Research Article ISSN 2639-9342

Gynecology & Reproductive Health

Oil-Based Hysterosalpingography İmproves Leukaemia İnhibitory Factor Expression İn Endometrial Flushing Samples

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Received: 20 Sep 2022; **Accepted:** 27 Oct 2022; **Published:** 01 Nov 2022

Citation: Nur Dokuzeylul Gungor, Arzu Yurci, Safak Hatırnaz, et al. Oil-Based Hysterosalpingography İmproves Leukaemia İnhibitory Factor Expression İn Endometrial Flushing Samples. Gynecol Reprod Health. 2022; 6(5): 1-4.

ABSTRACT

Objective: *The biological mechanism of the fertility-enhancing effect of hysterosalpingography (HSG) is not fully known. Leukemia inhibitory factor (LIF) is an important cytokine involved in implantation. This study was planned to investigate the effect of HSG on LIF mRNA expression in endometrial flushing samples.*

Methods: *Forty infertile patients scheduled for HSG were included in the study. Before HSG, endometrial flushing was performed in the mid-luteal phase, followed by contrast-medium infusion into the cavity. Second flushing was done in the mid-luteal phase of the next cycle. Ten patients who underwent diagnostic hysteroscopy were taken as the second control group. LIF mRNA levels were measured by RT-PCR in endometrial flushing samples collected from each group.*

Results: *The pre-HSG LIF mRNA levels of the infertile group were significantly lower than those of the fertile group. In infertile patients, LIF mRNA expression increased 3.2 times after HSG compared to baseline levels. LIF values reached fertile levels after HSG. While the LIF levels measured in the hysteroscopy group were significantly lower than in fertile patients, they were similar to the pre-HSG values of infertile patients. LIF levels of infertile patients after HSG were found to be significantly higher than those of the hysteroscopy group.*

Conclusions: *In infertile patients, HSG contributes to receptivity by increasing LIF mRNA synthesis.*

Keywords

HSG, LIF, RT-PCR, Endometrium, Receptivity.

Introduction

Although there is not very high-quality evidence, hysterosalpingography (HSG) is believed to have a fertility-enhancing effect for nearly half a century [1]. In a recent study conducted by Dreyer et al. [2] pregnancy rates after HSG were found to be significantly higher than water-based ones. However, there is a study reporting that HSG increases fertility independent of the contrast medium used [3]. Although the fertility-increasing effects of the HSG procedure and the medium useisre not known clearly, it is thought that they provide these effects through the following mechanisms: (i) due to the mechanical and flushing

effect of the contrast medium the removal of mucus plugs and cell debris from the proximal parts of the tubes [4], (ii) the facilitation of sperm and embryo transport due to increased ciliary activity in the fallopian tubes [5], (iii) immunological modulation in T cell and dendritic cell populations of endometrium and peritoneal microenvironment due to contrast medium [6,7].

Leukaemia inhibitory factor (LIF) is a 20 kDa protein synthesized by removing 22 amino acids from the N-terminal end of the 202 amino acid precursor [8]. LIF shows its effect on gp 130 and LIFRβ receptors. Although LIF shows close homology with IL-6, its receptors and functions are quite different [9]. Functional LIF receptors are located on many cell surfaces including the endometrium and between 100 and 400. LIF is expressed in endometrial cells during blastocystst implantation [10]. Increasing estrogen levels stimulate LIF expression [11]. LIF -/- female mice have been reported to be infertile due to blastocyst implantation defects [12]. Decreased midluteal phase endometrial LIF expression has been reported in women suffering from infertility, including endometrioma and hydrosalpinx [13-15]. Moreover, it has been reported that endometrial LIF release is restored after salpingectomy and endometrioma resections [14,15]. It is known that HSG positively affects short and long-term fertility in infertile women with ovulatory cycles [16]. The biological mechanism of the fertility-enhancing effect of hysterosalpingography (HSG) is not fully known. Leukemia inhibitory factor (LIF) is an important cytokine involved in implantation. This study was planned to investigate the effect of HSG on LIF mRNA expression in endometrial cells collected during endometrial flushing.

Material and Methods

Forty women who underwent hysterosalpingography during routine infertility examinations were included in the study. Inclusion criteria of patients in the HSG group; Patients aged 20 and 34 years, with regular menstrual cycles, who have not been able to conceive for 2 years and have an indication for HSG. Women with a high risk of pelvic inflammatory disease, iodine or contrast medium allergy, Asherman syndrome, hydrosalpinx, andout-of-dateendometrium were excluded. Women with endometrioma, endometrial polyp, submucous or intramural fibroids pressing on the endometrial cavity were also excluded. A pre-HSG pregnancy test was performed on the participants to exclude a possible pregnancy.

The control group consisted of two different patient populations. The first control group consisted of five fertile patients. The second control group was selected from infertile patients who were scheduled for diagnostic hysteroscopy. Endometrial flushing was performed in the mid-luteal phase of the cycle from both control groups. Thanks to this group, the possible effect of the media used during hysteroscopy on the receptivity molecules were investigated. With the findings to be obtained from the second control group, it was tested whether the liquid medium used in hysteroscopy had an effect on LIF expression. Endometrial flushing samples were collected at the end of the hysteroscopic procedures. Those who underwent endometrial injury, polypectomy, myomectomy, septum resection and adhesiolysis during hysteroscopy were not included in the study.

All patients in the HSG group underwent HSG in the mid-luteal phase of the cycle. Ovulation was followed by USG to calculate the midluteal phase. The midluteal phase was accepted 7-9 days after ovulation [17]. HSG was performed as stated in previous publications. After the cervical canal was held with a single tooth, the contrast medium was infused with a metal cannula. Approximately 5 to 10 ml of contrast medium was sufficient to fill both the cavity and fallopian tubes. By recording 4 to 6 radiocontrast images, the route of the contrast medium in the endometrial cavity tubes and peritoneum was evaluated. Radiographs were examined by a gynecologist or radiologist. Endometrial flushing was performed with a pipelle just before the contrast-medium infusion during the HSG procedure. Endometrial flushing was performed for the second time in the midluteal phase of the next cycle in all cases undergoing HSG. We evaluated the LIF mRNA expressions in the endometrial flushing samples obtained before HSG and respective flushing mterials after HSG. This study was performed with IRB approval.

RT-PCR

Flushing samples taken from patients in all three groups were stored in RNA later and at -20°C. Total RNA was isolated using Rneasy Mini Kits (QIAGEN). RNA levels and quality were evaluated spectroscopically. Complementary DNA (cDNA) was obtained using the Reverse Transcription Kit. β-Actin gene was used as a housekeeping gene of LIF gene. RT-PCR was performed using the Quantitect kit. The expression levels of the examined genes were given as Ct, ΔCt, and ΔΔCt. Each sample was studied at least three times in order to give the Ct value clearly. Relative gene expression was calculated by RT2 Profiler PCR Array method. Normalization of genes was done according to β-actin and given as Fold change. Fold change was accepted as up-regulation if it was greater than two, and as down-regulation if it was less than two.

Statistical Analysis

Statistical Package for Social Sciences software 18.0 for Windows package software was used for the analysis of the obtained mRNA data and other data. Whether the data were normally distributed or not was evaluated with the Kolmogorov-Smirnov test. Continuous variables were analyzed using the Mann-Whitney U test. Results are given as mean and standard deviation. P-value of <.05 was accepted as significant.

Results

The age of the fertile group (29.4 ± 0.44 years) was found to be higher than HSG (26.3 ± 1.33 years) and H/S (25.9 ± 4.3 years) control groups. Ages and duration of infertility of HSG and H/S groups were similar. The BMI of the fertile group was found to be significantly higher than both control groups. Hysterosalpingography showed bilateral tubal patency in 7 of 40 women. Bilateral tubal occlusion occurred in 4 women and unilateral tubal occlusion were detected in 3 women.

Pre-HSG LIF mRNA levels of the infertile group were significantly lower than those of the fertile group. In infertile patients, LIF mRNA expression increased 3.2 times after HSG compared to baseline levels. LIF values reached fertile levels after HSG. While the LIF levels measured in the hysteroscopy group were significantly lower than in fertile patients, they were similar to the pre-HSG values of infertile patients. LIF levels of infertile patients after HSG were found to be significantly higher than those of the hysteroscopy group.

Discussion

The underlying mechanism of the fertility-enhancing effect stimulated by HSG remains unclear. It is believed that the positive effects due to HSG are mostly due to the removal of mucus plugs in the tubas. Due to the increased intrauterine pressure during fat-based contrast medium flushing, all the obstructive materials in the fallopian tubes fall into the abdominal cavity from the proximal tubal ends and the passage is opened and pregnancy occurs. On the other hand, HSG may increase pregnancy rates by affecting endometrial receptivity [6,18,19]. HSG The reason that most of the studies are focused on fallopian tubes may be due to the lack of studies showing the effect of oil-based contrast medium on endometrial receptivity molecules. In an experimental study, it was reported that intrauterine infusion of oil-based contrast medium improved fertility by altering murine uterine dendritic cell populations [18]. In another experimental study, it was reported that oil-based contrast medium prevents sperm phagocytosis and contributes to pregnancy rates by regulating rat peritoneal macrophage and cytokine release [20]. All these data are clear evidence that studies investigating the effect of HSG on endometrial receptivity are needed to gain more insight into the fertility-enhancing mechanism of oil contrast.

Our study is the first study investigating the effects of oil based contrast used in HSG on LIF, one of the main molecules of endometrial receptivity. LIF mRNA expression was measured in endometrial flushing samples taken just before and one month after HSG in the midluteal phase. Since LIF expression peaked in the midluteal phase, HSG processing was performed during this period. Second endometrial flushing ws performed after HSG in the midluteal phase of the next cycle. Here, we rely on the work done by van Welie et al. [16]. These authors reported that the fertility-enhancing effect of oil-based contrast was at its highest level in the cycle immediately after HSG, then gradually decreased within one year and disappeared within two years [16].

LIF mRNA levels measured before HSG in infertile patients were significantly lower than in the fertile group. In the analyzes performed after HSG, LIF mRNA levels increased approximately 3.2 times. This finding is evidence that the HSG procedure itself or the oil-based contrast used in the procedure stimulates LIF expression. The lack of change in LIF mRNA levels in the H/S group is evidence that the main reason for increasing LIF expression is the oil-based contrast agent used in HSG. If the LIF mRNA increase was due to uterine and cervical manipulations during HSG, the same increase should have been detected in H/S where similar manipulations were performed. We do not know by what mechanism the insufflation of oil-based contrast into the cavity during HSG increases LIF mRNA levels. The increase in LIF mRNA may be induced due to the pressure created by the oil base contrast in the cavity. Similar to the endometrial scratching process, the contrast agent can stimulate inflammatory changes and increase LIF release [21]. Another possible mechanism for the positive effect of the contrast agent on endometrial LIF is the rearrangement of macrophage activity and cytokine release in the endometrial cavity similar to that in the peritoneum [18,20]. It has been reported that oil-based contrast insufflation positively affected the balance of CD205 + and CD1 + dendritic cells in the mice endometrium and increased fertility [18]. Some oil-based contrast mediums contain opium alkaloids. Since opioid receptor expression occurs cyclically in the endometrium, the contrast material we use in HSG may have increased the LIF mRNA expression by stimulating these receptors [22]. However, these mechanisms we have put forward must be confirmed by more comprehensive studies in order to avoid speculation.

A recent study by Deryer et al. [2] showed a substantial increase in ongoing pregnancy rates during the first 6 months following HSG. Similarly, van Welie et al. reported that the fertility-enhancing effect of HSG continued to decrease for 1 year [23]. These two comprehensive studies have suggested that the long-term fertility-enhancing effect after the use of oil-based contrast material is due to clearing the fallopian tubes from debris. Therefore, they argued that the changes occurring in the endometrium or peritoneum could not explain the long-term effects of HSG. When our study and these two studies [2,23] data are evaluated together, it is obvious that the fertility-enhancing effect of HSG occurs both in the endometrial and tubal environment. Cleaning of fallopian tubes from debris is the main mechanism for the continuation of long-term fertility enhancing effects, but positive changes in endometrial receptivity molecules after contrast agent may contribute to these effects. If we can determine when the increase in LIF mRNA levels started after HSG and how long it continues, we can better understand whether the contrast agent-induced LIF increase has a short or long-term effect.

Before starting this study we hypothesized that the fertility-enhancing effect of HSG may be in part mediated by altered expression of endometrial LIF, a cytokine implicated in early embryo implantation. The results we achieved support our hypothesis that tubal flushing using oil-based contrast improves endometrial receptivity by increasing LIF mRNA expression. The exact underlying LIF mRNA-enhancing mechanism of HSG is unclear, but we proposed that oil-based contrast flushing during HSG can improve the synthesis and release of receptivity molecules from the otherwise normal endometrial cavity. Our findings are clinically important since it is the first study to show a significant increase in endometrial LIF mRNA levels in infertile patients after HSG using oil-based contrast. More comprehensive studies are needed to investigate the mechanism by which the contrast agent

used in the HSG procedure increases receptivity.

Ethical Statement

The study protocol was approved by the Institutional Ethical Committee for Research on Human subjects.

Ethical Approval

All research procedures were evaluated and accepted by the Research Ethics Committee of Bahcesehir University Goztepe Medical Park Hospital (2021/16574) and were conducted in agreement with the ethical standards specified in the Declaration of Helsinki. Written and verbal informed consent was obtained from participating women prior to their participation in this study.

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