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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 markırının öneminin araştırılması amaçlandı.

Gereç ve Yöntem: Çalışmada ratlar her grupta 7'şer 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 markır 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 period[1]. Endometritis is seen as a component of pelvic inflammatory disease that causes infertility in women[2]. Endometritis or uterine inflammation evaluated in the pelvic inflammatory disease (PID) group may be acute or chronic[3]. 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 microabscesses[1]. They may also be found in lymphocytes, eosinophils, and other lymphoid aggregates[6]. White blood cells (WBC) are known to increase in cases of infection[7].

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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 nodes[8].

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 study[9]. 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 µl 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.

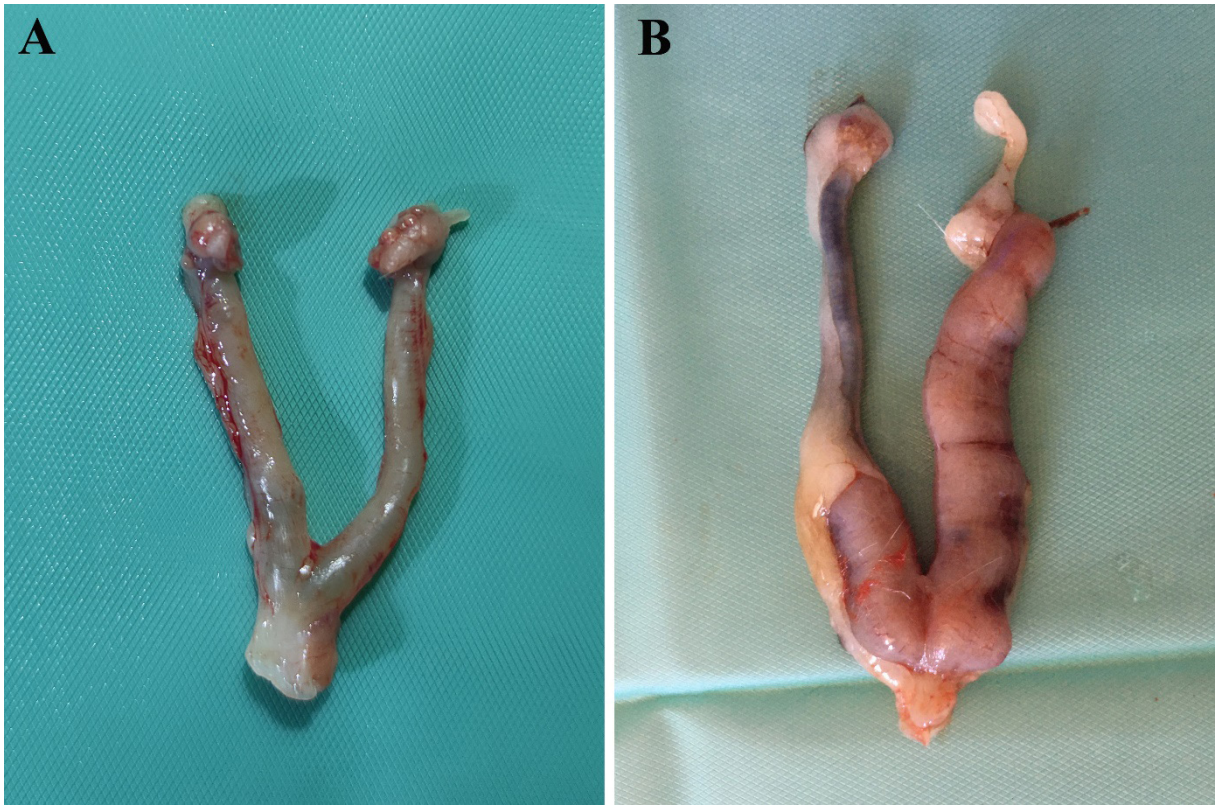

Figure 1: Macroscopic image of the uterus in the control (A) and endometritis (B) groups

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 childbirth[10]. 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 performance[11], whereas infertility is the ultimate consequence of the disease in humans[11–14]

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 infection[17]. 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*10^3/mm in a study [18]. The WBC value in the endometritis group in our study was 16.76*10^3/mm. Hale et al.[19] reported that WBC values of a

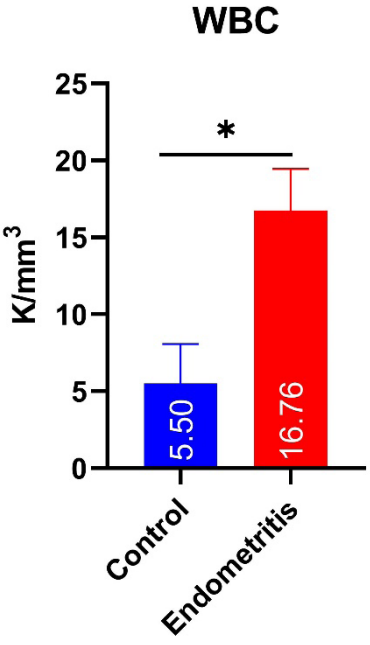

Figure 2: Change in WBC levels in the control and endometritis groups, * p=0.0004

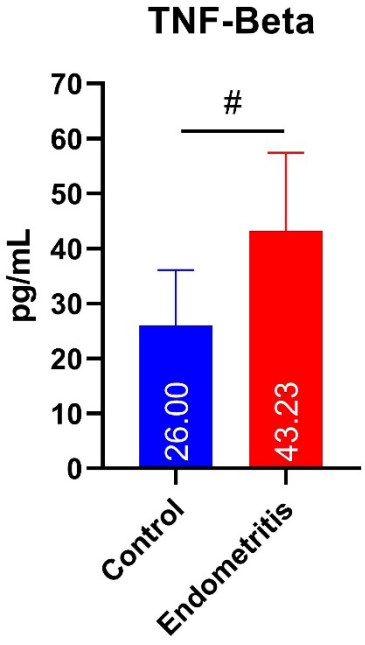

Figure 3: Change in TNF-beta level in the control and endometritis groups, # p=0.027

patient with necrotizing endometritis increased in their case report. Even subclinical endometritis in cattle showed an increase in WBC value in another study [20].

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 inflammation[21]. 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 group[22]. 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 study[23]. 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

Referance

1. Tambouret R. 17 - Gynecologic Infections. In: Kradin RL, editor. Diagnostic Pathology of Infectious Disease (Second Edition) [Internet]. Elsevier; 2018 [cited 2021 Jan 23]. p. 468–88. Available from: http://www.sciencedirect.com/science/article/pii/B9780323445856000175
2. Cohen CR, Manhart LE, Bukusi EA, Astete S, Brunham RC, Holmes KK, et al. Association between Mycoplasma genitalium and acute endometritis. The Lancet. 2002 Mar 2;359(9308):765–6.
3. Tulikangas PK, Schimpf MO. Chapter 22 - Genital and Urinary Tract Infections. In: Sokol AI, Sokol ER, editors. General Gynecology [Internet]. Philadelphia: Mosby; 2007 [cited 2021 Jan 23]. p. 523–42. Available from: http://www.sciencedirect.com/science/article/pii/B978032303247610022X
4. Ness R, Brooks-nelson D. 29 - Pelvic Inflammatory Disease. In: Goldman MB, Hatch MC, editors. Women and Health [Internet]. San Diego: Academic Press; 2000 [cited 2020 Aug 10]. p. 369–80. Available from: http://www.sciencedirect.com/science/article/pii/B9780122881459500334
5. Wiesenfeld H, Paavonen J. 6 - Pelvic Inflammatory Disease. In: Morse SA, Ballard RC, Holmes KK, Moreland AA, editors. Atlas of Sexually Transmitted Diseases and AIDS (Fourth Edition) [Internet]. London: W.B. Saunders; 2010 [cited 2020 Aug 10]. p. 94–110. Available from: http://www.sciencedirect.com/science/article/pii/B9780702040603000065
6. Kindelberger DW, Nucci MR. Chapter 6 - Benign Endometrium. In: Nucci MR, Oliva E, Goldblum JR, editors. Gynecologic Pathology [Internet]. Edinburgh: Churchill Livingstone; 2009 [cited 2021 Mar 3]. p. 197–232. Available from:https://www.sciencedirect.com/science/article/pii/B9780443069208500109
7. Şahin L, Gül HF, Dolanbay T, Makav M, Aras M. The Efficacy of Blood Parameters and Its Derivatives in the Diagnosis of Acute Cholecystitis. Medical Journal of Mugla Sitki Kocman University, 2020; 7(3), 135-140.
8. Ruddle NH. Lymphotoxin. In: Ratcliffe MJH, editor. Encyclopedia of Immunobiology [Internet]. Oxford: Academic Press; 2016 [cited 2021 Mar 3]. p. 466–75. Available from:https://www.sciencedirect.com/science/article/pii/B9780123742797100037
9. Cora MC, Kooistra L, Travlos G. Vaginal Cytology of the Laboratory Rat and Mouse: Review and Criteria for the Staging of the Estrous Cycle Using Stained Vaginal Smears. Toxicol Pathol. 2015 Aug 1;43(6):776–93.
10. Peterson M, Dabbs DJ, Weidner N. CHAPTER 37 - Uterus. In: Weidner N, Cote RJ, Suster S, Weiss LM, editors. Modern Surgical Pathology (Second Edition) [Internet]. Philadelphia: W.B. Saunders; 2009 [cited 2021 Mar 4]. p. 1295–340. Available from: https://www.sciencedirect.com/science/article/pii/B9781416039662000370
11. Demirel MA, Han S, Tokmak A, Gokay NE, Uludag MO, Ustun TY, et al. Therapeutic effects of resveratrol in Escherichia coli-induced rat endometritis model. Naunyn Schmiedebergs Arch Pharmacol. 2019;392(12):1577–89.
12. Adnane M, Kaidi R, Hanzen C, England GCW. Risk factors of clinical and subclinical endometritis in cattle: a review. Turk J Vet Anim Sci. :11.
13. Salah N, Yimer N. Cytological endometritis and its agreement with ultrasound examination in postpartum beef cows. Vet World. 2017 Jun;10(6):605–9.
14. Sheldon IM, Dobson H. Postpartum uterine health in cattle. Anim Reprod Sci. 2004 Jul 1;82–83:295–306.
15. Wilson DA, editor. White Blood Cells. In: Clinical Veterinary Advisor [Internet]. Saint Louis: W.B. Saunders; 2012 [cited 2021 Mar 4]. p. 972. Available from: https://www.sciencedirect.com/science/article/pii/B9781416099796004554
16. Bolliger AP, Everds N. Chapter 2.9 - Haematology of the Mouse. In: Hedrich HJ, editor. The Laboratory Mouse (Second Edition) [Internet]. Boston: Academic Press; 2012 [cited 2021 Mar 4]. p. 331–47. Available from: https://www.sciencedirect.com/science/article/pii/B9780123820082000143
17. Celkan TT. Hemogram bize neler söyler? Turk Arch Pediatr. 2020;55(2).
18. Sauer M, Altrichter J, Kreutzer H-J, Schmidt H, Nöldge Schomburg G, Schmidt R, et al. Safety Evaluation for a Cell-based Immune Support System in an Ex Vivo Rat Model of Gram-negative Sepsis. Ther Apher Dial. 2009;13(5):444–50.
19. Hale A, Kirby JE, Albrecht M. Fatal Spontaneous Clostridium bifermentans Necrotizing Endometritis: A Case Report and Literature Review of the Pathogen. Open Forum Infect Dis

[Internet]. 2016 Apr 1 [cited 2021 Mar 4];3(ofw095). Available from: https://doi.org/10.1093/ofid/ofw095

20. Heidarpour M, Mohri M, Fallah-Rad AH, Shahreza FD, Mohammadi M. Hematological changes before and after treatment in dairy cows with clinical and subclinical endometritis. 2014;5.

21. Nedwin GE, Naylor SL, Sakaguchi AY, Smith D, Jarrett-Nedwin J, Pennica D, et al. Human Lymphotoxin and tumor necrosis factor genes: structure, homology and chromosomal localization. Nucleic Acids Res. 1985 Sep 11;13(17):6361–73.

22. Temple SEL, Almeida CM, Cheong KY, Wunderink RG, Waterer GW. A diplotype in the lymphotoxin alpha gene is associated with differential expression of LTA mRNA induced by Gram-positive and Gram-negative bacteria. Int J Immunogenet. 2007;34(3):157–60.

23. Jin H, Uddin MS, Huang YL, Teo WK. Purification and renaturation of recombinant human lymphotoxin (tumour necrosis factor beta) expressed in Escherichia coli as inclusion bodies. J Chem Technol Biotechnol Oxf Oxfs 1986. 1994 Jan;59(1):67–72.

24. Buhrmann C, Shayan P, Aggarwal BB, Shakibaei M. Evidence that TNF- (lymphotoxin) can activate the inflammatory environment in human chondrocytes. Arthritis Res Ther. 2013 Nov 28;15(6):R202.