

Determination of TNF-beta Marker in Intrauterine *E. coli* Induced Endometritis Model in Rats

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Abstract

Objectives: This study aims to investigate the importance of tumor necrosis factor (TNF)-beta marker in the endometritis model created by *E. coli*.

Materials and Methods: Rats were divided into control and endometritis groups with 7 rats in each group in the study. All animals were fed as ad libitum. No application was made to rats in the control group. Progesterone (P4) was administered subcutaneously to rats in the experimental group at a daily dose of 16 mg/kg between the 0th and 4th days of the study. *E. coli* was administered intrauterinely at 25 µl and 1 × 10⁵ CFU/rat on the 3rd day. Blood samples were taken at the end of the study after the lives of all animals were terminated in accordance with ethical rules. Hemogram analysis was performed for white blood cell (WBC) levels from the blood samples taken. The TNF-beta analysis was performed with a commercial kit by separating serum from blood samples.

Results: WBC (p=0.0004) and TNF-beta (p=0.027) were statistically higher in the endometritis group compared to the control group.

Conclusion: Consequently, increased TNF-beta in endometritis may be a diagnostic marker for inflammations in the genital tract. However, its effectiveness may be better demonstrated by conducting comprehensive studies with the relevant marker.

Keywords: TNF-beta, White blood cell, Endometritis, Rat

Özet

Amaç: Çalışmanın amacı, *E. coli* ile oluşturulan endometritis modelinde tümör nekroz faktör (TNF) -beta markörünün öneminin araştırılması amaçlandı.

Gereç ve Yöntem: Çalışmada ratlar her grupta 7'er rat olacak şekilde kontrol ve endometritis grubuna ayrıldı. Tüm hayvanlar ad libitum olarak beslendi. Kontrol gruplarına herhangi bir uygulama yapılmamıştır. Deneysel gruba ise çalışmanın 0 ve 4. günleri arasında ratlara günlük subkutan 16 mg/kg dozunda progesteron (P4) uygulandı. 3. gün 25 µl, 1 × 10⁵ cfu/rat dozunda *E. coli* intrauterin olarak uygulandı. Çalışma sonunda tüm hayvanların yaşamına etik kurallara uygun bir şekilde son verildikten sonra kan örnekleri alındı. Alınan kan örneklerinden akyuvar (WBC) düzeyi için hemogram analizi yapıldı. Kan örneklerinden serum ayrıştırılarak ticari kit ile TNF-beta analizi yapılmıştır.

Bulgular: WBC (p=0.0004) ve TNF-beta (p=0.027) kontrol grubuna göre endometritis grubunda istatistiksel olarak daha yüksekti.

Sonuç: Sonuç olarak, endometritiste TNF-beta'nın artış göstermesi genital sistemdeki inflamasyonlar için tanı markırı olabilir. Fakat ilgili markir ile kapsamlı çalışmalar yapılarak etkinliği daha iyi ortaya konulabilir.

Anahtar kelimeler: TNF-Beta, Endometrit, Lökosit, Rat

Introduction

Endometritis is an infectious disease of the uterus, usually characterized by the passage of microorganisms through the endometrial cavity during menstruation, delivery, or postpartum period¹. Endometritis is seen as a component of pelvic inflammatory disease that causes infertility in women². Endometritis or uterine inflammation evaluated in the pelvic inflammatory disease (PID) group may be acute or chronic³. Anaerobic gram-negative bacteria generally cause endometritis. The most infectious microorganisms are *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Escherichia coli*, *Trueperella pyogenes*, *Fusobacterium necrophorum*, and *Staphylococcus aureus*^{2,4,5}.

Pelvic inflammatory disease is the most commonly associated-endometritis, chronic endometritis, or endometritis with dense plasma cells, as well as being more intense compared to lymphocytes and neutrophils. Histological results of chronic endometritis include plasma cells, glands, blood vessels, and lymphoid infiltrates in the stroma close to the surface epithelium. Plasma cells may not be numerous and may be distributed between lymphocytes and neutrophils alone. Neutrophils are found on the surface and in the glandular epithelium. They may also cause microabscesses¹. They may also be found in lymphocytes, eosinophils, and other lymphoid aggregates⁶. White blood cells (WBC) are known to increase in cases of infection⁷.

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Cytotoxic activity was discovered in culture supernatants of mitogen or specific antigen-induced lymphocytes. This is called Lymphotoxin alpha (LTA). LTAs have recently been named TNF-beta due to the difficulty in purification and confusion with the tumor necrosis factor (TNF) to which they are genetically linked. TNF-beta also plays an important role in the development of lymphoid organs. It also plays a positive role in autoimmunity against pathogens, chronic inflammation, and cancer by acting like lymph nodes⁸.

This study aims to determine the TNF-beta marker in rats with endometritis induced by intrauterine *E. coli* experimentally.

Materials and Methods

The study was started after the permission of the Kafkas University Animal Experiments Ethics Committee (KAU HADYEK-2021/003). The study used 14 albino rats. The rats used in the study were obtained from Kafkas University Experimental Animals Implementation and Research Center. Rats were divided into groups with 7 rats in each following the 15-day adaptation period. All animals were fed as *ad-libitum* with feed and drinking water.

The menstrual cycles of all rats were determined by vaginal cytology before starting the study⁹. Study groups were formed from rats determined to be in the diestrus. Blood was taken from the tail vein of rats found to be in the diestrus

period by vaginal cytology and centrifuged at 3000 RPM and serum progesterone concentration was measured on the same day. Serum progesterone concentration was also determined and those higher than 1 ng/ml were included in the study.

Group I (Control): Rats were fed with pellet feed and drinking water for 11 days, no other application was performed.

Group II (Intrauterine *E. coli*): Progesterone (P4) was administered subcutaneously to rats at a daily dose of 16 mg/kg between the 0th and 4th days of the study. *E. coli* was administered intrauterinely at 25 μ l and 1×10^5 CFU/rat on the 3rd day.

Rats were euthanized under ketamine hydrochloride (75 mg/kg) and xylazine (15 mg/kg) anesthesia and blood sampling was performed at the end of the study (7 days after *E. coli* administration). Blood samples were centrifuged at 3000 RPM and separated and stored at -20- until the day of measurement.

Biochemical analysis

WBC measurement was performed with a hemogram device (MS4, Vega Group) on whole blood samples taken at the end of the study on the same day. The serum samples were measured according to the TNF-beta procedure using a commercial ELISA (Elabscience[®]) kit.

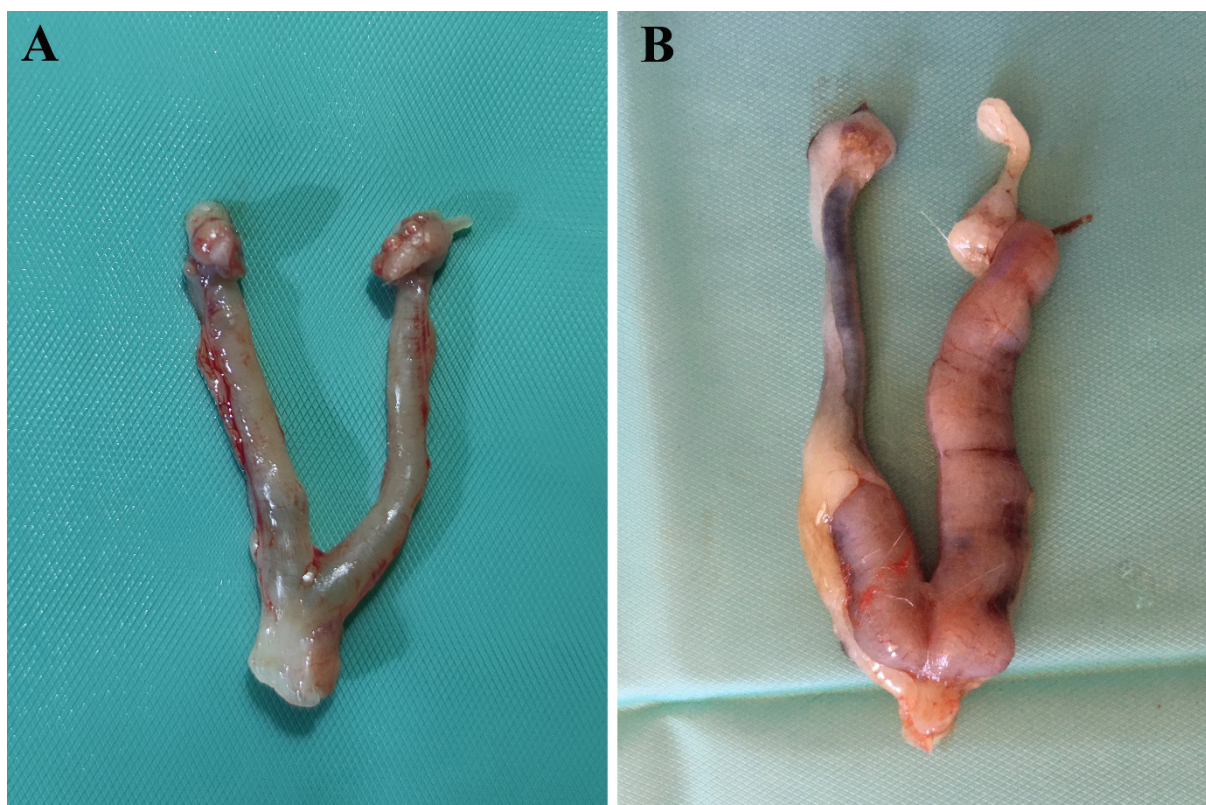


Figure 1: Macroscopic image of the uterus in the control (A) and endometritis (B) groups

Statistical analysis

Statistical analysis of the obtained data was performed with GraphPad 8.1 (GraphPad Prism 8/San Diego, CA) software. The differences between the groups were considered significant at $p < 0.05$. Analysis of unpaired t-test was conducted for all the biochemical parameters to test if there is a difference between the two groups.

Results

The case of endometritis is depicted in Figure 1. Edema and prudent discharge were detected as a result of the section made in the endometritis uterus shown in the figure. There was a statistically significant increase in WBC level in the endometritis group compared to the control group according to the analysis ($p = 0.0004$, Figure 2). A statistically significant difference was found in the endometritis group when TNF-beta obtained as a result of ELISA measurement was examined ($p = 0.027$, Figure 3).

Discussion

Endometritis can be an acute or chronic process or it can also be focal or common. Acute endometritis is an infection due to polymicrobial infection usually occurring in the

uterus during childbirth¹⁰. Anaerobic gram-negative bacteria generally constitute the infection. The most common microorganisms are *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Escherichia coli*, *Trueperella pyogenes*, *Fusobacterium necrophorum*, and *Staphylococcus aureus*^{2,4,5}. It not only causes infertility in livestock but also leads to serious economic losses by affecting milk yield and reproductive performance¹¹, whereas infertility is the ultimate consequence of the disease in humans¹¹⁻¹⁴

Leukocytes (WBC) originate in the bone marrow and are divided into two groups as granulocytes and agranulocytes. These cells play an active role both in innate immunity and in subsequently acquired immunity. WBC participates in the processes of immunity and inflammation. WBC, which is specifically divided into groups as neutrophils, eosinophils, basophils, lymphocytes, and monocytes, forms a response to antigenic stimuli^{15,16}. The increase in WBC in the blood suggests the presence of antigenic formation in this case. Increased WBC is known to be the body's acute phase response to infection¹⁷. Our study has created endometritis, one of the uterine infections, by intrauterinely administering *E. coli*. WBC differs statistically in the endometritis group compared to the control group as a result of the hemogram. This data supports the formation of infection in the uterus. WBC normal values were $2.1-15.54 \times 10^3/\text{mm}^3$ in a study¹⁸. The WBC value in the endometritis group in our study was $16.76 \times 10^3/\text{mm}^3$. Hale et al.¹⁹ reported that WBC values of a

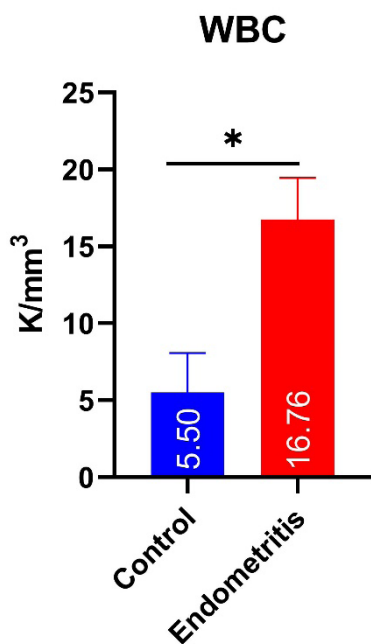


Figure 2: Change in WBC levels in the control and endometritis groups, * $p = 0.0004$

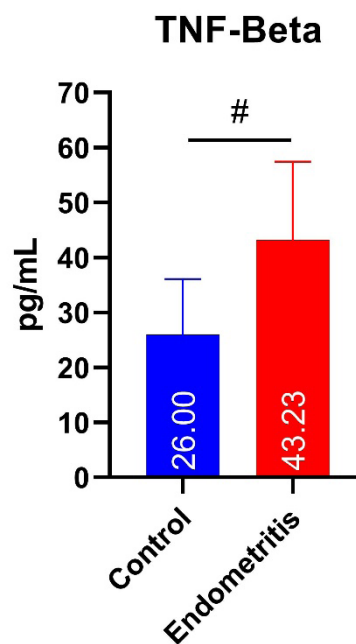


Figure 3: Change in TNF-beta level in the control and endometritis groups, # $p = 0.027$

patient with necrotizing endometritis increased in their case report. Even subclinical endometritis in cattle showed an increase in WBC value in another study²⁰.

Tumor necrosis factor-beta (TNF-beta), also known as lymphotoxin alpha (LTA), is a protein encoded by the lymphotoxin alpha gene. An increase in TNF-beta expression is observed in the case of inflammation²¹. LTA expression was performed in lipopolysaccharide (LPS), *E. coli*, *S. pneumonia* groups in one study, and it was reported as a result that a significant expression occurred in the first eight hours compared to the control group²². Our study has obtained a statistically significant result in LTA (TNF-beta) parameter in endometritis created by *E. coli*. Recombinant human tumor necrosis factor-beta (rhTNF-beta) was found to increase in *E. coli*-induced infections in another study²³. Expiration of TNF-beta due to *E. coli* has been demonstrated by studies. In addition, the effect of TNF on inflammation regulation is known. Our study has demonstrated an increase in TNF-beta in inflammation formed due to *E. coli* infection. Expression of TNF-beta due to *E. coli* in studies supports our study. Buhrmann et al.²⁴ reported that TNF-beta expiration increased in cytokine-induced inflammation.

There is an increase in TNF-beta marker due to infection-inflammation shaped in the endometritis model created by *E. coli*, as a result. Consideration should be given to wider studies on this.

Competing interests

The authors declare that they have no competing interests

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