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HEALTH SCIENCES

Tocilizumab is effective in preventing ovarian injury induced by ischemia- reperfusion in rats

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Abstract: Ovarian torsion can be defined as the bending of the ovaries on the supporting ligament, disrupting both venous and arterial blood circulation. Insufficient blood flow causes ovarian tissue hypoxia and leads to ischemia. This study aimed to investigate whether tocilizumab has a protective effect on ischemia-reperfusion injury due to ovarian torsion in rats. Eighteen female Wistar albino rats were divided into three equal groups (Sham (SG), ischemia-reperfusion (OIR), and ischemia-reperfusion+tocilizumab (OIRT)). Degeneration, necrosis, vascular dilatation/congestion, interstitial edema, hemorrhage, and polymorphonuclear lymphocyte (PMNL) infiltration scores were significantly different between the groups ($p=0.001$ for all parameters). Moreover, the OIRT group had a significant improvement in these criteria compared to the OIR group ($p<0.05$). Additionally, there was a considerable difference between OIRT and OIR groups in the number of primordial, developing, and atretic follicles groups ($p<0.05$), while there was no difference in the number of corpus luteum ($p=0.052$). Stress markers or cytokines, such as MDA, tGSH, NF-κB, TNF-α, IL-1β, and IL-6, were significantly different between groups ($p<0.05$). Furthermore, a significant improvement was found in the measured variables when the OIRT group was compared with the OIR group ($p<0.05$). Tocilizumab may be an alternative option for treating ischemia-reperfusion injury due to ovarian torsion.

Key words: interleukin-6, ovarian torsion, reperfusion injury, surgery, tocilizumab.

INTRODUCTION

Background/rationale

Ovarian torsion can be defined as the bending of the ovaries on the supporting ligament, disrupting both venous and arterial blood circulation. Insufficient blood flow causes ovarian tissue hypoxia and leads to ischemia (Somuncu et al. 2008). It is more common in the first three decades of women life, constitutes 2.7% of all gynecological emergencies, can lead to ischemia and consequent severe morbidity and mortality if not treated immediately (Renjit et al. 2008, Abdel-Gaber et al. 2020).

Early diagnosis and appropriate treatment in ovarian torsion are vital to prevent necrosis, infertility, and life-threatening sequelae (Beyazit et al. 2019). In this context, the first procedure to be performed is to provide reperfusion of the torsioned ovaries with detorsion. However, this action can cause overproduction of reactive oxygen species (ROS) and oxidative damage (Kalogeris et al. 2012). Increased ROS production with the introduction of abundant molecular oxygen to the ischemic tissue during reperfusion stimulates proinflammatory cytokine production and causes progressively worse tissue damage

(Minutoli et al. 2016). This situation can be defined as ischemia-reperfusion (IR) injury.

Ischemia-reperfusion injury is a pathological process that starts with tissue hypoxia, continues with ROS production, and expands with an inflammatory response (Yapca et al. 2013). Proinflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α), interleukin beta (IL-1β), and interleukin 6 (IL-6) and transcription factor - nuclear factor kappa B (NF-kB) play a role in this process (Bayir et al. 2016, Nayki et al. 2018).

In recent years, there has been an increase in studies on antioxidant agents that can protect or reduce the ovaries against reperfusion damage (Kirmizi et al. 2021, Akdemir et al. 2015, Ugurel et al. 2017).

Tocilizumab, an IL-6 receptor antagonist, is effective in inflammatory diseases (Scott 2017, Sheppard et al. 2017).

IL-6 induces the activation of NF-kB, which is a main proinflammatory pathway in intestinal inflammation. NF-kB activation is required for the induction of intercellular adhesion molecule 1 (ICAM-1) expression by IL-6. Also, suppressor of cytokine signaling 3 (SOCS-3), a classic inhibitor of the IL-6-induced phosphorylated signal transducer and activator of transcription 3 (phospho-STAT-3) pathway, abolishes the activation of the NF-kB pathway by IL-6. Thus, SOCS-3 not only suppresses cytokine-mediated Janus kinase/ signal transducer and activator of transcription (JAK/STAT) signaling, but also inhibits other pathways (NF-kB) that are triggered by the same receptor (Wang et al. 2003).

I/R injury significantly increases the plasma and spinal cord tissue TNF- α, total oxidant status (TOS), IL-6 levels, and decreases the plasma and spinal cord tissue total antioxidant status (TAS) and IL-10 levels. Tocilizumab treatment statistically significantly reduces the plasma and spinal cord tissue TNF-α, TOS, IL-6 levels, and increases plasma and tissue TAS and IL-10 levels (Karatas et al. 2019, Ibrahim et al. 2020, Avdeev et al. 2014). The levels of proinflammatory (IL-1b, -2, -6, -12, -15, -17, TNF-α) and growth factor (IL-7) dropped down by week 24 of the treatment of with tocilizumab (Avdeev et al. 2014).

This study investigated whether tocilizumab had a protective effect against IR-induced oxidative and inflammatory ovarian damage. We hypothesized that tocilizumab could prevent and treat ischemia-reperfusion injury due to ovarian torsion in rats.

MATERIALS AND METHODS

Study design

The study was conducted in an experimental design at the Atatürk University Experimental Animals Laboratory between April 2021 and June 2021. Study reporting was done in accordance with the PREPARE guideline (Smith et al. 2018). The study protocol was approved by the Atatürk University Animal Experiments Local Ethics Committee, Erzurum, Turkey (Number: E-75296309-050.01.04-2100107137-Date: 14.04.2021). All animals received care in compliance with the institution's guidelines, as outlined in the Guide for the Care and Use of Laboratory Animals, published by the National Institutes of Health.

Animals

Eighteen female Wistar albino rats weighing 225-269 g (4–6 months old, obtained from Atatürk University Medical Experimental Application and Research Center, Erzurum, Turkey) were used in the study. The animals were divided into three groups in equal numbers (Sham (SG), ischemia-reperfusion (OIR), and ischemia-reperfusion + tocilizumab (OIRT)). Before the experiment, the animals were fed standard rat chow and water ad libitum and housed in identical cages

with controlled temperatures and a 12-h light/dark cycle in a physical environment with 50% humidity for at least one week. A maximum of 4 rats was placed in a cage. Female rats were separated from male rats after weaning.

Anesthesia and surgical procedures

The number of follicles is affected by the sexual cycle, so during the experiment, every morning between 8:00 and 9:00 a.m. each animal cage was carried to the experimental room. Vaginal smear was collected with a plastic pipette filled with 10 µL of normal saline (NaCl 0.9%) by inserting the tip into the rat vagina, but not deeply. Vaginal smear samples taken from each rat were taken on a different slide and examined under a light microscope, without the use of the condenser lens, with 10 and 40x magnification. Three types of cells could be recognized: round and nucleated ones are epithelial cells; irregular ones without nucleus are the cornified cells; and the little round ones are the leukocytes. The proportion among them was used for the determination of the estrous cycle phases (Marcondes et al. 2002).

Ketamine was administered intraperitoneally at a dose of 60 mg/kg to all rats. Later, the lower abdomen of the rats was opened vertically with a length of 2-2.5 cm, and the ovaries were reached. Afterward, vascular clips were applied to the lower part of the right ovary of the OIRT and OIR group rats, and two hours of ischemia followed by two hours of reperfusion were performed (Turkler et al. 2018). Tocilizumab at a dose of 8 mg/kg was injected intraperitoneally to the OIRT group before the IR procedure was applied to the rat ovaries. Distilled water was applied as a solvent to the OIR and SG group rats in the same way (Fig. 1). In a previous experimental study, this dose of tocilizumab protected nerve tissues from oxidant and proinflammatory cytokine damage (Abdelrahman et al. 2019). We chose a 2-h duration of torsion based on the study of Kiremitli et al (Kiremitli et al. 2021).

Since adnexal torsion is more common on the right side (66% more than on the left side), the right ovary was preferred (Pansky et al. 2007). Since the parameters that cause oxidative and inflammatory damage in the ovary significantly increase during the first two hours (Unlubilgin et al. 2017), our protocol included two hours of ischemia followed by two hours of reperfusion. In the SG group, the abdomen was closed, taking care not to cause ischemia to the ovaries. After reperfusion, all animals were sacrificed with high-dose anesthesia (ketamine 120 mg/kg). Then, right ovaries obtained from each subject at the end of the experimental procedure were taken and placed in the fixation solution and examined histologically. The left

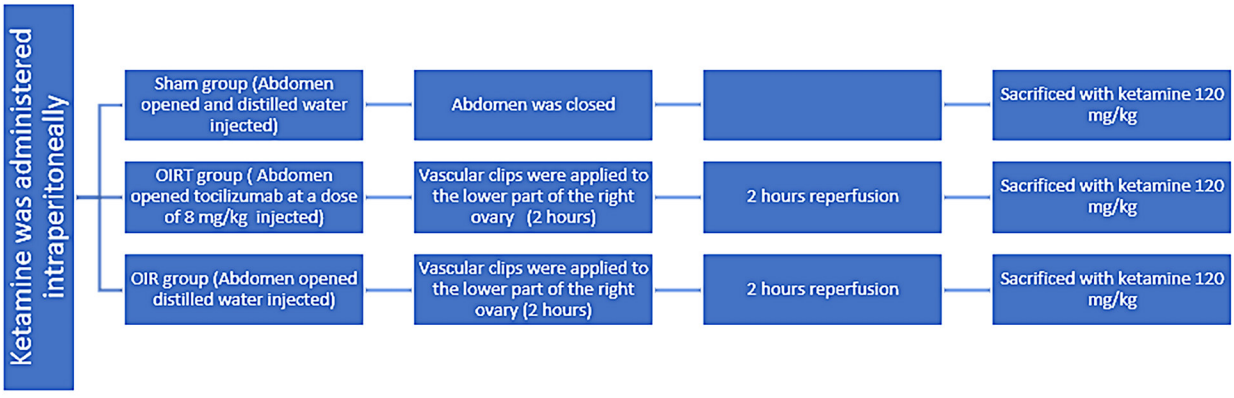

Figure 1. Timeline image of the experimental design.

ovaries are reserved for biochemical analysis. While performing histological grading, central and five peripheral regions were determined at 10x magnification in serial sections taken from each subject and counted in those regions.

Biochemical analysis

Preparation of samples

Prior to dissection, all tissues were rinsed with phosphate-buffered saline solution. The tissues were homogenized in ice-cold phosphate buffers (50 mM, pH 7.4) appropriate for the variable to be measured. The tissue homogenates were centrifuged at 5 000 rpm for 20 min. at 4°C, and the supernatants were extracted to analyze tGSH and MDA. All tissue results were expressed as gram / protein. All spectrophotometric measurements were performed via a microplate reader (Bio-Tek, USA).

Malondialdehyde (MDA) analysis

MDA measurements were based on the method used by Ohkawa et al. (Ohkawa et al. 1979), involving the spectrophotometric measurement of absorbance of the pink-colored complex formed by thiobarbituric acid and MDA. The absorbance of the supernatant was measured at 532 nm. The standard curve was obtained by using 1,1,3,3-tetramethoxypropane (Abdelrahman et al. 2019).

Total Glutathione (tGSH) analysis

According to the method defined by Sedlak J and Lindsay RH (Sedlak & Lindsay 1968), DTNB (5,5'-dithiobis [2-nitrobenzoic acid]) disulfide is chromogenic in the medium, and DTNB is reduced easily by the sulfhydryl groups (Turkler et al. 2018). The yellow color produced during the reduction is measured with spectrophotometry at 412 nm.

Nuclear factor-kappa B (NF-κB), tumor necrosis factor-alpha (TNF-α), interleukin 1 beta (IL-1β), and IL-6 analysis

The samples were weighted, rapidly frozen with liquid nitrogen, cut, homogenized by pestle and mortar, maintained at 2-8 °C after melting. We added PBS (pH 7.4), 1/10 (w/v), after that vortex for 10 seconds, centrifuged 20 min. at 10 000 xg, and collect the supernatants carefully. The levels of tumor necrosis factor α (TNF-α; ng/L), interleukin 1 beta (IL-1beta; pg/L), and interleukin 6 (IL-6; ng/L) were measured using a commercial kit supplied by Eastbiopharm Co. Ltd. ELISA kit, China. Tissue-homogenate NF-κB (a transcriptional factor) concentrations were measured using rat-specific sandwich enzyme-linked immunosorbent assay Rat Nuclear Factor Kappa B ELISA immunoassay kits (Cat. No: 201-11-0288; Shanghai Sunred Biological Technology Co. Ltd., Shanghai, PR China).

Histological evaluation

Tissue samples were fixed in 10% formaldehyde for 72 hours. They were washed under running water for 24 hours and then passed through 70%, 80%, 90%, and 100% ethyl alcohol. Following dehydration, they were immersed in xylene and embedded in paraffin. 4-5 µm cross-sections were taken from the samples with a microtome. The sections were stained with Hematoxylin Eosin (H&E) dual dye and evaluated and photographed using the Olympus DP2-SAL firmware program (Olympus® Inc. Tokyo, Japan).

In the serial sections taken, one central, five peripheral areas were selected at 100x magnification in six sections for each experimental group. Scoring was made for the criteria of degeneration, necrosis, and dilatation/congestion in the vessels, interstitial edema, hemorrhage, and polymorphonuclear cell infiltration. For tissue degeneration criteria,

scoring was done as follows: 0 = absent, 1 = mild, 2 = moderate, and 3 = severe. We chose a scoring system, degree of degeneration based on the study of Kiremitli et al. (2021).

Since there was a significant difference in the number of follicles developing in the ovarian tissues of the experimental groups, the developing follicles were classified and counted at 100x magnification in serial sections taken for each experimental group. We chose method of follicle counting based on the study of Gürgen et al. (2013).

Histopathological evaluation was performed by a histologist blinded to the study groups.

Statistical methods

The primary outcome variable of the study was IL-6 levels. The secondary outcome variables were biochemical (MDA, tGSH, NF-κB, TNF-α, IL-1β, and IL-6) and histological parameters (degeneration, necrosis, vascular dilatation/congestion, interstitial edema, hemorrhage, and PMNL infiltration). The independent variable was the rat groups.

Histopathological evaluations were made using semi-quantitative analysis methods. For each section, one central and five peripheral areas were selected. Mean values of the histopathological parameters (degeneration, necrosis, dilatation/congestion in the vessels, interstitial edema, hemorrhage, and polymorphonuclear cell infiltration) were scored semi-quantitatively. Scaling was done as follows:

A: absent.
B: minimal.
C: moderate.
D: severe.

The mean intensity values of the immune-reactivity were calculated with the following formula:

$$\text{Mean Immun Reactivity Intensity} = \frac{(Bx1)+(Cx2)+(Dx3)}{B+C+D}$$

Data were analyzed using the SPSS 25.0 software (SPSS Inc., Chicago, IL, USA). The results were presented as means and standard deviations. The normal distribution of the numerical data was analyzed by the Kolmogorov-Smirnov test. Binary comparisons were made using the Independent-Samples t-test or Mann-Whitney U test. For the multiple comparisons, the one-way analysis of variance (ANOVA) followed by Tukey's post hoc test or Kruskal-Wallis test was used followed by Tamhane post hoc test. A p-value of <0.05 was considered sufficient for statistical significance.

RESULTS

Histological results

In the histologic evaluation of the ovarian tissues of the sham group (SG), the ovarian cortex and medulla, developing follicle structures, the interstitial connective tissue, corpus luteum, and blood vessels were in normal (grade-0) histological arrangement (Fig. 2a).

Compared to the control group, there were fewer developing follicles, grade-3 degeneration, small amounts of necrotic cell mass in the follicles, widespread necrotized areas in the surrounding connective tissue, dense edema of the interstitial connective tissue, hemorrhage in the entire tissue and corpus luteum, as well as intense dilatation, and congestion in the blood vessels of the ovarian sections of the OIR group (Fig. 2b).

In the high-magnification examination, intense polymorphonuclear cell infiltration was detected around grade-3 dilated and congested blood vessels and in the deep interstitial connective tissue (Fig. 2c).

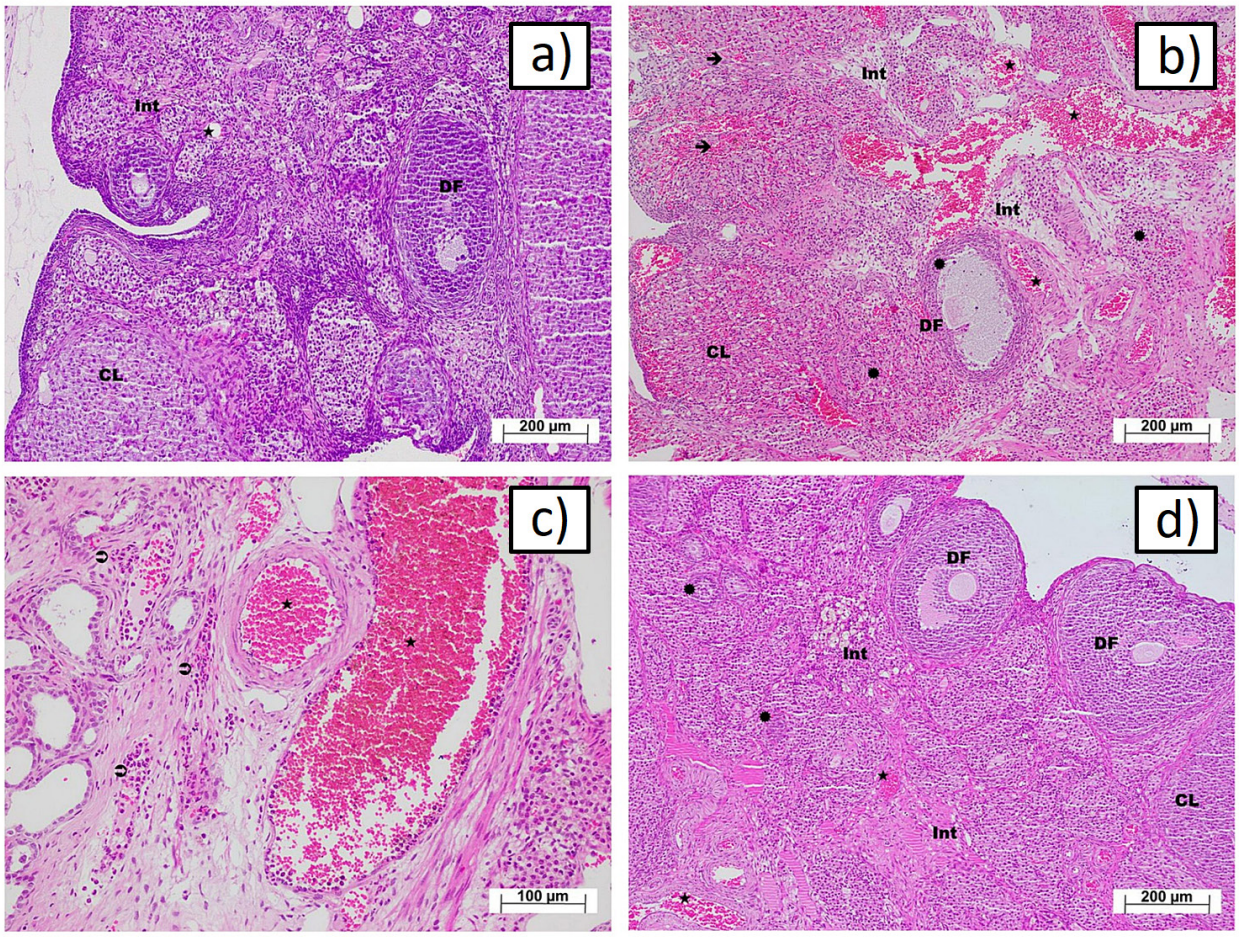

Figure 2. a) Hematoxylin and eosin-stained ovarian tissue in the control group DF: Developing follicle, Int: Interstitial area, CL: Corpus luteum (corpus luteum), ★: Blood vessel, (H&E x100). **b)** Ovarian tissue of the OIR group stained with hematoxylin and eosin. DF: Degeneration in developing follicles, ✷: Necrotized cell deposits in the follicles and tissues, Int: Dense edema in the interstitial area, CL: Hemorrhage in the corpus luteum, →: Tissue-wide hemorrhagic areas, ★: Densely dilated and congested blood vessels (H&E x100). **c)** Ovarian tissue of the OIR group stained with hematoxylin-eosin at high power magnification. ★: Densely dilated and congested blood vessel. ➲: Dense polymorphonuclear cell infiltration, x200. **d)** Ovarian tissue of the OIRT group stained with hematoxylin and eosin. DF: Normal developing follicle, Int: Mild to moderate edema in places in the interstitial area, CL: Corpus luteum, ✷: Small necrotic cell accumulations, ★: Mild to moderate congested blood vessels (H&E x100).

In the ovarian tissue samples of the OIRT group, developing follicles were usually in normal structure and morphology (grade 0) and mild (grade-1)-to-partly moderate (grade-2) edema was observed in the interstitial connective tissue; vascular structures were congested. Additionally, polymorphonuclear cell infiltrations were rarely seen in the OIRT group. However, small necrotized cell groups were noted (Fig. 2d).

Indicators of IR injury, such as degeneration, necrosis, vascular dilatation/congestion, interstitial edema, hemorrhage, and PMNL infiltration, were observed in different amounts between the OIR and OIRT groups; better outcomes were detected in the OIRT group. Mean IRI values were significantly lower for all indicators in the OIRT group compared to the OIR group with independent samples t-test ($p<0.001$

for all indicators). No IR indicators were found in the SG (Fig. 3).

Between the groups, there was a significant difference in primordial, developing, and atretic follicle counts, but no statistically significant difference was found in the corpus luteum numbers Table I. Post hoc analyses showed significant differences between the OIR and OIRT groups Table II.

Biochemical results

The levels of MDA, tGSH, NF-kB, TNF-α were significantly different between the groups at the level of p< 0.001, while the levels of IL-1β and IL-6 were significantly different at the level of p= 0.001 (Table III, Fig. 4).

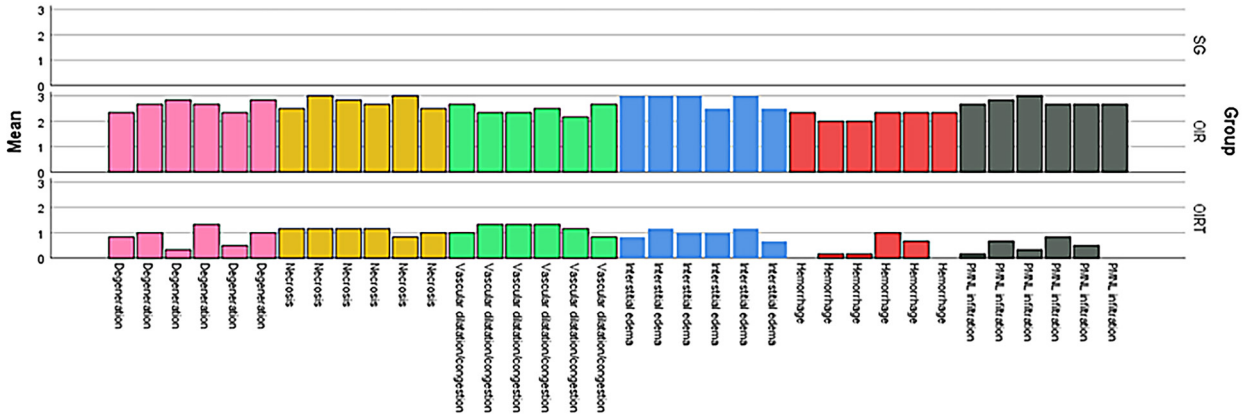

Figure 3. Comparison of the groups regarding ischemia-reperfusion injury. SG: Sham group, OIR: Ischemic reperfusion injury group, OIRT: Group treated with tocilizumab, PMNL: Polymorphonuclear leukocytes.

Table I. Comparison of groups concerning follicle numbers.

		n	Mean	SD	95% CI for Mean		Min	Max	p	
				LB	UB			F		
Primordial Follicles	SG	6	14.17	0.75	13.38	14.96	13	15	19.621	<0.001
	OIR	6	11.33	1.03	10.25	12.42	10	13		
	OIRT	6	13.83	0.75	13.04	14.62	13	15		
Developing Follicles	SG	6	23.17	1.47	21.62	24.71	21	25	120.153	<0.001
	OIR	6	13.50	1.38	12.05	14.95	12	15		
	OIRT	6	22.50	0.55	21.93	23.07	22	23		
Atretic Follicles	SG	6	3.67	1.03	2.58	4.75	2	5	23.632	<0.001
	OIR	6	7.67	1.21	6.40	8.94	6	9		
	OIRT	6	3.83	1.17	2.61	5.06	2	5		
Corpus Luteum	SG	6	12.83	0.75	12.04	13.62	12	14	3.275	0.066
	OIR	6	11.67	1.75	9.83	13.50	9	14		
	OIRT	6	13.50	1.05	12.40	14.60	12	15		

SG: Sham group, OIR: Ischemic reperfusion injury group, OIRT: Group treated with tocilizumab, F: one-way ANOVA test value, SD: Standard deviation, CI: Confidence interval, LB: Lower bound, UB: Upper bound.

DISCUSSION

Indicators of ischemia-reperfusion injury, such as degeneration, necrosis, vascular dilatation/congestion, interstitial edema, hemorrhage, and PMNL infiltration, were observed in different amounts between the groups. Moreover, the OIRT group had a significant improvement in these criteria compared to the OIR group. Furthermore, there was a significant difference in primordial, developing, and atretic follicle counts, but no statistically significant difference was found in corpus luteum numbers. Besides, there was a significant difference between the OIRT and OIR groups concerning the number of primordial, developing, and atretic follicles, while there was no difference in the numbers of corpus luteum. Stress marker or cytokine levels, such as MDA, tGSH, NF-κB, TNF-α, IL-1β, and IL-6, were significantly different between groups. Furthermore, a significant improvement in these indicators was observed when the OIRT group was compared with the OIR group.

Ovarian torsion causes IR damage to the ovarian tissue, which can lead to changes in histopathological parameters, such as degeneration, necrosis, vascular dilatation/congestion, interstitial edema, bleeding, and PMNL infiltration, as well as in biochemical parameters, such as MDA, tGSH, NF-κB, TNF-α, IL-1β and IL-6 (Kalogeris et al. 2016).

In our study, the significant change in all these parameters after ovarian torsion was accepted as proof of the success of the study methodology.

Acting as a mediator between pro-and anti-inflammatory reactivity, interleukin-6 can be produced by virtually all stromal and immune system cells, including T lymphocytes, B lymphocytes, monocytes, macrophages, mast cells, dendritic cells, and other non-lymphocytic cells, such as endothelial cells, fibroblasts,

Table II. Post Hoc tests of the groups compared according to the number of follicles.

Dependent Variable	(I) Group	(J) Group	MD (I-J)	p*	95% CI	
					LB	UB
Primordial Follicle	SG	OIR	2.83	<0.001	1.55	4.12
		OIRT	0.33	0.782	-0.95	1.62
	OIR	SG	-2.83	<0.001	-4.12	-1.55
		OIRT	-2.50	<0.001	-3.78	-1.22
Developing Follicle	SG	OIR	9.66	<0.001	7.86	11.48
		OIRT	0.66	0.614	-1.14	2.48
	OIR	SG	-9.66	<0.001	-11.48	-7.86
		OIRT	-9.00	<0.001	-10.81	-7.19
Atretic Follicle	SG	OIR	-4.00	<0.001	-5.71	-2.29
		OIRT	-0.16	0.965	-1.88	1.54
	OIR	SG	4.00	<0.001	2.29	5.71
		OIRT	3.83	<0.001	2.12	5.54
Corpus Luteum	SG	OIR	1.16	0.272	-0.72	3.05
		OIRT	-0.66	0.637	-2.55	1.22
	OIR	SG	-1.16	0.272	-3.05	0.72
		OIRT	-1.83	0.057	-3.72	0.05

SG: Sham group, OIR: Ischemic reperfusion injury group, OIRT: Group treated with tocilizumab, * Tukey's HSD (honestly significant difference) test, MD: Mean difference, CI: Confidence interval, LB: Lower bound, UB: Upper bound.

glomerular mesangial cells, and keratinocytes (Du et al. 2021, Hunter & Jones 2015).

Interleukin-6 is a multifunctional cytokine involved in acute and chronic inflammatory responses (Tanaka et al. 2014). Elevated IL-6 levels have been detected in serum, synovial fluid, and various tissues in inflammatory conditions, including amyloidosis (Tanaka et al. 2014), familial Mediterranean fever (Oktem et al. 2004), rheumatoid arthritis (Madhok et al. 1993), systemic sclerosis (Barnes et al. 2011), polychondritis (Stabler et al. 2004), giant cell arteritis (Weyand & Goronzy 2013), Castleman disease (Song et al. 2010), and adult-onset Still's disease (Mavragani et al. 2012). Additionally, in acute myocardial IR injury, IL-6 contributed to the expansion of infarct size in the early phase of reperfusion, independent of neutrophil influx, TNFα, IL-1β, tissue factor, and fibrin (Jong et al. 2016). These findings strengthened the idea that

Table III. Post Hoc tests of the groups compared according to oxidative stress markers.

Dependent Variable	(I) Group	(J) Group	MD (I-J)	p	95% CI	
					LB	UB
MDA*	SG	OIR	-2.56	<0.001	-3.05	-2.08
		OIRT	-0.23	0.433	-0.72	0.24
	OIR	SG	2.56	<0.001	2.08	3.05
		OIRT	2.33	<0.001	1.84	2.81
tGSH*	SG	OIR	3.72	<0.001	3.16	4.28
		OIRT	1.28	<0.001	0.72	1.84
	OIR	SG	-3.72	<0.001	-4.28	-3.16
		OIRT	-2.44	<0.001	-2.99	-1.88
NF-κB*	SG	OIR	-3.85	<0.001	-4.48	-3.22
		OIRT	-0.95	0.003	-1.57	-0.32
	OIR	SG	3.85	<0.001	3.22	4.48
		OIRT	2.90	<0.001	2.27	3.52
TNF-α*	SG	OIR	-3.36	<0.001	-3.71	-3.02
		OIRT	-0.29	0.103	-0.63	0.05
	OIR	SG	3.36	<0.001	3.02	3.71
		OIRT	3.07	<0.001	2.72	3.42
IL-1β**	SG	OIR	-3.84	<0.001	-4.58	-3.11
		OIRT	-0.48	0.116	-1.08	0.10
	OIR	SG	3.84	<0.001	3.11	4.58
		OIRT	3.36	<0.001	2.67	4.04
IL-6**	SG	OIR	-4.07	<0.001	-4.95	-3.20
		OIRT	-0.95	0.001	-1.39	-0.51
	OIR	SG	4.07	<0.001	3.20	4.95
		OIRT	3.12	<0.001	2.24	4.00

MDA: Malondialdehyde, tGSH: Total Glutathione, TNF: Tumor necrosis factor, IL: Interleukin, NF-κB: Nuclear factor kappa-light-chain-enhancer of activated B cells, SG: Sham group, OIR: Ischemic reperfusion injury group, OIRT: Group treated with tocilizumab, *: Tukey's HSD (honestly significant difference) test, **: Tamhane test, MD: Mean difference, CI: Confidence interval, LB: Lower bound, UB: Upper bound.

IL-6 has an important role in the pathogenesis of inflammatory events and provided motivation for investigating the effects of anti-IL-6 treatments.

The humanized monoclonal antibody, TCZ, inhibits the binding of IL-6 to its receptors. It reduces the proinflammatory activity of this cytokine by competing with both soluble and membrane-bound forms of the IL-6 receptor (Sheppard et al. 2017). Besides, circulating neutrophil levels, circulation of myeloid dendritic cells, monocytes levels, serum macrophage migration inhibitory factor levels, neutrophil infiltration into inflamed joints, and T-helper 17 levels are decreased by TCZ, while regulatory T-cells are increased (Rubbert-Roth et al. 2018). Moreover, it induces regulatory B-cell expansion, declines B-cell hyperactivity, and reduces the number of peripheral memory B-cells (Roll et al. 2011).

Tocilizumab ameliorated both histopathological and biochemical effects of ischemic reperfusion injury in nerve tissues (Karatas et al. 2019). Additionally, it also protects human cardiac myocytes against IR injury (Cheng et al. 2015). Moreover, it has been claimed that it may also be effective on IR injury occurring after cardiac transplantation (Falk et al. 2019).

TCZ protected renal tissue from IR induced oxidative stress and renal injury and considerably inhibited the increase in TNF-α, NF-kB, MDA, IL-1β, IL-6, blood urea nitrogen and creatinine levels and the decrease in total tGSH in IR damaged renal tissue (Erdem et al. 2022).

To the best of our knowledge and within our research, this is the first histological and biochemical study to investigate the effect of TCZ on IR injury caused by ovarian torsion. In line with previous studies, TCZ improved all biochemical parameters that we examined in our research and considered as inflammatory markers and thought to have a role in IR injury. The improvement in these markers was so great that most of the values in the treated group were similar to the values in the sham group. Furthermore, significant improvement was achieved in all histopathological findings due to IR injury. These results suggested that the use of TCZ may be a good alternative in IR injury due to ovarian torsion.

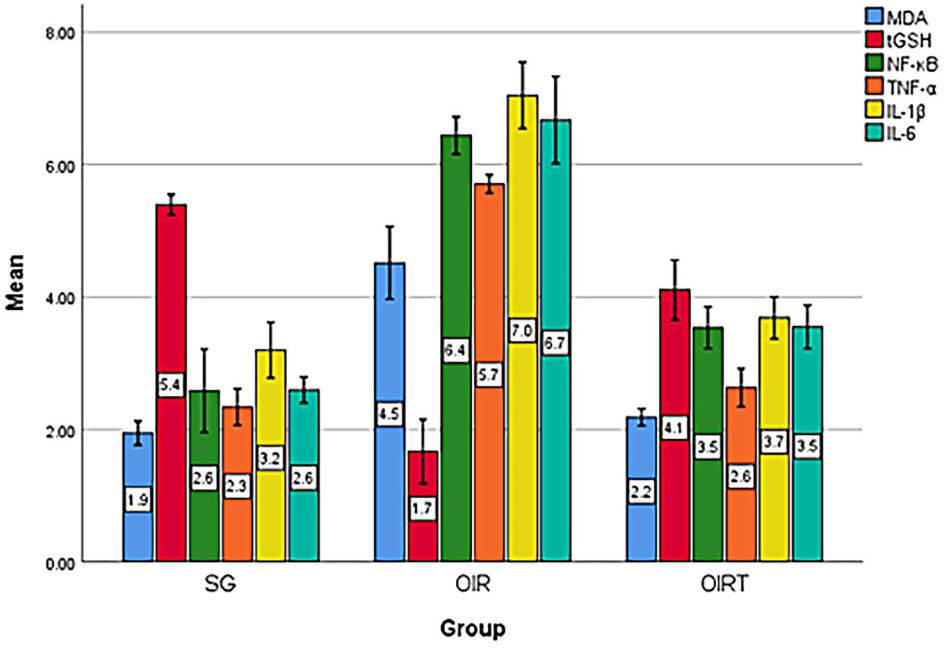

Figure 4. Comparison of the groups in terms of oxidative stress markers. MDA: Malondialdehyde, tGSH: Total Glutathione, TNF: Tumor necrosis factor, IL: Interleukin, NF-κB: Nuclear factor kappa-light-chain-enhancer of activated B cells, SG: Sham group, OIR: Ischemic reperfusion injury group, OIRT: Group treated with tocilizumab, Error bars: 95% Confidence interval.

This study has some limitations that deserve mention. First of all, ischemia-reperfusion damage in the ovaries was demonstrated with histopathological changes and biochemical parameters; infarct size or apoptosis were not evaluated. Also, the study was conducted with only one dose of TCZ. No previous study has been found in the literature on the effect of TCZ on ovarian ischemia-reperfusion. At last, the main limitations of our study are the timing of tocilizumab administration and the short-term analysis. Further research is needed for tocilizumab administration before ischemia and for late-term analysis after reperfusion.

In conclusion, tocilizumab may be an alternative option for the treatment of ischemia-reperfusion injury due to ovarian torsion. However, further experimental and clinical studies are needed to determine the appropriate dose and benefit-harm balance.

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