

Assessing Cytokine Levels of Infertile Patients after Induced Endometrial Scratching

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Abstract: Background: Both infertility and subfertility are defined as the inability to conceive after a certain period of time (the length of which vary), so often the two terms overlap. Cytokines are important in health and disease, specifically in host responses to infection, immune responses, inflammation, trauma, sepsis, cancer, and reproduction, these have been shown to act both in an autocrine and paracrine manner to regulate preimplantation embryo development and migration which is necessary for placental development. When local injury-induced in endometrial tissue this enhance an inflammatory reaction which is useful for favors implantation of embryo. Natural killer cells, macrophages, and dendritic cells are recruited to the injured site and increased quantities of cytokines, growth factors, and chemokines

Objectives: To assess the cytokine levels (IL6 & TNF- α) both in Serum & Endometrial secretions of infertile patients after induced Endometrial scratching.

Patients & Methods: The study was conducted in the Al-Alwia teaching hospital and a private center of IVF in Baghdad, from February 2016 till November 2017; 102 patients were involved in this study as they had unexplained infertility, then all patients underwent a hysteroscopy and endometrial scratching. Cytokines (TNF- α and IL6) were measured in serum & endometrial secretions, both before and after endometrial scratching.

Results: By measuring two of the most important cytokines (TNF- α and IL-6) in serum and endometrial secretions of patients involved in this study both before and after endometrial scratch, it was found that there was a highly significant difference in serum and endometrial TNF- α and IL-6 after scratching (both increased significantly).

Conclusion: Endometrial scratching causes a significant increment in concentrations of TNF- α and IL-6 both in serum & Endometrial secretions. Higher levels of TNF- α and IL-6 were associated with better pregnancy outcomes.

Keywords: endometrial scratching, Interleukin 6, Tumor necrosis factor, endometrial secretions, cytokines.

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I. Introduction

With the expansion of infertility interventions worldwide, including lower resource settings, the importance and value of a common nomenclature is critical, the close one is infertility is "a disease of the reproductive system defined by the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse (1). Both infertility and subfertility are defined as the inability to conceive after a certain period of time (the length of which vary), so often the two terms overlap (2). Infertility may occur at the level of pituitary or hypothalamus as well as at the level of the ovary. If there is amenorrhoea it should be investigated as such and oligomenorrhoea along similar lines (3). A hysteroscope is an endoscope that carries optical and light channels or fibers. It is introduced in a sheath that provides an inflow and outflow channel for insufflations of the uterine cavity. In addition, an operative channel may be present to introduce scissors, graspers or biopsy instruments (4). Cytokines (cyto, from Greek "cell" + kines, from Greek "kinisi" movement") are a broad and loose category of small proteins {~5–20 kDa (kilodalton)} that are important in cell signaling. Their release has an effect on the behavior of cells around them. It can be said that cytokines are involved in autocrine signaling (Cell signaling is part of a complex system of communication that governs basic activities of cells and coordinates cell actions, paracrine signaling and endocrine signaling as immunomodulation agents. Their definite distinction from hormones is still part of ongoing researches (5). They are important in health and disease, specifically in host responses to infection, immune responses, inflammation, trauma, sepsis, cancer, and reproduction, these have been shown to act both in an autocrine and paracrine manner to regulate preimplantation embryo development and migration which is necessary for placental development (6). IL-6 also plays a role in fighting infection and human endometrium expressed IL-6 at maximum concentrations in the mid- to late secretory phases, indicating a role in the regulation of endometrial function and in implantation (7).

Tumour necrosis factor- α (TNF- α) is known to be one of the most versatile cytokines. It serves as a normal mediator of tissue homeostasis, it has pathophysiological effects at high concentrations and it is expressed in various tissues. In the reproductive tract, TNF- α was detected in the ovaries, the oviduct, in preimplantation embryos and the endometrium. Tumor Necrosis Factor-alpha (TNF- α) plays an important role in the cyclic changes of the endometrium, regulated by the modulation of the different cell types- (8). However, increased expression of this cytokine can also cause pathophysiological effects reflected by its involvement in implantation failure, immunologically-mediated abortion and endometriosis (9). When local injury-induced in endometrial tissue this enhances an inflammatory reaction which is useful for favoring implantation of embryo. Natural killer cells, macrophages, and dendritic cells are recruited to the injured site and increased quantities of cytokines, growth factors, and chemokines (Chemokines mean movement they are a family of small cytokines, or signaling proteins secreted by cells) all are secreted, thus resulting in successful implantation (10). Endometrial injury in preceding cycle improves the implantation rate and live birth rate and reduces the miscarriage rate per clinical pregnancy in patients undergoing their first IVF/ICSI cycle. (10) Objectives: To assess the cytokine levels (IL6 & TNF-a) both in Serum & Endometrial secretions of infertile patients after induced Endometrial scratching.

II. Patients & Methods:

The study was conducted in the Al-Alwia teaching hospital and a private center of IVF in Baghdad, from February 2016 till November 2017; 102 patients were involved in this study as they had unexplained infertility, then all patients underwent hysteroscopy and endometrial scratching. Cytokines (TNF-a and IL6) were measured in serum & endometrial secretions, both before and after endometrial scratching. Patients' and their husbands' oral and informed consent were obtained regarding their participation in the study, we tried different methods of conception for infertile couples and they were followed up to know the outcome of conception. **Inclusion criteria:** Those with infertility more than 1 year. Types of infertility were either unexplained primary or secondary infertility. Age under 48 years. Body mass index from 19-35 kg/m². Normal basal hormonal study. Regular menstrual cycle. **Exclusion criteria:** Patients with hormonal disturbance. Polycystic ovary syndrome patients. Patient with endocrine disorders. Obvious pathological lesions as fibroid, polyps or congenital anomaly of female genital tract. **The Method of endometrial scratching:** Under general anesthesia, patient lied in lithotomy position, a speculum used so that we can see the neck of the (cervix). Then we passed the hysteroscopic catheter through the cervix into the uterus. This catheter of hysteroscope was connected to a camera and a TV monitor, which showed the inside of the uterus. Some fluid may be pumped into the uterus to make it swell a little, this makes it easier to see the lining of the uterus, when the camera showed no lesion in the uterus like fibroid or polyp we induced scratch that was generated on the posterior endometrium at midline 10-15 mm away from the fundus. After the procedure is completed the hysteroscope is gently removed. This procedure was done **at eighth day of menstrual cycle** (11 and 12) Venous blood samples and Endometrial secretions (to estimate the IL6 and TNF) were taken before endometrial induced injury by hysteroscopy and after 48 hours (from endometrial induced injury by hysteroscopy) and injury was done by an endosampler catheter (MedGyn Addison, IL60101 USA) connected to a 10ml syringe. Sample extraction was performed by manual vacuum application with the syringe. Aspirates were expelled into standard cryogenic tubes and immediately frozen at -80°C until processed (Bio-Cane 34™ Cryogenic Storage System, Thermo Scientific™, USA. Aspirate volumes varied from 5 to 10ml (13).

Ethical approval to perform this study was obtained from local ethical committee. All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent was obtained from all individual participants included in the study.

III. Results:

This study included 102 females suffering from infertility, all undergone endometrial scratching procedure with hysteroscopy, their demographic data were shown in table (1).

Table (1): Demographic data, and baseline hormonal study of patients involved in study

Parameter	Mean \pm SD
Total no. of females involved	102
Age (years)	34.39 \pm 6.15
BMI	25.57 \pm 5.87
Duration of infertility (year)	4.35 \pm 1.82
Baseline hormonal study	
	Mean \pm SD
FSH	5.35 \pm 3.44
LH	10.10 \pm 6.25
Prolactin	22.85 \pm 13.80

Testosterone	22.84±10.52
E2	38.94±18.68
Progesterone	14.19±5.60
BMI: Body mass index, FSH: follicular stimulating hormone, LH: Luteinizing Hormone, E2: Estradiol	

By measuring two of the most important cytokines(TNF-a and IL-6) in serum and endometrial secretions of patients involved in this study both before and after endometrial scratch, it was found that there was a highly significant difference in serum and endometrial TNF-a and IL-6after scratching (both increased significantly).As shown in table (2), figure (1).

Table (2): Comparison of TNF, and IL6 in serum and endometrial secretions before and after scratch

Variables		Before scratch	After scratch	P-value
		Mean±SD	Mean±SD	
TNF	Serum	3.84±1.62	17.35±8.28	0.000*
	Endometrial secretion	3.81±1.52	22.84±7.58	0.000*
IL6	Serum	4.27±1.96	16.46±3.18	0.000*
	Endometrial secretion	3.86±1.84	22.35±8.26	0.000*

TNF: Tumor necrosis factor, IL6: Interleukine-6.
*: highly significant<0.0001.

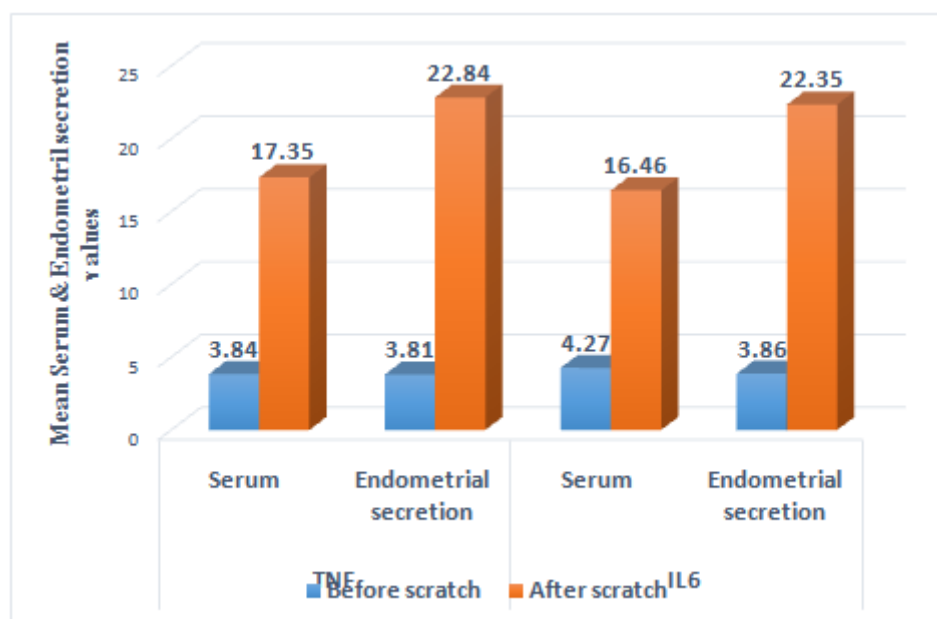


Figure 1:Comparison between serum & endometrial TNF, IL6 levels before & after Scratching

IV. Discussion:

One of the first studies that noted increased rates of implantation, clinical pregnancies, and live births following endometrial injury in women with recurrent implantation failure was published by (14).The mean age in this study was 34.39 ±7.33years, and this was comparable to (15) as the mean age for their study group was 29.4 ± 4.7 years (16) also showed close age group of 31.4 ± 4.0 years, similar to them (17) the mean age of the infertile group enrolled in their study was 32.6±4.5 years, it was shown that all the above mentioned studies had a mean age around 30 years, and it is known that age can affect women's fertility significantly (18). Meanwhile, the mean BMI calculation, revealed that the women enrolled in the study were slightly overweight with a mean BMI of 25.57± 3.88kg/m², which was less than BMI showed by (15), which was 30.0 ± 3.9 kg/m², while lower than our results were shown by (16) with 22.15 ± 2.99 kg/m², this could be explained by the cultural or social habits of each community. (19) stated that obesity affects the response to medication, thus reducing the number of follicles produced, but with adjustment of the dose according to body weight, this fact could be overcome.In this study the mean duration of infertility was 4.35 ±3.82years, and it was comparable to (20), also (15)showed it was 5.2 ± 3.5 years in their intervention group, at 2004; (21), linked the duration of infertility to depression and anxiety, which both can cause infertility, and should be handled efficiently.In the current study there was statistically significant increase in the concentrations of both TNF-aand IL-6 in serum & Endometrial secretions after scratching,same finding was reported by (22), they collected specimens of endometrium on day 21 in women undergone endometrial injury on day 8-13, of the same cycle, and found statistically significant

higher levels of TNF- α mRNA expression in these women compared to the control group, to the best of our knowledge there were no similar published data, but (23) had studied IL-6 immunohistochemistry of endometrial samples before and after scratching, and found that there was an increase in intensity of staining after scratching, and they concluded that IL-6 improved decidualisation and endometrial receptivity, also in vitro endothelial cells proliferation is stimulated by IL-6 and it contributed to regulation of the gestational tissues (24), while TNF- α production was not affected by Leukemia inhibitory factor and IL-6 (25) and (26) showed that serum and decidual TNF- α was abnormally elevated in patients with recurrent abortions, compared to healthy women, even more there is some evidence to support that giving immune therapy to decrease TNF- α /IL-10 ratio may increase success rate of IVF (27), Disturbances in cytokine production at the fetomaternal interface could be a cause of implantation failure. (28) had showed that cytokines increase after endometrial scratching help in preparing the endometrium for embryo implantation; patients selection and sample size might be affecting the results of these studies, there is a need for controlled randomized population based studies to limit this bias.

V. Conclusion:

Endometrial scratching causes a significant increment in concentrations of TNF- α and IL-6 both in serum & Endometrial secretions. Higher levels of TNF- α and IL-6 were associated with better pregnancy outcomes.

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