Endocrine gland–derived vascular endothelial growth factor concentrations in follicular fluid and serum may predict ovarian hyperstimulation syndrome in women undergoing controlled ovarian hyperstimulation

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Objective: To assess the predictive value of endocrine gland–derived vascular endothelial growth factor (EG-VEGF) concentrations in follicular fluid (FF) and serum for ovarian hyperstimulation syndrome (OHSS) in patients undergoing controlled ovarian hyperstimulation.
Design: Retrospective, case-control study.
Setting: University hospital, IVF center.
Patient(s): Seventeen women with OHSS and 61 controls.
Intervention(s): None.
Main Outcome Measure(s): FF and serum EG-VEGF and VEGF concentrations, IVF outcome.
Result(s): FF and serum EG-VEGF concentrations showed a significant negative correlation with serum E_2 concentration on the day of hCG administration. FF, but not serum, VEGF concentrations also showed a significant negative correlation with serum E_2 concentrations on hCG day. The FF EG-VEGF, FF VEGF, and serum EG-VEFG concentrations were significantly lower in the OHSS group than in the non-OHSS group. There was no significant difference in serum VEGF concentrations. Among FF and serum EG-VEGF and VEGF concentrations, only FF EG-VEGF concentrations were significantly lower in patients with moderate OHSS than in those with mild OHSS.
Conclusion(s): FF and serum EG-VEGF concentrations may predict OHSS occurrence. Furthermore, FF EG-VEGF concentrations were significantly correlated with OHSS severity; thus, EG-VEGF appears to be more valuable than VEGF for predicting OHSS. (Fertil Steril® 2011;95:673–8. ©2011 by American Society for Reproductive Medicine.)

Key Words: Endocrine gland–derived vascular endothelial growth factor, vascular endothelial growth factor, ovarian hyperstimulation syndrome, controlled ovarian hyperstimulation, ovarian response

Ovarian hyperstimulation syndrome (OHSS), an iatrogenic complication of ovulation induction, most frequently occurs in women undergoing controlled ovarian hyperstimulation (COH) for IVF-ET (1, 2). Severe OHSS is life threatening and difficult to treat because of our incomplete understanding of OHSS pathophysiology. Therefore, OHSS prevention is the key to reducing its mortality and morbidity. Effective prevention depends on reliable, early clinical and biochemical markers for OHSS, but few validated predictors are available. Although the precise pathophysiologic pathways to OHSS are unclear, vascular dysfunction, including abnormal angiogenesis, and impaired endothelial cell (EC) function are key factors (3). Vascular dysfunction caused by vasoactive substances released by the ovary appears important in OHSS (4), with vascular endothelial growth factor (VEGF) being the most important (5). Some studies have demonstrated that VEGF concentrations in peripheral blood and follicular fluid (FF) may predict the occurrence, severity, and progress of OHSS (6), whereas other studies showed them to be of limited predictive value (7, 8).

In 2001, LeCouter et al. (9) first reported a novel vascular endothelial growth factor, endocrine gland-derived vascular endothelial growth factor (EG-VEGF, also called *prokineticin*), which had a different structure but biologic functions similar to VEGF. EG-VEGF is produced by steroid-producing cells in the adrenal glands, testes, ovaries, and placenta. It is the mitogen of ECs in the microvascular beds of these tissues that regulate EC proliferation and function (10, 11). Unlike VEGF, EG-VEGF exerts its effect only on ECs of capillary vessels in endocrine glands. Ovarian EG-VEGF secretion is regulated by sex hormones, and its expression is periodic. The cellular localization and temporal and spatial expressions of EG-VEGF in ovarian tissue are complementarily correlated to VEGF (12, 13), suggesting that EG-VEGF participates in ovarian angiogenesis synergistically with VEGF, and together they are important in regulating the function of ovarian follicles or corpora lutea (14, 15).

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OHSS is associated with an enlarged ovary bearing multiple corpora lutea. OHSS has a peak incidence during the midluteal phase, when peak EG-VEGF production occurs. It has been reported that hCG stimulates EG-VEGF and VEGF mRNA expression in vitro (9). Therefore, we hypothesized that EG-VEGF acts synergistically with VEGF in the pathogenesis of OHSS and may have other more specific effects on ovarian tissue. However, no report has yet elucidated the relationship between FF and serum EG-VEGF expressions and OHSS incidence and ovarian response during COH.

In this study, the links between FF and serum EG-VEGF, VEGF, and OHSS were investigated. FF and serum EG-VEGF and VEGF concentrations were measured in women undergoing COH for IVF–intracytoplasmic sperm injection (ICSI). The EG-VEGF and VEGF concentrations of patients with and without OHSS were compared to identify correlations between these markers and the incidence of OHSS. Through these comparisons, we hoped to define parameters for EG-VEGF levels that could be used to predict OHSS in women undergoing IVF.

MATERIALS AND METHODS

Subjects

From January 2009 to July 2009, 78 women with infertility caused by tubal or male factors enrolled in our IVF program at Renji Hospital, and treated with COH for IVF-ICSI and ET, were included. All patients had a normal body mass index, regular menses, basal FSH <10 IU/L, no history of ovarian surgery, ultrasound-confirmed normal ovulation, a normal uterine cavity, and no hormonal drug use within 3 months before the study. Patients undergoing COH with low ovarian response (fewer than five follicles with diameter $\geq$16 mm and $E_2 \leq$1000 pg/mL on the day of hCG injection) were excluded. This study was approved by the ethics committee of Renji Hospital, and written informed consent was obtained from all patients.

The patients were classified into the OHSS group and the non-OHSS group, and OHSS group patients were further stratified into mild, moderate, and severe according to the Golan classification system (16).

IVF-ET Procedures

All patients were treated according to the routine practice of the center with the GnRHa (triptorelin [Decapeptyl]; Ferring, Kiel, Germany)–gonadotrophin (Gonal-F, Merck Serono, Geneva, Switzerland)–hCG (Lizhu, Zhuhai, China) long protocol for COH, gamete handling, embryo culture, and ET. The patients were monitored with Doppler ultrasonography (SSD-1400; Aloka, Tokyo, Japan) for follicle size and endometrial thickness and morphology during COH treatment. Serum E_2 concentrations were measured by chemiluminescence analysis (Immulite 1000; Siemens, Erlangen, Germany). When one dominant follicle reached 18 mm or two follicles reached 17 mm, or both, and serum E_2 levels corresponded to the number of follicles larger than 14 mm in size, hCG (5,000 IU) was injected. Oocyte retrieval (OR) was conducted under vaginal ultrasound guidance 34–36 hours later, and one to three embryos were transferred 2 or 3 days after OR.

FF and Serum Collection

During OR, FF was collected from follicles 16 to 20 mm in size. FF from the same patient was pooled and centrifuged immediately for 10 min at 2000 $\times$ *g* (Labofuge 400R; Thermo Heraeus, Wiesbaden, Germany). The supernatant was then transferred into an Eppendorf tube and immediately stored in a freezer at –80°C (MDF-382E(N); Sanyo, Gunma, Japan). The corresponding serum sample was collected 2 days after OR. Blood was drawn in the morning and centrifuged within 1 hour of collection. The centrifugation and storage methods for serum were the same as for FF. All measurements were conducted within 6 months after collection.

Measurement of EG-VEGF and VEGF with ELISA Assay

Total EG-VEGF and VEGF concentrations in FF and serum were measured by ABC-ELISA assay using commercially available ELISA kits (Quantikine; R&D Systems, Minneapolis, MN), and measurements were conducted according to the instructions with the ELISA Reader (Denley Dragon Wellscan MK3; Thermo, Vantaa, Finland). The sensitivity of the kits was 16 pg/mL for both recombinant and natural human EG-VEGF without any cross reaction with other cytokines. The interplate and intraplate coefficients of variation (CV) were all <10%. The sensitivity of the kits was 25 pg/mL for VEGF, with intraplate CV <10% and interplate CV <15%.

Statistics

All data were analyzed using SPSS version 13.0 software (SPSS Inc., Chicago, IL). The values are shown as mean $\pm$ SD where applicable. Linear regression analysis was used for correlation analysis. Intergroup differences were tested for significance using the independent samples *t* test for parametric variables and the Pearson chi-square test for categorical variables. Pearson's correlation coefficient was also calculated. Statistical significance was defined as $P<0.05$.

RESULTS

General Information

Of the 78 subjects, 52 had primary infertility, and 26 had secondary infertility. For the cause of infertility, 17 were tubal factors, 35 were male factors, and 26 were mixed factors. The subjects were 22–39 years old (30.15 $\pm$ 3.88 years). The duration of infertility was approximately 1–12 years (4.58 $\pm$ 3.02 years). All patients obtained transferable embryos, 38 with IVF and 40 with ICSI.

Correlation between FF and Serum EG-VEGF and VEGF Concentrations and Serum E_2 Concentrations on the Day of hCG Administration

The FF and serum EG-VEGF concentrations were 790 $\pm$ 351 pg/mL and 41 $\pm$ 20 pg/mL, respectively. The average FF/serum ratio of EG-VEGF was 21 $\pm$ 9. The FF and serum VEGF concentrations were 1,323 $\pm$ 484 pg/mL and 161 $\pm$ 91 pg/mL, respectively, and the average FF/serum ratio of VEGF was 11 $\pm$ 7. The average FF/serum ratio was significantly higher for EG-VEGF than for VEGF. In all subjects, the FF EG-VEGF, FF VEGF, and serum EG-VEGF concentrations were negatively correlated with serum E_2 concentrations on the day of hCG administration ($r = –0.621$, $P<0.01$; $r = –0.553$, $P<0.01$; $r = –0.478$, $P<0.01$, respectively; Fig. 1A–1C), whereas serum VEGF concentrations were not correlated with serum E_2 concentrations on the day of hCG administration. Furthermore, FF EG-VEGF concentrations were positively correlated with FF, but not serum, VEGF concentrations ($r = 0.417$; $P<0.01$; Fig. 1D).

Comparison between OHSS and Non-OHSS Groups

Of the 78 subjects, 17 developed OHSS. There were no significant differences between the OHSS and non-OHSS groups in average age, infertility subtype, cause of infertility, duration of infertility, gonadotrophin duration, and fertilization methods used. There were also no significant differences in the rates of oocyte maturation, fertilization, cleavage, and pregnancy. However, although gonadotrophin dosage was lower in the OHSS group than in the non-OHSS group, the serum E_2 on the day of hCG administration, the number of oocytes retrieved, and ET cancellation rate were significantly higher in the OHSS group than in the non-OHSS group ($P<0.01$; Table 1).

The FF EG-VEGF and FF VEGF concentrations were significantly lower in the OHSS group than in the non-OHSS group ($P<0.01$). Furthermore, the serum EG-VEGF concentrations were significantly lower in the OHSS group than in the non-OHSS group ($P<0.05$), whereas no significant difference in serum VEGF concentrations was observed ($P>0.05$; Table 2). In addition,