>0.05$, **Table 2**).

**Pairwise Comparison of CXCR4 Protein Expression Levels in the 3 Groups**

Pairwise comparisons between the groups showed that the placental CXCR4 protein expression level of the preeclampsia group was significantly higher than that of the normal group ($P<0.05$). The CXCR4 protein expression level of the gestational hypertension group was significantly higher than that of the normal group ($P<0.05$). The CXCR4 protein level of the gestational hypertension group was not significantly different from the level of the preeclampsia group ($P>0.05$).

**Severity of Preeclampsia and CXCR4/CXCR7 Protein Expression Levels**

Analysis of the 12 patients with preeclampsia showed that the placental CXCR4 protein level was significantly higher in patients with severe hypertension than in those with mild hypertension ($P<0.05$). However, there was no statistically significant difference in the CXCR7 protein expression level between the 2 groups ($P>0.05$). The CXCR4 and CXCR7 protein levels of patients with early-onset preeclampsia were significantly higher than those with late-onset preeclampsia ($P<0.05$). The expression levels of CXCR4 and CXCR7 proteins for patients with severe proteinuria (urinary protein $\geq 2.0$ g/24 h or random urinary protein ++) and patients with mild proteinuria were not significantly different ($P>0.05$). In addition, the CXCR4 and CXCR7 protein levels in patients with fetal growth restriction were not significantly different from the levels of those with normal fetal birth weight ($P>0.05$, **Table 3**).

**Correlation of CXCR4 and CXCR7 Protein Expression Levels with Lag Time of Preeclampsia**

Pearson’s correlation analysis showed that the expression levels of the CXCR4 and CXCR7 proteins were not correlated with the lag time of preeclampsia ($r=-0.006$, $P>0.05$; $r=-0.161$, $P>0.05$, respectively).

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**Table 1.** Comparison of general information of 3 groups of pregnant women.

|                  | n   | Maternal age (years) | Weight (kg) | Gravidity | Parity | Gestational age (weeks) |
|------------------|-----|----------------------|-------------|-----------|--------|-------------------------|
| Normal group     | 17  | 28.06±4.67           | 70.51±9.33  | 1.76±1.30 | 0.29±0.47 | 38.53±1.63              |
| Gestational hypertension group | 13  | 28.42±5.30           | 73.18±8.58  | 2.25±1.42 | 0.42±0.52 | 37.75±1.77              |
| Preeclampsia group | 12  | 32.46±7.45           | 72.65±6.69  | 2.69±1.32 | 0.69±0.95 | 37.54±2.44              |
| F value          |     |                      |             |           |        |                         |
| P value          | 0.103 |                    | 0.657      | 0.145    | 0.272  | 0.345                  |

**Table 2.** Comparison of CXCR4 and CXCR7 protein expression levels in placenta in the 3 groups (ng/g).

|                  | N     | CXCR4 (ng/g) | CXCR7 (ng/g) |
|------------------|-------|--------------|--------------|
| Normal group     | 17    | 42.81±10.06  | 12.81±2.75   |
| Gestational hypertension group | 13    | 51.53±9.62   | 13.44±3.03   |
| Preeclampsia group | 12    | 54.43±10.31  | 13.77±3.55   |
| F value          |       | 5.540        | 0.374        |
| P value          | 0.008 |              | 0.690        |
Table 3. Severity of preeclampsia and CXCR4/CXCR7 protein expression levels in placenta tissue (ng/g).

|                   | n (%) | CXCR4 (ng/g) | t value | P value | CXCR7 (ng/g) | t value | P value |
|-------------------|-------|-------------|---------|---------|-------------|---------|---------|
| Severe hypertension |       |             |         |         |             |         |         |
| Yes               | 7 (58.3) | 45.53±7.60 | 2.803   | 0.020   | 12.34±2.97 | 0.517   | 0.617   |
| No                | 5 (41.7) | 36.20±3.75 |         |         | 11.61±1.90 |         |         |
| Severe proteinuria |       |             |         |         |             |         |         |
| Yes               | 9 (75.0) | 41.88±7.79 | 0.161   | 0.883   | 12.04±2.04 | 0.012   | 0.991   |
| No                | 3 (25.0) | 40.93±9.20 |         |         | 12.01±4.22 |         |         |
| Fetal growth restriction |       |             |         |         |             |         |         |
| Yes               | 4 (33.3) | 47.46±7.82 | 1.946   | 0.109   | 13.67±2.97 | 1.501   | 0.202   |
| No                | 8 (66.7) | 38.73±6.21 |         |         | 11.21±1.94 |         |         |
| Early-onset preeclampsia |       |             |         |         |             |         |         |
| Yes               | 3 (25.0) | 50.91±4.48 | 4.538   | 0.015   | 14.94±1.87 | 3.108   | 0.044   |
| No                | 9 (75.0) | 38.55±5.84 |         |         | 11.06±1.87 |         |         |

Discussion

Currently, there is increasing interest in the pathogenesis of preeclampsia in the field of perinatal medicine. However, the etiology and pathogenesis of preeclampsia has not been fully explored [10]. Placenta-related physiological changes may play key roles in the occurrence of preeclampsia, as the clinical symptoms of preeclampsia gradually disappear after the placenta is delivered from the mother [11]. Studies have reported that the CXCR4/CXCR7 signaling pathway is important during pregnancy, especially in the placenta. Studies in reproductive biology report that the CXCR4/CXCR7 signaling pathway is involved in key processes, such as placenta formation, embryonic development, maternal-fetal immune tolerance, and vascular remodeling during early pregnancy. Dysregulated expression levels of CXCR4 and CXCR7 proteins are implicated in miscarriage, premature delivery, fetal growth restriction, and preeclampsia [12,13].

Chemokines are small secreted proteins produced by tissue cells and inflammatory cells that can move chemokine cells directionally. Chemokines bind to corresponding chemokine receptors and play a role in the migration of white blood cells, embryonic development, angiogenesis, and various physiological and pathological processes such as hematoipoiesis, atherosclerosis, tumor occurrence and metastasis, and HIV infection. CXCL12, also known as stromal cell-derived factor 1, is a member of the chemokine family [14]. CXCL12 is mainly secreted by stromal cells and bone marrow cells. It is expressed constitutively and can induce cells to move toward high concentrations. Chemokines and their receptors are widely expressed at the maternal-fetal interface and play a key role in immune response [15-17]. The chemokine CXCL12 is mainly derived from placental trophoblast cells, including trophoblast progenitor cells, syncytiotrophoblast cells, and extra villous trophoblast cells. Studies report that the expression level of the CXCL12 factor in placenta tissue and maternal blood of pregnant women with preeclampsia is significantly higher than that in women with normal pregnancies [18,19]. CXCR4 is one of the main receptors of the CXCL12 chemokine. Previous studies reported that CXCR4 was the only receptor for CXCL12. However, recent studies on the CXCL12/CXCR4 signaling pathway have identified CXCR7, formerly known as RDC1, as another G protein-coupled receptor that binds to CXCL12 with high affinity [20]. After CXCL12 binds to CXCR4 and CXCR7 receptors, the chemokine activates a variety of signaling pathways and regulates cell movement, differentiation, adhesion, and secretion functions [21]. CXCL12 and the CXCR4/CXCR7 signaling pathway can promote differentiation, invasion, and proliferation of placental trophoblast cells [19]. Lu et al reported that the CXCR4/CXCR7 signaling pathway is involved in the mediation of apoptosis of placental trophoblasts, which may be related to the occurrence and development of severe preeclampsia [22].

In the present study, the analysis of CXCR4 and CXCR7 proteins in the placental tissues of pregnant women with hypertension showed that the expression level of CXCR4 protein in placenta tissue of patients with hypertension in pregnancy, especially in patients with preeclampsia, was higher than the level in normal pregnancy. This finding indicates that the CXCR4 protein may be implicated in the occurrence and development of preeclampsia. In addition, significant increases in the CXCR4 protein level in preeclampsia patients with severe hypertension and in CXCR4 and CXCR7 protein levels in early-onset preeclampsia were observed. The results suggest
that levels of CXCR4 and CXCR7 proteins in the placenta have potential diagnostic value in the evaluation of severity of preeclampsia. In this study, CXCR4 and CXCR7 protein levels were not correlated with the lag time of preeclampsia. The approximate time and degree of early increase of CXCR4/CXCR7 protein expression in placental tissues of patients with preeclampsia should be further explored. The CXCR7 protein levels in the placental tissues of the 3 groups in this study were not significantly different (P>0.05). Further studies with a larger sample size should be conducted.

The research specimen in this study was postpartum placenta tissue and, therefore, its direct clinical application is limited. Further studies should explore the relationship between preeclampsia and the levels of CXCR4 and CXCR7 proteins in maternal serum to provide findings for clinical applications.

Conclusions

An increased level of CXCR4 protein in placental tissue was correlated with preeclampsia. Therefore, the CXCR4 protein level can be used for the diagnosis and evaluation of preeclampsia. Currently, the correlation between the CXCR4/CXCR7 signaling pathway and the pathogenesis of preeclampsia is not fully known, and further studies are needed to elucidate this relationship. The present data indicated the placental level of these substances and thus, at present, these data may not be of use in clinical practice. However, we believe that our results may illustrate the importance of CXCR4 and CXCR7 proteins in preeclampsia and may provide fundamental data for further research.

Conflicts of Interest

None.

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