

Endometrioma Cystic Fluid Aspiration and Retained Ethanol Sclerotherapy might improve the systemic Immune Milieu

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Abstract

Background: Ovarian endometrioma (OE) is in women of reproductive age, so its management must consider the preservation of ovarian reserve.

Objectives: This study investigated the effect of ultrasound-guided transvaginal aspiration and retained ethanol sclerotherapy (AEST) on antral follicular count (AFC) and serum levels of tumor necrosis factor- α (TNF- α) and interleukin (IL)-8 and 10.

Patients and methods: Design: Prospective interventional study. 69 women with OE were evaluated clinically and by transvaginal ultrasonography (TUV). AEST procedure was performed and the collected aspirate and pre-procedural blood samples were collected for estimation of cytokines' levels. At 6-m post-procedure, clinical evaluation and TUV were repeated and serum cytokines' levels were re-estimated.

Results: Post-procedural pain scores and diameter of the cyst remnants were significantly decreased. Cyst remnants were still large in 9 patients (13%). Post-procedural AFC was significantly increased, while serum AMH was non-significantly increased. Cystic aspirate cytokines' levels were significantly higher than the pre-and post-procedural serum levels. Post-procedural serum TNF- α and IL-8 levels were significantly decreased by 30.9% and 34.5%, respectively, while serum IL-10 levels were significantly increased by 160%. Statistical analyses defined high TNF- α and low IL-10 in cystic fluid as the predictors for small AFC, while a post-procedure high percentage of decrease in serum IL-8 as the predictor for the presence of post-procedural ≥ 5 follicles.

Conclusion: AEST improved ovarian reserve and systemic immune milieu and reduced pain severity in patients who had OE. Sclerotherapy spared the need for surgery and resource.

Keywords: Ovarian endometrioma; Ethanol Sclerotherapy; Ovarian reserve; inflammatory cytokines.

DOI: 10.21608/svuijm.2023.185178.1485

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Received: 3 December,2022.

Revised: 20 December,2022.

Accepted: 1 January,2023.

Cite this article as: Ahmed M. Hagras, Maha M. Hagras, Amr Sharafeldein.(2023). Endometrioma Cystic Fluid Aspiration and Retained Ethanol Sclerotherapy might improve the systemic Immune Milieu. *SVU-International Journal of Medical Sciences*. Vol.6, Issue 1, pp: 484- 4 .

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Introduction

Endometriosis (EM) is a common gynecological disorder that affects about 10% of the general female population and 38% of infertile women (Namazi et al., 2021). Endometriosis is often associated with dysmenorrhea, adenomyosis, infertility, and altered endometrial receptivity (Mikhaleva et al., 2021). Pathologically, endometriosis is characterized by the presence of endometrial-like tissue in the extrauterine location especially the ovaries causing the development of ovarian endometrioma (OE) (Karadağ et al., 2020). OE is a cystic lesion of ovaries that originates from endometrial glands and stroma and is identified in 17-44% of patients with endometriosis (Jiang and Nie, 2020).

The OE is commonly seen in women of reproductive age who may wish to preserve their ovarian function (Vignali et al., 2020); however, its management is still challenging due to the lack of correlation between ultrasonographic and serological evaluation data (Muzii et al., 2018), and ovarian reserve (Kuroda et al., 2012) on one-side and the choice of therapeutic modality on the other side (Cosma and Benedetto, 2020).

Medical therapy of symptomatic endometrioma can control pain, but its effect on fertility is questionable (Miller, 2021). Laparoscopic drainage or complete removal of OE is the treatment of choice for associated pain (Muzii et al., 2018), but damage to the ovarian reserve limited surgical intervention for cysts that are unresponsive to medical treatment (Vignali et al., 2020).

Aspiration and sclerotherapy of simple ovarian cysts using variant sclerosing materials were tried in earlier studies that reported no

recurrence (AbdRabbo and Atta, 1995; Kafali et al., 2003). However, in the case of OE, the recurrence rate of 28.9-91.5% was detected after one US-guided aspiration, but can be reduced on repeated aspiration down to 5.4% in the 6th trial (Gonçalves et al., 2016). Another meta-analysis reported a significantly lower recurrence rate after alcohol sclerotherapy with ethanol retention than with ethanol wash (Cohen et al., 2017). The effect of ultrasound-guided transvaginal aspiration of OE cystic fluid and retained ethanol injection on cytokines' serum levels and antral follicular count (AFC) was investigated.

Patients and methods

All patients underwent transvaginal ultrasonography (TVU) to select patients with OE and determine the cyst characters. Collected data included age, pain type, location, and severity as judged by 10-points visual analogue scale (VAS), duration of pain, mode of analgesia used to get pain relief, fertility status, number of living offspring, and other associated clinical manifestations. Then, blood samples were collected from the selected patients for estimation of baseline levels of routine and study investigations.

Design: Multicenter prospective interventional study

Setting: Departments of Obstetrics & Gynecology and Clinical Pathology, Faculty of Medicine, Tanta and Benha Universities

Ethical considerations: The study protocol was approved by the Local Ethical Committee at Tanta Faculty of Medicine by approval number: RC: 35560/6/22 and registered by ClinicalTrials.gov Identifier: NCT05470972.

Exclusion criteria: Women with recurrent OE, a cyst that was suspicious of being malignant, diabetes

mellitus, polycystic ovary syndrome, body mass index (BMI) of >30 kg/m², previous surgical interference that resulted in pelvic adhesions, history of previous (within 12 months) or current abuse of alcohol or drugs were excluded from the study.

Inclusion criteria : Women with OE of a mean diameter of >3 cm, free of exclusion criteria, and signed written fully informed consent to undergo the study protocol were included in the study.

Pre-procedural preparation

All women were asked to follow routine vaginal toilet using povidone-iodine vaginal wash (Betadine Vaginal Wash, Nile Co for Pharmaceutical & Chemical Industries, Alamiria, Cairo, Egypt) and to insert Fluconazole tab 150 mg (Diflucan, Pfizer Co, NY, USA) once daily for two days before the procedure. On arrival at the hospital, antibiotic prophylaxis was provided as ceftriaxone 2gm slowly intravenous (Epicephin, E.P.I.CO, 10th Ramadan City, Egypt)

Aspiration and ethanol sclerotherapy (AEST)

The AEST procedure was performed under intravenous anesthesia as an outpatient surgical procedure. With the patient in lithotomy position, TVU was performed (Hitachi EUB-5500) to localize the endometrioma. The AEST procedure was performed as described previously (Messalli et al., 2003; Yazbeck et al., 2012); briefly as follows: vaginal walls were cleansed using vaginal povidone-iodine, a 17 gauge, 30-cm length needle was inserted through the posterior vaginal fornix into the pouch of Douglas, and the cyst was aspirated till complete disappearance of the cyst on the ultrasound scanner. The collected cystic fluid was collected into a plastic

tube without an anticoagulant. The needle was maintained in its place, the syringe was removed and the cyst was flushed with saline solution until obtaining a clear liquid. Then, 96% ethanol was injected as 60% of the volume of the aspirated fluid to guard against over distension or rupture of the cyst and/or ethanol diffusion into the pelvis. The collected fluid was divided into three sterile tubes for cytological and bacteriological examinations and the study investigations. Patients were allowed to be completely recovered and were discharged home.

Post-procedural care

Patients were prescribed local vaginal wash to be used trice daily and antimicrobial vaginal tablets to be inserted before sleep. Systemic treatment was prescribed as ciprofloxacin 750 mg once daily and diclofenac sodium 50 mg tab twice daily for three days. All patients were asked to attend the outpatient clinic every 14 days to undergo TVU for assessment of the cyst remnant size for three assessments. At the one-month PO visit, blood samples were collected to estimate post-procedural levels of the studied cytokines.

Sample processing

1. The collected cystic fluid was centrifuged at 2000xg for 10 min at 4°C and the supernatant was kept frozen at -20°C within 1 h of collection until the time of analysis.
2. Collected pre and post-procedural blood samples (S1 & S2) were allowed to clot, centrifuged at 1500xg for 15 min and the serum samples were collected in a clean dry Eppendorff tube to be stored at -20°C until being assayed.

Investigations

Sample levels of studied cytokines were estimated for levels of

human TNF- α (Coughlan et al., 2001), IL-8 (Spasova et al., 2005) and IL-10 (Poll, 1996) using ELISA kits (Abcam Inc., San Francisco, USA; catalog no. ab46087, ab214030 & 46087, respectively)

Outcomes

1. The primary outcome was the effect of AEST procedures on patients' clinical, ultrasonographic, and laboratory data.
2. Secondary outcomes
 - a. The relation between cystic aspirate and serum levels of the studied cytokines.
 - b. The relation between the change of serum cytokines' levels and changes in AFC at 6-m after the procedure.

Sample size

According to the results of a meta-analysis, the lowest recurrence rates of OE on aspiration alone or with sclerotherapy were 29.8% and 13.3%, respectively, (Gonçalves et al., 2016).

The current study supposed a recurrence rate of <10%, so 68 women were studied to give the study a power of 80% with α value of 0.05 and β value of 0.2.

Statistical analysis

Results were analyzed using One-way ANOVA, paired-t and Chi-square (X² test) tests using IBM® SPSS® Software (Version 22, 2015; Armonk, USA). The performance of the studied parameters for prediction of outcome studied using the ROC curve and Regression analyses. P-value at the cutoff point of <0.5 indicates significance of the value.

Results

The study included 76 women with OE, 7 women were excluded and 69 women were enrolled in the study. Clinical data of the enrolled women were shown in (Table.1).

Table 1. Clinical data of enrolled women

Data		Findings
Age (years)		32±3.6
Body mass index (kg/m ²)		30.5±1.8
Family history of endometriosis		14 (20.3%)
Infertility		80 (72.5%)
Pelvi-abdominal tenderness		48 (69.6%)
Pain	Frequency	69 (100%)
	Score	7.2±3.2
Duration (years)		5.5±3.1
Hematochezia		5 (7.2%)
Hematuria		2 (2.9%)
Serum AMH (ng/ml)		3.58±1.34
Antral follicle count		5.5±2.1
Cyst's greatest diameter (mm)		46.9±8.8
Cyst calculated volume (cc)		29.5±5.5

Mean procedural duration was 21.2±3.3 min and the mean volume of the injected ethanol was 17.7±3.3 ml. During the follow-up period, pain severity gradually decreased and at the 6th month after the procedure, the

frequency of patients still had pain and mean values of pain score; total and according to the type of pain were significantly lower with significant reduction of the use of analgesia than pre-procedural scores. Moreover, the

frequency of the associated clinical manifestations was significantly reduced. Objectively, despite of the significant reduction of the mean diameter of the cyst remnants compared to pre-procedural diameter,

9 patients still having large cysts. Further, post-procedural AFC was significantly increased than pre-procedural count by 27.4% ($\pm 14.9\%$), while serum AMH levels were non-significantly increased (**Table 2**).

Table 2. Follow-up clinical and laboratory data determined at 6-m after the procedure

Data		Pre-procedural	6-m post-procedural
Pain scores	Dysmenorrhea	4.7 \pm 1.4	1.6 \pm 2
	Dyspareunia	1.8 \pm 1.2	0.3 \pm 0.6
	NMPP	0.7 \pm 0.8	0.2 \pm 0.4
	Total	7.3 \pm 3.2	2.1 \pm 2.6
Frequency of pain according types	Dysmenorrhea	69 (100%)	21 (30.4%)
	Dyspareunia	59 (85.5%)	15 (21.7%)
	NMPP	39 (56.5%)	16 (23.2%)
Analgesia	No	0	39 (56.5%)
	Oral NSAIDs	8 (11.6%)	17 (24.6%)
	Injectable NSAIDs	52 (75.4%)	11 (16%)
	Others	9 (13%)	2 (2.9%)
Associated manifestations	No	13 (18.8%)	50 (72.5%)
	Pelvic tenderness	32 (46.4%)	15 (21.7%)
	Abdominal tenderness	17 (24.6%)	3 (4.3%)
	Hematuria	2 (2.9%)	0
	Bleeding per rectum	5 (7.3%)	1 (1.5%)
Greatest cyst diameter (cm)	Total patients (n=69)	4.7 \pm 0.9	1.9 \pm 0.5
	Recurrent cysts (n=9)	4.6 \pm 1	2.8 \pm 0.4
AFC		5.5 \pm 2.2	6.9 \pm 2.6
Serum AMH (ng/ml)		3.12 \pm 1.4	3.58 \pm 1.3

Cystic aspirate levels of estimated cytokines were significantly higher than the pre-procedural serum levels. Post-procedural serum TNF- α and IL-8 levels were significantly decreased in comparison to pre-procedural serum levels by 30.9%

($\pm 14\%$) and 34.5% (10.9%), respectively. On contrary, post-procedural serum IL-10 levels were significantly increased in comparison to pre-procedural levels by 160% ($\pm 77\%$) as shown in (**Table 3**).

Table 3. Follow-up laboratory data determined at 6-m after the procedure

Material Cytokines	Cystic aspirate	Serum		P-value
		Pre-procedural	6-m post-procedural	
TNF- α (pg/ml)	5.22 \pm 4.19	1.19 \pm 0.37	0.8 \pm 0.21	<0.001
	P1 value <0.001			
IL-8 (pg/ml)	32.8 \pm 20.5	5.07 \pm 1.2	3.28 \pm 0.8	<0.001
	P1 value <0.001			
IL-10 (pg/ml)	3 \pm 1.1	1.76 \pm 0.62	4.23 \pm 1	<0.001
	P1 value <0.001			

Pre-procedural AFC is negatively correlated with cystic aspirate levels of TNF- α and IL-8, while is positively correlated with cystic aspirate levels of IL-10. The percentage of change in post-procedural AFC concerning the pre-

procedural count showed a positive correlation with the percentage of change in post-procedural serum cytokines' levels, but the correlation was significant with TNF- α and IL-10 as shown in (Table 4).

Table 4. Correlation analysis of laboratory data in relation to AFC

Dependent variable	Independent variables	Pearson's correlation coefficient	P-value
Pre-procedural AFC	Cystic aspirate TNF- α level	-0.445	<0.001
	Cystic aspirate IL-8 level	-0.279	0.019
	Cystic aspirate IL-10 level	0.281	0.020
	Serum AMH level	0.069	0.573
Post-procedural % of change in AFC	% of change of serum TNF- α	0.348	0.003
	% of change of serum IL-8	0.222	0.066
	% of change of serum IL-10	0.271	0.024
	% of change of serum AMH	0.123	0.315

Regression analysis of the cytokines' levels estimated in cystic aspirate as predictors for AFC defined high TNF- α (β = 0.568, p <0.001) and low IL-10 (β = 0.517, p <0.001) as the predictors for a small number. For prediction of the extent of increase in AFC after AEST, Regression analysis defined a higher percentage of

decreased serum TNF- α as the significant predictor (β = 0.348, p =0.003). Prediction of the minimal AFC, ROC curve analysis high TNF- α (β = 0.568, p <0.001) and low IL-10 (β = 0.517, p <0.001) in cystic aspirate as the predictors for the presence of ≤ 3 follicles, while at 6-m post-procedure high percentage of decrease in serum

IL-8 as the predictor for the presence of ≥ 5 follicles (Table 5).

Table 5. ROC curve analysis of laboratory data for prediction of pre-procedural AFC and its change at 6-m after the procedure

Dependent variables	Independent variables		AUC (SE)	P	95 CI
Pre-procedural AFC	Cystic aspirate level of	TNF- α	0.788 (0.076)	0.002	0.639-0.937
		IL-8	0.427 (0.075)	0.429	0.281-0.573
		IL-10	0.729 (0.088)	0.013	0.557-0.901
	Serum AMH level		0.398 (0.098)	0.271	0.207-0.590
Post-procedural % of change in AFC	% of change of serum of	TNF- α	0.642 (0.096)	0.222	0.453-0.831
		IL-8	0.772 (0.071)	0.019	0.632-0.912
		IL-10	0.594 (0.096)	0.415	0.407-0.782
		AMH	0.461 (0.087)	0.735	0.291-0.630

Discussion

Aspiration of OE cystic fluid and retained intra-cystic ethanol injection significantly reduced pain severity and the need for analgesia. The procedural duration allowed it to be applied as an outpatient procedure and thus spared the need for surgery with cost reduction to a minimum. Despite the insignificant increase in serum AMH, the AFC was increased significantly and this might increase the chances of pregnancy. Post-procedural TVU detected large cyst remnant in 9 cases (13%) and these cases could be due either to insufficient aspiration or recurrence of the cyst.

In hand with the obtained results, recent comparative studies between OE sclerotherapy and surgery detected well-preserved ovarian function with a shorter hospital stay, no complications, and non-significant change in serum AMH levels with sclerotherapy, while postoperative

serum AMH levels were decreased (Koo et al., 2021; Martinez-Garcia et al., 2021; Antonaci et al., 2022). Additionally, one study detected significant increases in AFC after sclerotherapy than after surgery (Martinez-Garcia et al., 2021). A recent meta-analysis concluded that OE sclerotherapy might be safe and effective management procedure with special regard to pain resolution, recurrence, and pregnancy rate (Kim et al., 2022).

The reported diminution of the size of OE could be attributed to the previously documented, using experimental animals, efficacy of 95% ethanol for induction of shrinkage of endometrial glands, incomplete epithelial and glandular epithelial cell structure, and formation of fibrotic hyperplasia due to induction of collagen I expression (Sun et al., 2019; Zhang et al., 2021). Clinically, a case accessory cavitated uterine mass

was successfully managed using ethanol sclerotherapy and post-procedural hysteroscopy detected a normal uterine cavity without communication with the mass (Merviel et al., 2020).

Ethanol retention or re-aspiration is still a matter of debit; earlier studies documented a low recurrence rate and pain scores with higher rates for cyst reduction and increase in AFC with ethanol retention than aspiration (Cohen et al., 2017; Chang et al., 2013). Thereafter, another study reported a marginally higher pregnancy rate and lower recurrence rate with ethanol retention than aspiration (Aflatoonian and Tabibnejad, 2020). Moreover, the duration of ethanol exposure before re-aspiration is also variable between 10 and 20 minutes (Miquel et al., 2021; Nardone et al., 2020; Lee et al., 2022), but one study documented that exposure time >10 min was more effective than <10 minutes (Kim et al., 2022) and another study did not define exposure time but recommended aspiration to get effective preservation of ovarian reserve and (Huang et al., 2021).

Cystic aspirate cytokines' levels showed a significant difference compared to serum levels. Baseline AFC was negatively correlated with aspirate levels of TNF- α and IL-8, while was positively correlated to levels of IL-10. Thus, the disturbing local cystic cytokines' balance in the inflammatory direction deleteriously affected the ovarian reserve. In hand with this, a previous study found a reduction of follicular fluid cytokine levels in women with EM who received GnRH-a, is associated with significant improvement in embryo quality, implantation, and clinical pregnancy rates (Kaponis et al., 2020). Also, increased numbers of

macrophages with higher levels of inflammatory cytokines were detected in follicular fluid of patients with ovarian EM than in patients with tubal factor infertility and these findings allowed concluded that the follicular immune microenvironment affects the development and fertilization of oocytes and is negatively associated with the cumulative birth rate in IVF (Zhou et al., 2022).

Increased cystic fluid cytokines' levels could be attributed to the experimental findings that TNF- α and monocyte chemotactic protein 2, which recruits and activates monocytes, were expressed in both eutopic and ectopic endometria and may be involved in the pathogenesis of endometriosis (Aksak et al., 2021). Another suggestion was the local synthesis of C3 promotes the engraftment of the endometriotic cysts and C3a participates in an auto-amplifying loop leading to mast cell infiltration and activation, which is pathogenic in EM (Agostinis et al., 2021). Thereafter, upregulation of expression levels of IL-17 was detected in serum, peritoneal fluid, and EM biopsies and assured the role of inflammatory cytokines in the growth and invasion of these ectopic lesions (Shi J et al., 2022). Another recent study detected increased expression levels of the nucleotide-binding oligomerization domain-containing protein 1 and inflammatory cytokines in EM tissue samples (Wei et al., 2022).

Thus, the elevated serum cytokines' levels may be a consequence of elevated cystic fluid levels as evidenced by the significant changes in serum cytokines' levels at 6-m after aspiration. Similarly, various lines of bioactive therapies resulted experimentally in improved cytokine levels with EM lesion diminution (Miao et al., 2022; Cordaro et al.,

2021; Ding et al., 2022; Lu et al., 2022). A previous study, using NimbleGen human gene expression microarrays analysis, detected high expressions of IL6, CD36, JUNB, B4GALT1, HES1, and NR4A1 in blood samples of OE patients and significant down-regulation of these genes after ethanol sclerotherapy (Wang et al., 2015).

The reported relation between improved serum immune milieu and improved AFC assured the role of inflammatory disturbance in reduction of the ovarian reserve and equalization of inflammatory/anti-inflammatory ratio in anti-inflammatory direction improves or increases the ovarian reserve. In support of this assumption, the difference in serum AMH levels at 6-m was non-significant concerning baseline levels. These observations are evidence of the success of AEST as a safe and effective procedure for OE management, especially in women seeking pregnancy. Similarly, multiple recent studies reported no significant difference in AMH levels before and after sclerotherapy but detected an increased pregnancy rate compared to surgery (Antonaci et al., 2022; Kim et al., 2022; Lee et al., 2022).

The reduced pain severity and need for medications could be attributed to the reduced local and systemic levels of inflammatory cytokines as previously detected where blocking of Neuropeptide S receptor 1 signaling, significantly reduced TNF- α release, and monocyte chemotaxis leading led to a significant reduction of inflammatory cell infiltrate and abdominal pain in EM animal model (Tapmeier et al., 2021).

Conclusion

Ethanol sclerotherapy improved ovarian reserve and systemic immune milieu and reduced pain

severity in patients who had OE. Sclerotherapy spared the need for surgery and resource.

Limitation

Follow-up for a longer duration to evaluate the pregnancy outcomes and detect any more recurrence is the study limitation.

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