Granulocyte Colony-stimulating Factor: Is there an Association between Follicular Fluid Levels in the Largest Follicle and In Vitro Fertilization Outcome?

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Abstract

Background: It has been suggested that the level of granulocyte colony-stimulating factor (G-CSF) in follicular fluid (FF) might act as a noninvasive biomarker of oocyte competence and assist in embryo selection during in vitro fertilization (IVF) cycles. The aim of this study was to evaluate the G-CSF concentration in the FF of the largest follicle as a prognostic factor in IVF cycles.

Methods and findings: This was a prospective cohort analysis comprising 40 infertile women undergoing IVF treatment. Fluid was collected from the first (largest) follicle aspirated. Levels of G-CSF were determined by enzyme-linked immunosorbent assay. The findings were compared between women who achieved pregnancy (n = 15) and those who did not (n = 25).

The mean concentration of G-CSF in FF was 85.7 ± 7.5 pg/ml. No significant differences were found between the concentration in women who achieved pregnancy and those who did not (92.3 ± 15.6 pg/ml vs. 81.8 ± 7.7 pg/ml; p = 0.45).

Conclusions: The concentration of G-CSF in the FF of the largest follicle during oocyte retrieval was not correlated with the IVF pregnancy rate in this study.

Keywords: Granulocyte colony-stimulating factor; In Vitro fertilization; Embryo; Implantation

Introduction

Granulocyte colony-stimulating factor (G-CSF) is a cytokine that stimulates the proliferation and differentiation of hematopoietic cells of the neutrophilic granulocyte lineage [1]. Monocytes and macrophages are the main source of G-CSF, but cells of mesodermal origin can also produce this factor [2].

There is considerable evidence that G-CSF regulates reproductive processes at different times during a woman’s reproductive life. It promotes blastomere cell division by inhibiting apoptosis and decreasing DNA fragmentation in the embryo [3]. Additionally, G-CSF has positive effects on various processes such as cell proliferation, progression to blastocyst, zona pellucida hatching and embryo implantation [4]. Sjöblom et al. suggested that addition of G-CSF to the embryo culture medium might improve the yield of implantation-competent blastocysts on in vitro fertilization (IVF) programs [5].

Analysis of preimplantation embryo morphology is not enough to predict reproductive success [6] and current research is oriented to metabolomic analysis of the follicular fluid (FF) as a more objective way of embryo quality [7]. Salmassi et al. did not find significant differences in G-CSF concentration in FF between follicles with fertilized oocytes and those with unfertilized oocytes [8]; however, in 2008 a study was performed with the aim of identifying a predictor of embryo quality in the FF of each oocyte. G-CSF was one of the cytokines quantified and it showed a positive correlation with the rate of production of embryos of good quality and implantation potential when its levels were above 20 pg/ml [9].

In another study, Lédée et al. found that the FF G-CSF level was highly predictive of subsequent implantation. They classified embryos by their FF G-CSF concentration, and concluded that embryos from follicles with a FF G-CSF concentration of over 30 pg/ml had the highest positive predictive value for implantation [10].

The cost of measuring individual G-CSF concentrations in FF as a predictor of success is an economic obstacle. Therefore, an alternative method to reduce costs is to analyze the concentration in the largest follicle observed during oocyte retrieval. There have been no studies correlating this measurement as a predictor of success in IVF. Therefore, the aim of this study was to evaluate the FF G-CSF concentration in the largest follicle as a prognostic factor for successful pregnancy in IVF cycles.
Materials and Methods

Study subjects and data collection

This prospective cohort study was performed in the assisted reproduction clinic of National Institute of Perinatology in Mexico City from June to December 2012. The study was approved by the ethics and research committees with research project number 212250-03031. A total of 40 patients were recruited from infertile patients undergoing IVF treatment. Written informed consent was obtained from all patients enrolled in the study.

Ovarian stimulation

Ovarian hyperstimulation was initiated on day 2 of the menstrual cycle, employing two alternative schemes: (1) recombinant follicle stimulating hormone (rFSH; GONAL-F; Merck Serono, Germany), or (2) rFSH and urinary menotropins (Merapur, Ferring Pharmaceuticals, Switzerland).

The ovarian stimulation protocol was selected by each treating physician as follows: (1) a long standard protocol with gonadotropin-releasing (GnRH) agonist treatment (Lucrin; Abbott USA) in only three patients, or (2) a flexible antagonist protocol (Cetrotide; Merck Serono) in 37 patients. The response level of patients to hyperstimulation was classified as low (≤5 oocytes), moderate (6-10 oocytes) or high (>10 oocytes). Oocytes were retrieved by transvaginal ultrasound-guided aspiration 36 h after a choriogonadotropin alfa (Ovidrel; Merck Serono, Mexico) injection, using a 17-g needle (Cook Medical, Bloomington, IN, USA).

Fluid was collected from the first follicle aspirated and further flushing was not performed. Samples contaminated with blood were excluded. The oocytes were analyzed in the laboratory to define their morphological status and allowed to incubate for about 4 h before insemination based on the physician’s selected technique. Standard IVF and intracytoplasmic sperm injection (ICSI) was performed.

The percentage of mature oocytes was calculated based on the number of metaphase II oocytes among all those collected. The percentage of fertilized oocytes was based on the number of zygotes with two pronuclei at 24 h among all oocytes collected. On day 3 prior to embryo transfer, we carried out morphological evaluation of the embryos using the Lucinda Veeck classification [11]. They were judged as top quality embryos (grades 1 and 2) and others (grades 3-5). After embryo transfer, patients were treated with micronized progesterone vaginally (600 mg daily) for luteal support. Pregnancy or successful implantation was considered in patients who were positive for beta human chorionic gonadotropin (1 mIU/ml) 14 days post-transfer of embryos.

Measuring the concentration of G-CSF

The samples were collected in a separate tube to avoid contamination of blood and culture medium. These were centrifuged for 15 min at 3000 rpm and stored at −80°C until analyzed together. Levels of G-CSF in the FF were determined by enzyme-linked immunosorbent assay (ELISA, catalog number DC550, R & D Systems, Inc., Minneapolis, MN, USA) following the supplier’s instructions. The assay does not cross-react with other cytokines and recognizes both G-CSF in its native or recombinant form.

Briefly, 100 μl aliquots of standards, controls and samples were placed in 96-well plates and incubated for 2 h at room temperature. Thereafter, three washes were done with buffer and 200 microliters of a conjugate of G-CSF was added for 2 h at room temperature. Finally, 50 μl of stop solution was added to each well and the optical density was determined using a microplate reader system (GloMax, Promega, Madison, WI, USA) at 450 nm wavelength. Recombinant G-CSF (R & D Systems) was used as a control. G-CSF levels ranged between 39-2,500 pg/ml, with a sensitivity of 20 pg/ml. The intra-assay precision was 5% and inter-assay precision 10%.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics Software (20.0, SPSS Inc., Chicago, IL, USA). Shapiro-Wilk’s test was used to test the distribution of variables. The sociodemographic variables of the population were characterized using descriptive statistics. Correlations between variables were analyzed using Spearman’s correlation coefficient. Differences between groups were compared with the Mann-Whitney nonparametric U test for independent samples. Hormone levels between comparison groups were analyzed by analysis of variance and a least significant difference post hoc test. Statistical significance was assumed at p < 0.05. The results are expressed as the mean ± Standard Deviation (SD).

Results

The 40 study participants were divided into groups with a successful IVF pregnancy (n=15), or without success (n=25). The patients’ general characteristics are presented in Table 1. The overall mean concentration of G-CSF in FF was 85.7 ± 7.5 pg/ml. No difference was found between the two groups (pregnant group 92.3 ± 15.6 pg/ml; non pregnant group 81.8 ± 7.7 pg/ml). No statistically significant correlation was found between patient age and the concentration of G-CSF in FF.

All patients were stimulated using GnRH analogues. On the day of choriogonadotropin alfa injection, patients had a median of 5 follicles >18 mm (range 3-21) and a serum estradiol level of 1671 ± 137.2 pg/ml. No significant correlation was found between the levels of estradiol on the day of administration of choriogonadotropin alfa and C-CSF levels in the FF. There were no significant differences in the mean concentrations of G-CSF between groups of patients with levels of estradiol under 1500 pg/ml (82.5 ± 8.5 pg/ml), levels of 1501-3500 pg/ml (88.4 ± 13.8 pg/ml) or levels >3500 pg/ml (66.5 ± 23.7 pg/ml).

No significant differences were found in the mean concentrations of G-CSF between groups of patients with a
low response (70.8 ± 8.1 pg/ml), a moderate response (95 ± 15 pg/ml) or a high response (88.2 ± 12.5 pg/ml). A median of 7.5 (range 2-22) oocytes was obtained per patient. The percentage of mature oocytes ranged from 38 to 100% with a mean of 85% and the percentage of fertilized oocytes per patient varied from 15 to 100% with a mean of 76%. No significant correlations were found between the concentration of G-CSF and the percentages of fertilized oocytes or mature oocytes were found. No significant difference was found in the concentrations of G-CSF in FF when comparing top-grade embryos and others (55.1 ± 6.4 vs. 72.7 ± 7.6, respectively).

Table 1: Basal characteristics of patients included in this study.

<table>
<thead>
<tr>
<th></th>
<th>Pregnancy n = 15</th>
<th>No pregnancy n = 25</th>
<th>U</th>
<th>z</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years): median</td>
<td>34 (27-37)</td>
<td>35 (22-39)</td>
<td>129</td>
<td>-1.4</td>
<td>0.14</td>
</tr>
<tr>
<td>BMI (kg/m²): median</td>
<td>24 (17-32)</td>
<td>24 (18-30)</td>
<td>137</td>
<td>-1.24</td>
<td>0.22</td>
</tr>
<tr>
<td>Years of infertility:</td>
<td>7 (3-10)</td>
<td>6 (1-18)</td>
<td>169</td>
<td>-0.3</td>
<td>0.76</td>
</tr>
<tr>
<td>FSH Day 3 (mIU/mL):</td>
<td>5.6 (4.4-11.8)</td>
<td>7.9(1.5-15.7)</td>
<td>130</td>
<td>-0.64</td>
<td>0.53</td>
</tr>
<tr>
<td>Estradiol Day 3 (pg/mL):</td>
<td>34 (26-111)</td>
<td>36 (16-102)</td>
<td>115</td>
<td>-1.4</td>
<td>0.15</td>
</tr>
<tr>
<td>Endometriosis: n (%)</td>
<td>2</td>
<td>7</td>
<td>151</td>
<td>-1.1</td>
<td>0.4</td>
</tr>
<tr>
<td>Follicles &gt;18 mm:</td>
<td>7 (2-12)</td>
<td>5 (3-14)</td>
<td>121</td>
<td>-1.7</td>
<td>0.09</td>
</tr>
<tr>
<td>IVF n (%)</td>
<td>5 (33)</td>
<td>12</td>
<td>150</td>
<td>-1</td>
<td>0.39</td>
</tr>
<tr>
<td>ICSI n (%)</td>
<td>10 (66)</td>
<td>13</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

U Mann Whitney test. BMI: Body Mass Index; hCG: Human Chorionic Gonadotropin. No statistically significant differences were found between the two groups.

Discussion

In this study, the concentration of G-CSF in FF of the largest follicle aspirated during oocyte retrieval had no clinical use as a prognostic factor for successful pregnancy in IVF. Therefore, quantification of each FF might be required as recommended by Lédée et al. [10]. All of our patients had a FF concentration of G-CSF >30 pg/ml, but we used ELISA while Lédée et al. used a microbead assay; this might explain the differences in the mean concentrations of G-CSF between the two studies.

Kahyaoglu et al. also quantified G-CSF levels in single follicles in patients with polycystic ovarian syndrome [12]. However, despite increased levels of this cytokine both in serum and the follicular microenvironment, no association could be demonstrated between G-CSF levels and a good ovarian response or clinical pregnancy rate.

Lédée et al. reported that patients aged <30 years had a higher content of G-CSF in FF than patients aged >37 years (p = 0.03) [9]. However, in the present study, no statistically significant correlation was found between age and the concentration of G-CSF.

Foster et al. reported that follicles from patients subjected to hyperstimulation with menotropins had higher G-CSF concentrations than those where only FSH was used [13]. However, our results showed no significant difference in G-CSF concentration when comparing patients given only FSH with those given both types of drugs for hyperstimulation.

Salmassi et al. found a positive and significant correlation between the concentrations of G-CSF and serum estradiol on the day of oocyte retrieval (r = 0.37; p = 0.05) [8]. Our data showed no significant correlation between the serum estradiol level on the day of chorionic gonadotropin alfa trigger and FF G-CSF concentration. Dividing our patients into groups based on their estradiol level on the day of chorionic gonadotropin alfa trigger also showed no significant correlation with the FF G-CSF level. There was a trend towards higher G-CSF concentrations as estradiol levels increased up to 3500 pg/ml, however, despite only two patients having a higher level of estradiol, the levels of this cytokine decreased in their FF. Joo et al. also found that very high concentrations of estradiol have deleterious effects on endometrial receptivity and can impair the embryo directly [14].

Salmassi et al. found differences in the G-CSF levels in patients with low, moderate and high ovulatory response (p = 0.001) [8]. However, in the present study no significant difference was found in the concentration of G-CSF between different grades of embryos.
Salmassi et al. found that the mean FF G-CSF level in patients who underwent intracytoplasmic sperm injection (ICSI) was significantly higher than those given IVF (p = 0.02) [8], probably related to better oocyte quality in patients with only a male factor, but in our study no significant difference was found between these groups (87.8 ± 8.6 pg/ml vs. 86 ± 12.2 pg/ml) probably because in our population the indication for ICSI was not limited to male factor infertility.

The metabolite analysis approach is a potential powerful tool in the study of FF as a predictor of success in IVF. However, we found that the concentration of G-CSF in the FF of the largest follicle during oocyte retrieval did not correlate with IVF pregnancy rates.

References


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