Original Article

Is There any Role for Granulocyte Colony Stimulating Factor in Improvement of Implantation in Intrauterine Insemination? A Prospective Double-Blind Randomized Control Trial

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Abstract

Background: Granulocyte colony stimulating factor (GCSF) has been introduced as an immunomodulatory agent by increasing implantation rate *in vitro* fertilization (IVF) patients but it has not been studied in intrauterine insemination (IUI) patients. The aim of this study is to answer the role of GCSF in implantation rate of IUI.

Materials and Methods: In this prospective double-blind randomized control trial, 320 eligible patients were enrolled, who were referred to the referral infertility clinic of Shiraz University of Medical Sciences from February 2018 till the end of 2019. They were divided into two groups randomly. After collecting the demographic data, all patients received clomiphene citrate from the 5th day of the menstruation cycle for 5 days. 50-150 units of recombinant purified follicle-stimulating factor (FSH) were started from the 8th day of the cycle. Follicle monitoring was done by transvaginal sonography till a mature follicle of 18 mm or more was developed. Human chorionic gonadotropin (HCG) injection was done in both groups with intrauterine administration of 300 µg GCSF in the case group and normal saline in the control group simultaneously. After 36 hours, IUI was performed. The clinical pregnancy, miscarriage, and ongoing pregnancy rates of both groups were calculated by SPSS software.

Results: The results showed improvement of clinical pregnancy rate [15.38% vs. 13.81% OR=1.17 (0.62-2.21)], miscarriage rate [3.84% vs. 5.26% OR=0.74 (0.25-2.20)] and ongoing pregnancy rate [11.53% vs. 8.55% OR=1.37 (0.65-2.92)] in the GCSF group compared to the control. However, the results revealed no statistically significance (P>0.05).

Conclusion: Although it was not statistically significant, 300 µg Intrauterine GCSF administration simultaneously with hCG injection in standard IUI procedure might increase the pregnancy outcomes. Further studies are warranted (registration number: IRCT201212079281N2).

Keywords: Embryo Implantation, Granulocyte Colony-Stimulating Factor, Pregnancy Rates

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Introduction

Nowadays, unexplained subfertility is an issue of concern in infertility clinic visits among 30-50% of couples (1, 2). Expectant management controlled ovarian hyper-stimulation with intrauterine insemination (IUI) as a less invasive method, or the more aggressive technique of *in vitro* fertilization (IVF) are the accepted practices for managing unexplained subfertility (3-5). Although treatment strategies should be selected individually, some authors recommend stimulated IUI as the first method of therapy with a success rate of 12% per cycle that is followed by IVF after three cycles of failure (1, 2). In addition, some authors indicated that the success rate of IUI is defined to be more similar to IVF than previously recognized (6).

It is logical to manage unexplained subfertility patients stepwise and gradually start with inexpensive, less invasive, and low-risk treatments (2). As IUI is less invasive and more economic than IVF with considerable benefits, it is reasonable to improve the success rate of IUI in these patients. Normal semen analysis and patent uterine tubes of unexplained subfertility patients highlight the role of the uterus as the main target of therapy for IUI improvement of success rate by affecting the implantation rate (7).

Granulocyte colony stimulating factor (GCSF) is introduced as an effective cytokine in reproduction and fertility via overcoming immunologic factors by the final consequence of altering the implantation rate (8, 9). This cytokine is derived from the bone marrow and cells like the monocyte, macrophage, and fibroblasts; it triggers the proliferation of the neutrophils and promotes releasing them into the blood circulation (10). It plays a role in inflammatory prohibition, angiogenesis, and prevention

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of apoptosis (8, 11). Also, GCSF is responsible for advancing ovarian function, promoting oocyte maturation, regulating the endometrium by increasing receptivity, and improving embryo implantation (8, 12, 13). Although there are controversies, GCSF is introduced as a successful immunotherapy modality in IVF for advancing fertility in Recurrent Implantation Failure (RIF) patients by impacting the implantation process (8, 9, 11, 14-17). Also, GCSF is found in endometrial and fetal cells which may bold the possible role of this cytokine to improve pregnancy outcome (18). A noticeable point is the minimal harm of administration of GCSF for pregnancy outcome (19, 20).

To the best of our knowledge, there are limited data on IUI improvement by immunotherapy, especially on the effect of GCSF on IUI. Considering multiple aspects of IUI including low cost, less invasiveness, and patient-friendly points, and recognizing the uterus as the possible cause of IUI failure, we were encouraged to conduct this survey to evaluate the possible effects of intrauterine GCSF administration on the pregnancy success rate among patients with recurrent IUI failure to avoid the burden of IVF in unexplained subfertility.

Materials and Methods

The study protocol and setting

In this randomized control prospective study, we aimed to evaluate the effect of GCSF on the IUI success rate by measuring chemical and clinical pregnancy as primary outcome and miscarriage and ongoing pregnancy rates as secondary outcomes. It was approved by the Ethics Committee of Shiraz University of Medical Sciences following the Declaration of Helsinki Guideline (IR.SUMS.MED.REC.1395.60) and registered at the Iranian Registry of Clinical Trials (IRCT201212079281N2). To calculate the sample size based on a previous study (21), the success rate for the control and case groups was determined to be 19.6% and 44.6%, respectively. Considering the confidence interval of 95%, power of 80%, and type one and two errors of 0.05 and 0.20 respectively, the sample size was set to be 87 patients in each group (22). In previous studies on GCSF efficacy which were carried out on IVF protocol, the number of embryos was more than the patients due to the transfer of more than one embryo for most patients. Since this study was performed on the IUI protocol with an almost equal ratio of patients and embryos in each cycle, we increased the total studied samples to 320 eligible patients who were referred to the referral infertility clinic of Shiraz University of Medical Sciences from February 2018 till the end of 2019.

Patients were recruited after filling out the informed consent. Demographic data and basic fertility characters were checked. Randomization was done exactly performing IUI by a web-based software, considering each block size to be 4 (160 patients in each arm study). It should be mentioned that all laboratory tests of participants were done at the laboratory of our center, and the staff was blind to the study groups too. Also, all patients' endometrial thickness was examined by an expert sonographer using the Voluson E8 machine who was blind to allocations.

Inclusion and exclusion criteria

The inclusion criteria were a mean age of 20-40 years, normal body mass index, and anti-Mullerian hormone level of 2-3.5 ng/ml, patent tubes in hysterosalpingography, and normal hormonal assay including follicle-stimulating factor (FSH), luteinizing hormone (LH), thyroid stimulating hormone (TSH), and prolactin. The patients should have subfertility subtype of primary unexplained infertility for less than three years with normal endometrium thickness in women. The husband should have been examined by the urologist of our center in order to have a normal physical exam and normal laboratory studies including semen analysis with no medical diagnosis. It is emphasized that they should have a total motile count of more than 10 million in semen analysis. The exclusion criteria were the participants who had thin endometrium (less than 7 mm) on the day of human chorionic gonadotropin (HCG) injection, any chronic disease (like malignancy, chronic hypertension, Diabetes Mellitus, thyroid or kidney disease, anemia, polycystic ovarian disease), history of previous surgery on the uterus, ovulatory dysfunction, any contraindication for GCSF administration (patients with allergy to E. coli-derived proteins or previous history of severe side effects), severe male factor infertility, any stages of endometriosis, or unwillingness to continue the project.

Treatment protocol and outcome

All patients had a basal evaluation of antral follicular count (AFC) on the second day of their cycle by transvaginal sonography. The enrolled patients received 100 mg clomiphene citrate (Iran Hormone Laboratory, Tehran, Iran) daily from the 5th day of the menstruation cycle for 5 days. In addition, starting from the 8th day of the cycle, 50-150 units of recombinant purified FSH (Gonal-F, Merck Serono, Switzerland) were prescribed individually. Then, on the 11th day of the cycle, transvaginal sonography was done by an assigned gynecologist who was blind to the group of patients by using the Voluson E8 machine. Based on the number and size of the dominant follicles, FSH dosage was adjusted for the next days till at least one mature follicle with a diameter of 18 mm or more was developing. At this time, 5000 units of hCG intramuscular injection (Choriomon, IBSA, Switzerland) was injected. Meanwhile, to make the study blind to the patients and remove the distributing factors, we inserted the IUI catheter (Prince medical, France) for all patients. Then, an

intrauterine injection of 300 µg of GCSF (1 cc, single-dose vial of Neupogen, Roche, Switzerland) was done for the case group, while 1 cc normal saline was injected in the control group in the same manner of the case group (23). Saline was in a bottle exactly like GCSF with the material the same in color and odor. There was an assigned staff in charge of preparing the syringe for injection of GCSF or saline after opening the sealed envelope of the patient group's allocation. The gynecologist who performed the procedure was blind to the group allocation and type of the substance in the syringe. 36 hours later, IUI was done by an expert gynecologist blinded to the group allocations by the standard local protocol method with swim-up technique of sperm preparation (24). After two weeks, the serum pregnancy test was done. Pregnancy was clinically established by transvaginal sonography at 6 weeks of gestational age in the patients with positive serum tests. The clinical pregnancy rate was calculated by dividing the number of patients with the presence of gestational sac in sonography divided into the total number of patients in each group. Also, miscarriage rate was defined as pregnancy loss before 12 weeks of gestational age. The ongoing pregnancy rate was calculated by subtracting the miscarriage rate from the total clinical pregnancy rate.

Statistical analysis

Quantitative data were presented as mean ± SD while qualitative data were presented as number (n) and percentage. The comparison between two groups with quantitative data and normal distribution was done by using an independent Student t test while the Mann-Whitney U-test was used only with non-parametric data. Logistic regression analysis was used to assess the odds ratio of factors related to birth rates between two groups. Statistical analysis was carried out using SPSS version 21 (SPSS IBM, Armonk, NY, USA). P<0.05 was considered statistically significant.

Results

As shown in Figure 1, 156 cases received GCSF (3 cases did not complete their follow up, one case had a technical problem in the administration of GCSF), and 152 control patients that not received GCSF (all omitted cases with not availability for follow up after IUI procedure) were enrolled at the end of the study. Six patients out of the GCSF group and 8 patients out of the control group had a miscarriage. In this study, all the ongoing pregnancies had live births. In the pregnancy course, one patient of each group (case at 27 weeks of gestation and control at 25 weeks of gestation) had alive premature birth that the neonates of both groups expired due to prematurity. Except for developing leukemia in one of the infants of the control group, no other specific event was notable in their follow-up. The demographic data of each group is presented in more detail in Table 1.

As demonstrated, both groups were not statistically different in age, endometrial thickness, number of follicles, parity, AFC, and body mass index (BMI).

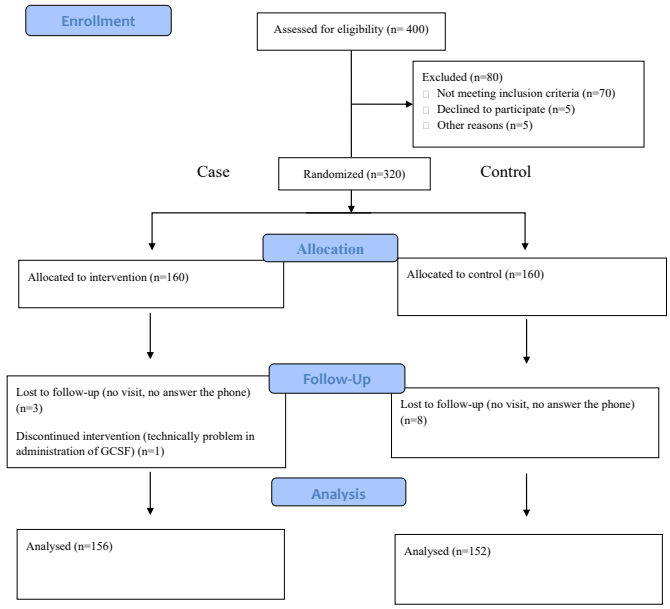

Fig.1: Flow chart of patients enrollment in the study that were randomly divided into groups of case and control.

Table 1: Demographic data of the case and control group

Characteristics	Groups		P value*
	GCSF (n=156)	Control (n=152)	
Age (Y)	30.01 ± 5.18	29.05 ± 6.32	0.145
ET (mm)	7.47 ± 1.46	7.63 ± 1.49	0.38
Follicle number (n)	2.39 ± 0.82	2.33 ± 1.05	0.358
Parity (n)	0.2 ± 0.49	0.16 ± 0.44	0.546
AFC on the second day of cycle (n)	9.38 ± 3.31	9.42 ± 3.28	0.45
BMI (kg/m^2)	22.48 ± 2.43	22.5 ± 2.41	0.315

Data are presented as mean ± SD. GCSF; Granulocyte colony stimulating factor, ET; Endometrial thickness, AFC; Antral follicular count, BMI; Body mass index, and *; Two-tailed t test.

The pregnancy rate in the GCSF group was 24 out of 156 patients (15.38%) in comparison to 21 out of 152 patients (13.81%) calculated for the control groups. Although the data showed an improved pregnancy rate documented by sonography in the GCSF group, it was not significant (P=0.63). No specific side effects were seen among the case and control groups. Also, non-significant improvement in ongoing pregnancy and miscarriage is shown in the GCSF group (Table 2, P>0.05).

Table 2: IUI outcome in case and control group

Characteristic	GCSF group (n=156)	Control group (n=152)	OR (95%CI)	P value
Clinical pregnancy rate	24 (15.38)	21 (13.81)	1.17 (0.62-2.21)	0.63
Miscarriage rate	6 (3.84)	8 (5.26)	0.74 (0.25-2.20)	0.55
Ongoing pregnancy rate	18 (11.53)	13 (8.55)	1.37 (0.65-2.92)	0.38

Data are presented as n (%). IUI; Intrauterine insemination, GCSF; Granulocyte colony stimulating factor, OR; Odds ratio, and CI; Confidence interval.

Discussion

The results of this study showed no statistically significant improvement in fertility rate in patients who received GCSF on the day of hCG injection in the IUI cycle. To the best of our knowledge, we found no previous study on testing GCSF to improve the IUI success rate study. There are some articles in the literature focusing on GCSF in assisted reproductive techniques (ART) success among patients suffering from recurrent miscarriage (10, 25) or thin endometrium in ARTs (9, 26) although there are some non-specific side effects like nausea and vomiting, anorexia, and headache; moreover, chest pain, hypoxemia, and syncope are mentioned as its side effects (12).

There is a controversy on GCSF efficacy to treat RIF patients (20). Kamath et al. (27), in a recent systematic review, Kalem et al. (23) in a randomized control study on intrauterine administration of GCSF in normal endometrium patients (23), and Davari Tanha et al. (28), in a randomized double-blind placebo control trial presented GCSF as an ineffective treatment in RIF patients. They are all in line with the Practice Committee of the American Society for Reproductive Medicine which believes there is no effect of GCSF considering insufficient study on the issue (29). In contrast, the following mentioned studies indicated that GCSF was beneficial. Zhang et al. (15) revealed the positive effect of GCSF in either systematic or intrauterine root administration in RIF patients. Also, the potency of GCSF to increase fertility in RIF patients is shown in a systematic review as well as other immunotherapy methods (10). Zhao et al. (30), in a systematic review and meta-analysis presented this cytokine as a beneficial method of fertility improvement. These controversies occur due to national, ethical, and genetic variations as well as different sample sizes and study design studies, the dosage of administration, and root of injection (8, 31). In line with the Practice Committee of the American Society, Davari Tanha et al. (28), we found no significant improvement in the fertility rate although it was more in the groups that received GCSF. It may be attributed to the very short lag between the administration of GCSF and insemination (36 hours). More time might be needed to present the positive effects of GCSF. Also, we perfused GCSF once in the uterine cavity, with possible benefit in more times of administration of the cytokine.

The outstanding root of GCSF administration is uncertain. Zeyneloglu et al. (14) demonstrated the benefits of dual subcutaneous and intrauterine administration of GCSF in patients with recurrent implantation failure in the intracytoplasmic sperm injection process. Patients received GCSF subcutaneously for 15 days starting from the oocyte retrieval day. The intrauterine dose was injected on the day of ovulation induction. The result of the study revealed the effectiveness of combination therapy of GCSF as the best method of prescription. Kalem et al. (23) showed no effectiveness in intrauterine administration of GCSF daily on hCG. Recently, a systematic review emphasized the effectiveness of GCSF in both intrauterine and subcutaneous administration with more success for subcutaneous method (8). Cavalcante et al. (10) in a systematic review showed the subcutaneous root as the method of choice for recurrent miscarriage treatment purposes, while the intrauterine root was a suitable choice for RIF or thin endometrium. In a systematic review, the beneficial effect of GCSF was attributed to the subcutaneous root of administration (30). Incongruently, Xie et al. (32) presented the effectiveness of intrauterine administration of GCSF in patients suffering from thin endometrium. In the present study, although we presented a better outcome in patients who received intra-uterine GCSF, this improvement was not statistically significant in patients with normal endometrium thickness. Effects on the patients with thin endometrium were not studied in this survey, so the possible intrauterine positive effect of GCSF might have been ignored. The potential effects of systematic administration of GCSF on normal endometrium patients should be investigated in further studies.

The strength of our study is its large population with the study design of a double-blind randomized control trial. Sonographer, laboratory, and IUI performer were the same among all participants, leading to a reduction in bias. Also, to the best of our knowledge, there is limited data on the effect of GCSF administration on the IUI success rate. We focused on the possible effects of GCSF that could lead to altering the protocols of subfertility management. Finally, it is concluded that less expensive modalities with less invasive procedures should be used. Performing this study only on patients with normal endometrial thickness is the limitation of our study. It is recommended that further studies be conducted considering the thin endometrium group and those with normal endometrium. Also, considering different lags between GCSF prescription and insemination should be examined in future studies to evaluate the possible positive effects of the cytokine prescribed in systemic, intra-uterine, or both methods.

Conclusion

Intrauterine 300 µg GCSF administration simultaneously with hCG injection in standard IUI procedure has increased the pregnancy outcome although it was not statistically significant. More studies are warranted that focus on the root and day of administration and studied population.

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Authors' Contributions

S.A., Z.Sh., N.N.; Contributed to conception and design. F.J.; Contributed to all experimental work, data and statistical analysis, and interpretation of data. S.A., Z.Sh; Were responsible for overall supervision. N.N.; Drafted the manuscript, which was revised by F.J., S.A. All authors read and approved the final manuscript.

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