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Original Research

Protective Effect of Ursodeoxycholic Acid in Experimental Endometriosis Induced Rat Model

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Abstract

Background: Considering the presence of an inflammatory process in the pathogenesis of endometriosis, anti-inflammatory agents could be an alternative option. The study aimed to elucidate the curative efficacy of Ursodeoxycholic acid (UDCA) on the experimental rat model of endometriosis. **Methods**: This experimental research included a total of 60 mature female Wistar albino rats (250 $\pm$ 50 g) with no pregnancy. They were grouped as Standard (n: 20), Laparoscopic Pretreatment (n: 10), Laparoscopic Posttreatment (Sham) (n: 10), UDCA-Pretreatment (n: 10) and UDCA-Posttreatment (n: 10). Transforming growth factor β1 (TGF-β1), matrix metallo-proteinases-2 (MMP-2), Tissue inhibitor of metalloproteinase-1 (TIMP-1), Tumor necrosis factor-α (TNF-α) were analyzed. **Results**: In the UDCA post-treatment group, endometriotic focal volume (43.3 $\pm$ 24.04 mm^3) was lower than the pre-treatment values (165.7 $\pm$ 21.7 mm^3) (p = 0.005). There was no significant change UDCA group before and after the treatment in terms of MMP-2, TGF-β1, TIMP-1 and TNF-α levels ($p > 0.05$). Comparing the posttreatment values of the Sham srugery group and the UDCA group, while the endometriotic focal volume was 251 $\pm$ 51 mm^3 in the Sham group, it decreased to 43.3 $\pm$ 24 mm^3 in the UDCA ($p < 0.0001$). Histological scoring decreased from 2.6 $\pm$ 0.51 to 1 $\pm$ 0.81 after the treatment (p = 0.001). **Conclusions**: The pre-treatment laparotomy group exhibited elevated TNF-α levels, indicating an inflammatory response. UDCA treatment reduced endometriotic focal volume and histological scoring, indicating a potential therapeutic benefit.

Keywords: endometriosis; ursodeoxycholic acid; metalloproteinase; tumor necrosis factor-α

1. Introduction

Endometriosis, stroma outside the uterine-cavity, is a chronic condition characterized by the ectopic location of the functional endometrial gland [1]. It is the main reason of chronic pelvic related pains in premenopausal females [2]. In addition, it is one of the important causes of infertility in women, and due to its adhesive effect in oviducts, it also causes decreased ovarian reserve and embryo quality and implantation possibility [3]. Although many theories about the pathogenesis of endometriosis have been proposed, its origin is not fully clarified [4].

Cytokines and chemokines play an important role in its pathophysiology proving that endometriosis is a chronic inflammatory process [5]. The increase of macrophages causes the growth of ectopic endometrial lesions and angiogenesis leading to chronic pain and infertility. Another evidence can be stated as the demonstration of increased plasma cells and activated macrophages in endometriotic lesions in immunohistochemical studies [6]. Agostinis *et al*. [7] explored the anti-inflammatory and proapoptotic effect of this combination on human endometriotic endothelial cells and mice treated with N-acetylcysteine presented a lower number of cysts, smaller in size, compared to untreated mice. As another molecule, Alpha-Lipoic acid is a natural antioxidant synthetized by plants and animals, identified as a catalytic agent for oxidative decarboxylation of pyruvate and α-ketoglutarate. According to Di Tucci *et al*. [8], it can be safely used for treatment of neuropathic pain and as a dietary support during pregnancy. In different research, Salehpoor *et al*. [9] investigated the effects of pentoxifylline on inflammatory and apoptotic pathways in the rat model of induced endometriosis, and reported it can induce enhancing effect on suppression of endometriosis and enhancing apoptosis.

Ursodeoxycholic acid (UDCA) has attracted attention due to its effectiveness in the treatment of primary biliary cholangitis. UDCA has a cytoprotective effect in cholestatic liver disease with an anti-apoptotic mechanism as well as an anti-inflammatory effect due to its glucocorticoid receptor [10]. Apart from the liver and biliary tract dis-

eases, UDCA showed a protective effect in inflammatory disease due to its anti-inflammatory effect. Considering the presence of an inflammatory process in the pathogenesis of endometriosis, the treatment with anti-inflammatory agents could be an alternative option for certain patients. The study aimed to investigate the curative efficacy of UDCA on the experimental rat model.

2. Materials and Methods

2.1 Experimental Design

This animal trial was assessed and approved by the Animal Studies Ethical Board of the Dumlupinar University (ID: 20151208-date:22/12/2022) and was compliant with "Principles of laboratory animal care - 1985". This research included a total of 60 mature Wistar albino rats (weight range: 250 $\pm$ 50 g) in the female gender with no pregnancy. They were grouped as Standard group (n: 20), Laparoscopic-Pretreatment (n: 10), Laparoscopic-Posttreatment (Sham) (n: 10), UDCA-Pretreatment (n: 10) and UDCA-Posttreatment (n: 10). The rats were transferred to the research center one week before the research and maintained at humidity 60 $\pm$ 10%, constant of 23 $\pm$ 2 °C, and light/dark (12_h/12_h) cycle.

2.2 Experimental Protocols

Anesthesia of all animals has been performed by administering ketaminehydrochloride (70 mg/kg, Ketas; Eczacibasi, Turkey), xylazinehydrochloride (7 mg/kg, Rompun: Bayer Company, Istanbul, Turkey) intraperitoneally under aseptic conditions. Skin antisepsis has been administered to all rats via 10% povidone-iodine solution during the operation time. While the rats, which were determined to be in the estrus stage, were taken into surgery, vaginal smear samples were examined with Papanicolaou.

The same researcher was interested in the surgical procedures applied to the rats in the study. A vertical incision was made in the lower midline region of the rats in a 5 cm wide abdominal laparotomy. An autologous part of uterine was located into the inner-surface of the right abdominal wall to achieve the experimental endometriosis procedure and thus surgically induced in rats. The endometrial part of the right uterus was transplanted into the right peritoneal cavity with the inner surface of the endometrium as opposed to the peritoneum and fixed on two sides. Then, 2 mL saline was injected into the abdominal-cavity to eliminate the possibility of drying on the abdominal wall and to prevent adhesion formation. The anterior abdominal wall was tapped as 2 layers using vicryl 3/0, prolene 4/0, respectively.

Endometrial implants were created in rats in the first laparotomy. Rats were found to have successfully developed endometrial implant focus at the end of the four-week waiting period were included in the study. 3 rats died after the first laparotomy. A second laparotomy was applied to the rats to understand whether endometriosis had occurred. In the second laparotomy approach, firstly, peritoneal fluid samples were taken from all rats. While rats were found to have endometriosis were included in the study, 5 rats without endometriotic focus were excluded from the study. After measuring the volumes of endometriosis-developing tissues as length, width and height, they were calculated according to the formula (V (mm^3) = 0.52 $\times$ Length $\times$ Width $\times$ Height) and these values were recorded. Tissue samples were taken from these areas for histopathological examination.

2.3 Experimental Rat Groups

After the second laparotomy, the rats were divided into two groups. While 11 rats were included in the study group, 11 rats were included in the control group. 150 mg/kg/day UDCA (Galenica SA, Athens, Greece) was dissolved in 1 mL sterile saline solution and administered orally for 40 days to the rats in the study group. In the control group, 1 mL of saline without active substance was administered orally for 40 days. During the period from the second laparotomy to the third laparotomy, a total of 2 rats, 1 rat from the study group and 1 rat from the control group, died. The study was continued with a total of 20 rats, 10 rats in the control and 10 rats in the UDCA. At the end of 40 days, the rats in both groups underwent a third laparotomy. During the third laparotomy, peritoneal fluid samples were taken from the rats and tissue samples were taken from the foci previously found to be endometriosis for histopathological examination, and the procedure was terminated. The rats were euthanized by giving a lethal dose of Ketamine after the procedure.

2.4 Peritoneal Fluid Samples

During the first, second, and third laparotomy, peritoneal fluid was obtained and centrifuged at 200 g for 5 minutes and the supernatants were separated for storage at –80 °C. The electrochemiluminescence immunoassay (ELISA) kits used in this study to measure tumor necrosis factor-α (TNF-α), transforming growth factor-β1 (TGF-β1), matrix metalloproteinase-2 (MMP-2), and metalloproteinase-1 tissue inhibitor (TIMP-1) (Quanterix Corporation, Biotek, Billerica, MA, USA). The kits were utilized following the manufacturer's instructions. Samples were added to microplates pre-coated with antibodies specific for each target molecule. After incubation and washing to remove unbound substances, a detection antibody was added, forming an antibody-antigen sandwich. A substrate solution was then added, and a luminescent signal was generated. All assays were performed in duplicate, and the results were averaged. Data were analyzed using the standard curve method, and concentrations were expressed in pg/mL for TNF-α, ng/mL for TGF-β1, ng/mL for MMP-2, and ng/mL for TIMP-1.

Table 1. Comparison of the standard and the pretreatment groups.

Variables	Standard	Laparotomy (pre-treatment)	p-value
MMP-2, pg/mL	0.705 $\pm$ 0.595	0.968 $\pm$ 0.466	0.120
TGF-β1, pg/mL	1.005 $\pm$ 0.541	1.161 $\pm$ 0.388	0.359
TIMP-1, pg/mL	1.043 $\pm$ 0.547	0.920 $\pm$ 0.398	0.455
TNF-α, pg/mL	0.322 $\pm$ 0.256	0.461 $\pm$ 0.271	0.015

All data were given as Mean $\pm$ Standard Deviation. TGF-β1, Transforming growth factor β1; MMP-2, matrix metallo-proteinases-2; TIMP-1, Tissue inhibitor of metalloproteinase-1; TNF-α, Tumor necrosis factor-α.

2.5 Histopathological Assessments

Tissue samples taken during the second and third laparotomy were fixed in a 10% neutral buffered formaldehyde solution. Then, the samples went through the dehydration stage and paraffin blocks were prepared from the tissue samples. 4-micron thick sections were prepared from paraffin blocks with the help of a microtome and stained with hemotoxylineosin. Samples were analyzed under a light microscope (Nikon Eclipse Ni light microscope was used in the study. Nikon DS-Ri2 imaging system on the same microscope was used to view histopathological specimens). In this study, histological assessment was based on visualization of the endometrial stroma and glandular. Scoring after the histopathological examination was made with the following criteria: 3: Well-preserved epithelial tissue, 2: Moderately preserved epithelial and leukocyte infiltration, 1: Small amount of epithelial cell, 0: Cell is not visible [11].

2.6 Statistical Analysis

Data analysis was done using the SPSS v23.0 (IBM Corp., Armonk, NY, USA), the Statistical program for Windows, while graph drawings were performed using the Graph-Pad Prism Software v9.1 (GraphPad Software, Inc., San Diego, CA, USA). Normality analysis was completed with the Kolmogorov-Smirnov test. For comparisons of the groups, the "Independent Sample T-Test" was used for the two groups, and the "Paired T-Test" compared the values before & after the treatment. The Sample-T-Test was used to examine whether there is a difference among MMP-2, TGF-β1, TIMP-1, and TNF-α values between groups in the study. Chi-square was performed to compare the histological score. The results were considered statistically significant when the p-value was less than 0.05.

3. Results

As given in Table 1 and Fig. 1, in the comparison of the pre-treatment laparotomy and the standard groups, MMP-2, TGF-β1, and TIMP-1 levels were similar between the groups, while TNF-α values were higher in the laparotomy group (p = 0.015).

The data evaluation of the control and the UDCA-treated group before and after the treatment are separately given in Tables 2,3. Accordingly, the MMP-2 value after treatment (1.378 $\pm$ 0.475 pg/mL) in the control was higher than before the treatment (0.974 $\pm$ 0.443 pg/mL) (p = 0.028). Similarly, the endometriotic focal volume after the treatment (251 $\pm$ 51.1 mm^3) was significantly higher than before the treatment (169 $\pm$ 24.5 mm^3) (p = 0.007). TGF-β1, TIMP-1 and TNF-α levels did not differ ($p > 0.05$).

When comparing the pre-treatment and post-treatment values of the UDCA group, the post-treatment Endometriotic focal volume (43.3 $\pm$ 24.04) was significantly lower than the pre-treatment values (165.7 $\pm$ 21.7) (p = 0.005). MMP-2, TGF-β1, TIMP-1 and TNF-α levels did not differ ($p > 0.05$). As given in Table 4 and Fig. 2, when comparing the post-treatment values of the Sham and the UDCA group, while the endometriotic focal volume was 251 $\pm$ 51.1 in the Sham group, it decreased to 43.3 $\pm$ 24.046 in the UDCA group ($p < 0.0001$). Similarly, histological scoring decreased from 2.6 $\pm$ 0.516 to 1 $\pm$ 0.816 after the treatment (p = 0.001).

4. Discussion

In this study, we evaluated the effects of pre-treatment laparotomy and UDCA treatment on endometriosis-related markers and clinical outcomes. Our results show that the pre-treatment laparotomy group exhibited elevated TNF-α levels, indicating an inflammatory response. In the control group, MMP-2 values and endometriotic focal volume increased post-treatment, suggesting disease progression. Conversely, UDCA treatment significantly reduced endometriotic focal volume and histological scoring, indicating a potential therapeutic benefit.

Endometriosis is a chronic inflammatory disease with unclear pathogenesis. It is an estrogen-dependent disease defined by the presence and growth of functional endometrial-like tissue, glands and stroma, outside the uterine cavity. A study by Laganà *et al.* [12] showed that macrophages are broadly classified into pro-inflammatory macrophages, which have selective anti-inflammatory and pro-fibrotic activities and are able to induce immunotolerance and angiogenesis. According to Ni *et al.* [13], the abnormal fecal metabolites, which are influenced by dysbacteriosis, may be the characteristics of Endometriosis mice and can be the potential important indices to distinguish the disease. Similarly, D'Alterio *et al.* [14] reported that endometriosis appears to be associated with elevated lev-

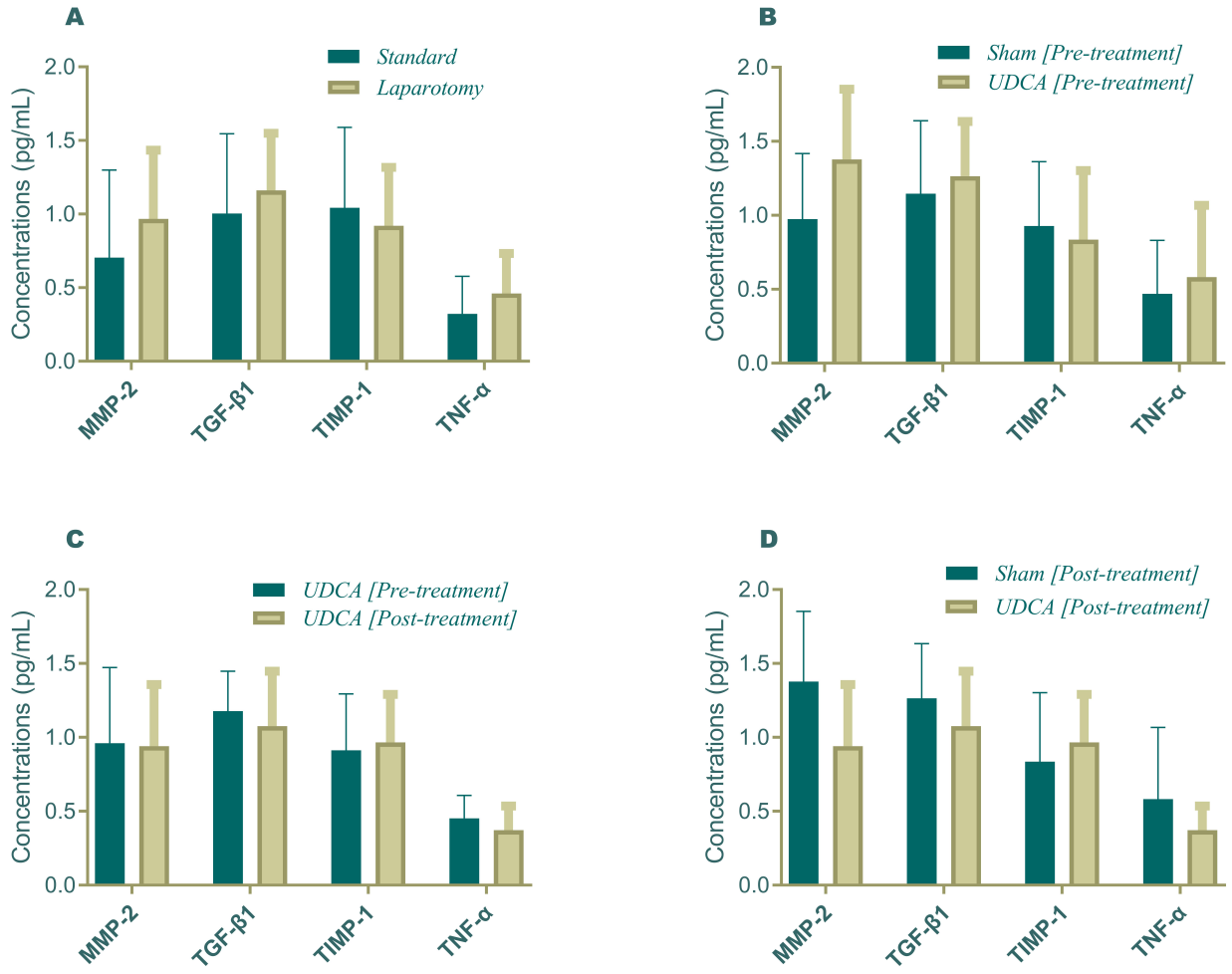

Fig. 1. Results of transforming growth factor β1 (TGF-β1), matrix metallo-proteinases-2 (MMP-2), tissue inhibitor of metalloproteinase-1 (TIMP-1), and Tumor necrosis factor-α (TNF-α) in rat models. (A) Comparisons of Standard and Laparotomy groups; (B) Comparisons of Sham pre-treatment and Ursodeoxycholic acid (UDCA) pre-reatment groups; (C) Comparisons of UDCA pre-treatment and UDCA post-treatment groups; (D) Comparisons of Sham post-treatment and UDCA post-reatment groups.

els of different microorganisms across various microbiome. An ineffective immune response seems to play a role in its pathogenesis, and there is some scientific proof to state that the immune response may be modulated by the microbiome. Laboratory and clinical investigations indicate that hosts' microbiome profiles with and without endometriosis can be different. After the initiation of endometriosis treatment, the symptoms can recur when the treatment is completed [15].

Endometriosis is defined as a chronic inflammatory disease, treatment with anti-inflammatory agents appears to be prominent. TNF-α levels have been shown to increase in peritoneal fluid in endometriosis cases [16]. If one conducts desktop research, there are many studies about the successful results in endometriosis and increased fertilization rates with the use of TNF-α-blocking agents. A TNF-α inhibitor infliximab has been shown to ameliorate endometriosis and chronic pain [17]. A similar outcome has been achieved with etanercept that and chronic pain. In another study, the endometriotic foci used in the treatment of TNF-α inhibitor etanercept endometriosis regressed and the fertility rate increased [18]. Increased TNF-α level in peritoneal and follicular fluids in endometriosis causes autophagy, apoptosis, cell death, and follicle atresia and decreases the success rate of fertilization [19]. Blocking the TNF-α with etanercept is thought to increase the success rate of fertilization. In our study, we found that TNF-α was higher in the laparotomy group, while it did not differ in other subgroups.

MMP-2 is in the matrix metalloproteinases enzyme group and this enzyme group plays a regulatory role in many physiological functions such as angiogenesis, inflammation, ovulation, embryogenesis [20]. The matrix metalloproteinase (MMP) enzyme group plays a role in extracellular matrix remodeling, which is associated with the

Table 2. Comparison of pre-treatment and post-treatment values of the control group.

Variables	Pre-treatment	Post-treatment (Sham)	*p*-value
MMP-2, pg/mL	0.974 $\pm$ 0.443	1.378 $\pm$ 0.475	0.028
TGF-β1, pg/mL	1.145 $\pm$ 0.494	1.265 $\pm$ 0.369	0.180
TIMP-1, pg/mL	0.928 $\pm$ 0.435	0.836 $\pm$ 0.466	0.726
TNF-α, pg/mL	0.470 $\pm$ 0.361	0.582 $\pm$ 0.485	0.611
Endometriotic Volume (mm^3)	169 $\pm$ 24.518	251 $\pm$ 51.153	0.007

All data were given as Mean $\pm$ Standard Deviation. TGF-β1, Transforming growth factor β1; MMP-2, matrix metallo-proteinases-2; TIMP-1, Tissue inhibitor of metalloproteinase-1; TNF-α, Tumor necrosis factor-α.

Table 3. Comparison of pre-treatment and post-treatment values of the UDCA group.

Variables	Pre-treatment	Post-treatment	*p*-value
MMP-2, pg/mL	0.961 $\pm$ 0.512	0.941 $\pm$ 0.417	0.917
TGF-β1, pg/mL	1.177 $\pm$ 0.270	1.076 $\pm$ 0.372	0.752
TIMP-1, pg/mL	0.912 $\pm$ 0.382	0.965 $\pm$ 0.325	0.678
TNF-α, pg/mL	0.452 $\pm$ 0.156	0.372 $\pm$ 0.164	0.109
Endometriotic Volume (mm^3)	165.7 $\pm$ 21.762	43.3 $\pm$ 24.046	0.005

All data were given as Mean $\pm$ Standard Deviation. UDCA, Ursodeoxycholic acid; TGF-β1, Transforming growth factor β1; MMP-2, matrix metallo-proteinases-2; TIMP-1, Tissue inhibitor of metalloproteinase-1; TNF-α, Tumor necrosis factor-α.

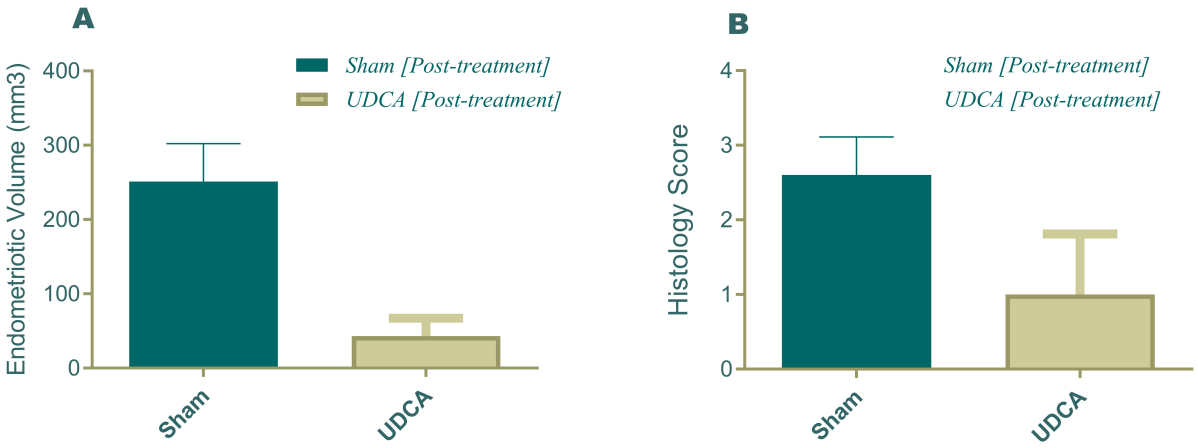

Fig. 2. Endometriotic volume and histology scores in groups of Sham and Ursodeoxycholic acid (UDCA) rat models. (A) Comparisons of Endometriotic volume between the Sham post-treatment and UDCA post-reatment groups; (B) Comparisons of Histology scores between the Sham post-treatment and UDCA post-reatment groups.

proteolytic enzyme family, as well as in various pathologies such as tumor invasion [21]. Additionally, the high proteolytic activities of MMP enzymes play an important role in the pathogenesis of endometriosis [22]. In a study of endometriosis cases, MMP-2 was found to be high in peritoneal endometrial implants and it was thought that increased proteolytic activity affected pathogenesis [23]. Previously, it was determined that the level of MMP-2 increased in proportion with the severity of endometriosis as the disease progressed, more local and systemic MMP-2 levels increased, and more tissue remodeling has occurred, and MMP-2 increased invasion and progression further increased [24]. MMP-2 levels have been high in peritoneal fluid and serum of endometriosis patients. While the estrogen hormone increased MMP-2 on the other hand the progesterone hormone has prevented the development of endometriosis by decreasing MMP-2 [17]. Studies have revealed that MMP-2 was also associated with angiogenesis, tumor growth, invasion, and metastasis. MMP-2 has also been shown to play an important role in tumor growth, invasion, and metastasis in many types of cancer such as gastric cancer [25], lung cancer [26], and suppression of MMP2 is

Table 4. Comparison of post-treatment values of Sham and UDCA group.

Variables	Sham	UDCA	*p*-value
MMP-2, pg/mL	1.378 ± 0.475	0.941 ± 0.417	0.049
TGF-β1, pg/mL	1.265 ± 0.369	1.076 ± 0.372	0.290
TIMP-1, pg/mL	0.836 ± 0.466	0.965 ± 0.325	0.226
TNF-α, pg/mL	0.582 ± 0.485	0.372 ± 0.164	0.705
Endometriotic Volume (mm^3)	251 ± 51.153	43.3 ± 24.046	<0.0001
Histology score	2.6 ± 0.516	1.0 ± 0.816	0.001

All data were given as Mean ± Standard Deviation. UDCA, Ursodeoxycholic acid; TGF-β1, Transforming growth factor β1; MMP-2, matrix metalloproteinases-2; TIMP-1, Tissue inhibitor of metalloproteinase-1; TNF-α, Tumor necrosis factor-α.

thought to be important for cancer treatment. In our study, MMP-2 following the treatment in the control was higher than before the treatment.

In our research, TIMP-1 has shown similarity in all of the groups. In the previous literature with endometriosis cases [27]. TIMP-1 levels decrease while MMP increase in breast cancer [28], on the contrary, there are also studies showing that TIMP-1 increases. In adenomyosis cases, MMP-2 and TIMP-1 was shown to increase together and it has determined that increase in TIMP-1 level developed secondary to MMP-2 increase, therefore preventing the invasive effect of MMP-2 [29]. The increase in TIMP-1 affects cell growth, angiogenesis, apoptosis, oncogenesis with a cytokine-like effect [30]. In a trial, the elevation of TIMP-1 was shown to indicate poor prognosis in colorectal cancer as a result of increased MMP in tumor invasion and metastasis, which had an effect of increasing tumor growth [31].

In our data evaluation, the endometriotic focal volume showed a signficant alteration by the treatment. Comparing the pre-treatment and posttreatment values of the UDCA group, the post-treatment endometriotic focal volume was significantly lower than the pre-treatment values.

As a minor limitation, we can notice the number of rats. Although a total of 60 mature female rats with no pregnancy were included in our research, it was the relatively low number of experimental animals in the present study.

5. Conclusions

As a result, the post-treatment endometriotic focal volume and histological scoring decreased with UDCA. There was no significant change UDCA group before and after the treatment in terms of MMP-2, TGF-β1, TIMP-1 and TNF-α levels. According to the results of the study, UDCA may be considered an effective alternative in the treatment of endometriosis. Further prospective trials with a large number of human participants are needed to achieve more data on this area.

Availability of Data and Materials

The data supporting this study's findings are available from the corresponding author upon reasonable request.

Author Contributions

AS, SKK, UE and AND designed the research study. AS and UE performed the research. SKK and AND provided help and advice on the experiments. UE analyzed the data. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

This animal trial was assessed and approved by the Animal Studies Ethical Board of the Dumlupinar University (ID: 20151208/date: 22/12/2022) and is compliant with "Principles of laboratory animal care - 1985".

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Conflict of Interest

The authors declare no conflict of interest.

References

[1] Child TJ, Tan SL. Endometriosis: aetiology, pathogenesis and treatment. Drugs. 2001; 61: 1735–1750.

[2] Fukunaga M. Uterus-like mass in the uterine cervix: superficial cervical endometriosis with florid smooth muscle metaplasia? Virchows Archiv. 2001; 438: 302–305.

[3] Macer ML, Taylor HS. Endometriosis and infertility: a review of the pathogenesis and treatment of endometriosis-associated infertility. Obstetrics and Gynecology Clinics of North America. 2012; 39: 535–549.

[4] Rolla E. Endometriosis: advances and controversies in classifi-

cation, pathogenesis, diagnosis, and treatment. F1000Research. 2019; 8: F1000 Faculty Rev-529.

[5] Greene AD, Lang SA, Kendziorski JA, Sroga-Rios JM, Herzog TJ, Burns KA. Endometriosis: where are we and where are we going? Reproduction. 2016; 152: R63–R78.

[6] Hever A, Roth RB, Hevezi P, Marin ME, Acosta JA, Acosta H, *et al*. Human endometriosis is associated with plasma cells and overexpression of B lymphocyte stimulator. Proceedings of the National Academy of Sciences of the United States of America. 2007; 104: 12451–12456.

[7] Agostinis C, Zorzet S, De Leo R, Zauli G, De Seta F, Bulla R. The combination of N-acetyl cysteine, alpha-lipoic acid, and bromelain shows high anti-inflammatory properties in novel in vivo and in vitro models of endometriosis. Mediators of Inflammation. 2015; 2015: 918089.

[8] Di Tucci C, Di Feliciantonio M, Vena F, Capone C, Schiavi MC, Pietrangeli D, *et al*. Alpha lipoic acid in obstetrics and gynecology. Gynecological Endocrinology. 2018; 34: 729–733.

[9] Salehpoor Z, Jahromi BN, Tanideh N, Nemati J, Akbarzade-Jahromi M, Jahromi MK. High intensity interval training is superior to moderate intensity continuous training in enhancing the anti-inflammatory and apoptotic effect of pentoxifylline in the rat model of endometriosis. Journal of Reproductive Immunology. 2023; 156: 103832.

[10] Chapman RW. Cost effectiveness of using ursodeoxycholic acid to treat primary biliary cholangitis. British Journal of Hospital Medicine. 2018; 79: 460–464.

[11] Taskin MI, Gungor AC, Adali E, Yay A, Onder GO, Inceboz U. A Humanized Anti-Interleukin 6 Receptor Monoclonal Antibody, Tocilizumab, for the Treatment of Endometriosis in a Rat Model. Reproductive Sciences. 2016; 23: 662–669.

[12] Laganà AS, Salmeri FM, Ban Frangež H, Ghezzi F, Vrtačnik-Bokal E, Granese R. Evaluation of M1 and M2 macrophages in ovarian endometriomas from women affected by endometriosis at different stages of the disease. Gynecological Endocrinology. 2020; 36: 441–444.

[13] Ni Z, Sun S, Bi Y, Ding J, Cheng W, Yu J, *et al*. Correlation of fecal metabolomics and gut microbiota in mice with endometriosis. American Journal of Reproductive Immunology. 2020; 84: e13307.

[14] D'Alterio MN, Giuliani C, Scicchitano F, Laganà AS, Oltolina NM, Sorrentino F, *et al*. Possible role of microbiome in the pathogenesis of endometriosis. Minerva Obstetrics and Gynecology. 2021; 73: 193–214.

[15] Keely SJ, Steer CJ, Lajczak-McGinley NK. Ursodeoxycholic acid: a promising therapeutic target for inflammatory bowel diseases? American Journal of Physiology. Gastrointestinal and Liver Physiology. 2019; 317: G872–G881.

[16] Önalan G, Tohma YA, Zeyneloğlu HB. Effect of Etanercept on the Success of Assisted Reproductive Technology in Patients with Endometrioma. Gynecologic and Obstetric Investigation. 2018; 83: 358–364.

[17] Huang HF, Hong LH, Tan Y, Sheng JZ. Matrix metalloproteinase 2 is associated with changes in steroid hormones in the sera and peritoneal fluid of patients with endometriosis. Fertility and Sterility. 2004; 81: 1235–1239.

[18] Chae U, Min JY, Kim SH, Ihm HJ, Oh YS, Park SY, *et al*. Decreased Progesterone Receptor B/A Ratio in Endometrial Cells by Tumor Necrosis Factor-Alpha and Peritoneal Fluid from Patients with Endometriosis. Yonsei Medical Journal. 2016; 57: 1468–1474.

[19] Creus M, Fábregues F, Carmona F, del Pino M, Manau D, Balasch J. Combined laparoscopic surgery and pentoxifylline therapy for treatment of endometriosis-associated infertility: a preliminary trial. Human Reproduction. 2008; 23: 1910–1916.

[20] Bałkowiec M, Maksym RB, Włodarski PK. The bimodal role of matrix metalloproteinases and their inhibitors in etiology and pathogenesis of endometriosis (Review). Molecular Medicine Reports. 2018; 18: 3123–3136.

[21] Matrisian LM. The matrix-degrading metalloproteinases. BioEssays. 1992; 14: 455–463.

[22] Chung HW, Wen Y, Chun SH, Nezhat C, Woo BH, Lake Polan M. Matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-3 mRNA expression in ectopic and eutopic endometrium in women with endometriosis: a rationale for endometriotic invasiveness. Fertility and Sterility. 2001; 75: 152–159.

[23] Chung HW, Lee JY, Moon HS, Hur SE, Park MH, Wen Y, *et al*. Matrix metalloproteinase-2, membranous type 1 matrix metalloproteinase, and tissue inhibitor of metalloproteinase-2 expression in ectopic and eutopic endometrium. Fertility and Sterility. 2002; 78: 787–795.

[24] Malvezzi H, Aguiar VG, Paz CCPD, Tanus-Santos JE, Penna IADA, Navarro PA. Increased circulating MMP-2 levels in infertile patients with moderate and severe pelvic endometriosis. Reproductive Sciences. 2013; 20: 557–562.

[25] Wang T, Hou J, Jian S, Luo Q, Wei J, Li Z, *et al*. miR-29b negatively regulates MMP2 to impact gastric cancer development by suppress gastric cancer cell migration and tumor growth. Journal of Cancer. 2018; 9: 3776–3786.

[26] Wu DM, Deng SH, Liu T, Han R, Zhang T, Xu Y. TGF-β-mediated exosomal lnc-MMP2-2 regulates migration and invasion of lung cancer cells to the vasculature by promoting MMP2 expression. Cancer Medicine. 2018; 7: 5118–5129.

[27] Collette T, Bellehumeur C, Kats R, Maheux R, Mailloux J, Villeneuve M, *et al*. Evidence for an increased release of proteolytic activity by the eutopic endometrial tissue in women with endometriosis and for involvement of matrix metalloproteinase-9. Human Reproduction. 2004; 19: 1257–1264.

[28] Têtu B, Brisson J, Wang CS, Lapointe H, Beaudry G, Blanchette C, *et al*. The influence of MMP-14, TIMP-2 and MMP-2 expression on breast cancer prognosis. Breast Cancer Research. 2006; 8: R28.

[29] Yang JH, Wu MY, Chen MJ, Chen SU, Yang YS, Ho HN. Increased matrix metalloproteinase-2 and tissue inhibitor of metalloproteinase-1 secretion but unaffected invasiveness of endometrial stromal cells in adenomyosis. Fertility and Sterility. 2009; 91: 2193–2198.

[30] Ries C. Cytokine functions of TIMP-1. Cellular and Molecular Life Sciences. 2014; 71: 659–672.

[31] Holten-Andersen M, Christensen IJ, Nilbert M, Bendahl PO, Nielsen HJ, Brünner N, *et al*. Association between preoperative plasma levels of tissue inhibitor of metalloproteinases 1 and rectal cancer patient survival. A validation study. European Journal of Cancer. 2004; 40: 64–72.