

ORIGINAL ARTICLE

Evaluation of Laparoscopic Intervention versus Ultrasound Guided Aspiration of Bilateral Hydrosalpinges on Endometrial LIF mRNA Expression Level and Pregnancy Outcome after IVF-ET Cycle

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ABSTRACT

Key words:

Laparoscopy, Hydrosalpinx, LIF, Implantation

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Background: Hydrosalpinx is a common gynecological disease. 30% of infertile women undergoing in vitro fertilization-embryo transfer (IVF-ET) have hydrosalpinx. Leukemia inhibitory factor (LIF) mRNA expression level is lower in the endometrium during the implantation window in the presence of bilateral hydrosalpinges. If the hydrosalpinx is removed, LIF mRNA expression returns to normal values. **Objectives:** This study was designed to evaluate laparoscopic intervention of bilateral hydrosalpinges versus transvaginal ultrasound guided aspiration on LIF mRNA expression level during implantation window and pregnancy outcome after IVF-ET cycle. **Methodology:** Sixty patients who had bilateral hydrosalpinges were classified into two groups; 30 women underwent laparoscopic salpingectomy or proximal tubal occlusion and 30 women underwent transvaginal ultrasound guided aspiration. Determination of LIF mRNA expression level in endometrial samples was performed preoperatively and in the 3rd postoperative cycle. In addition, pregnancy outcome was evaluated in the first IVF-ET cycle. **Results:** Endometrial LIF mRNA expression level in laparoscopic group significantly increased postoperatively than transvaginal ultrasound guided aspiration group with a significant difference ($P=0.03$). In addition, there is increased levels in patients undergoing laparoscopic and ultrasound guided aspiration groups after treatment than before with a significant difference ($P=0.005$) and ($P=0.04$); respectively. As regards pregnancy outcome, it is more favorable in laparoscopic group than ultrasound guided aspiration with statistically significant difference ($P=0.013$). **Conclusion:** Our findings showed that endometrial LIF mRNA expression level was increased and pregnancy outcome was more favorable after laparoscopic intervention versus transvaginal ultrasound guided aspiration for treatment of bilateral hydrosalpinges patients.

INTRODUCTION

Hydrosalpinx is a common gynecological disease resulting from a series of pelvic inflammatory diseases (PID), which can lead to pelvic pain and infertility. About 30% of infertile women undergoing in vitro fertilization-embryo transfer (IVF-ET) treatment have hydrosalpinx diagnosed by transvaginal ultrasound scanning (TVS) or hysterosalpingography (HSG)¹. Many studies have demonstrated the embryotoxicity of hydrosalpinx fluid².

Successful embryo implantation requires a receptive endometrium. In each menstrual cycle, the human endometrium exhibits a limited period to accept the implanting embryo, known as the "window of implantation" (day 21-24 of the menstrual cycle)³. During this period, the endometrium undergoes a complex series of distinct cellular and molecular changes

initiated by ovarian steroid hormones, which render it receptive to conception⁴.

Some studies have focused on the morphological and biochemical characteristics of the receptive endometrium. At the morphological level, endometrial receptivity can be evaluated by histopathological investigation or the appearance of pinopod structures⁵.

The biochemical markers of receptive endometrium include estrogen receptors (ERs), progesterone receptors (PRs), interleukins (ILs), glycodefin, vascular endothelial growth factor (VEGF), mucin 1 (MUC1), integrins and leukemia inhibitory factor (LIF)⁶.

Leukemia inhibitory factor belongs to the interleukin (IL)-6 family. It is a secretory protein, composed of 180 amino acids and glycosylated at asparagine residues. It binds to its receptors, which may influence the reproductive activities, including follicular development, embryonic development, blastocyst

implantation and maintenance of pregnancy⁷. LIF regulates the blastocyst implantation via the regulation of the invasive ability of the trophoblasts and by influencing the immune tolerance⁸.

Leukemia inhibitory factor expression may be found in the natural killer cells in the decidua and chorion. It has been well established that LIF mRNA expression in the endometrium is high during the mid-to late-secretory phase (from days 18 to 28) with a peak at day 20 of the menstrual cycle⁹.

In the presence of hydrosalpinges, the expression of LIF is reduced in the endometrium during the window of implantation, compared with patients without hydrosalpinx. Moreover, if the hydrosalpinges are removed, LIF expression returns to normal level in these patients¹⁰.

Several management options for hydrosalpinges as transvaginal ultrasound guided aspiration, hysteroscopic tubal occlusion by Essure micro-inserts, or medical treatment (antibiotics and/or corticosteroids) were suggested as alternatives to laparoscopic salpingectomy or proximal tubal occlusion. Transvaginal ultrasound guided aspiration of hydrosalpinx fluid is the best alternative because it is simple, safe and inexpensive¹¹. On the other hand, the occlusion of fallopian tube with Essure micro-inserts is expensive, delays IVF-ET cycle for 3 months and its risk to the patients who become pregnant and their fetuses is not known¹².

The aim of this study was to evaluate laparoscopic intervention of bilateral hydrosalpinges versus transvaginal ultrasound guided aspiration on LIF mRNA expression level during implantation window and pregnancy outcome after IVF-ET cycle.

METHODOLOGY

This prospective, two arm, allocation concealed, randomized study was conducted at the Obstetrics and Gynecology Department, Faculty of medicine, Zagazig university Hospitals and Medical Microbiology and Immunology Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt during the period between August 2013 and May 2017. The study protocol was approved by the institutional review board (IRB) of Faculty of Medicine, Zagazig University. The patients were counseled about the benefits and risks of both management options and written informed consent was obtained.

One hundred infertile women attending the endoscopy and cytogenetic unit were recruited to this study. The following inclusion and exclusion criteria were used in this study:

Inclusion criteria:

The patients had regular menstrual cycles, no history of smoking, and did not receive any hormonal treatment for the previous 3 months. **Exclusion criteria:**

The patients who had any abnormalities of the uterus or ovaries, endometriosis, hyperprolactinemia or polycystic ovary syndrome.

Hence, forty patients were out of this study; 12 patients were not meeting inclusion criteria and 28 were declined to participate (**Flow chart**).

Sixty patients, who were fulfilled the inclusion criteria of the study, had bilateral hydrosalpinges (diagnosed by hysterosalpinogram (HSG) and transvaginal ultrasound scan) were allocated to two groups; 30 of them underwent laparoscopic salpingectomy or proximal tubal occlusion and 30 patients underwent transvaginal ultrasound guided aspiration. The patients were randomly allocated to laparoscopy group or ultrasound guided aspiration group using computer generated randomization list and sequentially numbered sealed envelopes containing allocation information written on a card. The sealed envelopes were prepared by statistician not involved in the study. Study nurse opened the sequentially numbered envelopes to allocate patients to the assigned group.

In laparoscopic group, after confirmation of hydrosalpinx and associated severe tubal disease, salpingectomy was done on both sides where the mesosalpinx was coagulated as close as possible to the fallopian tubes using bipolar electrocoagulation to avoid any compromise in the ovarian blood supply. Proximal tubal occlusion and linear salpingostomy of hydrosalpinges were done in cases with extensive pelvic adhesions.

In transvaginal ultrasound guided aspiration group, an aspiration needle was inserted into the hydrosalpinx and suction was applied to aspirate the hydrosalpinx fluid completely.

The patients were subjected to preoperative endometrial sampling that obtained during cycle day 20 and on the corresponding menstrual cycle day of the 3rd post-operative cycle. The day of the menstrual cycle was established from the patient's menstrual history. The Novak's curette was used for all endometrial biopsies.

Patients in this study were referred to Assisted Reproductive Technology (ART) centre for IVF-ET trial and were undergone a follow up for the occurrence of pregnancy and its outcome (clinical pregnancy; ongoing pregnancy and spontaneous abortions) after IVF-ET cycle for up to 24 weeks of pregnancy.

Detection of LIF mRNA expression level in endometrial biopsies:

RNA extraction:

Endometrial biopsies were lysed by RLT buffer (QIAGEN, Germantown, MD). The lysates were further prepared for total RNA extraction using the RNeasy mini kit (QIAGEN, Germantown, MD) according to the manufacturer's instructions. DNase was applied to avoid DNA contamination. The RNA extract was stored

at -80°C until future use. RNA purity, yield, and concentration were determined through dual spectrophotometry (Beckman, USA), and 1 μg of RNA was run on a 1% agarose gel (Roche, Castle Hill, Australia) to ensure integrity of the RNA.

Quantitative RT-PCR (qRT-PCR):

Reverse transcriptase (RT) reaction mixture using High Capacity Reverse Transcriptase kit (Applied Biosystems, USA) containing: 1 μg total RNA from each sample, 0.5 μg random primer, 5X RT buffer, 2.5 mmol/L dNTP, 20 U RNase inhibitor and 200 U moloney murine leukemia virus (M-MLV) reverse transcriptase in a total volume of 25 μl was incubated at 37°C for 60 minutes, then heated to 95°C for 5 minutes to inactivate MMLV. Minus RT for each sample was applied as negative control.

Reverse transcriptase (RT) reaction was followed by qPCR, 50 ng of cDNA was added to 5 X Fast-Start SYBR green master mixes with Rox (Roche Diagnostics, Indianapolis, IN) and 200 ng of primer mix (Sigma).

The reaction was carried out in micro-optical plates (Applied Biosystems, USA) and analyzed using StepOne real-time PCR system (Applied Biosystems, USA). The PCR running method was as follows: 10 min at 95°C for enzyme activation followed by 40 cycles of 15 s at 95°C , 20 s at 55°C , and 30 s at 72°C for the amplification step. The primers used in the qRT-PCR evaluation were specific for LIF gene: Forward: 5'-TGGTTCTGCACTGGAAACATG-3' and Reverse: 5'-GTAATAGAGAATAAAGAGGGCATTGG-3' and GAPDH housekeeping gene: Forward: 5'-CCTCTACTGGCGCTGCCAAGGCT-3' and Reverse: 5'-GTCCACCACTGACACGTTGG-3'¹³. Relative mRNA expression was calculated by the comparative cycle threshold method ($\Delta\Delta C_t$) as outlined in the manufacturer's user manual with GAPDH. The fluorescence was plotted versus PCR cycle number for reaction, and each sample was indicated.

Sample size

Comprehensive sampling was used in this study and it means studying and observing all the studied population in the study setting.

Statistical Analysis

It was performed using an intention to treat and per protocol analysis. Data were computerized and analyzed using Statistical Package for Social Science program (SPSS) version 20. Qualitative data was expressed as number and percentage and Chi square test was used to compare these data. Quantitative data was expressed as

mean \pm SD. Student t- test was used for comparison of means of quantitative variables in the two groups and paired T-test was used for comparing means of quantitative variables in the same group before and after treatment. *P* value less than 0.05 and 0.01 was considered statistically significant and highly significant; respectively.

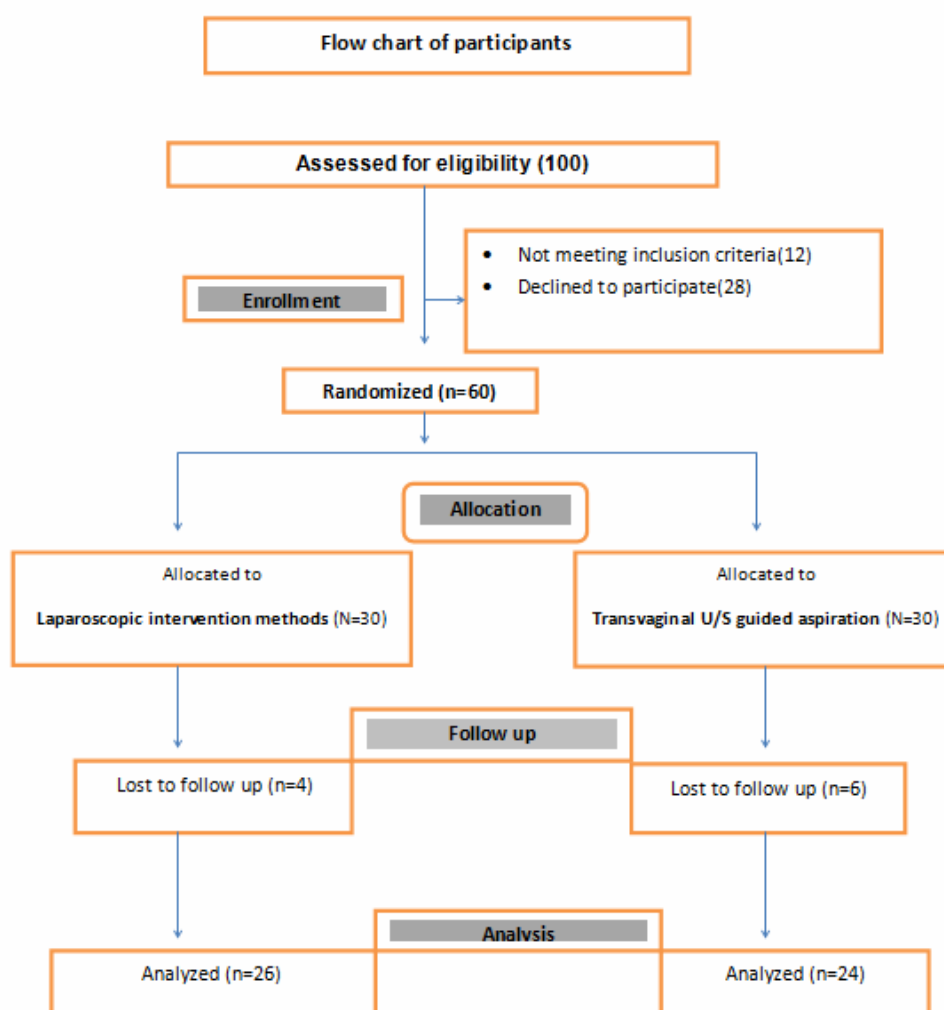
RESULTS

There were no significant differences as regards age, BMI, duration of infertility and basal FSH level between patients undergoing laparoscopic intervention and those undergoing transvaginal ultrasound guided aspiration (table 1). Before treatment of hydrosalpinges patients, they were 60 and after treatment, their number was reduced to 50 as 10 patients were dropped during follow up (**Flow chart**).

In table (2), there is no significant difference in LIF mRNA expression level before treatment between patients undergoing laparoscopic intervention (7.91 ± 0.43) and those undergoing transvaginal ultrasound guided aspiration (6.561 ± 0.23) ($P=0.67$). After treatment, it is higher in patients undergoing laparoscopic intervention (16.22 ± 1.38) than those undergoing ultrasound guided aspiration (14.43 ± 1.836) with a significant difference ($P=0.03$).

In addition, there was an increased LIF mRNA expression levels in patients undergoing laparoscopic intervention before (7.9 ± 0.43) and after (16.22 ± 1.38) treatment with a significant difference ($P=0.005$). Also, there was an increased LIF mRNA expression levels in patients undergoing ultrasound guided aspiration before (6.56 ± 0.23) and after (14.43 ± 1.36) treatment with a significant difference ($P=0.04$).

In table (3), there was an increased clinical pregnancy rate after IVF-ET cycle between patients undergoing laparoscopic intervention (50.0%) and those undergoing ultrasound guided aspiration (33.3%) with a significant difference ($P=0.013$). While the ongoing pregnancy rate in patients undergoing laparoscopic intervention decreased (42.3%) and those undergoing ultrasound guided aspiration (20.8%) with a significant difference ($P=0.005$). The spontaneous abortions rates in patients undergoing laparoscopic intervention was (7.69%) decrease and those undergoing ultrasound guided aspiration was (12.5%) with a significant difference ($P=0.023$).



(1): Flow chart of participants

Table 1: Demographic data of the hydrosalpinges patients

	<i>Laparoscopic group (N=30)</i>	<i>U/S guided aspiration group (N=30)</i>	P value
Age (yrs)	29.15±3.57	27.65± 3.55	0.1081
BMI (Kg/m ²)	24.6± 2.05	24.2± 1.91	0.4385
Duration of infertility(yrs)	3.56 ±1.74	3.34 ±1.75	0.6272
Basal FSH(IU/L)	5.37 ± 1.98	5.06 ± 1.69	0.5168

NS: *P value* >0.05

Table 2: LIF mRNA expression level in hydrosalpinges patients before and after treatment using two methods

	<i>Laparoscopic group (N=30)</i>	<i>U/S guided aspiration group (N=30)</i>	P value
Before treatment	(N=30)	(N=30)	
Mean±SD	7.91±0.43	6.561±0.23	0.67 ^a
After treatment	(N=26)	(N=24)	
Mean±SD	16.22±1.38 ^c	14.43±1.36 ^d	0.03 ^b

^a*P value*: Laparoscopic versus U/S guided aspiration group before treatment

^b*P value*: : Laparoscopic versus U/S guided aspiration after treatment

^c*P value*: Laparoscopic group before versus after treatment (*P*=0.005).

^d*P value*: : U/S guided aspiration group before versus after treatment (*P*=0.04).

Table 3: Reproductive outcome after one cycle of IVF-ET treatment in both studied groups

	<i>Laparoscopic group (N=26)</i>	<i>U/S guided aspiration group (N=24)</i>	P value
Clinical pregnancy rate	13 (50%)	8 (33.3%)	0.013 ^a
Ongoing pregnancy rate	11 (42.3%)	5 (20.8%)	0.005 ^b
Spontaneous abortions	2 (7.69%)	3 (12.5%)	0.023 ^c

^aP value: Laparoscopic versus U/S guided aspiration group as regards clinical pregnancy rate

^bP value: : Laparoscopic versus U/S guided aspiration after treatment as regards ongoing pregnancy rate

^cP value: Laparoscopic versus U/S guided aspiration after treatment as regards spontaneous abortions

DISCUSSION

Leukemia inhibitory factor (LIF) expression is markedly increased in the endometrial epithelial cells during blastocyst implantation; middle and late secretory phase and early pregnancy¹⁴. Its expression in the endometrial secretory phase was 22-fold higher than that of the proliferative phase in females with a past history of pregnancy¹⁵.

Low endometrial concentrations of LIF protein during the window of implantation are associated with unexplained infertility and a high risk of implantation failure after IVF-ET¹⁶. Treatment with recombinant human LIF prior to IVF-ET has been shown to improve pregnancy rates¹⁷.

Several studies have showed that salpingectomy for hydrosalpinx before IVF therapy results in increased pregnancy rates. However, it requires hospitalization and use of general anesthesia. In contrast, transvaginal ultrasound-guided aspiration of hydrosalpinx fluid is less invasive, inexpensive, simple, and does not require hospitalization. However, it has been associated with an increase in the recurrence rate¹⁸.

In this work, after treatment, LIF mRNA expression level was higher in patients undergoing laparoscopic intervention than those undergoing ultrasound guided aspiration with a significant difference. In addition, there is an increased LIF mRNA expression levels in patients undergoing both techniques before and after treatment with a significant difference.

This is in agreement with the findings of Seli *et al*¹⁹ who found that its expression during the implantation window was increased after laparoscopic salpingectomy.

In this study, the clinical pregnancy and ongoing pregnancy rates per IVF-ET cycle were higher in the laparoscopic group compared with the ultrasound guided aspiration group (50% vs. 33.3% and 42.3% vs. 20.8%; respectively) with statistically significant difference. On the other hand, spontaneous abortions rates were lower in the laparoscopic group compared with the ultrasound guided aspiration group with statistically significant difference (15.3% vs 37.5% respectively).

Fouda *et al*²⁰ showed that the implantation rate was higher in the salpingectomy group compared with the

ultrasound guided aspiration group (40% vs. 27.5% and 18.95% vs. 12.82%, respectively). However, these differences failed to reach a statistical significance. It might be attributed to that rapid re-accumulation of the hydrosalpinx fluid which has a negative impact on implantation and pregnancy rates.

Also, a small retrospective study revealed that the aspiration of hydrosalpinx fluid improved the outcomes of IVF-ET²¹.

In contrast, a study by Na *et al.*²² showed that the clinical pregnancy rate was comparable between ultrasound guided aspiration of hydrosalpinx fluid and sclerotherapy compared with laparoscopic salpingectomy in the management of patients with hydrosalpinx undergoing IVF-ET (38% vs. 40%). In addition, a retrospective study by Sowter *et al.* concluded that the drainage of hydrosalpinx fluid had no beneficial effect on the outcome of IVF-ET²³.

In conclusion, this work revealed that hydrosalpinx reduced endometrial LIF mRNA expression level and compromised the endometrial receptivity. On the other hand, treatment either with laparoscopic intervention or transvaginal ultrasound guided aspiration of bilateral hydrosalpinges increases endometrial mRNA LIF level and improve pregnancy outcome after IVF-ET cycle. Also, laparoscopic treatment is associated with decreased spontaneous abortions after IVF-ET cycle. However, prospective, randomized controlled studies with large sample sizes would be required to confirm our findings.

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