Journal of Kerman University of Medical Sciences

JKMU

Journal of Kerman University of Medical Sciences, 2019; 26 (5): 390-397

Does Subcutaneous Administration of Granulocyte Colony Stimulating Factor Improve Pregnancy Outcome in Patients Undergoing Intracytoplasmic Sperm Injection?

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Received: 29 June, 2019 Accepted: 11 October, 2019

ARTICLE INFO

Article type:
Original Article

Keywords:
Granulocyte Colony Stimulating Factor
Intracytoplasmic Sperm Injections
Pregnancy

Abstract

Background: Expression of granulocyte colony stimulating factor (G-CSF) and its receptors in embryo and endometrium implicates the involvement of this glycoprotein on implantation process. In the present study, we aimed to evaluate the impact of routine use of subcutaneous administration of G-CSF on pregnancy outcomes in intracytoplasmic sperm injection (ICSI) patients.

Methods: In this retrospective study, ICSI outcomes were compared between two groups of patients: the first group (n=108) who received subcutaneous G-CSF (300 mcg) two hours before the embryo transfer and the second group (n=110) who did not receive it. Pregnancy outcome was compared between the two groups. P-value<0.05 was considered statistically significant.

Results: There was no significant difference between G-CSF and control groups with respect to the rate of implantation (respectively, 23%vs. 23%, p=0.49), chemical (respectively, 43.5%vs. 50%, p=0.34) and clinical (respectively, 40.7% vs. 46.4%, p=0.23) pregnancy. In logistic regression analyses, subcutaneous G-CSF administration was not associated with clinical pregnancy in both crude and adjusted odds ratios (OR) with 95% confidence interval (CI) (crude OR: 0.8, CI: 0.47-1.36, p=0.4, and adjusted OR: 0.99, CI: 0.48-2.07, p=0.99).

Conclusion: In the present study, subcutaneous G-CSF did not improve pregnancy outcomes in patients undergoing ICSI; therefore, the routine use of this cytokine is not suggested for all patients.

Citation: Kabodmehri P, Mehrafza M, Zare Yousefi T, Saghati Jalali S, Sedaghat F, Hosseinzadeh E, Raoufi A, Samadnia S. Does Subcutaneous Administration of Granulocyte Colony Stimulating Factor Improve Pregnancy Outcome in Patients Undergoing Intracytoplasmic Sperm Injection? *Journal of Kerman University of Medical Sciences*, 2019; 26 (5): 390-397

Introduction

In spite of the progress in assisted reproductive techniques (ART), the implantation rate still remains low. Evidences indicated that immunological mechanisms play a critical function during implantation processes via production and release of different cytokines and growth factors by decidual cells (1, 2).

The immunological relationship between the mammalian fetus and its mother during pregnancy has been considered similar to that between a transplanted allograft and its recipient (3, 4). However, recent evidence indicates that implantation might involve predominantly a novel allogeneic recognition system based on natural killer (NK) cells rather than T cells. NK cells express receptors that are specific for human leukocyte antigen (HLA-C) molecules (5).

Granulocyte-colony stimulating factor (G-CSF) is a hematopoietic cytokine that is constructed by a number of cells including decidual cells (6), chorionic villous trophoblast (7) and NK cells exclusively uterine cells (8). Identification of G-CSF receptors in some kinds of cells including human trophoblast cells, placental membranes (9), hematopotetic progenitors, macrophages, NKs, T cells and platelets (10) implicated the impact of this glycoprotein on implantation process (11).

Clinical applications of G-CSF has been reported for oocyte maturation (12), thin endometrium (13), recurrent spontaneous abortions and repeated implantation failure (RIF) in ART treatments (11). It was indicated that intrauterine infusion of G-CSF may increase endometrial thickness and pregnancy outcome in patients with inadequate endometrial growth (14). Several studies implicated the value of G-CSF administration on the rate of implantation and pregnancy in RIF patients (15-18); however, there are also studies that failed to show improvement in pregnancy outcomes following G-CSF administration (19, 20). Therefore, its efficacy in infertile patients has not yet been proven. This inconsistence might be owing to the heterogeneity in administration route (systemic or intrauterine infusion) or clinical conditions among studies. These studies came into controversial conclusions, so both clinicians and infertile women are in an awkward position of whether the G-CSF should be given. In the present retrospective study, we aimed to further evaluate whether systemic G-CSF administration has beneficial effect on the outcome of ART in normal infertile women.

Materials and Methods

The present retrospective study was conducted at Mehr Fertility Research Center, Guilan University of Medical Sciences, Rasht, Iran between 2015 and 2017.All women with less than two failed embryo transfer cycles were included in the study. However, since G-CSF is contraindicated in patients with known sickle cell disease, thrombocytopenia, leukocytosis and malignancies, the mentioned cases were excluded from the study.

All patients were evaluated according to basal hormonal screening, ultrasonography, hysterosalpingography and hysteroscopy if indicated. Pituitary suppression was achieved with either gonadotropin releasing hormone (GnRH) agonist or antagonist. In GnRH agonist cycles, decapeptyl (1.87 mg; Ferring, Germany) was administrated on the day 21 of the preceding menstrual cycle. According to the antagonist protocol, when the leading follicle diameter reached 14mm, cetrotide (0.25 mg/day; Merck-Serono, Germany) was administrated daily until the hCG day.

Ovarian stimulation was started with human recombinant follicle stimulation (rFSH, Gonal-F, Merck-Serono, Germany) and/or human menopausal gonadotropin (hMG, Menopur, Ferring, Germany) on the second day of menstrual cycle and continued until the day of triggering final oocyte maturation with hCG (10000 IU, Daroupakhsh, Iran) or GnRH agonist (Decapeptyl, 0.1 mg; Ferring, Germany).

Ovarian response was monitored with estradiol measurement and ultrasonography. Transvaginal ultrasound guided oocyte retrieval was done under light general anesthesia. After denudation of oocyte-cumulus complexes, ICSI was performed by fresh sperm prepared on the same day.

Luteal phase was supported by 400mg intravaginal and 100mg intramuscular progesterone (Aburaihan, Iran) and 2.5mg estrogen (Aburaihan, Iran). Based on the administration of G-CSF on the day of embryo transfer, patients were divided into the two groups: G-CSF and control groups.

In the G-CSF group, a single dose of 300 mcg G-CSF (PD grastim, PooyeshDarou, Iran) was administrated subcutaneously, two hours before the embryo transfer. While, patients in the control group did not receive any G-CSF treatment prior to the embryo transfer.

Biochemical pregnancy was defined as positive serum β-hCG test, two weeks after the embryo transfer. Clinical pregnancy was assessed with observation of fetal heart by transvaginal ultrasonography in the sixth to seventh weeks of pregnancy.

Statistical analysis was performed using statistical package for the social sciences (SPSS) version 21 (SPSS Inc. Chicago, IL, USA). Data were analyzed using student´s t-test or Mann-Whitney U test and presented as mean ± standard deviation (SD), median (minimum, maximum) and percentage. Chi-square test was used for categorical variables. According to univariate logistic regression, variables with p-value less than 0.2 were considered as confounding and evaluated by multivariate logistic regression. Logistic regression was used to calculate crude and adjusted odds ratios for clinical pregnancy. P-value less than 0.05 was considered statistically significant.

Results

A total of 218 patients were included in the present study: 108 were in the G-CSF group and 110 were in the control group. The mean and standard deviation of age of women was 31.94±5.81 years. The baseline characteristics of patients are presented in table 1. No significant difference was found between the two groups with respect to the age, body mass index (BMI), number of agonist and antagonist cycles and primary or secondary infertility.

Table 1. The baseline characteristics of patients

Characteristics	G-CSF Group (n=108)	Control Group (n=110)	P-value
Age (year)	31.96±6.03	31.93±5.60	0.96*
Body mass index (kg/m²)	25.62 (17.58, 33.59)	25.97 (18.56, 103.21)	0.11**
Type of infertility **Primary (%)** **Secondary (%)**	 73/105 (69.5) 32/105 (30.5)	 83/108 (76.9) 25/108 (23.1)	0.23***

Data were presented as mean ± standard deviation, median (minimum, maximum) and percent.
* t-Test, ** Mann-Whitney Test, *** Chi-Square Test

The number of transferred embryos and top quality transferred embryos were significantly higher in the control group than those in the G-CSF group (2.82±0.85 versus 2.38 ± 0.87; P<0.0001 and 2.21±0.89 versus 1.67±0.72; P<0.0001). There were no statistically significant difference between the two groups with regards to the rate of fertilization, implantation, chemical pregnancy and clinical pregnancy (table 2).

Table 2. The stimulation outcomes of patients

Characteristics	G-CSF Group	Control Group	P-value
Pituitary suppression			
Agonist (%)	78/108 (72.2)	90/110 (81.8)	0.09***
Antagonist (%)	30/108 (27.8)	20/110 (18.2)	
Fertilization rate (%)	715/1200 (60)	612/986 (62)	0.63**
Transferred embryos	2.38 ± 0.87	2.82 ± 0.85	0.000*
Top quality transferred embryos	1.67 ± 0.72	2.21 ± 0.89	0.000*
Implantation rate (%)	59/257 (23)	68/296 (23)	0.49**
Chemical pregnancy (%)	47/108 (43.5)	55/110 (50)	0.34***
Clinical pregnancy (%)	44/108 (40.7)	51/110 (46.4)	0.23***

Data were presented as mean ± standard deviation and percent. * t-Test, ** Mann-Whitney Test, *** Chi-Square Test

According to the univariate logistic regression, age, number of transferred embryos and top quality transferred embryos were considered as confounding variables (p-value less than 0.2). In both crude and adjusted models, no significant association was found between systemic G-CSF injection and pregnancy outcome (table 3).

Table 3. The cruds and adjusted odds ratios of clinical pregnancy for confounding variables

Variables	p-value*	Crude OR	95% C.I Lower-Upper	p-value*	Adjusted OR	95% C.I Lower-Upper
G-CSF method	0.4	0.8	(0.47,1.36)	0.995	0.998	(0.48,2.07)
Age	0.015	0.94	(0.9,0.99)	0.1	0.95	(0.89,1.01)
Transferred embryos	0.02	1.47	(1.07,2.01)	0.46	1.2	(0.74,1.96)
Top quality transferred embryos	0.03	1.45	(1.04,2.01)	0.06	1.61	(0.98,2.66)

C.I: confidence interval, OR: odds ratio, * Logistic regression analysis

Discussion

G-CSF is a glycoprotein synthesized in several different tissues. It has been indicated that G-CSF may affect oocyte maturation and implantation and consequently the reproduction process. G-CSF concentration gradually increases during the follicular phase in normal menstrual cycles and attains its peak at ovulation (21).Follicle development and ovulation is under the influence of G-CSF and there is a positive linkage between the concentration of G-CSF in follicular fluid (FF) and IVF outcomes (22).Follicular concentration of G-CSF, as an important biomarker before fertilization, can improve the

synchronization between uterine milieu and embryo development (23, 24).

The presence of G-CSF receptors in the trophoblastic and decidual cells indicates the importance of this glycoprotein in implantation mechanism (25).The impact of recombinant human G-CSF (rhG-CSF) on expression of critical endometrial genes involving in implantation process has been described in an Ex-Vivo study which suggested that the expression of G-CSF receptor, plasminogen activator urokinase receptor, integrine alpha-V/beta-3, thymidine phosphorylase, CD40 and CD40L increased during addition of rhG-CSF to endometrial biopsies culture (26).

Our results did not show any improvement in pregnancy outcome between the control and G-CSF groups. Like ours, Barad et al. examined the effect of intrauterine G-CSF administration on endometrial thickness and pregnancy outcomes in normal IVF patients and they reported no improvement in endometrial thickness, implantation and pregnancy rates (20). Kunicki et al. indicated that intrauterine infusion of G-CSF in frozen-thawed blastocyst transfer cycles with thin endometrium can improve the thickness of the endometrium but cannot improve clinical pregnancy and live birth rate (27). Also, the results of a study by Eftekhar et al. on normal IVF patients with normal endometrial thickness showed that the intrauterine infusion of G-CSF did not improve pregnancy outcomes (28). In our study, contrary to the studies mentioned above, the procedure of prescribing G-CSF was systemically and was 2 hours before the embryo transfer; thus, the effect of G-CSF on the thickness of the endometrium was not evaluated.

However, some studies showed the benefit of G-CSF administration on pregnancy outcome. In a nonrandomized clinical trial by Tehrannejad et al., the effect of intrauterine infusion of G-CSF on the day of oocyte pick-up or 5 days before the embryo transfer in fifteen patients undergoing embryo transfer and with the history of cycle cancellation due to thin endometrium were studied. Their results demonstrated that G-CSF may increase endometrial thickness (14). Xu et al. showed that intrauterine infusion of G-CSF in patients with thin endometrium significantly increased embryo implantation and clinical pregnancy rate during frozen embryo transfer cycles. However, the difference in endometrial thickness between two groups was not statistically significant (29). In Aleyasin et al. study, subcutaneous administration of G-CSF in RIF patients improved implantation and pregnancy outcomes (15). Eftekhar et al., also, indicated that intrauterine infusion of G-CSF can significantly improve implantation rate and pregnancy in patients with RIF (16). The difference between those studies and ours was in study population. In the mentioned studies, only RIF patients were included, while in our study all patients except RIF were studied.

In a study by Wurfel et al., systematic administration of G-CSF to patients with RIF who lacked the three activating KIR genes was beneficial (11). It seems that in these patients, the interactions between embryonic trophoblast (through the expression of HLA-C) and uterine NK cells are impaired, resulting in failure in the implantation or abortion. It appears that the prevalence of this defect is significantly higher in the population of RIF patients. Therefore, the routine use of G-CSF seems to be ineffective in all infertile patients.

In our study, more than 90% of embryos in the control group were transferred in the fresh cycle, which had a significant difference with the G-CSF group. Also, the number of transferred embryos and the number of high-quality

transferred embryos were significantly higher in the control group. After adjusting these factors by logistic regression, no significant relationship was observed between G-CSF prescription and pregnancy outcomes. In the present study, the number of participants was limited and we cannot draw a firm conclusion because of the retrospective nature of our study which is considered as its main limitation.

In summary, we concluded that subcutaneous administration of G-CSF in infertile women cannot affect implantation outcomes and the routine use of G-CSF may not applicable to all patients. More studies are required to find out the mechanisms of G-CSF effects on implantation and the best approach of G-CSF administration to improve the success rate.

Acknowledgements

We would like to thank Clinical Development Research Unit of Ghaem Hospital, Guilan University of Medical Sciences, Rasht, Iran for their kind cooperation.

Conflict of interest

We declare that there is no conflict of interest.

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