Endometriosis Regression in Rats Treated by Colchicine: Histopathological Evaluation and Assessment of TNF-α Levels

Raziye Keskin Kurt, Neslihan Soylu, Atilla Karateke, Ayşe GÜLER OKYAY,

Dilek Benk Silfeler, Aynur Albayrak, Seyda Özdemir, Ali Ulvi Hakverdi

Mustafa Kemal University
Department of Obstetrics and Gynecology

Hatay/Turkey

Objective

To assess the efficacy of colchicine

in an experimental rat endometriosis model.

Materials and methods-1

- Approval of Mustafa Kemal University Animal Laboratory Ethical Committee

- In all procedures which were applied to the animals, local ethical committee laboratory rules and rules of Guidelines for the Care and Use of Laboratory Animals of the US National Institutes of Health (Washington, DC) were obeyed.

Materials and methods-2

- 20 Wistar-albino rats
- Endometriosis model by Vernon and Wilson method during estrous phase
- 4 weeks
- Group 1(colchicine) ($n = 8$) and Group 2 (control) ($n = 8$), 4 were excluded
- İmplant volumes were measured
- Peritoneal fluid samples were taken
- 0.1 mg/kg colchicine, po, same amount of serum physiologic
- 4 weeks

Materials and methods-3

- 2 pieces of endometrial implants for
  - histological examination
  - Biological assessment
- TNF-alpha levels were measured in peritoneal fluid samples and endometrial tissue samples.

Materials and methods-4
Histological Evaluation

- The persistence of epithelial cells in endometrial autografts was evaluated semiquantitatively. The pathologic evaluation of the uterine autografts was performed according to a previously published method as follows:

  - well preserved epithelial layer=score 3
  - moderately preserved epithelium with leukocyte infiltrate=score 2
  - poorly preserved epithelium=score 1
  - no epithelium = score 0

Islimye M, Kilic S, Zulfikaroglu E, Topcu O, Zergeroglu S, Batioglu S. Regression of endometrial autografts in a rat model of endometriosis treated with etanercept. European journal of obstetrics, gynecology, and reproductive biology. 2011;159(1):184-9. Epub 2011/07/12.

Statistics

Continuous variables→ mean ± SD

The differences between numeric variables→Kruskal-Wallis test

Mann-Whitney U test for post-hoc analysis.

$p<0.05$ was recognized as statistically significant.

SPSS 20.0 (SPSS Inc, Chicago, IL, USA) package program.

Findings

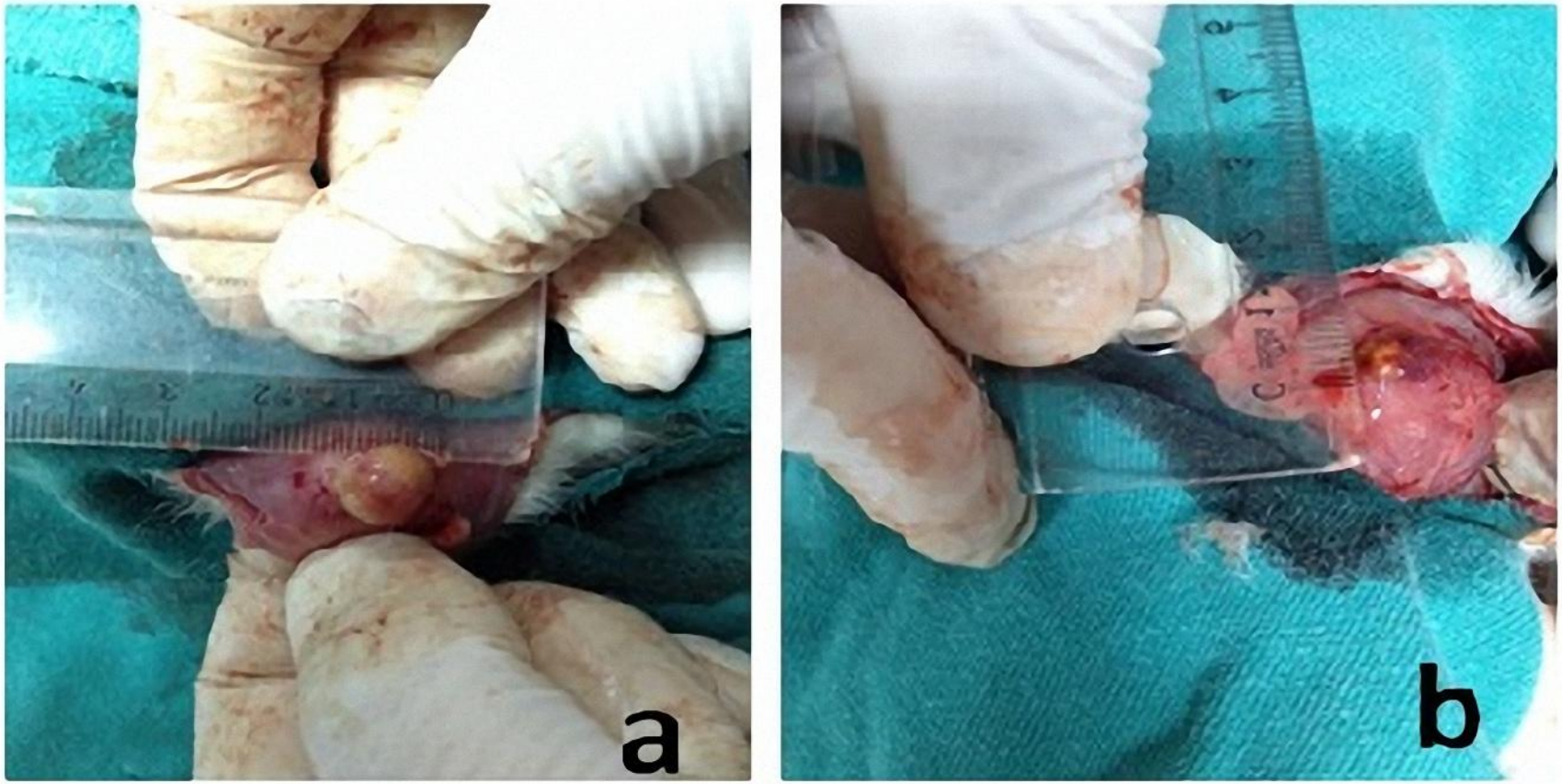
- Rat weights

	before	after	
colchicine	230±11.2 g.	234±9.4 g.	($p>0.05$)
control	221±9.8 g.	231±9.23 g.	($p>0.05$)

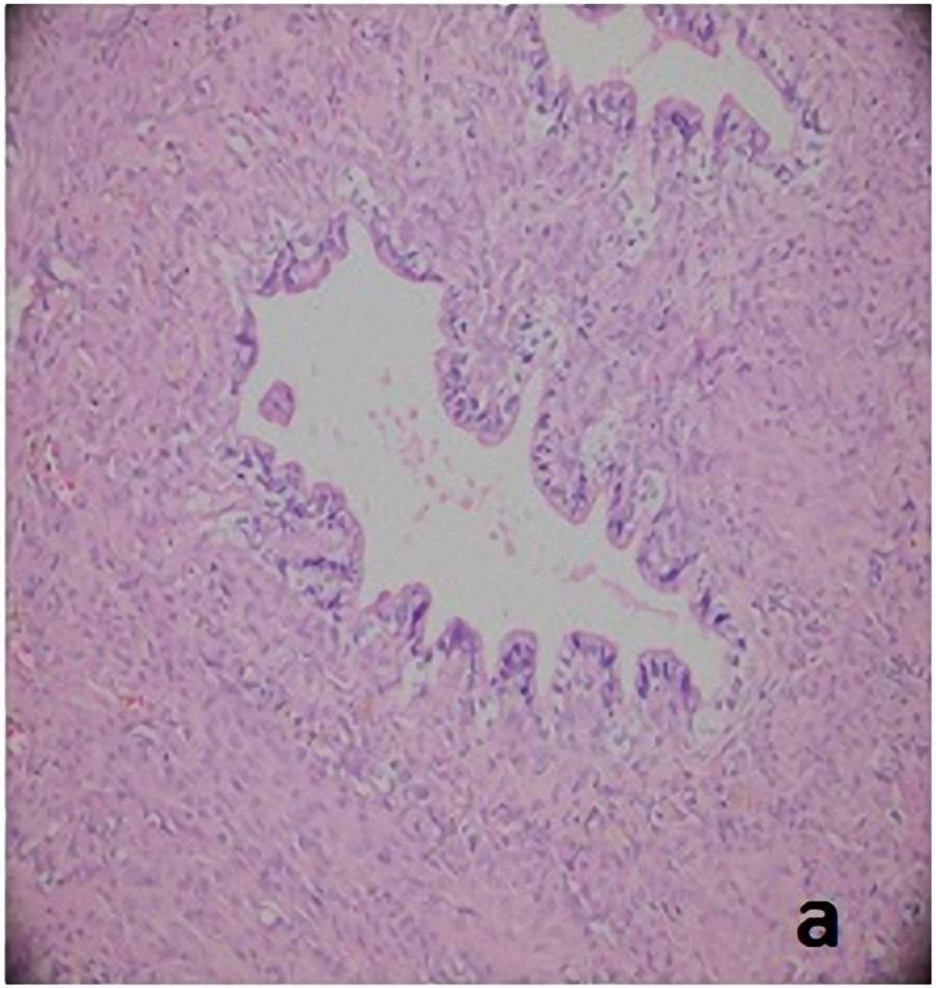
- Implant volumes

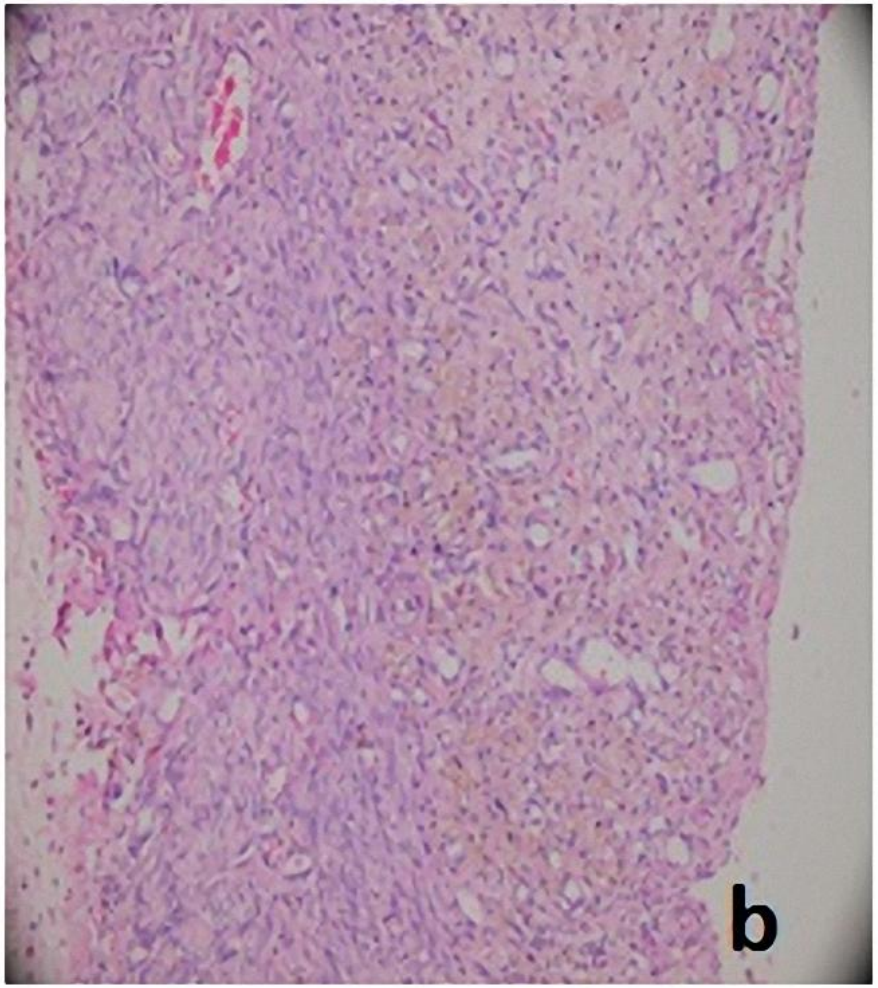
	pre-treatment	post-treatment	
colchicine	89 mm^3	35 mm^3	($p<0.05$)
control	85 mm^3	110mm^3	($p<0.05$)

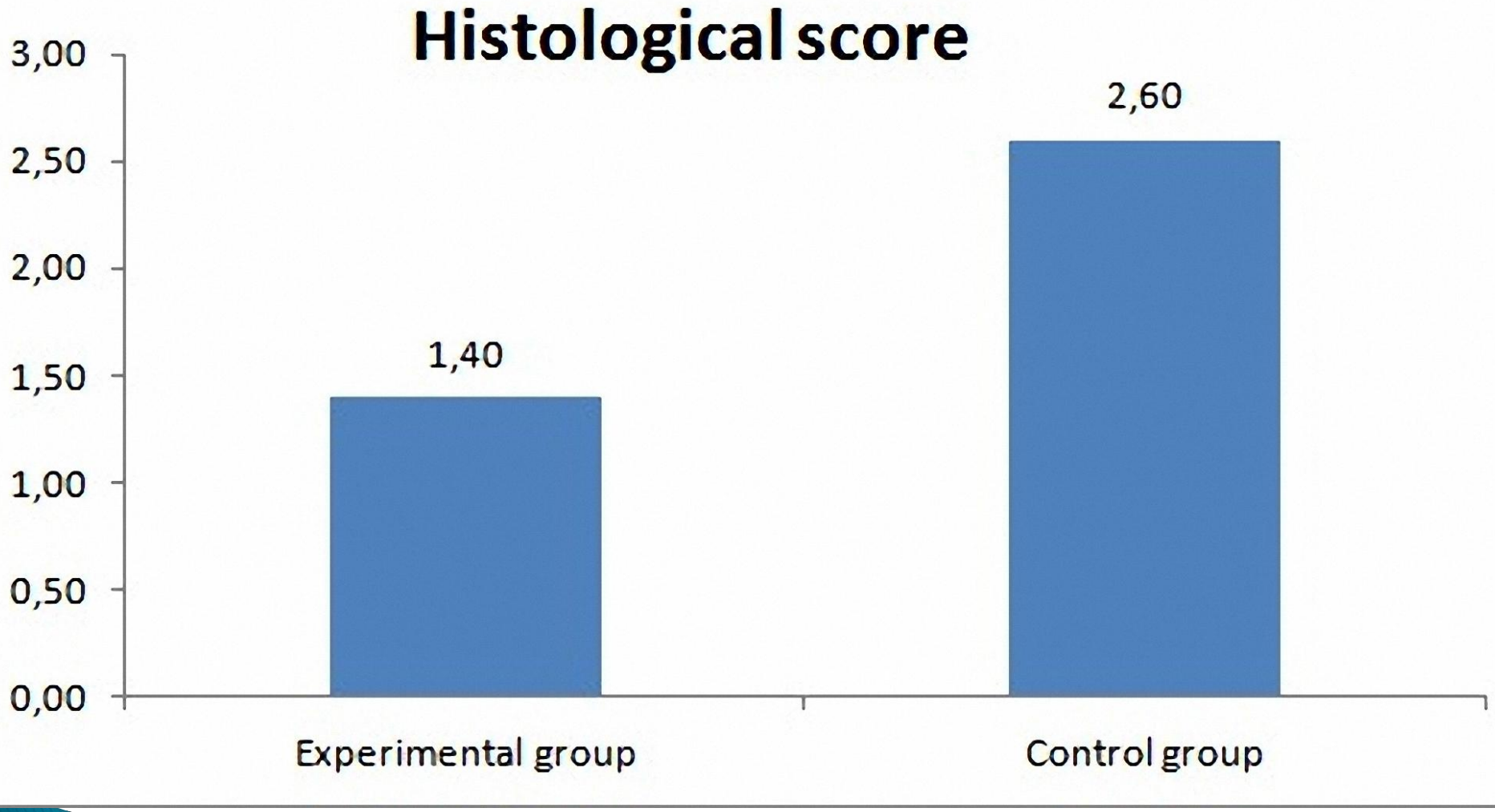
Implant volumes before (a) and after (b) treatment

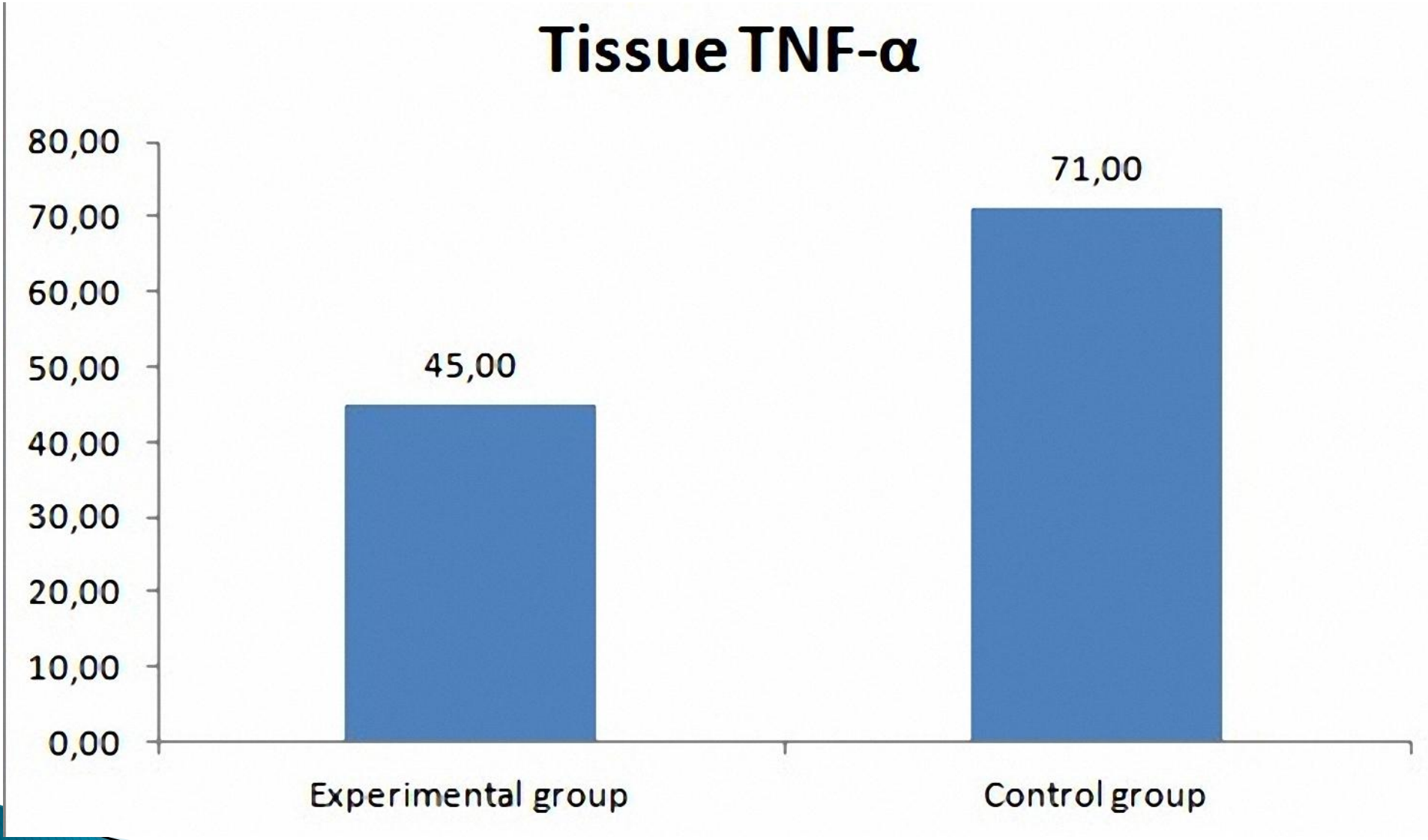


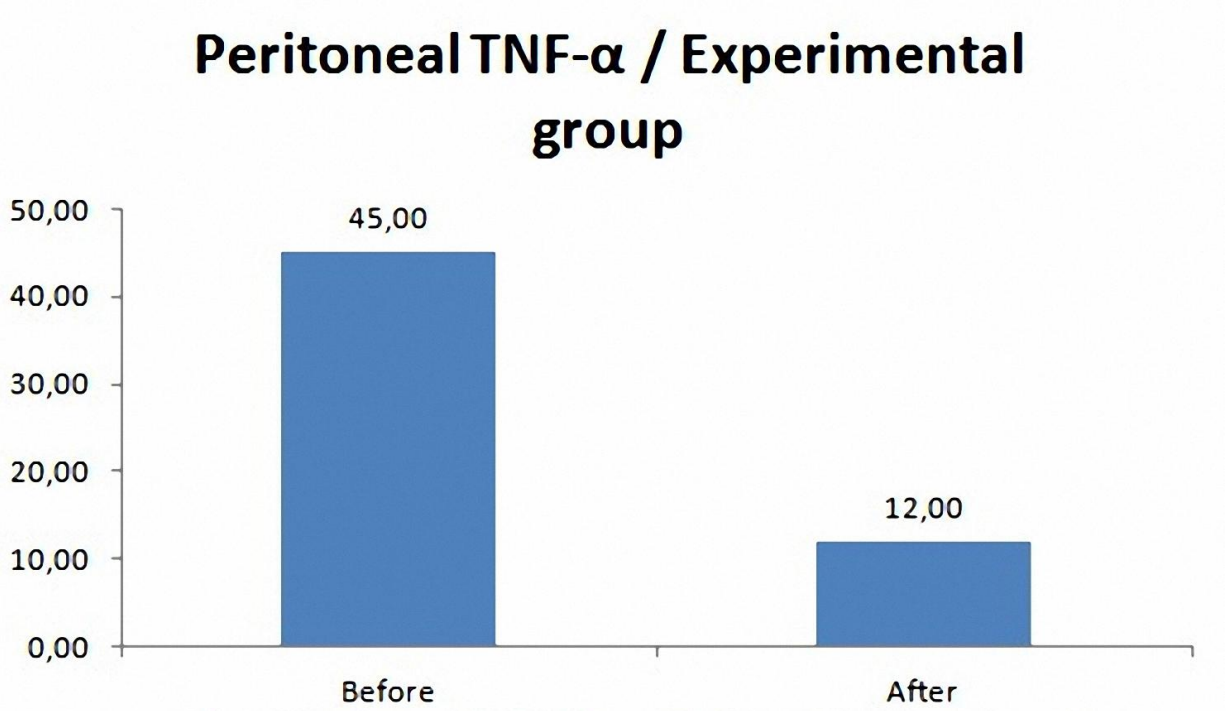
Histopathologic pictures of endometriotic implants in control (a) and colchicine (b) groups






Histological score
3,00
2,50
2,00
1,50
1,00
0,50
0,00
1,40
2,60
Experimental group
Control group


Tissue TNF-α
80,00
70,00
60,00
50,00
40,00
30,00
20,00
10,00
0,00
45,00
71,00
Experimental group
Control group


Peritoneal TNF-α / Experimental group
50,00
40,00
30,00
20,00
10,00
0,00
45,00
12,00
Before
After

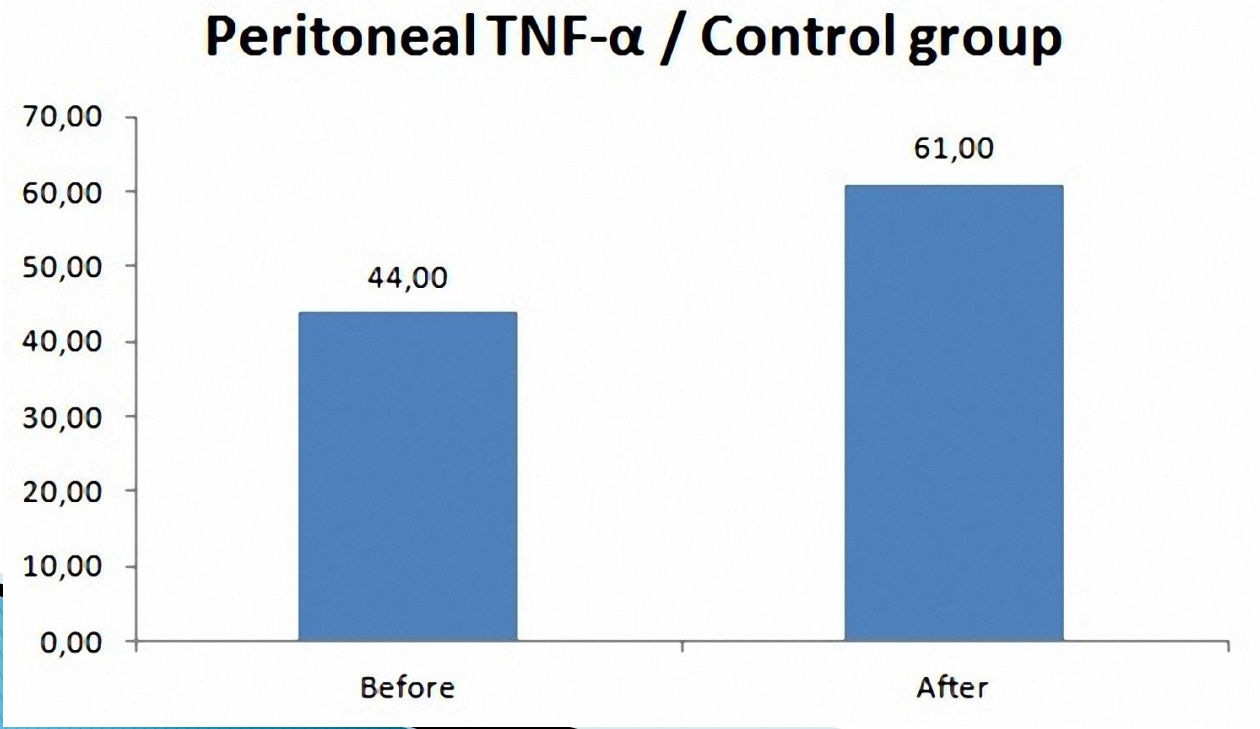

Peritoneal TNF-α / Control group
70,00
60,00
50,00
40,00
30,00
20,00
10,00
0,00
44,00
61,00
Before
After

Table-1: Comparison of implant volumes, histopathologic scores and tissue TNF-α levels between colchicine and control groups

	Colchicine Group (n=8)	**Control Group (n=8)**	**P**
Weight (g)	230±11.2	221±9.8	NS
Initial implant volume (mm^3)	89	85	NS
Post-treatment implant volume (mm^3)	35	110	<0.05
Histopathologic score	1.4±0.2	2.6±0.4	<0.001
Tissue TNF-α levels (pg/ml)	45±8.6	71±11.2	<0.001

Table-2: Peritoneal TNF-α levels before and after treatment in groups

	Colchicine Group			**Control group**		
	Before	**After**	**p**	**Before**	**After**	**p**
Peritoneal TNF-α levels (pg/ml)	45±5	12±5	**<0.001**	44±3	61±12	**<0.001**

Discussion-1

- Activated macrophages, inflammatory cytokines, chemokines and prostaglandins are increased in peritoneal fluid of endometriosis.

  Koninckx PR, Kennedy SH, Barlow DH.
  Endometriotic disease: the role of peritoneal fluid. Human reproduction update.
  1998;4(5):741-51

- Decreased cytotoxic T cells and natural killer cell activity are observed in peritoneal fluid of the patients with endometriosis.

  Sikora J, Mielczarek-Palacz A, Kondera-Anasz Z.
  Role of natural killer cell activity in the pathogenesis of endometriosis.
  Current medicinal chemistry. 2011;18(2):200-8.

- Increased levels of IL-1, IL- 6, IL-8, IL-18 and TNF-α were detected during the inflammatory process of endometriosis.

  Lebovic DI, Mueller MD, Taylor RN.
  Immunobiology of endometriosis. Fertility and sterility. 2001;75(1):1-10.
  Eisermann J, Gast MJ, Pineda J, Odem RR, Collins JL.
  Tumor necrosis factor in peritoneal fluid of women undergoing laparoscopic surgery.
  Fertility and sterility. 1988;50(4):573-9

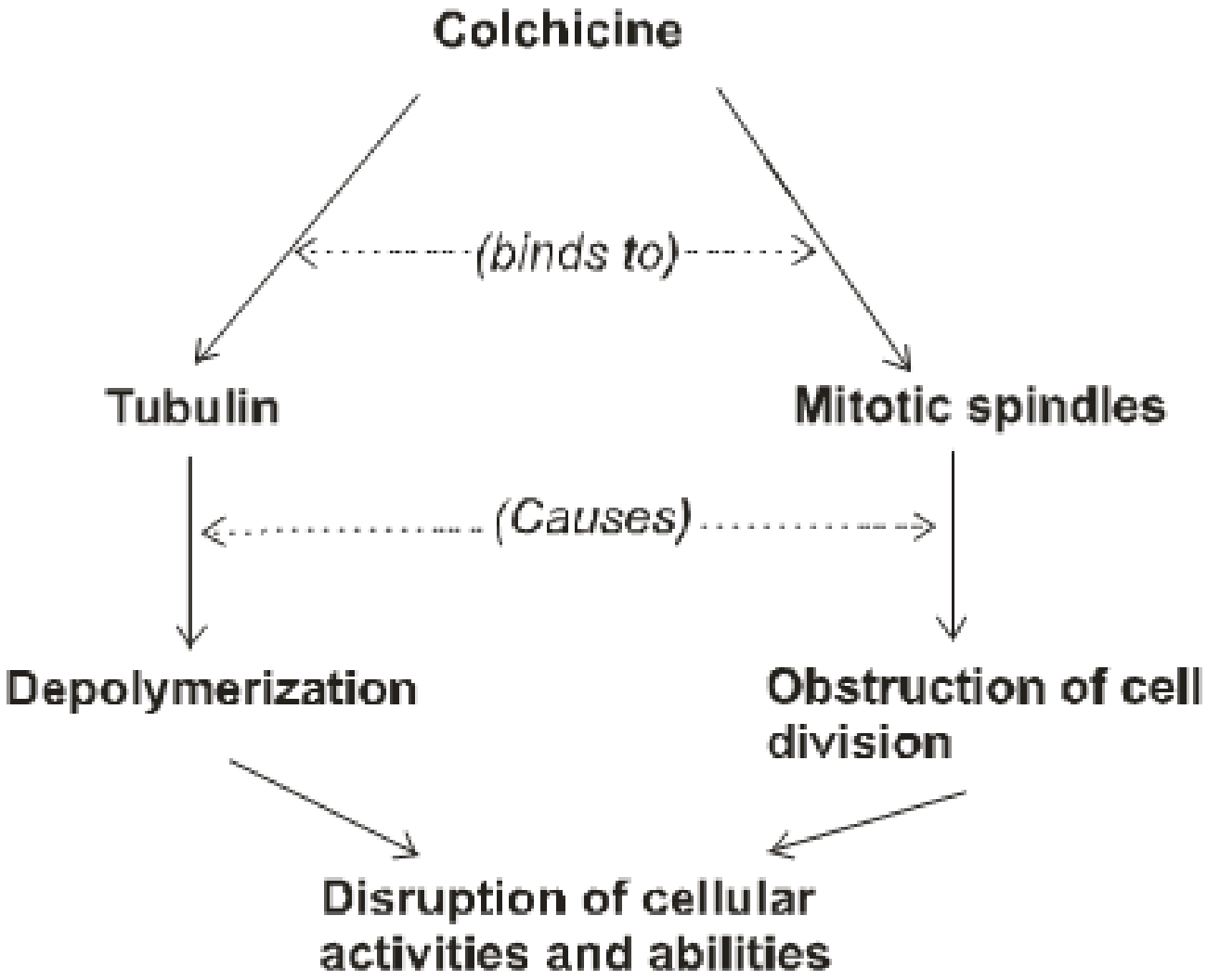
Discussion-2

- TNF-α secreted from active macrophages modulates the secretion of other cytokines and plays a crucial role in pathogenesis of endometriosis.

Islimye M, Kilic S, Zulfikaroglu E, Topcu O, Zergeroglu S, Batioglu S. Regression of endometrial autografts in a rat model of endometriosis treated with etanercept. European journal of obstetrics, gynecology, and reproductive biology. 2011;159(1):184-9. Epub 2011/07/12.

- A relation between TNF-α levels and the severity of endometriosis has been established.

Richter ON, Dorn C, Rosing B, Flaskamp C, Ulrich U. Tumor necrosis factor alpha secretion by peritoneal macrophages in patients with endometriosis. Archives of gynecology and obstetrics. 2005;271(2):143-7. Epub 2004/01/28.



Mechanism of Action of Colchicine
(Jeepakistan.blogspot.com)

Discussion-3

According to the results of our experiment, we propose that the main effect of colchicine on endometriosis model is its inhibition of TNF-α mediated immune response.

Discussion-4

- Many immunomodulator drugs such as imiquimod, levamizole, etanercept decrease in endometrial implant volume.

Islimye M, Kilic S, Zulfikaroglu E, Topcu O, Zergeroglu S, Batioglu S.
Regression of endometrial autografts in a rat model of endometriosis treated with etanercept. European journal of obstetrics, gynecology, and reproductive biology. 2011;159(1):184-9.

Burney RO.
The genetics and biochemistry of endometriosis. Current opinion in obstetrics & gynecology. 2013;25(4):280-6.

Koninckx PR, Kennedy SH, Barlow DH.
Endometriotic disease: the role of peritoneal fluid. Human reproduction update. 1998;4(5):741-51.

- Less toxic treatment modalities are needed

- TNF alpha blocker drugs may cause infection and cancer.

Ceyhan ST, Onguru O, Fidan U, Ide T, Yaman H, Kilic S, et al.
Comparison of aromatase inhibitor (letrozole) and immunomodulators (infliximab and etanercept) on the regression of endometriotic implants in a rat model. European journal of obstetrics, gynecology, and reproductive biology. 2011;154(1):100-4.

- Colchicine is currently being used safely in many diseases.

Discussion-4

- Some limitations to perform this treatment in a similar manner in humans.
- Colchicine is good for endometriosis in rats
- human studies are needed.

Many thanks for your attention