Kallistatin in follicular fluid of women with endometriosis and its correlation with IVF outcome

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ABSTRACT

Background: Endometriosis (EM) affects 10% women of reproductive age and alters fertility. Its management is still debated notably the timing of surgery and ART in infertility. Kallistatin (KS) is an endogenous protein that regulates differential signaling pathways and biological functions. However, the function and the underlying molecular mechanism in EM and its correlation with in vitro fertilization (IVF) outcome have not been determined. The purpose of this study was to evaluate KS concentrations in follicular fluid (FF) of women with EM and controls women without EM who underwent IVF with embryo transfer (IVF–ET).

Methods: FF KS concentrations from 40 patients with EM and 40 non-EM patients were measured by ELISA.

Results: Compared with the non-EM patients, patients with EM had lower KS levels in FF (281.67 ± 104.60 vs. 490.70 ± 216.33 pg/ml). The rates of fertilization (61.64 ± 22.42 vs. 71.00 ± 24.39%), available embryo (45.96 ± 19.83 vs. 50.61 ± 26.26%), and top-quality embryo (12.71 ± 21.01 vs. 16.04 ± 16.87%) were significantly lower in the EM group than in the control group. The KS concentrations in the FF of women who conceived consequent to the treatment were significantly higher than those from women who did not in the combined EM and control groups.

Conclusions: These results indicate that the KS concentration in FF could be used as a predictor for IVF–ET outcomes. This may contribute to the pathologic mechanism responsible for the poor outcome of IVF in patients with EM.

Background

Endometriosis (EM) is a common chronic gynecologic disorder defined by the ectopic occurrence of endometrium-like tissue which is associated with pelvic pain and infertility [1]. It has been estimated at 10% in women of reproductive age [2]. The causes of infertility in women with EM may range from anatomical distortions due to adhesions and fibrosis to abnormalities and immunological disturbances [3,4]. Approximately 25–50% of infertile women have EM, and 30–50% of women with EM are infertile [5]. Assisted reproductive technology (ART) may be required with EM-related infertility. Accumulating evidence suggests that EM affects oocyte and embryo development as well as endometrial receptivity, resulting in poor in vitro fertilization (IVF) outcomes [6–10]. However, the mechanism is unclear.

Follicular fluid (FF) provides an environment that controls oocyte growth and maturation, and plays a key role in ovulation, subsequent fertilization, and early embryo development [11]. Both cellular and secreted mediators are aberrantly expressed in the peritoneal fluid (PF) and plasma of EM patients [12]. Some studies have shown that angiogenesis and tissue growth factor are increased in the serum and PF of women with EM [13,14].

For these reasons, we analyzed the concentrations of KS in the FF of women undergoing IVF to identify factors related to EM and to further elucidate its correlation with IVF outcome. KS is an endogenous protein that regulates differential signaling pathways and biological functions inhibiting inflammation, angiogenesis, oxidative stress, apoptosis, tumor growth, and metastasis in animal models and cultured cells [15–22]. Plasma levels of KS are reduced in patients with sepsis, liver disease, and obesity [23,24]. Our preliminary data showed that KS in EM patients’ endometrial tissue and blood were much lower than control patients without EM and the decrease of KS is correlated with the severity of EM. However, the concentrations of KS in the FF of EM patients and its correlation with IVF outcome have not been determined.

The aim of this study was to investigate KS in FF of women with EM and its correlation with IVF outcome. This information may form the basis of strategies to control these factors and improve the IVF outcomes in patients with EM.
Methods

Subjects

A total of 80 patients who underwent IVF-ET between July 2017 and August 2018 in the Center for Reproductive Medicine, Third Affiliated Hospital of Guangzhou Medical University, were recruited for this study. EM patients with regular menstrual cycles who had undergone laparoscopic ovarian cystectomy for the treatment of ovarian endometriotic cysts (40 patients, aged 28–33 years). Control patients who had undergone hysterectomy because of myoma or other benign diseases without EM (40 patients, aged 26–33 years). None of the patients had received hormonal treatments for at least 6 months before surgery. Women with polycystic ovary syndrome, diabetes, hypertension, dyslipidemia, HIV infection, or any active infection and autoimmune diseases were excluded. All the Forty women with EM had ovarian EM and underwent laparoscopic ovarian cystectomy for making a definite diagnosis. The average size of ovarian cysts is 6.8 ± 1.6 cm in diameter. FF was extracted from both groups.

ART procedures

Women were monitored and managed according to the hospital’s clinical protocols. Various controlled ovarian stimulation (COS) protocols were used, with 150–450IU/d of recombinant FSH or human menopausal gonadotropin in a gonadotropin-releasing hormone antagonist protocol, a long agonist protocol, or a short agonist protocol. The protocols were determined according to each patient’s characteristics (age, body mass index [BMI], AFC, and AMH). Transvaginal oocyte retrieval was scheduled 35–36 h after hCG injection ART was performed per standard operating procedure of the hospital. Fertilization was assessed by the appearance of two pronuclei. Cleavage stage embryos were graded as per the Istanbul consensus. Fresh embryo transfer (ET) was performed 2–3 or 5 d later. Embryos were vitrified frozen on day 3, 5, or 6. The luteal phase was supported by vaginal administration of micronized progesterone (P) (400 mg/d) started on the day of ovarian puncture.

Follicular fluid collection

FF was preserved at oocyte retrieval, by collecting the liquid aspirated from the follicle into the suction tube, to avoid contamination by blood. FF samples from each follicle were pooled for each patient for measurement of KS concentrations. Pooled FF was extracted from both groups.

KS and hormone assays

The FF KS concentrations were collected and determined measured by enzyme-linked immunosorbent assay (human Kallistatin ELISA kits, Catalog Number: DY1669; R&D Systems, Minneapolis, MN) according to the manufacturer’s protocol. The concentrations of serum estradiol, FSH, LH, P, testosterone (T), and AMH, and FF estradiol and P, were measured by chemiluminescence (Roche, Basel, Switzerland).

Outcome measure

The reproductive outcome of this study included implantation, clinical pregnancy, live birth rate (LBR), and spontaneous miscarriage rates. Neonatal outcomes included preterm birth, stillbirth, birth weight, low birth weight, and congenital anomalies. Live birth was defined as the delivery of any viable neonate who was 28 weeks of gestation or older, and twins delivered by one mother were calculated as one live birth. Clinical pregnancy was defined as the presence of gestational sac on ultrasound at 6–8 weeks of gestation; low birth weight was defined as the birth weight less than 2500 g and very low birth weight less than 1500 g.

Statistical analysis

The statistical analysis was performed using the Statistical Package for Social Science (SPSS) version 22.0 (SPSS, Inc., Chicago, IL). The baseline characteristic was expressed as the mean ± SD (standard deviation) and differences in variables were compared by means of Student’s t-test. Categorical variables were described as frequencies and percentages, and compared using chi-square test. A p value of .05 was considered significant.

Results

Patient characteristics

Eighty women were selected. Baseline characteristics of these women are shown in Table 1. No significant differences were observed between the EM and control groups regarding age, BMI, and duration of sterility. In the control group, there were fourteen patients with infertility due to tubal factor (10%), two cases of ovulatory dysfunction (5%), 20 cases of male factor (50%), and 14 cases of unexplained (35%). Thirty patients in the control group were given IVF protocols, while 10 underwent ICSI procedures. In the EM group, IVF protocols were given to 70% women and 30% underwent ICSI procedures.

KS expression was decreased in FF of EM patients

Serum concentration of base LH, FSH, P, T, E2, and AMH showed no difference between the EM and control groups. Moreover, The FF KS concentrations in the EM patients were 281.67 ± 104.60 pg/ml and lower than those in the control group (490.70 ± 216.33 pg/ml) (p < .05) (Table 2).

Table 1. Clinical pathological characteristics of EM and control subjects.

| Outcome measure | Control (n = 40) | EM (n = 40) | p Value |
|-----------------|----------------|------------|---------|
| Age (y)         | 30.45 ± 2.45   | 29.58 ± 3.26 | .18     |
| Body mass index (BMI, kg/m²) | 20.97 ± 0.68 | 21.22 ± 1.62 | .39     |
| Duration of sterility (y) | 4.55 ± 1.32 | 4.68 ± 1.03 | .07     |
| Infertility factor | Tubal factor [n (%)] | 4 (10%) | 0 | – |
|                 | Ovulatory dysfunction [n (%)] | 2 (5%) | 0 | – |
|                 | Uterine factor [n (%)] | 0 | 40 (100%) | – |
|                 | Male factor [n (%)] | 20 (50%) | 0 | – |
|                 | Unexplained [n (%)] | 14 (35%) | 0 | – |
| Fertilization techniques | IVF | 30 (75%) | 28 (70%) | – |
|                 | ICSI | 10 (25%) | 12 (30%) | – |
**ART cycle characteristics in EM and control subjects**

Comparisons of ART cycle characteristics between the EM and control groups are summarized in Table 3. The AFC, dosage of Gn, endometrial thickness, No. of embryos retrieved, the rates of cleavage, and No. of embryos transferred were similar between the two FET groups. Meanwhile, the rates of fertilization, available embryo, and top-quality embryo were significantly lower in the EM group than in the control group.

**KS concentrations in follicular fluid from subjects with different pregnancy outcomes**

The clinical pregnancy rate in the EM group was 61.54% (24/39), which was significantly lower than that in the control group (67.57%, 25/37). In both the EM (Figure 1(B)) and control groups (Figure 1(A)), the KS concentrations in the FF of women who conceived consequent to the treatment were significantly higher than those in women who did not. This was determined with the highest specificity and sensitivity set as the optimal prediction point (Figure 2). Optimal prediction point, sensitivities, specificities, and AUC values were 292.78 pg/ml, 71.7%, 65%, and 0.7279, respectively. The present results indicate that the KS concentration in FF could be used as a predictor for IVF–ET outcomes.

**Discussion**

FF composition is complex and is basically derived from plasma exudation and ovarian secretion [25]. It is an important environment for oocyte survival and directly affects oocyte maturation and quality. FF contains a large number of cytokines, a variety of hormones, oxidation/antioxidant systems, and metabolic products [13].

In this study, the analysis of the levels of KS in FF of women with EM and controls who underwent IVF with embryo transfer (IVF–ET). Data clearly show that, compared with the non-EM patients, patients with EM had lower KS levels in FF. This indicates that low KS concentrations in FF may play a key role in folliculogenesis and the pathogenesis of EM. However, there were no differences in the concentrations of LH, FSH, P, T, E2, and AMH between the EM and control groups. This means that the KS changes of follicular microenvironment caused by EM are independent of these six factors.

KS is an endogenous protein that regulates differential signaling pathways and biological functions. Our preliminary data showed that KS in EM patients’ endometrial tissue and blood were much lower than control patients without EM and the decrease of KS is correlated with the severity of EM. However, the concentrations of KS in the FF of EM patients and its correlation with IVF outcome have not been determined. This study found that the rates of fertilization, available embryo, and top-quality embryo were significantly lower in the EM group than in those who conceived consequent to the treatment and those who did not (\(p < .05\)).
the control group. This result is consistent with the worse prognosis of EM reported in previous literature. We supposed that KS can significantly improve the quality of oocytes and embryos.

However, whether KS can act as a biomarker for predicting the clinical pregnancy of EM remain unknown. ROC curves were plotted in our study, result showed that the AUC values were 0.7279. These results indicate that the KS concentration in FF could be used as a predictor for IVF–ET outcomes.

Based on the findings of our study, we conclude that KS is associated with the microenvironment of FF in patients with EM. The change in the regulation network in EM influences oocyte development, resulting in poor IVF outcomes. However, the results in this study should be validated in a large sample in the future.

Conclusions

Our study provides a theoretical basis for the development of strategies to improve the outcome of IVF in patients with EM. This evidence may help to clarify the pathology of EM and seek new treatments for EM patients.

Acknowledgments

The study was performed under the auspices of the IVF unit of the Third Affiliated Hospital of Guangzhou Medical University.

Ethical approval and consent to participate

This study was approved by the ethics committee of the Third Affiliated Hospital of Guangzhou Medical University.

Author contributions

All authors have contributed significantly to this manuscript and each author has contributed to the manuscript as follows: Yuling Mao and Rui Chen designed the project. Yuling Mao, Shaoquan Zhan collected clinical samples and performed the ELISA experiments. Jingda Qiao and Lei Li analyzed the data. Yuling Mao wrote the manuscript. Hanyan Liu and Rui Chen revised the manuscript. All authors read and approved the final manuscript.

Disclosure statement

The authors have declared that no conflict of interest exists.

Funding

This study was supported by National Nature Science Foundation of China [Grant Number 81871211].

Data availability statement

The data sets used and/or analyzed during this study are available from the corresponding author on reasonable request.

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Figure 2. ROC curve analysis to assess the predictive powers of clinical pregnancy.
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