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Review Article

Granulocyte colony-stimulating factor: A relation between serum and follicular fluid levels and in-vitro fertilization outcome in patients with polycystic ovary syndrome

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ABSTRACT

Evidence is accumulating in the literature about the potential role of serum and follicular fluid (FF) granulocyte colony-stimulating factor (G-CSF) as a non-invasive biomarker of oocyte competence and embryo selection in in-vitro fertilization (IVF) cycles. In this study, we aimed to evaluate the effect of serum and FF G-CSF levels on IVF outcome in non-hyperandrogenic, non-obese patients with polycystic ovary syndrome (PCOS). Twenty-two patients with PCOS (Group I), and 22 patients with the etiology of male factor infertility (Group II) undergoing IVF treatment were included. Demographic features, controlled ovarian stimulation parameters, neutrophil count (NC), neutrophil/leukocyte (N/L) ratio, serum and FF G-CSF levels of the two groups were compared. Serum E2 level on the day of hCG (2982.5 ± 171.4 vs. 2279.0 ± 207.2 pg/mL), total number of retrieved oocytes (14.7 ± 0.9 vs. 11.5 ± 1.3) and mature oocytes (11.6 ± 0.8 vs. 9.1 ± 1.1) were significantly higher in group I when compared to group II ($p < 0.05$). On the day of oocyte retrieval, both the mean serum (54.8 ± 1.7 vs. 48.1 ± 0.9 pg/mL) and FF G-CSF levels (48.8 ± 1.4 vs. 44.1 ± 0.5 pg/mL), NC (4.4 ± 0.2 × 10^3 vs. 3.6 ± 0.3 × 10^3/μL) and N/L ratio (63.6 ± 1.4 vs. 56.1 ± 1.7) in group I were found to be significantly higher than group II ($p < 0.05$). Despite the increased levels of G-CSF both in the serum and follicular microenvironment in patients with PCOS, a relation between G-CSF and good ovarian response or clinical pregnancy rates could not be demonstrated in this study.

1. Introduction

Ovarian function is primarily regulated by gonadotropins, although cytokines are increasingly recognized as potential modulators of their function either by autocrine or paracrine mechanisms [1,2]. There has been a growing body of literature showing that lympho-hemopoietic cells and their cytokine networks have an important role in many reproductive events [3]. Granulocyte colony-stimulating factor (G-CSF) is a pleiotropic cytokine best known for its specific effects on the proliferation, differentiation, and activation of hematopoietic cells of the neutrophilic granulocyte lineage. Besides hematopoietic cells, several non-hematopoietic cell types, such as osteoblasts, smooth muscle, endothelial and epithelial cells, as well as reproductive tissue cells, have also been shown to produce G-CSF [4–8]. Luteinized granulosa cells are also one of the cell types that express G-CSF and its receptors [9]. A possible role in ovulation has been demonstrated in previous studies [10]. Moreover, the role of G-CSF in in-vitro fertilization (IVF)/intracytoplasmic sperm injection (ICSI) cycles has been studied widely in recent years. An increase in its levels during ovarian stimulation [11] and the relation between G-CSF and the degree of ovarian response have previously been demonstrated by many researchers [8]. Levels of G-CSF in follicular fluid (FF) was also shown to be a non-invasive marker of oocyte competence in both stimulated and natural IVF/ICSI cycles [12,13] and proposed as a predictor of implantation potential of embryos [14].

G-CSF was studied previously in patients with endometriosis [8], previous IVF failure [13] or mixed male and female infertility [14], but its role in polycystic ovary syndrome (PCOS) has not yet been studied extensively. Although follicular fluid m-RNA of M-CSF was shown to not be increased in PCOS patients when compared to non-PCOS cases in one study [15], an increase in various other inflammatory markers, such as C-reactive protein, white blood cells and several other pro-inflammatory cytokines in PCOS

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was confirmed in several other studies [16–23], supporting the chronic low-grade inflammation that is an important contributor to the pathogenesis of the disease [16–23].

Therefore, this study was designed to investigate the relation between serum and follicular fluid G-CSF levels and IVF/ICSI outcome in patients who were non-hyperandrogenic, normal weight women.

2. Materials and methods

2.1. Study subjects and data collection

This case control study was performed in the assisted reproduction clinic of a tertiary research and education hospital. A total of 44 patients were recruited from infertile patients undergoing IVF treatment. The study group consisted of 22 patients who were non-obese and non-hyperandrogenic diagnosed as PCOS according to the Rotterdam criteria (Group I) [24] and a control group of 22 patients with the etiology of male factor infertility (Group II). Patients with severe male factor infertility, PCOS patients with the evidence of either biochemical or clinical hyperandrogenism or BMI > 30 kg/m^2 were excluded. This study was approved by the Institutional Review Board of the hospital. Written informed consent was obtained from all patients.

2.2. In vitro fertilization stimulation

All patients underwent a GnRH agonist long luteal down-regulation protocol with the administration of the GnRH agonist leuprolide acetate (Lucrin, Abbot, Turkey) in the midluteal phase of the previous cycle until the day of hCG administration. After onset of menstrual bleeding, when satisfactory pituitary desensitization was achieved (serum E2 level <50 pg/ml, endometrial thickness <5 mm, serum LH levels <5 IU/ml), GnRH agonist dose was reduced to half and gonadotropin administration was started with a daily use of recombinant FSH (Gonal-F; Merck Serono, Istanbul, Turkey or Puregon, Organon, Istanbul, Turkey), which was individualized according to the patient's age, baseline serum FSH concentration on day 3 and body mass index (BMI). The dose was adjusted according to the individual ovarian response. Response was monitored by serial E2 measurements and transvaginal ultrasonography. Recombinant hCG (250 micrograms sc., Ovitrelle, Serono, Istanbul, Turkey) was administered when at least three follicles showed a mean diameter of 17 mm. Oocytes were retrieved by transvaginal ultrasound-guided aspiration 36 h after the hCG injection. Single embryos were transferred to all women. Luteal support was given by vaginal progesterone (Crinone 8% gel, Serono, Istanbul). Pregnancy was determined by β-hCG levels in blood tests performed 14 days after embryo transfer and clinical pregnancy was defined as the presence of a gestational sac with accompanying fetal heartbeat.

2.3. Collection of follicular fluid samples

Follicular fluid was collected from one follicle of ⩾17 mm from which an oocyte was retrieved. Fluid was collected from the first follicle aspirated and flushing was not performed. Samples contaminated with blood were excluded.

2.4. Granulocyte colony-stimulating factor assay in serum and follicle Fluid

Blood and FF were taken from 44 patients on the day of follicle puncture. After oocyte pick-up (OPU), the FF underwent the same treatment as the blood. Samples were centrifuged for 15 min at 3000 rpm and stored at −80 °C for subsequent analysis. Each sample was masked to be analyzed blindly.

G-CSF levels in serum and FF were measured with Instant Human G-CSF kit (Bender Med Systems, Vienna, Austria) used for the quantitative detection of human G-CSF in cell culture supernatants, human serum, plasma, or other body fluids. Granulocyte colony-stimulating factor levels ranged between 7.8 and 500 pg/ml, with a sensitivity of 0.6 pg/mL. The calculated overall intra- and inter-assay coefficient of variation was 8.6% and 7.7%, respectively.

2.5. Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics Software (17.0, SPSS Inc., Chicago, IL, USA). Shapiro–Wilks test was used to test the distribution of variables. Continuous variables were presented as mean ± standard deviation values and compared using the Independent Samples *t* test and Mann–Whitney *U* test. Categorical variables were compared with Fisher's exact or Pearson chi-square tests when available. Pearson's correlation coefficient (r) was applied to investigate the correlation between variables. Statistical significance was assumed with a probability error of $p < 0.05$.

3. Results

The levels of G-CSF in serum and FF were detected in 44 infertile patients, 22 diagnosed as non-obese, non-hyperandrogenic PCOS (Group 1) and 22 as male factor infertility, undergoing IVF treatment. No difference was found between the two groups regarding age, BMI and basal hormonal profile (Table 1) ($p > 0.05$). Regarding the duration of stimulation and total dose of gonadotropins used, no significant difference was found between PCOS and control groups ($p > 0.05$) (Table 1). Serum E2 level on the day of hCG (2982.5 ± 171.4 vs. 2279.0 ± 207.2 pg/mL), total number of retrieved oocytes (14.7 ± 0.9 vs. 11.5 ± 1.3) and mature oocytes (11.6 ± 0.8 vs. 9.1 ± 1.1) were significantly higher in group I when compared to group II ($p < 0.05$). However fertilization rate and clinical pregnancy rate did not differ ($p > 0.05$). On the day of oocyte retrieval, both the mean serum and FF G-CSF levels in group I were found to be significantly higher than group II (54.8 ± 1.7 vs. 48.1 ± 0.9 and 48.8 ± 1.4 vs. 44.1 ± 0.5 pg/mL, respectively) ($p < 0.05$). No significant correlation was found between serum and FF G-CSF levels and clinical pregnancy rates, serum E2 levels on the day of hCG or the number of mature oocytes ($p > 0.05$). Although no difference was found between the two groups regarding leukocyte count, neutrophil count (NC) and neutrophil/leukocyte (N/L) ratio were both significantly higher in group I than group II ($p < 0.005$). A moderate but significant correlation was found between both NC and N/L ratio and serum G-CSF levels ($r = 0.377$, $p = 0.012$, $r = 0.368$, $p = 0.014$), but not FF G-CSF levels ($p > 0.05$).

4. Discussion

The result of this study demonstrated that the measured G-CSF concentrations in FF and serum on the day of oocyte retrieval was significantly higher in patients with PCOS than in patients with the etiology of male factor infertility undergoing IVF treatment. Although E2 levels on the hCG day, number of retrieved oocytes and mature oocytes were significantly higher in the PCOS group, no difference was found between fertilization or clinical pregnancy rates. Neutrophil count and neutrophil/leukocyte ratio were found to be significantly higher in the PCOS group and a significant correlation was found between these two parameters and serum G-CSF levels, indicating the inflammatory nature of the disease.

Granulocyte colony-stimulating factor is a cytokine that stimulates neutrophilic granulocyte proliferation and differentiation that

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