Research Article

Preoperative TNF-α Reduction in Endometriosis Lesions with Combined Oral Contraceptives.

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Abstract

Objective: To compare the effects of COC therapy with two newer endometriosis treatments: oral Dienogest 2 mg and injectable Leuprorelin acetate 3.75 mg on TNF-α levels in serum and peritoneal fluid.

Methods: Forty subjects were divided into four groups: three groups receiving medical therapy—COC (0.03 mg ethinylestradiol and 0.15 mg levonorgestrel, taken one active tablet per day continuously), oral Dienogest 2 mg, and intramuscular Leuprorelin acetate 3.75 mg every four weeks—and a control group of endometriosis patients who had not received any hormonal therapy. Peripheral venous blood samples were taken before surgery, and peritoneal fluid was collected at the start of surgery in the pelvic cavity. TNF-α levels were analyzed using the ELISA method. Data analysis was performed using the Kruskal-Wallis test with a significance level of 0.05.

Results: The Kruskal-Wallis test showed significant differences between treatment groups in TNF-α levels in peritoneal fluid (p=0.023), specifically between the COC group and the control group, and between the Dienogest group and the control group.

Conclusion: Preoperative hormonal therapy for estradiol suppression provides limited benefit in reducing systemic inflammatory agents but is effective locally, as evidenced by reduced TNF-α levels in the peritoneal fluid after preoperative COC and Dienogest therapy.

Keywords: COC, dienogest, leuprorelin acetate, peritoneal fluid , serum, TNF-α.

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INTRODUCTION

Endometriosis is a local inflammatory process in the pelvic region with functional changes in cells related to the immune system, causing serum in women with endometriosis to increase the number of active macrophages that release products such as growth factors and cytokines [1] Endometriosis can cause pain and lead to infertility. Endometriosis is estimated to affect 10-11% of women of reproductive age worldwide, or around 176-190 million women in the global population as of 2017[2,3]. Dysmenorrhea is the most common complaint experienced by women with endometriosis, with a prevalence of approximately 64% [4]. Almost all major signs of inflammation are found in patients with endometriosis, including calor, rubor, tumour, dolor, and function loss. Both the cellular and humoral immune systems are involved in the process of endometriosis. One method to measure the severity of the inflammation is by assessing the presence of inflammatory agents such as pro-inflammatory cytokines [5].

Cytokines are inflammatory mediators whose concentration increases in plasma or other bodily fluids, indicating the presence, duration, and extent of tissue inflammatory lesions. Several

studies have reported a relationship between higher cytokine levels and the development and stability of endometriosis, which is often accompanied by infertility or pelvic pain. The peritoneal fluid of endometriosis patients directly experiences the effects, with deposits rich in pro-inflammatory agents such as cytokines, prostaglandins, and growth factors[6]. Natural and acquired immune system dysfunction is the basic concept of endometriosis progression and the advancement of its therapeutic response[7].

Currently, there is no single anti- or pro-inflammatory biomarker that is strong for the diagnosis and monitoring of endometriosis. According to Arwan & Hendri[1]TNF is increased in the peritoneal fluid and serum of patients with endometriosis, and it is said that TNF is an essential factor in the pathogenesis of endometriosis. The importance of TNF in the pathogenesis of endometriosis is further supported by the proliferative effects of TNF observed in endometrial cells of women with endometriosis but not in healthy female cells as a control. In addition, high concentrations of TNF appear to affect sperm motility in vitro and can have embryotoxic effects. These findings suggest that TNF may have an additional role in the development of infertility associated with endometriosis. In addition, the examination of the TNF-α peritoneal fluid sample is one of the evaluation aids for the diagnosis of endometriosis in addition to surgery, as a marker of increased cellular and humoral immunity signals for endometriosis[8]. Tumor Necrosis Factor (TNF) can stimulate the proliferation of endometrial ectopic and inhibit its clearance function. TNF regulation can be suppressed by inhibiting its production, preventing binding to receptors, and breaking its signal transmission pathway.[9,10]

TNF-alpha, as a key pro-inflammatory cytokine, plays a crucial role in the body's response to infections, and other chronic inflammatory conditions. On the other hand, hormonal treatment, such as hormonal contraception or hormone replacement therapy, is a medical approach that utilizes hormones to regulate bodily functions, manage certain medical conditions, and support reproductive health. Recent research demonstrates the potential interaction between the hormonal system and the inflammatory response regulated by TNF-alpha, which could have significant implications in the development of new therapies and holistic approaches to healthcare.

The goal of hormonal therapy is to reduce inflammatory reactions such as overcoming pain, suppressing the progressive growth of tumour masses, preventing further impairment of fertility function, and preventing postoperative recurrence. The treatment of endometriosis medicamentosa in several Asian countries is still primarily centered around three main preparations, considered as the first and second lines of treatment: the progestin group, currently dominated by dienogest, the group of oral contraceptive tablets combining estrogen and progesterone, and the GnRH agonist group [11]. The revised HIFERI consensus in 2023 strongly recommends progestins and combined contraceptive pills (COC) as first-line therapy for pain management, with GnRH agonists as second-line therapy due to their associated side effects (ESO). This consensus does not recommend the use of preoperative hormonal therapy [12].

The current condition at the obstetric polygyn of Fertility, Endocrinology, and Reproduction (FER) of Dr. Soetomo Hospital Surabaya, after the patient was diagnosed with endometriosis and has been decided to undergo surgical therapy, is not immediately carried out. The limitation of time, energy, and surgical facilities compared to the increase in the number of patients causes the queue time for the surgery schedule to be 3-6 months. During the surgery queue, patients receive hormonal suppression therapy to reduce complaints or symptoms. These conditions and challenges are quite interesting for researchers to test the effectiveness of anti-inflammatory therapy through the measurement of differences in cytokine levels caused by the administration of various hormonal therapies given at Dr. Soetomo Hospital. As a comparison, it can be used for those who do not receive preoperative therapy at several educational network hospitals outside Dr. Soetomo Hospital or from executive class patients. This research is expected to be a form of monitoring and evaluation of pharmaceutical services in the field of gynecology, especially preoperative endometriosis medical therapy. This study also ensures the effectiveness of the use of preoperative hormonal therapy to suppress inflammation through comparison of the results of measuring the cytokine level profile in the serum and peritoneum of endometriosis patients. So this study aims to assess the effectiveness of several types of short-term hormonal therapy given to current endometriosis patients. The effectiveness in question is the severity of

inflammatory conditions systemically (serum) and locally in the peritoneal fluid by objectively measuring the levels of pro-inflammatory cytokines. The parameters used are the main types of pro-inflammatory cytokines in endometriosis, namely: TNF-α.

METHODS

This study is a quasi-experimental design with a non-equivalent post-test-only control group. The sample was divided into four groups: the first group received preoperative Combined Oral Contraceptive (COC) therapy (estrogen + progesterone), the second group received preoperative Dienogest tablets, the third group received Leuprorelin Acetate therapy, and the fourth group served as a control without hormonal therapy. Measurements were taken post-treatment on serum (peripheral venous blood) and peritoneal fluid during surgery. The study was conducted at Dr. Soetomo General Hospital Surabaya and Darmo Hospital Surabaya. Serum samples were taken when the patients were admitted for surgery, while peritoneal fluid was collected during laparotomy or laparoscopy. The diagnosis of endometriosis was confirmed through observation by the operator and the research team during surgery, and anatomical pathology was used to rule out malignancy and infection. A total of 40 patients were sampled (10 patients per group). All patients provided informed consent for participation. They were clinically and medically diagnosed with endometriosis and had undergone preoperative therapy for at least three months. The control group had not received any hormonal therapy for at least three months prior. Patients with chronic infectious diseases, metabolic disorders, or those taking medications that influence TNF-α cytokine receptors were excluded. Other exclusion criteria included using other hormonal therapies affecting the hypothalamus-pituitary-ovary (HPO) axis, intraoperative findings of infectious masses, and malignancy confirmed by histopathology results. The variables measured were TNF-α cytokine levels in serum and peritoneal fluid. TNF-α sampling was performed using a Human TNF-α ELISA Kit, with samples collected from peripheral venous serum and peritoneal fluid. Samples were collected and stored at the immunology laboratory of Dr. Soetomo General Hospital. Statistical analysis began with a normality test using the Shapiro-Wilk test. If the data were normally distributed, a One-way ANOVA analysis was performed. If not, the non-parametric Kruskal-Wallis test was used. A post hoc test was conducted if significant differences were found, with a confidence level of $p < 0.05$

RESULTS

Before statistical testing was carried out, homogeneity testing was carried out on the characteristics of research subjects between groups including age, parity, Body Mass Index (BMI), level of pain before therapy based on the Visual Analog Scale (VAS) pain scale, duration of hormonal therapy (calendar month), leukocyte levels in peripheral blood during preoperative (units/dl), stagging of endometriosis surgery according to r-ASRM in 2005.

The calculation results showed that all patient characteristics across the groups were homogeneous, as they had a p-value greater than 0.05. The normality test for TNF-α data, using the Shapiro-Wilk method, indicated an abnormal data distribution; therefore, the Kruskal-Wallis test was used, followed by further analysis with the Mann-Whitney test.

Table 1. Characteristics and Homogeneity Test of Characteristics Between Groups

Characteristics	1 (N = 10)	2 (N = 10)	3 (N = 10)	4 (N = 10)	*P-value*
Age	35 (24 - 47)	32 (23 - 41)	31 (25 - 43)	35.5 (25 - 49)	0.506
	34.3 + 6.634	31.7 + 5.417	32.9 + 5.724	36.1 + 8.634	
BMI	23.55 (18.7 - 28.5)	24.85 (19.1 - 30.8)	22.3 (18.2 - 23.1)	23.8 (20.8 - 31.3)	0.141
	23.67 + 3.419	24.7 + 3.841	21.65 + 1.583	24.16 + 2.954	
VAS Score	7 (5 - 9)	7 (6 - 8)	6.5 (4 - 9)	6 (5 - 8)	0.177
	7.2 + 1.135	7 + 0.816	6.5 + 1.581	6.2 + 0.919	
Length of Therapy	4 (3 - 6)	4.5 (3 - 7)	4 (3 - 5)	0 (0 - 0)	0.154
	3.9 + 0.994	4.9 + 1.37	4 + 0.816	0 + 0	
Leukosit	8055 (5700 - 10100)	7390 (5910 - 10210)	7250 (5000 - 9830)	8025 (5580 - 18380)	0.777
	7986 + 1292.991	7759 + 1539.917	7392 + 1719.786	8728 + 3709.294	

Paritas	0	9 (90)	9 (90)	8 (80)	7 (70)	0.592
	1	1 (10)	1 (10)	2 20)	3 (30)	
Stagging	III	4 (40)	3 (30)	4 (40)	5 (50)	0.841
	IV	6 (60)	7 (70)	6 (60)	5 (50)	

Group 1 : Preoperative therapy of Combination Birth Control Pills (PKK), namely estrogen + progesterone (levonorgestrel 0.15 mg and ethinyl estradiol 0.03 mg) 1 tablet per day.
Group 2 : Preoperative therapy of Dienogest tablets (2 mg peroral per day).
Group 3 : Leuprorelin acetate therapy (3.75 mg intramuscularly every 4 weeks).
Group : control group (not getting hormonal therapy)

Table 2. Results of the Difference Test between Groups

Variable	1 (N = 10)	2 (N = 10)	3 (N = 10)	4 (N = 10)	*P-value*
TNF-α serum	13.91 (2.86 - 84.3) 30.403 + 31.01	22.395 (1.47 - 39.17) 19.609 + 14.15	18.245 (0.42 - 55.34) 25.193 + 18.65	31.16 (7.29 - 153.26) 54.668 + 56.29	0.457
TNF-α peritonium	5.99 (0.16 - 62.9)[a] 17.047 + 22.43394	15.555 (1.29 - 990.8)[b] 112 + 308.97669	26.76 (2.42 - 243.99)[c] 50.467 + 73.08569	56.01 (11.38 - 1074)[cd] 170.728 + 326.15096	0.023

Notasi a, b, c, d = Post hoc test

The difference test results for TNF-α showed a p-value greater than 0.05, indicating no significant difference in serum TNF-α between the treatment and control groups. Based on the median values, Dienogest therapy resulted in the lowest serum TNF-α levels, followed by Leuprorelin Acetate and, lastly, the combined oral contraceptive (COC) group. This suggests that Dienogest therapy reduces serum TNF-α levels.

The TNF-α peritoneal test results showed a p-value of less than 0.05, indicating a significant difference in peritoneal TNF-α between the treatment and control groups. Based on the median values, the combined oral contraceptive group showed the lowest peritoneal TNF-α levels, followed by Dienogest, Leuprorelin Acetate, and the control group with the highest levels. The post hoc test results revealed significant differences between the combined oral contraceptive, Dienogest, and Leuprorelin Acetate groups, indicating that these three therapies cannot be directly compared, with the combined oral contraceptive showing the best response. The study also showed that the therapeutic response was more significant in the peritoneum compared to serum, and conventional therapy with combined oral contraceptives had a greater impact on TNF-α levels in both serum and peritoneum.

DISCUSSION

The comparison of TNF-α levels in serum and peritoneal fluid can be seen in Table 2. The results indicate that the local inflammatory reaction is more dominant than the systemic intravascular response (in serum) in patients with severe endometriosis (grade 3 and 4) according to rASRM criteria. The impact of the presence and production of pro-inflammatory TNF-α by immunological cells is higher in response to inflammation of endometriosis lesions locally in the pelvic/abdominal cavity. This aligns with the findings of Ramirez and colleagues, who demonstrated the accumulation of macrophages in the peritoneal cavity of women with endometriosis [13]. This is also consistent with the findings of Wank and colleagues, whose ELISA tests showed that TNF-α levels in the peritoneal fluid of endometriosis patients with infertility complications were significantly higher compared to non-endometriosis patients based on the peritoneal fluid examination [14]. Additionally, the research demonstrated a significant increase in TNF levels in peritoneal fluid compared to serum [13].

The abundant TNF-α levels in peritoneal fluid, in line with the chronic process occurring, provide a significant opportunity for evaluating differences. This is consistent with the study by Velho and colleagues in 2021, which showed that the therapeutic response to hormonal suppression had an earlier impact on the peritoneal fluid of patients with severe or chronic endometriosis. The study highlighted the increased extravasation, differentiation, and adaptation of immune macrophage cells in endometriosis tissue. In addition to type 2 macrophages, which are the primary producers of cytokines, peritoneal mesothelial cells, fibroblasts, NK cells, lymphocytes in the tissue, and endothelial cells also contribute to cytokine

production[15].

TNF-α is an angiogenic mediator for the angiogenic activity of macrophages by inducing endothelial cell migration, which in turn stimulates the production of VEGF for the formation of new microvessels around the lesion, and has a vasodilator effect by no stimulation in the macrophages. TNF-α is produced continuously on all lesion surfaces in the peritoneum as well as the ectopic endometrial stroma. Tumor necrosis factor α (TNF-α), a key factor in the inflammatory immune response, is involved in the development of endometriosis. TNF-α is reported to be abundant in the peritoneal cavity of patients with endometriosis, and the levels are much higher in the early stages of endometriosis pathology [13].

TNF-α levels in the control group were higher compared to the groups that received hormonal therapy. The mean and median results of the samples showed that local TNF-α levels in peritoneal fluid varied. TNF has been identified as a key regulator of the inflammatory response. It interacts with two distinct receptors, TNFR1 and TNFR2, which are differentially expressed on cells and tissues, initiating distinct yet overlapping signal transduction pathways. These diverse signaling cascades result in a range of cellular responses, including cell death, survival, differentiation, proliferation, and migration. Vascular endothelial cells respond to TNF by undergoing several pro-inflammatory changes that enhance leukocyte adhesion, transendothelial migration, vascular leakage, and increased thrombosis. The central role of TNF in inflammation has been demonstrated by the effectiveness of agents that inhibit TNF activity in treating various inflammatory conditions [16].

Although the implantation of ectopic endometriosis lesions is not entirely dependent on hormones, estrogen plays a crucial role in cell survival, cell proliferation, and the inflammatory response in endometriosis lesions. This occurs because ectopic lesions respond to the cyclic secretion of ovarian steroid hormones, particularly estrogen, despite significant evidence of progesterone resistance. Current hormonal therapies are designed to induce amenorrhea. The heterogeneity in the distribution of estrogen receptors (ER) and progesterone receptors (PR) in the lesions causes varying therapeutic responses, including in the immunological reactions to the inflammation that occurs[17]. This is one of the factors contributing to the unevenly distributed preoperative hormonal therapy response observed in this study.

A substantial body of research has explored the comparative effects of hormonal therapies on endometriosis. The most commonly compared hormonal treatments include combined oral contraceptives (COC), progestins (oral tablets, injections, or intrauterine devices/IUDs), GnRH agonists, aromatase inhibitors, and danazol. The outcomes assessed in these comparisons typically include clinical features such as pain severity, improvement in radiological findings, levels of adhesion or lesion stage progression, and the impact on infertility.

In this study, a more objective assessment was used by measuring TNF-α levels in both peritoneal fluid and serum. The results showed a significant difference, with lower cytokine levels in the combined oral contraceptive (COC) therapy group compared to the control group that did not receive any therapy and in the dienogest therapy group compared to the control group. These findings are consistent with several clinical studies, such as the prospective research which demonstrated significant clinical improvement in pain and regression of mass size during serial sonographic evaluations [18]. The results showed that COC therapy was statistically more effective than dienogest in patients with endometriosis and adenomyosis[(18)]. The suspected dual biological effects of combined oral contraceptives (COC) include inhibiting the implantation of endometrial cells while also providing a protective effect against early macrophage necrosis in endometriosis lesions[19].

The clinical differences between endometriosis patients treated with combined oral contraceptives (COC) and those treated with dienogest, specifically regarding lesion size and pain reduction, were studied by Casper and colleagues. The results indicated that progestins are more effective as a first-line therapy compared to COC in reducing pain and suppressing endometriosis lesions anatomically[20] .

Progestins harm cell proliferation, inflammatory processes, neovascularization, and neurogenesis in endometriosis[(21,22)]. In this study, the administration of the progestin dienogest showed a significant difference compared to the control group in the examination of the pro-inflammatory cytokine TNF-α. This finding is related to the research which revealed the multi-directional effects of progestins on cytokine production, depending on several factors, such as their structure [21]. Some synthetic progestins

were found to stimulate TNF-α production, while endogenous progesterone does not affect TNF-α due to its suppressive effect on the hormone axis in the presence of estrogen[23].

The study provides important considerations regarding progestin therapy[22]. It showed that high levels of progesterone in peritoneal fluid were reduced with progestin therapy, such as norethisterone, while estradiol levels remained unaffected. Additionally, there was no difference in FSH or LH levels between untreated women and those receiving treatment[22]. This indicates that the impact of local intraperitoneal estrogen may remain relatively unchanged, even though progestin hormonal therapy also works through central mechanisms via negative feedback. Leuprorelin acetate therapy in endometriosis is associated with its ability to reduce GnRH pulsatility, suppress the gonadotrope axis, and prevent estrogen stimulation on the ovaries and ectopic glands in endometriosis lesions. Due to the severe hypoestrogenic effects it can cause, Leuprorelin acetate therapy is considered a second-line treatment for endometriosis. In this study, the impact of preoperative Leuprorelin acetate injections on pro-inflammatory TNF-α expression showed lower levels in the peritoneum compared to the control group. However, this difference was not statistically significant when compared to the control group or among the treatment groups. These findings are inconsistent with the study which reported that GnRH agonist therapy appeared to have a direct effect on local endometrial cells by increasing the percentage of apoptotic cells and reducing the release of pro-mitogenic cytokines such as IL-1 and VEGF[23]. Their research suggested that GnRH agonists are beneficial in treating endometriosis by reducing cell proliferation and promoting apoptosis in eutopic endometrium. The direct effect of GnRH agonists on the release of pro-inflammatory cytokines and angiogenic factors from ectopic endometrial tissue in endometriosis patients remains unclear[23]. The multicenter clinical study reported that oral dienogest 2 mg/day demonstrated equivalent efficacy to depot leuprorelin acetate 3.75 mg administered intramuscularly every 4 weeks [24]. After 24 weeks of therapy in endometriosis patients, the degree of pain reduction due to endometriosis-related inflammation showed no significant difference between the two treatments following therapy.

The failure of GnRH agonist therapy may be attributed to various factors, including paracrine and autocrine effects, the microenvironment, cytokines, or the potential role of endocrine disruptors in regulating the growth of severe endometriosis lesions. Autonomous molecular abnormalities in endometriotic lesions contribute to increased local estradiol concentrations. These abnormalities include the upregulation of aromatase, which converts C19 steroids to estrogen, and a deficiency in 17-hydroxysteroid dehydrogenase type 2, which converts estradiol to estrone in the local endometrial stroma in response to progesterone. This was clinically reported by Anaf and colleagues, where therapy failure in deep infiltrating endometriosis (DIE), extending to the ureter, occurred in patients receiving GnRH agonists. However, clinical improvement was achieved with aromatase inhibitor therapy[25].

CONCLUSIONS, LIMITATIONS, AND SUGGESTIONS

There was no significant difference in pro-inflammatory cytokine TNF-α levels in the serum between the treatment groups (COC tablets, dienogest, and leuprorelin acetate injections) during the preoperative phase compared to the control group (without preoperative hormonal therapy). Similarly, no significant difference was observed in serum TNF-α levels among the three preoperative therapy groups (COC, dienogest, and leuprorelin acetate injections). However, there was a significant difference in pro-inflammatory TNF-α levels in the peritoneal fluid of the groups receiving preoperative COC tablets and dienogest compared to the control group without preoperative hormonal therapy. No significant difference was observed in the group receiving leuprorelin acetate injections compared to the control group. Furthermore, no significant difference in TNF-α levels in the peritoneal fluid was found among the three preoperative therapy groups (COC tablets, dienogest, and leuprorelin acetate injections).

The results of this study may inform clinical decision-making to optimize the effectiveness and efficiency of endometriosis treatment. Medicinal therapy for endometriosis remains relevant with the use of combined oral contraceptives and dienogest, which aligns with current first-line endometriosis treatment protocols. Future research should continue with studies that measure cytokine levels at the beginning and end of therapy, use samples from patients with

early-stage endometriosis, and assess both pro-inflammatory and anti-inflammatory cytokines.

RESEARCH ETHICS

The research ethics certificate was obtained from the Ethics and Clinical Research Committee of Dr. Soetomo Hospital Surabaya, in October 2023.

CONFLICTS OF INTEREST

The authors state that there are no interests involved or competing in this research

REFERENCES

1. Arwan B, Hendri D. Role of TNF-a and Interleukin 6 Serum against Ovarian Reserve in Endometriosis Cysts. Indones J Obstet Gynecol 2021;157–161. doi: 10.32771/inajog.v9i3.1243.
2. Patel BG, Lenk EE, Lebovic DI, Shu Y, Yu J, Taylor RN. Pathogenesis of endometriosis: Interaction between Endocrine and inflammatory pathways. Best Pract Res Clin Obstet Gynaecol. 2018;50:50–60. doi: 10.1016/j.bpobgyn.2018.01.006. Cited: in: : PMID: 29576469.
3. Shafrir AL, Farland LV, Shah DK, Harris HR, Kvaskoff M, Zondervan K, Missmer SA. Risk for and consequences of endometriosis: A critical epidemiologic review. Best Pract Res Clin Obstet Gynaecol. 2018;51:1–15. doi: 10.1016/j.bpobgyn.2018.06.001. Cited: in: : PMID: 30017581.
4. Puspasari B, Baziad A, Hestiantoro A. Karakteristik Pasien Endometriosis di Rumah Sakit Dr. Cipto Mangunkusumo Selama Periode 1 Januari 2000-31 Desember 2005 [Internet]. Jakarta; 2007 [cited 2024 Sep 11]. Available from: https://www.inajog.com/index.php/journal/article/view/119.
5. Malvezzi H, Hernandes C, Piccinato CA, Podgaec S. Interleukin in endometriosis-associated infertility-pelvic pain: systematic review and meta-analysis. Reproduction. 2019;158:1–12. doi: 10.1530/REP-18-0618.
6. Nazri HM, Imran M, Fischer R, Heilig R, Manek S, Dragovic RA, Kessler BM, Zondervan KT, Tapmeier TT, Becker CM. Characterization of exosomes in peritoneal fluid of endometriosis patients. Fertil Steril. 2020;113:364–373. doi: 10.1016/j.fertnstert.2019.09.032. Cited: in: : PMID: 32106990.
7. Fedotcheva TA, Fedotcheva NI, Shimanovsky NL. Progesterone as an Anti-Inflammatory Drug and Immunomodulator: New Aspects in Hormonal Regulation of the Inflammation. Biomolecules [Internet]. 2022;12. doi: 10.3390/biom12091299. Cited: in: : PMID: 36139138.
8. Bedaiwy MA, Falcone T, Sharma RK, Goldberg JM, Attaran M, Nelson DR, Agarwal A. Prediction of endometriosis with serum and peritoneal fluid markers: a prospective controlled trial. Human Reprod. 2002;17:426–431. doi: 10.1093/humrep/17.2.426. Cited: in: : PMID: 11821289.
9. García-Gómez E, Vázquez-Martínez ER, Reyes-Mayoral C, Cruz-Orozco OP, Camacho-Arroyo I, Cerbón M. Regulation of Inflammation Pathways and Inflammasome by Sex Steroid Hormones in Endometriosis. Front Endocrinol (Lausanne). 2020;10. doi: 10.3389/fendo.2019.00935.
10. Taylor HS, Pal L, Seli E. Speroff's Clinical Gynecologic Endocrinology and Infertility. Lippincott Williams & Wilkins, a Wolters Kluwer business; 2020.
11. Kim HJ, Kim SH, Oh YS, Lee SR, Chae HD. Dienogest May Reduce Estradiol- and Inflammatory Cytokine-Induced Cell Viability and Proliferation and Inhibit the Pathogenesis of Endometriosis: A Cell Culture- and Mouse Model-Based Study. Biomedicines. 2022;10:2992. doi: 10.3390/biomedicines10112992.
12. HIFERI. KONSENSUS TATA LAKSANA NYERI ENDOMETRIOSIS Revisi Pertama Himpunan Endokrinologi Reproduksi dan Fertilitas Indonesia (HIFERI) Perkumpulan Obstetri dan Ginekologi Indonesia (POGI) 2017. 2023.
13. Ramírez-Pavez TN, Martínez-Esparza M, Ruiz-Alcaraz AJ, Marín-Sánchez P, Machado-Linde F, García-Peñarrubia P. The role of peritoneal macrophages in endometriosis. Int J Mol Sci. MDPI; 2021.
14. X.-M. WANG1 Z-YMNS. Inflammatory cytokines and peritoneal fluid flora in endometriosis patients. 2018.
15. Velho RV, Halben N, Chekerov R, Keye J, Plendl J, Sehouli J, Mechsner S. Functional changes of immune cells: signal of immune tolerance of the ectopic lesions in endometriosis? Reprod Biomed Online. 2021;43:319–328. doi: 10.1016/j.rbmo.2021.04.012. Cited: in: : PMID: 34103260.
16. Bradley J. TNF–mediated inflammatory disease. J Pathol. 2008;214:149–160. doi: 10.1002/path.2287. Cited: in: : PMID: 18161752.
17. Brichant G, Laraki I, Henry L, Munaut C, Nisolle M. New Therapeutics in Endometriosis: A Review of Hormonal, Non-Hormonal, and Non-Coding RNA Treatments. Int J Mol Sci. 2021;22:10498. doi: 10.3390/ijms221910498. Cited: in: : PMID: 34638843.
18. Torres PC, Martínez-Zamora MÁ, Ros C, Rius M, Mensión E, Gracia M, Carmona F. Clinical and sonographic impact of oral contraception in patients with deep endometriosis and adenomyosis at 2 years of follow-up. Sci Rep. 2023;13:2066. doi: 10.1038/s41598-023-29227-2. Cited: in: : PMID: 36739298.
19. Schindler AE. Dienogest in long-term treatment of endometriosis. Int J Womens Health. 2011;175. doi: 10.2147/IJWH.S5633. Cited: in: : PMID: 21792339.
20. Casper RF. Progestin-only pills may be a better first-line treatment for endometriosis than combined estrogen-progestin contraceptive pills. Fertil Steril. 2017;107:533–536. doi: 10.1016/j.fertnstert.2017.01.003. Cited: in: : PMID: 28162779.
21. Pavlik TI, Shimanovsky NL, Zemlyanaya OA, Fedotcheva TA. The Effect of Progestins on Cytokine Production in the Peripheral Blood Mononuclear Cells of Menopausal Women and Their Luminol-Dependent Chemiluminescence. Molecules. 2023;28:4354. doi: 10.3390/molecules28114354. Cited: in: : PMID: 37298830.

22. Kim-Björklund T, Landgren B-M, Hamberger L. Peritoneal fluid volume and levels of steroid hormones and gonadotrophins in peritoneal fluid of normal and norethisterone-treated women. Hum Reprod. 1991;6:1233–1237. doi: 10.1093/oxfordjournals.humrep.a137518. Cited: in: : PMID: 1752923.
23. Meresman GF, Bilotas MA, Lombardi E, Tesone M, Sueldo C, Barañao RI. Effect of GnRH analogues on apoptosis and release of interleukin-1beta and vascular endothelial growth factor in endometrial cell cultures from patients with endometriosis. Hum Reprod. 2003;18:1767–1771. doi: 10.1093/humrep/deg356. Cited: in: : PMID: 12923126.
24. Strowitzki T, Faustmann T, Gerlinger C, Seitz C. Dienogest in the treatment of endometriosis-associated pelvic pain: a 12-week, randomized, double-blind, placebo-controlled study. Eur J Obstet Gynecol Reprod Bio. 2010;151:193–198. doi: 10.1016/j.ejogrb.2010.04.002.
25. Anaf V, Bossche MV, Simon Ph, Riera C, Fayt I, Noel J-C. Persisting active and proliferative intrinsic ureteral endometriosis under GNRH agonists therapy: a case with immunohistological study. Eur J Obstet Gynecol Reprod Bio. 2001;97:250–254. doi: 10.1016/S0301-2115(00)00531-5. Cited: in: : PMID: 11451560.