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Research Article

# NF-κB expression in endometriosis induced rat uterine tissue

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## Abstract

**Objective:** Our aim in this study is to examine the effects of endometriosis on uterine tissue.

**Materials and methods:** Two groups were formed, with eight rats in each group. Sham group was subjected only administration of 1% corn oil for 7 days. The endometriosis (EM) model was induced with estradiol benzoate (EB). In the EM group, EB was given to rats for 7 days. At the end of the experiment, blood was taken from the animals and serum total antioxidant status (TAS) and total oxidant status (TOS) values were studied. Uterine tissues were fixed in formalin and embedded in paraffin blocks. NF-κB 3 immune staining were performed on uterine sections. The results were examined under the microscope.

**Results:** Compared to sham group, TAS values were significantly decreased and TOS values were significantly increased in serum of rats belonging to EM group. In the analysis of NF-κB expression in uterine tissue of sham group, the expression was slight and found only in connective tissue cells and inflammatory cells. NF-κB expression was mainly negative. After EM induction, NF-κB expression was increased in degenerated epithelial cells and gland cells, inflammatory cells around the glands and blood vessel endothelial cells and lamina propria.

**Conclusion:** We think that NF-κB signal may be a determinant in the treatment of endometriosis.

**Keywords:** endometriosis, immunohistochemistry, NF-κB, biochemistry, rat

## INTRODUCTION

Endometriosis is a common, benign, inflammatory gynecological disease involving the presence and growth of dysfunctional endometrial-like glands and stroma, often with reactive fibrosis and muscle metaplasia outside the uterus. Endometriosis affects 5-10% of all women of reproductive age and causes a variety of symptoms including chronic pelvic pain, dysmenorrhea and dyspareunia. Endometriosis is associated with infertility and an increased risk of epithelial ovarian cancer (EOC) [1, 2].

Chronic inflammation in the peritoneal cavity is important in the progression of endometriosis. Inflammatory mediators such as IL-1β and TNF-α activate the transcription factor NF-κB, which produces a positive appearance to increase various inflammatory mediators including TNF-α, IL-1, IL-6 and IL-12 [3, 4]. The inflammatory state can cause the formation of endometrial cell adhesion sites due to the tissue damage process. NF-κB activation is a determinant of intracellular survival and tissue specificity. The NF-κB protein helps regulate a variety of post-translational modifications that affect their stability, degradation, affinity for binding sites, and interactions within the dimer and with other transcription factors [5].

In our study, it was aimed to examine the effect of the cytokine transcription factor NF-kB activation on the inflammation process of the changes in the uterine region after endometriosis.

## MATERIALS AND METHODS

Before starting the experimental procedure, all animals were given general anesthesia with 90 mg/kg intramuscular ketamine hydrochloride (Ketalar; Pfizer, Istanbul, Turkey) and 8 mg/kg xylazine (Rompun; Bayer, Istanbul, Turkey). Two groups were formed, with eight rats in each group. To create an endometriosis model, estradiol benzoate (EB) was formed by dissolving it in 1% corn oil.

1. **Sham group (n=8):** After the estrus stages of female rats were determined, no further intervention was done and animals were given 1% corn oil.
2. **Endometriosis (EM) group (n=8):** After the estrus stages of female rats were determined, 3 µg/kg EB was administered subcutaneously to the rats for 7 days.

After all experimental stages were completed (at the end of the 7th day), the animals were sacrificed by intracardiac blood collection under general anesthesia.

### Biochemical Analysis

Biochemical analysis was performed according to Durgun et al [6]. After centrifugation at 12.000 rpm for 5 minutes to separate serum from intracardiac blood samples, supernatants were used in assays to measure total antioxidant status (TAS) and total oxidant status (TOS). Measurements were measured spectrophotometrically using a biochemical autoanalyzer (AU5800; Beckman Coulter, Inc., Brea, CA, USA). TAS values

were recorded as µmol H2O2 Eq/L and TOS values were recorded as µmol Trolox Eq/L.

### Histological Tissue processing

The abdomen of the rats was opened and the uterine tissue was taken and 10% formalin was taken for routine histology. After fixation (24 hours), tissues will be washed (1 night), increased alcohol series (50%, 70%, 80%, 90%, 96% and absolute ethyl alcohol series) and clearing (3x30 minutes in xylene) followed by 58°C. C was paraffin infiltration. Then, the tissues were embedded in paraffin blocks and 5 µm thick sections were taken from the blocks for immunohistochemical staining with the help of a microtome (catalog no: Leica RM2265, Wetzlar, Germany) [7].

### Immunohistochemical Staining

Uterine sections taken from paraffin blocks were placed in a water bath set at 37°C and then on polylysine slides. Sections were kept in an oven at 58-62°C for 6 hours to dissolve excess paraffin on the slide. Sections were deparaffinized in xylene for 3x15 minutes. Sections were passed through decreasing alcohol series (100%, 96%, 90%, 70%, 50% ethyl alcohol) for 10 minutes and brought to distilled water for 5 minutes. Sections were washed 3x5 minutes in phosphate buffer solution (PBS). Sections were taken into EDTA buffer solution (pH: 8.0, catalog no: ab93680, Abcam, Cambridge, USA) and heat-induced epitope retrieval was performed. Sections left at room temperature for 20 minutes were taken back into PBS. Hydrogen peroxide solution (catalog no: TA-015-HP, ThermoFischer, Fremont, CA, USA) was dripped onto the sections and incubated for 20 minutes. Then, they were washed with PBS for 3X5 minutes and kept in Ultra V Block (catalog no: TA-015-UB, ThermoFischer, Fremont, CA, USA) solution for 7 minutes. Sections were overnight at +4°C with NF-κB (AFG bioscience, US, 1/100) antibodies. Biotin-containing secondary antibody (catalog no: TP-015-BN, ThermoFischer, Fremont, CA, USA) was dripped onto the sections washed with PBS and incubated for 14 minutes. Then, streptavidin-peroxidase (catalog no: TS-015-HR, ThermoFischer, Fremont, CA, USA) will be dripped and waited for 15 minutes. Diaminobenzidine (DAB) (catalog no: TA-001-HCX, ThermoFischer, Fremont, CA, USA) was dripped onto the sections and the reaction was observed under the microscope and stopped with PBS. After counterstaining with Harris hematoxylin, the sections were covered with entellan (catalog no: 107961, Sigma-Aldrich, St. Louis, MO, United States) and evaluated and visualized under a Zeiss Imager A2 photomicroscope [8].

### Statistical analysis

All statistical analyzes were performed with the IBM SPSS Statistics version 25 software program. Data distribution was made using the Kolmogorov Smirnov test. Comparisons between groups were made with Student t test. Data were recorded as median (min-max). A p value of <0.05 was considered statistically significant.

## RESULT

Total antioxidant status (TAS) and total oxidant status (TOS) values in sham and endometriosis (EM), groups are shown in Table 1. TAS was decreased and TOS was increased significantly in EM group compared to sham group (p=0.012).

Table 1: Comparison of TAS and TOS values between groups

| **Groups** | **TAS** (µmol H2O2 Eq/L) median (min-max) | **TOS** (µmol Trolox Eq/L) median (min-max) | **p** |
|---|---|---|---|
| **Sham** | 1.45 (0.76 – 1.65) | 15.73 (10.34 – 55.18) | 0.012 |
| **EM** | 0.95 (0.58 – 1.11) | 35.80 (21.76 – 88.85) | |

**EM:** endometriosis, **TAS:** total antioxidant status, **TOS:** total oxidant status

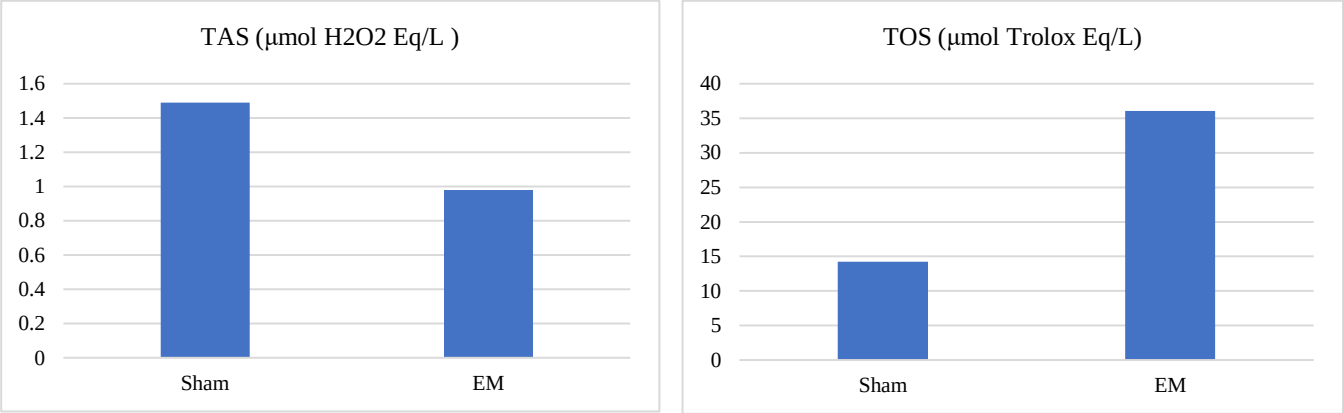


Figure 1: Distribution of TAS and TOS data by groups

NF-κB expression in uterine tissue was shown in Figure 1 by immunohistochemistry method. In sham group, the uterine sections showed slight NF-κB expression in macrophages near the basement membrane, in plasma cells and a few connective tissue cells (Figure 2a). When the endometriosis group was examined, a significant increase in NF-κB expression was observed in degenerated epithelial cells and gland cells, and a significant increase in NF-κB expression in solitary and aggregated inflammatory cells around the glands and blood vessel endothelial cells and lamina propria (Figure 2b).

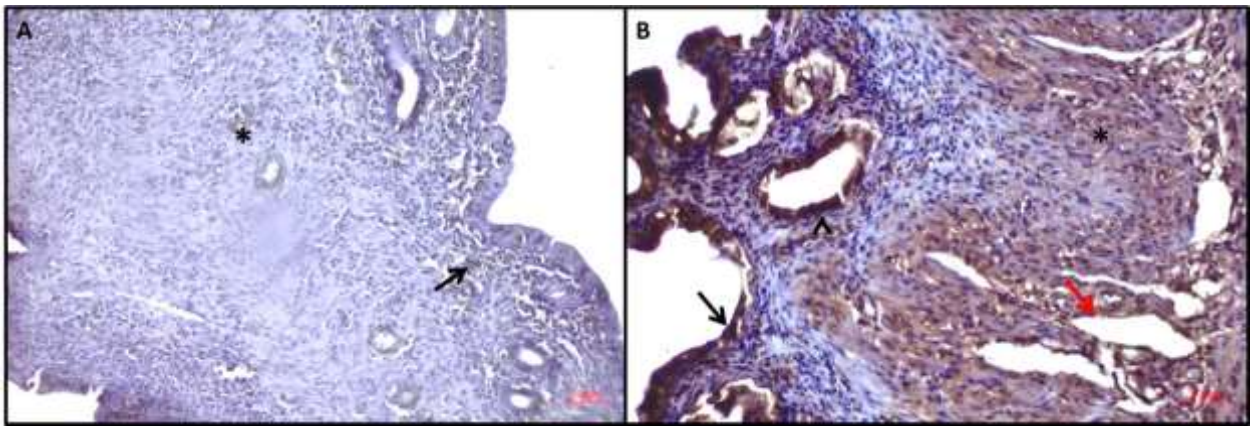


Figure 2. NF-κB immune staining. **a) Sham group:** Positive NF-κB expression in macrophages and connective tissue cells (asterisk). **b) Endometriosis group:** Increased NF-κB expression in epithelial cells (arrow) and gland cells (arrowhead), inflammatory cells (asterisk) and blood vessel endothelial cells (red arrow). Scale bar: 50µm, magnification: 20X

## DISCUSSION

Endometriosis is a common, benign, inflammatory gynecological disease with the development of dysfunctional endometrial-like glands and stroma, often with reactive fibrosis and muscle metaplasia outside the uterus. The proliferation and growth of the ectopic endometrium causes chronic pelvic pain, infertility, dysmenorrhea and other clinical symptoms in endometriosis patients [9, 10]. In addition to increased inflammatory responses, activation of proinflammatory factors and signaling pathways in ectopic endometrial tissues, this response is closely associated with the pathogenesis of endometriosis induced by increased infiltration of macrophages and immune cells [11-13]. The severity of vaginal hyperalgesia after endometriosis parallels estradiol (E2) levels during the estrous cycle. E2 has been reported to modulate hyperalgesic severity [14].

Endometriosis change the histology of uterine tissue. Kennedy et al [15] stated that morphology of endometriosis is superficial "powder burn" or "gunshot" lesions; black, dark-brown, or bluish puckered lesions, nodules or small cysts with hemorrhage and increased fibrosis. Mashele et al [16] showed that endometriosis causes pathology in tissues such as topographic sites, endometrial glands, hemorrhage, endometriotic cysts, ciliated metaplasia.

The NF-κB signaling pathway is a major transcription factor that plays an important role in many chronic inflammatory diseases such as endometriosis [17]. Increased NF-κB activation in endometriosis leads to the maintenance and progression of endometriosis lesions, making NF-κB a potential drug target in endometriosis. NF-κB proteins are normally retained in the cytoplasm by inhibitory proteins called members of the IκB family. Increased NF-κB activation in endometriosis has led to the maintenance and progression of endometriosis lesions, downgrading NF-κB as a potential pathway in the condition of endometriosis [18]. Various factors such as estrogen, progesterone, oxidative stress and non-coding RNAs can regulate NF-kB signaling in endometriosis, while proinflammatory cytokines such as TNF-α, IL-1β, IL-6 and IL-8 induce degradation of NκB. May cause nuclear translocation of NF-κB members [19]. Binding of NF-κB members to the DNA promoter has been reported to help induce the expression of proinflammatory cytokines, intercellular adhesion molecules and angiogenesis factors [18, 20].

In our study, an increase in NF-KB expression was observed in cells in the basal region of the uterine epithelium and in solitary and aggregated communities in the areas where the lamina propria is located after endometriosis. In degenerative gland cells after endometriosis, NF-KB expression areas were observed in blood vessels, endothelium and surrounding inflammatory cells (Figure 2). As a result of accelerating endometriosis, NF-kB suppression monocytes/macrophages enhance M2 macrophage polarization and inhibit M1 macrophage polarization, developing a pro-repair environment causes neovascularization in ectopic lesions[21, 26].

## CONCLUSION

It has been observed that the expression of NF-κB signal is activated and increased in endometriotic lesions which may cause affects other tissues with the onset of endometriosis and its progression. We think that NF-κB signal may be a determinant in the treatment of endometriosis.

## Conflict of Interest

The author declared that there was no conflict of interest during the cause of this study and producing and submitting this manuscript for publication.

## Author contribution

I.S.E, E.D. and F.A contributed equally to manuscript drafting, writing, data collection, conceptualization and observation. All authors read and approved the final version of the manuscript.

## Data availability

All generated data were presented in this study.

## Ethical approval:

Ethical approval was taken from Dicle University, Animal Experiments Local Ethical Committee (date:24/02/2022 and number:2021/42).

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## REFERENCES

1. Sacco K, Portelli M, Pollacco J, Schembri-Wismayer P, Calleja-Agius J. The role of prostaglandin E2 in endometriosis. Gynecol Endocrinol. 2012; 28(2):134-8. https://doi.org/10.3109/09513590.2011.588753

2. Ahn SH, Monsanto SP, Miller C, Singh SS, Thomas R, Tayade C. Pathophysiology and Immune Dysfunction in Endometriosis. Biomed Res Int. 2015; 2015:795976. https://doi.org/10.1155/2015/795976

3. de Ziegler D, Borghese B, Chapron C. Endometriosis and infertility: pathophysiology and management. The Lancet. 2010; 376(9742):730-8. https://doi.org/10.1016/S0140-6736(10)60490-4

4. Stilley JA, Birt JA, Sharpe-Timms KL. Cellular and molecular basis for endometriosis-associated infertility. Cell Tissue Res. 2012; 349(3):849-62. https://doi.org/10.1007/s00441-011-1309-0

5. Liu J, Sudom A, Min X, Cao Z, Gao X, Ayres M, et al. Structure of the nuclear factor κB-inducing kinase (NIK) kinase domain reveals a constitutively active conformation. J Biol Chem. 2012; 287(33):27326-34. https://doi.org/10.1074/jbc.M112.366658

6. Durgun C, Aşir F. Effect of ellagic acid on damage caused by hepatic ischemia reperfusion in rats. Eur Rev Med Pharmacol Sci. 2022; 26(22):8209-15.

7. Aşır F, Nergiz Y, Pala A. Vitamin E protected the mouse adrenal gland against immobilization stress. Pol J Vet Sci. 2022; 25(3):447-54.

8. Durgun C, Aşır F. Daidzein alleviated the pathologies in intestinal tissue against ischemia-reperfusion. Eur Rev Med Pharmacol Sci. 2023; 27(4):1487-93.

9. Vercellini P, Viganò P, Somigliana E, Fedele L. Endometriosis: pathogenesis and treatment. Nature Reviews Endocrinology. 2014; 10(5):261-75. https://doi.org/10.1038/nrendo.2013.255

10. Kapoor R, Stratopoulou CA, Dolmans MM. Pathogenesis of Endometriosis: New Insights into Prospective Therapies. Int J Mol Sci. 2021; 22(21). https://doi.org/10.3390/ijms222111700

11. Jiang L, Yan Y, Liu Z, Wang Y. Inflammation and endometriosis. Front Biosci (Landmark Ed). 2016; 21(5):941-8. https://doi.org/10.2741/4431

12. Vallvé-Juanico J, Houshdaran S, Giudice LC. The endometrial immune environment of women with endometriosis. Hum Reprod Update. 2019; 25(5):564-91. https://doi.org/10.1093/humupd/dmz018

13. Symons LK, Miller JE, Kay VR, Marks RM, Liblik K, Koti M, et al. The Immunopathophysiology of Endometriosis. Trends Mol Med. 2018; 24(9):748-62. https://doi.org/10.1016/j.molmed.2018.07.004

14. Cason AM, Samuelsen CL, Berkley KJ. Estrous changes in vaginal nociception in a rat model of endometriosis. Horm Behav. 2003; 44(2):123-31.

https://doi.org/10.1016/S0018-506X(03)00121-1

15. Kennedy S, Bergqvist A, Chapron C, D'Hooghe T, Dunselman G, Greb R, et al. ESHRE guideline for the diagnosis and treatment of endometriosis. Hum Reprod. 2005; 20(10):2698-704. https://doi.org/10.1093/humrep/dei135

16. Mashele T, Reddy Y, Pather S. Endometriosis: Three-year histopathological perspective from the largest hospital in Africa. Ann Diagn Pathol. 2020; 45:151458. https://doi.org/10.1016/j.anndiagpath.2019.151458

17. Liu T, Zhang L, Joo D, Sun S-C. NF-κB signaling in inflammation. Signal Transduction and Targeted Therapy. 2017; 2(1):17023. https://doi.org/10.1038/sigtrans.2017.23

18. Oeckinghaus A, Ghosh S. The NF-kappaB family of transcription factors and its regulation. Cold Spring Harb Perspect Biol. 2009; 1(4):a000034.

https://doi.org/10.1101/cshperspect.a000034

19. Liu Y, Wang J, Zhang X. An Update on the Multifaceted Role of NF-kappaB in Endometriosis. Int J Biol Sci. 2022; 18(11):4400-13. https://doi.org/10.7150/ijbs.72707

20. Fischer OM, Kaufmann-Reiche U, Moeller C, Fuhrmann U. Effects of dienogest on surgically induced endometriosis in rats after repeated oral administration. Gynecol Obstet Invest. 2011; 72(3):145-51. https://doi.org/10.1159/000331642

21. Huang YL, Zhang FL, Tang XL, Yang XJ. Telocytes Enhances M1 Differentiation and Phagocytosis While Inhibits Mitochondria-Mediated Apoptosis Via Activation of NF-κB in Macrophages. Cell Transplant. 2021; 30:9636897211002762. https://doi.org/10.1177/09636897211002762

22. Hou X-X, Wang X-Q, Zhou W-J, Li D-J. Regulatory T cells induce polarization of pro-repair macrophages by secreting sFGL2 into the endometriotic milieu. Communications Biology. 2021; 4(1):499. https://doi.org/10.1038/s42003-021-02018-z

23.Haznedar B, Deveci E, Gök E. Effect of Mancozeb Inhalation on the Olfactory Mucosa in Rats. International Journal of Otolaryngology and Head & Neck Surgery. 2022; 11(5):227-33. https://doi.org/10.4236/ijohns.2022.115024https://doi.org/10.4236/ijohns.2022.115024

24. Haznedar B, Ertugrul G, Deveci E. Effect of Cadmium Chloride Toxicity on Olfactory Mucosa. Int J Morphol. 2022; 40(6):1574-8. https://doi.org/10.4067/S0717-95022022000601574

25. Deveci S, Deveci E, Deveci S, Deveci E. Histopathological changes in incisive teeth of the newborn pups of cadmium-applied female rats during pregnancy. Int j morphol. 2010; 28(4):1131-4. https://doi.org/10.4067/S0717-95022010000400023

26. Özevren H, İrtegün S, Deveci E, Aşır F, Pektanç G, Deveci Ş. Ganoderma Lucidum Protects Rat Brain Tissue Against Trauma-Induced Oxidative Stress. Korean Journal of Neurotrauma. 2017; 13(2):76-84 https://doi.org/10.13004/kjnt.2017.13.2.76