

Hydrosalpingeal fluid leads to subfertility *via* endometrial TNF- α and IL-7 and NF- κ B signaling pathways

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Abstract. – OBJECTIVE: To determine the concentrations of the nuclear factor kappa B (NF- κ B), tumor necrosis factor-alpha (TNF- α) and interleukin-7 (IL-7) in midluteal phase endometrial samples of infertile patients diagnosed with uni or bilateral hydrosalpinx (HX).

PATIENTS AND METHODS: A total of 24 patients who decided to undergo laparoscopic salpingectomy were included in the study. Salpingectomy indications consisted of patients with a diagnosis of hydrosalpinx (n=12) or ectopic pregnancy (n=12). Twelve healthy patients who underwent Pomeroy-type tubal ligation were considered as the second and healthy control group. The diagnosis of hydrosalpinges was made by transvaginal 2D ultrasonography or HSG. All patients in the hydrosalpinges or ectopic pregnancy group underwent laparoscopic salpingectomy. Just before salpingectomy, endometrial samples were obtained from all patients by Pipelle cannula. Endometrial sampling was performed 7-9 days after the LH surge in the control group. IL-7, NF- κ B and TNF- α concentrations were measured by ELISA method in the endometrial samples of all three groups.

RESULTS: The endometrial IL-7 concentration before salpingectomy of the patients in the hydrosalpinx group was 44.6 ± 6.65 ng/mg wet-tissue. The IL-7 levels of the HX group were significantly higher than those of the patients in the ectopic pregnancy group (19.3 ± 3.06 ng/mg wet tissue versus 44.6 ± 6.65 ng/mg wet tissue, $p<0.04$). Similarly, IL-7 levels of the HX group were significantly higher than those of the tubal ligation group (6.08 ± 1.48 ng/mg wet tissue vs. 44.6 ± 6.65 ng/mg wet tissue, $p<0.03$). The endometrial TNF- α concentration of the patients in the hydrosalpinx group was 33.20 ± 5.40 ng/mg wet-tissue. The TNF- α value detected in the hydrosalpinx group was significantly higher than both the TNF- α value in the ectopic pregnancy group (11.8 ± 1.07 ng/mg wet-tissue vs. 33.20 ± 5.40 ng/mg wet-tissue, $p<0.01$) and the TNF- α value in the tubal ligation group (5.30 ± 1.22 ng/mg wet-tissue vs. 33.20 ± 5.40 ng/mg wet-tissue, $p<0.01$). The pre-salpingecto-

my endometrial NF- κ B concentration of the patients in the hydrosalpinx group was 6.38 ± 1.40 ng/mg wet-tissue. This value is higher than endometrial NF- κ B levels in the ectopic pregnancy group (3.67 ± 0.41 ng/mg wet-tissue vs. 6.38 ± 1.40 ng/mg wet-tissue, $p<0.02$) and NF- κ B levels in the tubal ligation group (1.07 ± 0.38 ng/mg wet-tissue vs. 6.38 ± 1.40 ng/mg wet-tissue, $p<0.01$).

CONCLUSIONS: The presence of hydrosalpinx prevents successful implantation by increasing the levels of endometrial proinflammatory cytokines TNF- α , IL-7 and NF- κ B.

Key Words:

Hydrosalpinx, Ectopic pregnancy, IL-7, NF- κ B, TNF- α .

Introduction

Tubal factor infertility mainly causes fertilization defect by preventing sperm-oocyte interaction and is one of the main reasons for the emergence of IVF treatment. Tubal diseases are responsible for approximately 20% of the cases in the infertile population¹. Hydrosalpinx, which is an inflammatory pathology with multifactorial etiology of the fallopian tubes, occurs as a result of the accumulation of fluid with altered chemical properties by occlusion of the distal tubes. This serious disease reduces implantation rates as well as fertilization defects. Hydrosalpinges may impair endometrial receptivity through different mechanisms. In the presence of hydrosalpinx, the fluid accumulated in the Fallopian tubes may be alkaline or toxic, or it may be infected with various microorganisms^{1,2}. Reaching the endometrium through the tubal ostia of the chemically altered fluid can lead to both receptivity defect and decidualization failure. The flow rate and amount

of the fluid into the cavity may prevent its attachment by creating a mechanical effect on the embryo at the implantation stage³.

Hydrosalpinges can cause receptivity defects by disrupting the expression of endometrial receptivity genes or by increasing the production of inflammatory cytokines in the endometrial microenvironment^{2,3}. Although the endometrium of patients with hydrosalpinges shows normal histological maturation, they do not show integrin expression and most of the cases have out-of-phase histology. Since most cases of hydrosalpinx do not show integrin expression and have out-of-phase histology, the disease is considered to cause both type I and type II receptivity defects. It has been reported⁴ that receptivity defects returned to normal in endometrial biopsies performed after salpingectomy. It has also been shown² that NF- κ B expression, a dimer that triggers inflammation, is increased in both luminal and glandular cells of women with hydrosalpinx. Similarly, hydrosalpinx fluid has been shown⁵ to reduce the expression of HOXA10, the transcription factor responsible for receptivity. Similarly, it has been known³ for a long time that leukemia inhibitory factor (LIF), another cytokine involved in implantation, shows decreased expression in hydrosalpinx. Since salpingectomy largely restores the endometrial receptivity defects mentioned above, the idea of removing ultrasonographically visible hydrosalpinges before IVF has gained attention. However, in cases of hydrosalpinges with preserved mucosa, reconstructive surgery can be tried⁶. While the clinical pregnancy rate with salpingectomy in visible hydrosalpinges was 36.6%, this rate was reported as 23.9% in cases without any intervention. In cases of visible bilateral hydrosalpinges on ultrasound, salpingectomy increased birth rates approximately 3.5 times⁷. Recent Cochrane review¹ suggested that clinical pregnancy rates were significantly higher in patients who had a pre-IVF salpingectomy compared to those who did not have a surgical procedure. However, it was stated that this result obtained from 11 randomized controlled trials was moderate quality.

Although hydrosalpinx is presented as an inflammatory disease, no comprehensive study has been conducted on the effect of the disease on inflammatory pathways in the endometrium². Since the coordinated relationship between NF- κ B and COX-2 is important in maintaining healthy decidualization, it has been suggested⁸ to investigate the role of inflammatory molecules in implantation. In addition to NF- κ B expression, TNF- α

and IL-17 released from endometrial stromal cells create an inflammatory microenvironment to increase trophoblast invasion and receptivity^{9,10}. There are not enough studies on how these proinflammatory cytokines are expressed in the endometrium of patients with hydrosalpinges. This study was designed to detect the changes in NF- κ B, TNF- α and IL-7 levels in midluteal phase endometrial samples of patients diagnosed with uni- or bilateral hydrosalpinges.

Patients and Methods

A total of 24 patients who decided to undergo laparoscopic salpingectomy were included in the study. Participants in the salpingectomy group consisted of patients with a diagnosis of hydrosalpinx (n=12) or ectopic pregnancy (n=12). All hydrosalpinx cases were infertile. The patients in the ectopic pregnancy group consisted of fertile (n=5) or infertile (n=7) cases. With the ectopic pregnancy control group, we had the opportunity to detect the changes in the endometrium caused by non-hydrosalpinx tubal pathologies and to compare them with the study group. Twelve healthy patients who underwent Pomeroy type tubal ligation were considered as the second control group. The diagnosis of unilateral or bilateral hydrosalpinges was made by transvaginal 2D ultrasonography or HSG. All women in hydrosalpinx group underwent laparoscopic salpingectomy. The patients whose diagnosis of hydrosalpinges was confirmed during laparoscopy remained in the study, while the patients whose diagnosis of hydrosalpinx was not confirmed were excluded from the study. Endometrial sampling was performed with a pipelle cannula from all hydrosalpinx patients just before salpingectomy. Endometrial sampling was performed 7-9 days after the LH surge in the mid-luteal phase from the control group. Since ectopic pregnancy is a clinical emergency, salpingectomy and endometrial sampling were performed regardless of the cycle phase. The endometrial samples were divided into two groups. The first group of samples were fixed with formalin and embedded in paraffin. These blocks were used for endometrial dating¹¹, for which the Noyes criteria were considered. A difference of three days or more between chronological and histological days was considered out-of-date histology¹². The second samples were washed twice with sterile saline solution to remove blood, clots and debris. Cleaned samples were placed in RNA later and stored at -20°C until analysis.

Patients with a diagnosis of endometrioma, endometriosis, adenomyosis, polycystic ovary syndrome, submucous uterine fibroids and endometrial polyps that may cause tubal or endometrial inflammatory pathology were not included in the study. Those with a history of Asherman syndrome, those with mechanical endometrial injury, and individuals with systemic inflammatory disease were excluded from the study. Those with a history of previous abdomino-pelvic surgery and those whose hydrosalpinx diagnosis was not confirmed during laparoscopy were also excluded from the study. Patients with male factor infertility and those who had previously undergone dilatation and curettage were also excluded from the study. The study was initiated in compliance with the Declaration of Helsinki and after obtaining patient consent.

Measurement of IL-7, TNF- α and NF- κ B Concentration in Endometrial Supernatants

Each sample was washed with phosphate-buffered saline (PBS) to remove blood and debris from them. The wet weights of the samples were then measured and recorded. All biopsy specimens were homogenized using a TissueLyser (Qiagen, Hilden, Germany) and 1 mL of PBS. The samples, which were kept overnight at -20°C , were then thawed and frozen twice to break the integrity of the cell membrane. The obtained homogenates were centrifuged at 4,000 rpm for 10 minutes and the supernatants formed were frozen and kept until the analysis period. TNF- α , IL-7 and NF- κ B concentrations in endometrium supernatants were measured by enzyme-linked immunosorbent assay (ELISA) using human immunoassay kits (Sunred Biotechnology Company, Shanghai, China). These kits are used to measure the NF- κ B, IL-7 and TNF- α concentrations in homogenates and cell culture supernatants as well as biological fluids. The detection range of the NF- κ B kit (assay range) was 0.15 to 40 ng/mL and the minimum measurable level (sensitivity) was 0.146 ng/mL. The intra- and inter-assay coefficients of variation were $<10\%$ and $<12\%$, respectively. The detection range of the TNF- α kit was 3 to 900 ng/L and the minimum measurable level was 2.82 ng/L. The intra- and inter-assay coefficients of variation were $<10\%$ and $<12\%$, respectively. The detection range of the IL-7 kit was 0.8 to 200 ng/L and the minimum measurable level was 0.716 ng/L. The intra- and inter-assay coefficients of variation were $<10\%$ and $<12\%$, re-

spectively. The results of IL-7, TNF α and NF- κ B concentrations detected by ELISA kit were divided by the wet weight of endometrial tissue. The results were presented as ng/mg wet-tissue for IL-7, TNF α and NF- κ B.

Statistical Analysis

Statistical analysis was performed by the use of Statistical Package for the Social Sciences for Windows version 21 (IBM Corp., Armonk, NY, USA). Normality of the distribution was assessed by using Kolmogorov Smirnov test. While Student's *t*-test was used for normally distributed variables and Mann-Whitney test for abnormal variables. Results were presented as mean \pm standard deviation. Categorical variables were expressed in counts (percentages). A *p*-value <0.05 was accepted as statistically significant.

Results

Age and body mass index (BMI) values of the patients in the hydrosalpinx group were significantly lower than those in the ectopic pregnancy and tubal ligation group. Uni or bilateral salpingectomy was successfully performed in both the hydrosalpinx group and the ectopic pregnancy group. Unilateral salpingectomy was performed in all women with ectopic pregnancy. Eight of the hydrosalpinx cases went to unilateral and 4 of them went to bilateral salpingectomy. Pomeroy style tubal ligation was performed in all patients in the control group. Endometrial sampling was successfully performed with a pipelle cannula under anesthesia from all patients just before the laparoscopy (Table I).

The endometrial IL-7 concentration before salpingectomy of the patients in the hydrosalpinx group was 44.6 ± 6.65 ng/mg wet-tissue (Table II). The IL-7 levels of the HX group were significantly higher than the patients in the ectopic pregnancy group (19.3 ± 3.06 ng/mg wet tissue vs. 44.6 ± 6.65 ng/mg wet tissue, $p < 0.04$). Similarly, IL-7 levels of the HX group were significantly higher than the tubal ligation group (6.08 ± 1.48 ng/mg wet tissue vs. 44.6 ± 6.65 ng/mg wet tissue, $p < 0.03$). The patients in the ectopic pregnancy group had significantly higher endometrial IL-7 levels before salpingectomy compared to the control group (19.3 ± 3.06 ng/mg wet-tissue vs. 6.08 ± 1.48 ng/mg wet-tissue, $p < 0.02$). The endometrial TNF- α concentration of the patients in the hydrosalpinx group was 33.20 ± 5.40 ng/mg wet-tissue. The

Table I. Demographic characteristics of each group.

	Hydrosalpinx (n=12)	Ectopic pregnancy (n=12)	Tubal ligation (n=12)
Age (years)	24.3±2.04*	26.9±5.07	32.1±8.30
BMI (kg/m ²)	24.9±5.22*	26.8±9.23	27.1±10.3
Infertile/fertile, n (%)	12 (100%)/0	7 (58.3%)/5 (41.6%)	0/12 (100%)
Unilateral/bilateral salpingectomy, n (%)	8 (66.6%)/4 (33.3%)	12 (100%)/0	12 (100%)/0

*: $p < 0.05$ compared to ectopic pregnancy or tubal ligation groups

Table II. Comparison of endometrial IL-7, TNF- α , NF- κ B concentrations in women with hydrosalpinges and control groups.

	IL-7 (ng/mg- wet tissue)	TNF- α (ng/mg- wet tissue)	NF- κ B (ng/mg- wet tissue)
I- Salpingectomy due to hydrosalpinges (n=12)	44.6±6.65	33.20±5.40	6.38±1.40
II- Salpingectomy due to ectopic pregnancy (n=12)	19.3±3.06	11.8±1.07	3.67±0.41
III- Fallopiian Tubal Ligation (n=12)*	6.08±1.48	5.30±1.22	1.07±0.38
<i>p</i> -values			
I vs. II	0.04	0.01	0.02
I vs. III	0.03	0.01	0.01
II vs. III	0.02	0.02	0.03

*: Pomeroy type.

TNF- α value detected in the hydrosalpinx group was significantly higher than both the TNF- α value in the ectopic pregnancy group (11.8±1.07 ng/mg wet-tissue vs. 33.20±5.40 ng/mg wet-tissue, $p < 0.01$) and the TNF- α value in the tubal ligation group (5.30±1.22 ng/mg wet-tissue vs. 33.20±5.40 ng/mg wet-tissue, $p < 0.01$). Before salpingectomy, endometrial TNF- α levels of the patients in the ectopic pregnancy group were significantly higher than the control group (11.8±1.07 ng/mg wet-tissue vs. 5.30±1.22 ng/mg wet-tissue, $p < 0.02$).

The pre-salpingectomy endometrial NF- κ B concentration of the patients in the hydrosalpinx group was 6.38±1.40 ng/mg wet-tissue. This value is higher than endometrial NF- κ B levels in the ectopic pregnancy group (3.67±0.41 ng/mg wet-tissue vs. 6.38±1.40 ng/mg wet-tissue, $p < 0.02$) and NF- κ B levels in the tubal ligation group (1.07±0.38 ng/mg wet-tissue vs. 6.38±1.40 ng/mg wet-tissue, $p < 0.01$). The patients in the ectopic pregnancy group had significantly higher endometrial NF- κ B levels before salpingectomy compared to the control group (3.67±0.41 ng/mg wet-tissue vs. 1.07±0.38 ng/mg wet-tissue, $p < 0.03$). ELISA test results did not change depending on whether the hydrosalpinx was uni or bilateral.

Discussion

While hydrosalpingeal fluid has a toxic effect on the gametes and embryos in the tuba in natural cycles, it may damage the embryo development in early embryogenesis by disrupting the endometrial microenvironment in IVF cycles¹. Hydrosalpingeal fluid disrupts implantation by inhibiting many physiological processes that try to make the endometrial microenvironment suitable for embryo implantation^{13,14}. Since the hydrosalpingeal fluid is rich in inflammatory cells and its intense proinflammatory cytokine content will impair the decidualization ability of endometrial cells, it prevents the formation of a receptive endometrium^{2,3,5}. Injecting embryo culture supernatant into the cavity prior to salpingectomy or embryo transfer neither increases nor adversely affects clinical pregnancy rates¹⁵. Heterotopic pregnancy cases in which intrauterine and extrauterine pregnancy occur simultaneously should be considered in the differential diagnosis of hydrosalpinx cases. Early diagnosis may be helpful in preserving intrauterine pregnancy¹⁶.

The presence of hydrosalpinx creates an inflammatory microenvironment in the endometri-

um, preventing both decidualization and successful implantation. In a recent study², it was shown that hydrosalpingeal fluid initiates inflammatory reactions by increasing endometrial NF- κ B synthesis, and salpingectomy returns the endometrium to its physiological conditions. In the present study, we found that the endometrial NF- κ B levels in the hydrosalpinx group were approximately twice as high as in the ectopic pregnancy group and six times higher than in the control group. It is quite reasonable to directly attribute this increase in endometrial NF- κ B levels to the presence of hydrosalpinx. Despite the intra-tubal conception product, endometrial NF- κ B levels were not as high as hydrosalpinx in the ectopic pregnancy group. The endometrial NF- κ B levels of the ectopic pregnancy group were higher than the control group and lower than the hydrosalpinx group. This finding is evidence that hydrosalpinx is a unique disease. Many of intratubal pathologies have not the same clinical findings as hydrosalpinx. Hydrosalpingeal fluid contributes to the proinflammatory environment in the endometrium. Increased endometrial NF- κ B levels in cases of ectopic pregnancy may have developed mostly due to the secondary effects of pregnancy-related hormones on the endometrium. If the conception product located in the tube had a hydrosalpinx-like clinic, endometrial NF- κ B levels would have been similar in both groups. In conclusion, the increase in endometrial NF- κ B expression in the presence of hydrosalpinx is mainly due to the disease-related fluid collection and its flow into the endometrium. Endometrial NF- κ B expression is regulated during the menstrual cycle under the influence of estrogen and progesterone^{2,17,18}. NF- κ B contributes to the development of healthy decidua by working in coordination with prostaglandins and COX-2⁸. Increased endometrial NF- κ B in the presence of hydrosalpinges may lead to impaired decidualization and implantation defect^{17,18}. Freezing of eggs collected before salpingectomy with open or closed vitrification protocol has similar success rates¹⁹.

Inflammatory reaction at the physiological level plays a pivotal role for both decidualization and successful trophoblast invasion. Macrophages and natural killer cells accumulating in the endometrium during the decidualization stage form an inflammatory microenvironment²⁰. TNF- α is one of the proinflammatory cytokines whose expression increases in the endometrium in successful pregnancies²¹. TNF- α plays a role in intercellular communication in the endometri-

um in accordance with the cycle phase. TNF- α , which is weakly expressed in the proliferative phase, exhibits increased expression during the secretory phase. If pregnancy occurs, all glandular, stromal, and decidual cells express TNF α intensely²². In our patients with a diagnosis of hydrosalpinx, endometrial TNF- α levels before salpingectomy increased approximately three times that of ectopic pregnancies, and approximately 7 times higher than in the control group. Abnormal increase in endometrial TNF- α levels may be due to hydrosalpingeal fluid reaching the cavity and potentiating the inflammatory process. The increase in endometrial NF- κ B in the presence of hydrosalpinx suggests that alkaline and toxic fluid activates endometrial inflammatory pathways^{2,17,18}. Hydrosalpingeal fluid reaching the cavity may lead to the activation of macrophages and epithelial cells, increasing TNF- α expression²³. Since the expression of macrophages and TNF- α receptors will increase in the presence of endometrial inflammation, an increase in the expression of receptivity genes can be expected²¹. However, the continuous and pathological flow of alkaline fluid may disrupt the positive correlation between TNF- α and receptivity genes, leading to failed implantation and early pregnancy loss²⁰. Consistent with this, You et al⁹ showed that physiological TNF- α expression plays a role in early implantation by regulating trophoblast migration and invasion. When all these data and our results are evaluated together, TNF- α of macrophage or decidual origin is overexpressed in the presence of inflammatory hydrosalpinx, thus impairing trophoblast migration and expression of receptivity genes. Endometrial TNF- α expression above physiological limits can lead to implantation defects or early pregnancy loss.

In addition to NF- κ B and TNF- α expression, endometrial interleukins are also involved in decidualization and trophoblast migration^{24,25}. The significant increase in decidual IL-7 expression in spontaneous and recurrent pregnancy loss is evidence of the importance of this cytokine in implantation. Increasing IL-7 levels create an inflammatory environment, disrupt feto-maternal communication and lead to implantation defects¹⁰. You et al⁹ reported that endometrial TNF- α expression caused IL-17 activation and stimulated trophoblast migration. Wu et al²⁴ showed that the IL-7/IL-7R signaling pathway increases inflammatory Th17 cells and leads to early or recurrent pregnancy losses. We found that IL-7 expression in the endometrium of women with hydrosalpinx was approximately twice as high as

in the ectopic pregnancy group and approximately seven times higher than in healthy controls. This massive increase in IL-7 levels before salpingectomy may be due to hydrosalpingeal fluid reaching the cavity. The potentiation of the inflammatory effect of alkaline hydrosalpingeal fluid in the endometrium may lead to inadequate decidualization and implantation failure.

Conclusions

Despite the small number of participants, this is the first study to show that more than one endometrial inflammatory pathway is activated in women with hydrosalpinx. The presence of two different control groups in our study allowed the results to be interpreted more clearly. It has been suggested that the increase in endometrial NF- κ B in women with hydrosalpinx causes decreased expression of receptivity genes and subfertility. We concluded that the increase in endometrial inflammation in patients with hydrosalpinx is not only due to the increase in NF- κ B, but also due to the increase of other proinflammatory cytokines such as TNF- α and IL-7. Overexpression of IL-7, NF- κ B, and TNF in the presence of hydrosalpinx may induce a proinflammatory environment in the endometrium, resulting in implantation defect. Whether the hydrosalpinx fluid affects oocyte or germ cell quality remains an unanswered question²⁶.

Conflict of Interests

The authors declare that they have no conflict of interests.

Informed Consent

Informed consent was obtained from participants.

Ethics Approval

The authors declare that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008. The Ethical Committee of the Kayseri City Hospital approved the study protocol (2023/786).

Authors' Contributions

Both authors contributed to the study conception and design. Material preparation was performed by Bertan Demir and Seyma Daglituncezdi Cam. The first draft was written by Bertan Demir. The article was edited and finalized by Bertan Demir and Seyma Daglituncezdi Cam. All authors approved the final version of the manuscript.

Funding

None.

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