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Original Research Article

CXCR4/CXCL12 GENE EXPRESSION IN WOMEN WITH ENDOMETRIOMAS: A POTENTIAL DIAGNOSTIC BIOMARKER

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ABSTRACT

Background: Endometriomas, a form of ovarian endometriosis, are associated with chronic pelvic pain and infertility. The CXCR4/CXCL12 signaling pathway plays a important role in cell migration, angiogenesis, and inflammation, making it a potential biomarker for endometriosis. Our study evaluates the expression of CXCR4/CXCL12 in the blood of women with endometriomas. **Materials and Methods:** This case-control study included women between 20-49 years of age, diagnosed with endometriomas and healthy controls. Quantitative real-time PCR (qRT-PCR) was used to measure CXCR4/CXCL12 gene expression in blood samples. Statistical analyses were performed to assess the correlation between gene expression levels and severity of the disease. **Result:** CXCR4 and CXCL12 expression levels were significantly increased in the blood of women with endometriomas compared to controls ($p<0.05$). Higher expression levels correlated with increased disease severity. **Conclusion:** The upregulation of CXCR4/CXCL12 in peripheral blood suggests its potential as a non-invasive biomarker for diagnosing endometriomas. Further validation in larger cohorts is recommended.

INTRODUCTION

Endometriomas are sign of a more progressed stage of endometriosis. Endometriomas can cause chronic pelvic pain, dyspareunia and infertility and are frequently treated surgically.[1,2] Endometrioma of the ovary appears to be a predictor of more extensive pelvic and intestinal illness (hallmark of deep endometriosis).[3] The patients with ovarian endometriomas frequently present with endometriosis-related symptoms such as pelvic discomfort, dysmenorrhea, dyspareunia, and infertility. Endometrioma is describes as a cyst that develops when ectopic endometrial glands and stroma in the ovary bleeds. These endometriotic cysts in the ovary frequently include a viscous dark brown fluid (chocolate fluid) made of hemosiderin produced from past intraovarian bleeding. In up to 50% of patients, these cysts are bilateral. Chemokines are a family of small chemotactic cytokine proteins (<15 kDa) produced by various cell types upon stimulation or expressed constitutively. They are classified into four categories:

- CXC, CC, C, and CX3C in regard to the number and position of conserved cysteine residues (cysteine motif) in the N-terminal region of the protein. They trigger functional outcome by binding to their corresponding receptors.
- CXCL12, also described as stromal cell-derived factor 1 (SDF-1), is a chemokine ligand and considered to be the best characterized chemokines in mobilization of BM-derived stem cells in etiology of cancer and inflammation.
- In the endometriosis niche, CXCL12 causes epithelial and glandular cell proliferation via autocrine/paracrine mechanisms, vasculogenesis, and angiogenesis.

CXCR4 is a G protein-coupled receptor (GPCR) for CXCL12 that is expressed in mesenchymal stem cells generated from bone marrow, solid tumours, and endometriosis implants. CXCL12-CXCR4 signaling is increased in endometriosis patients and has been proven as a critical signal for bone marrow-derived cells (BMDCs) migration to endometriosis5. Flow cytometry was used to identify DsRed+ cells in the blood of endometriotic animals. CXCR4 and mesenchymal stem cell (MSC) indicators were

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expressed in circulating donor cells, but not hematopoietic stem cell markers. Following this research, the role of CXCR7 in an endometriosis mice model was determined. CXCR7 expression is elevated during pathological inflammation and tumour formation, resulting in increased CXCR7 expression.[4]

MATERIALS AND METHODS

This pilot study was conducted at the Department of Obstetrics and Gynecology with the Departments of Biochemistry and Radiodiagnosis from November 2021 to October 2023. The study aimed to assess CXCR4/CXCL12 gene expression in blood samples of women with and without ultrasonographic ally diagnosed endometriomas.

A total of 30 participants (women aged 20-49 years) were enrolled, with 15 cases and 15 age-matched controls. Inclusion criteria for cases included radiologically diagnosed endometriomas based on ultrasound findings, while controls were women with gynaecological complaints other than endometriomas. Women with malignancies, HIV, preeclampsia, or autoimmune diseases were excluded.

Following ethical approval and informed consent, participants underwent general, abdominal, and pelvic examinations. Blood samples (2 mL) were collected in EDTA vials and processed for mRNA isolation using TRIzol. RNA integrity was assessed spectrophotometrically, and cDNA synthesis was done. Then Quantitative real-time PCR (qRT-PCR) was used to analyze CXCR4/CXCL12 expression, with GAPDH as the housekeeping gene. Statistical analyses included t-tests, chi-square tests, and ROC curve analysis to assess diagnostic potential.

The primary outcome was the number of women with altered gene expression, while secondary outcomes included relative fold change and correlation with endometrioma size.

Statistical analysis: The data was recorded in MS EXCEL spreadsheet and was analysed using (SPSS) version 21.0. The categorical variables were shown in numbers and percentages (%) and continuous variables were shown as mean ± SD and median. Normality of data was tested by Kolmogorov-Smirnov test and when normality was rejected, then nonparametric test was applied.

Statistical tests were used as below:

- Independent t test/Mann-Whitney Test were used to compare quantitative variables (when the data sets were not normally distributed) between the two groups and ANOVA/Kruskal Wallis test between more than two groups.
- Chi-Square test/Fisher's Exact test were used to compare qualitative variables.
- Spearman rank correlation coefficient was applied to assess the correlation of true fold change of CXCR4/CXCL12 gene with other parameters.
- A p-value of <0.05 was statistically significant.

RESULTS

The mean (SD) of CXCR4 Delta ct of cases and controls patients were 4.36 (0.95) and 5.00 (0.90), respectively. The difference between means of cases and controls was statistically insignificant (p=0.0686). [Table 1].

Table 1: Comparison of serum CXCR4 Delta ct levels between cases and controls

CXCR4 Delta CT	Cases [N=15]	Controls [N=15]	P-Value
Mean (SD)	4.36 (0.95)	5.00 (0.90)	t=1.894 p=0.0686

p<0.05 is considered statistically significant

The mean (SD) of true fold change of serum CXCR4 levels of cases and controls was 1.87 (1.3) and 1.00 (0.00), respectively. The difference between means of true fold change of serum CXCR4 levels of cases and controls was statistically significant (p=0.0150). [Table 2].

The expression of CXCR4 was significantly upregulated in cases as compared to control.

Table 2: Comparison of true fold change of CXCR4 between cases and controls

TRUE fold change of CXCR4	Cases [N=15]	Controls [N=15]	P-VALUE
Mean (SD)	1.87 (1.3)	1.00 (0.00)	t=2.592 p=0.0150*

p<0.05 is considered statistically significant

The mean (SD) of CXCL12 Delta ct of cases and controls was 5.06 (0.88) and 5.85 (1.09) respectively. The difference between means of CXCL12 Delta ct of cases and controls was statistically significant (p=0.0375*). [Table 3].

Table 3: Comparison of CXCL12 Delta ct between cases and controls

CXCL12 Delta ct	Cases [N=15]	Controls [N=15]	P-value
Mean (SD)	5.06 (0.88)	5.85 (1.09)	t=2.184 p=0.0375*

p<0.05 is considered statistically significant

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The mean true fold change of CXCL12 between cases and controls were 2.13±1.46 and 1.00±0.0, respectively. The mean difference was statistically significant (p=0.0057). [Table 4].

The expression of CXCL12 was significantly upregulated in cases as compared to control.

Table 4: Comparison of mean true fold change of CXCL12 between cases and controls

True fold Change of CXCL12	Cases [N=15]	Control [N=15]	P-value
Mean (SD)	2.13 (1.46)	1.00 (0.0)	t=2.998 p=0.0057*

p<0.05 is considered statistically significant

DISCUSSION

Endometriosis, a chronic inflammatory disease, has been associated with various chemokine signaling pathways, particularly CXCR4/CXCL12. Recent studies have highlighted the role of these chemokines in disease progression, lesion development, and immune evasion. Our findings demonstrate an upregulation of CXCR4 and CXCL12 in the blood of patients with endometriomas, reinforcing the hypothesis that these biomarkers could serve as potential diagnostic indicators.

CXCR4/CXCL12 Axis in pathogenesis of Endometriosis

Emerging evidence suggests that the CXCR4/CXCL12 axis plays a significants role in the recruitment of bone marrow-derived stem cells to endometriotic lesions, contributing to disease propagation. Pluchino et al. (2020) reported that CXCR4 and CXCR7 antagonists effectively reduced lesion size by disrupting bone marrow cell traffickingally,[5] Koo et al. (2021) demonstrated that CXCL12 enhances endometrial receptivity, further implicating its role in endometriosis-associated infertility.[6]

In Mediators and Lesion Development

Chronic inflammation in endometriosis is characterized by increased chemokine and cytokine activity. Tal et al. (2021) observed that loss of CXCR4 in endometriotic lesions led to reduced proliferation and an increase in intraepithelial lymphocyte infiltration, indicating a potential immune-modulating effect.[7]

Clinical Implications and Diagnostic Potential

Our study found a statistically significant increase in CXCR4 and CXCL12 expression in the blood of patients with endometriomas. These findings are supported by Zhao et al. (2020), who demonstrated that protein kinase CK2 facilitates endothelial progenitor cell homing to endometriotic lesions through an SDF-1 (CXCL12)-CXCR4-dependent mechanism.[8] The potential ro4/CXCL12 as a non-invasive biomarker is further strengthened by Bianchi et al. (2020), who highlighted its involvement in cell proliferation and tissue regeneration.[9]

Limitations: While our results indicate a promising role for CXCR4/CXCL12 in endometriosis diagnosis, the study is limited by a small sample size. Larger, multicentric studies are needed to establish definitive cut-off values for clinical use. Additionally, therapeutic interventions targeting CXCR4/CXCL12 should be explored, as suggested by recent studies on chemokine receptor antagonists in endometriosis management.

1. CXCR4 Expression:
   - No significant difference in CXCR4 Delta Ct between cases and controls (p=0.0686).
   - True Fold Change of CXCR4 was significantly higher in cases (p=0.0150), suggesting an association with endometrioma.
   - ROC curve for True Fold Change of CXCR4 was significant (p=0.0294), indicating diagnostic potential.
2. CXCL12 Expression:
   - CXCL12 Delta Ct was significantly different between cases and controls (p=0.0375).
   - True Fold Change of CXCL12 was significantly upregulated in cases (p=0.0057).
   - ROC curve for True Fold Change of CXCL12 was highly significant (p=0.0006), making it a promising biomarker for endometrioma.
3. Overall Diagnostic Potential:
   - CXCL12 appears to be a stronger predictor of endometrioma than CXCR4.
   - True Fold Change of CXCR4 and CXCL12 are better diagnostic markers than Delta Ct values.
   - Larger studies are needed to validate these findings due to the small sample size.

CONCLUSION

- True Fold Change of CXCR4 and CXCL12 could serve as potential predictive biomarkers for endometrioma.
- CXCL12 True Fold Change showed the highest diagnostic significance (p=0.0006), making it a strong candidate for future studies.
- Further large-scale, multi-center studies are recommended to validate these findings.

Clinical Significance

Our study found a statistically significant increase in CXCR4 and CXCL12 expression in the blood of patients with endometriomas. These findings are supported by Zhao et al. (2020)[8], who demonstrated that protein kinase CK2 facilitates endothelial progenitor cell homing to endometriotic lesions through an SDF-1 (CXCL12)-CXCR4-dependent mechanism. The potential ro4/CXCL12 as

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