Effect of metformin and detorsion treatment on serum anti-Müllerian hormone levels and ovarian histopathology in a rat ovarian torsion model

Sema KARAKAŞ¹, Cihan KAYA²*, Hakan GÜRASLAN², Damla SAKIZ², Sema SÜZEN ÇAYPINAR², Hüseyin CENGİZ², Murat EKİN², Levent YAŞAR²

¹Department of Obstetrics and Gynecology, University of Health Sciences, Gaziosmanpaşa Taksim Training and Research Hospital, Istanbul, Turkey
²Department of Obstetrics and Gynecology, University of Health Sciences, Bakırköy Dr. Sadi Konuk Training and Research Hospital, Istanbul, Turkey
³Department of Pathology, University of Health Sciences, Bakırköy Dr Sadi Konuk Training and Research Hospital, Istanbul, Turkey

Background/aim: Adnexal torsion is a common gynaecological emergency, and considered to be a problem mostly in reproductive-age women. To evaluate the effect of metformin and detorsion treatment on reducing ovarian reserve in an ovarian torsion model.

Materials and methods: Twenty-four nonpregnant, Wistar Hannover rats were included in the study. Animals were divided into 3 groups: the control group, the detorsion only group, and the metformin + detorsion group. The first group received only laparotomy. In the second group, ovaries were fixed to the abdominal wall after performing 360° ovarian torsion, followed by detorsion after a 3-h period of ischemia. The third group underwent the same torsion and detorsion procedures as the second group, and received 50 mg/kg metformin by gavage for 14 days. Ovarian damage scores, follicle counts, and AMH levels were evaluated.

Results: The total damage score was significantly increased in the detorsion only group compared to the metformin+detorsion and control groups. Pre-operative/post-operative AMH decreases were statistically significant in negative direction in the detorsion only group when compared to the metformin+detorsion and control groups (P = 0.001).

Conclusion: Metformin+detorsion treatment may be effective in protecting the ovarian reserve after ovarian torsion.

Key words: anti-Müllerian hormone, detorsion, metformin, ovarian reserve, ovarian torsion

1. Introduction

Ovarian torsion is defined as partial or complete rotation of the ovary around its pedicle or vascular axis [1,2]. Torsion of adnexal structures can result in massive parenchymal congestion, infarcts, and haemorrhagic necrosis after arterial and venous blockade [2]. Adnexal torsion is the fifth most common gynaecological emergency, with a reported incidence of 2.7% in the United States [3,4]. Although ovarian torsion is considered to be a problem mostly in reproductive-aged women, it can occur from early foetal life to the postmenopausal period [5].

Owing to its nonspecific symptoms, such as nausea, vomiting, and pelvic pain, there is almost always a delay in diagnosis. Ultrasound imaging may be helpful in the diagnosis. However, even Doppler sonography is successful in diagnosing only 40% of surgically confirmed cases [6]. This difficulty in diagnosing ovarian torsion leads to loss of ovarian tissue and function. A total of 50%–90% of adnexal torsion cases are caused by physiological cysts, endometriosis, dermoid cysts, fibromas, and other benign or malignant ovarian neoplasms [7,8]. Traditionally, the suggested treatment is salpingoophorectomy or oophorectomy. However, evaluating tissue perfusion intraoperatively and leaving the tissue in its anatomical place is a common and reliable approach, especially for reproductive-aged women [9,10]. There is no relation between the variable colours (purple to black) of the adnexal structures and tissue viability.

It has also been reported that detorsion of the adnexa has no effect on the risk of thromboembolism [10, 11]. After the detorsion of tissue, ischemia may resolve, resulting in tissue recirculation and reperfusion. However, this procedure also has adverse effects, including reperfusion injury [12]. Ischemia followed by reperfusion of the tissue...
Metformin, a biguanide group agent, is used as an insulin sensitizer for the treatment of diabetes. In a few reported studies, it has been speculated that metformin has antioxidant and anti-inflammatory effects [14]. It enhances the use of glucose in peripheral tissues and increases cyclic adenosine mono phosphate kinase (AMPK) which plays a major regulatory role in the balance of cellular energy by switching cells from the anabolic state to the catabolic state [15]. It has been reported that metformin has an ability to decrease inflammation by reducing ROS, by decreasing the activity of mitochondrial complex I [16]. Besides, its anti-inflammatory effects rely on inhibition of the activation of NF-κB and activation of AMPK [17].

In this study, we aimed to evaluate the efficacy of metformin therapy in addition to detorsion for preserving ovarian reserve and ovarian structure.

2. Materials and methods
This study was performed at a tertiary medical centre named Bağcılar Training and Research Hospital Experimental Laboratory, İstanbul after the approval by the ethics committee of the animal studies of the same institution. A power analysis was performed to calculate the minimum sample size required for animal studies, considering anti-Müllerian hormone (AMH) results, (alpha error: 0.05; beta: 0.8) suggested that ≥12 ovaries were required for each study group. Because 10% of study animals are lost during procedures, we included 16 ovaries per surgery group. We planned to include 8 rats in each group, and 24 female, nonpregnant, Wistar Hannover rats (reproductive age, 8 weeks; weight, 180–260 g; with 2 menstrual cycles in humans, 1 mL of blood samples were drawn from the jugular vein of the each rat for a second AMH analysis. Laparotomy was performed in each rat, and bilateral oophorectomies were performed for histopathological analysis. Each rat was euthanized by means of cervical dislocation after the operations.

2.1. Histopathology
The excised ovaries were kept in 10% formalin solution and evaluated after 24 h by a pathologist from Bakirköy Dr Sadi Konuk Training and Research Hospital who was blinded to the study groups. The tissue samples were embedded in paraffin blocks, cut into 4-μm slices, and prepared for haematoxylin and eosin staining. Follicle counting was performed according to a study by Ozler et al. [19]. At least 5 microscopic areas were evaluated with light microscopy (Nikon Eclipse 80i AS Amstelveen, The Netherlands). The follicles were divided into 4 groups according to diameter: primordial (<20 μm),
preantral (20–220 μm), small antral (221–310 μm), and large antral (311–370 μm). Atretic follicles were defined according to the study by Osman et al. [20]. The ovarian damage score was evaluated on the basis of the following parameters: follicle cell degeneration, vascular congestion, haemorrhage, and inflammation for both ovaries (0: none, 1: mild, 2: moderate, 3: severe) (Figure 3,4).

2.2. AMH analysis
All blood samples were centrifuged for 10 min at 4000 rpm to obtain serum samples. The samples were kept at −80 °C in Eppendorf tubes until analysis. Serum AMH levels were analysed with an automatic ELISA kit (Omentin ELISA kit; Hangzhou Eastbiopharm Co., Hangzhou, China).

2.3. Statistical analysis
Statistical analyses were performed by using NCSS (Number Cruncher Statistical System) 2007 statistical software (Kaysville, UT, USA). Descriptive statistical analysis, such as mean, standard deviations, and one-way analysis of variance, was used for continuous data with a normal distribution. A valid chi-squared test is used for categorical variables. The Tukey test was used for post hoc analysis of normally distributed parametric data. A paired sample t-test was used to evaluate the preoperative and postoperative AMH levels. The Kruskal–Wallis test was used to compare continuous data with skewed distribution, and the Dunn test was used for post hoc analysis. The chi-
square test was used for qualitative data. P values of <0.05 were considered statistically significant.

3. Results
The ovarian damage scores were evaluated for each rat, and there was a statistically significant difference in the follicular damage scores between the control, detorsion only, and metformin and detorsion groups (P = 0.048). There was a mildly increased damage score in the control and metformin and detorsion groups. However, a severe score was observed in the detorsion only group, which was statistically significant in comparison with the other groups. There was also a statistically significant difference in the inflammation scores among the study groups (P = 0.002). No inflammation was observed in the control and metformin and detorsion groups. However, moderate and severe scores were observed in the detorsion only group. The total damage scores were statistically different among the study groups. The total damage scores were higher in the detorsion only group than in the control and metformin and detorsion groups (P = 0.005 and P = 0.021, respectively). However, there was no statistical difference in the total damage score between the control group and the metformin and detorsion group. There was no statistically significant difference in vascular congestion and haemorrhage scores among the study groups (Table 1, 2).

The study groups were also evaluated for follicle counts. There was a statistically significant difference in the numbers of preantral follicles, large antral follicles, and corpora lutea among the control, detorsion only, and metformin and detorsion groups (P = 0.0001, P = 0.041, P = 0.023, respectively) (Table 3). The preantral follicle, large antral follicle, and corpora lutea counts were lower in the detorsion only group than in the control and metformin and detorsion groups. There was no significant difference in preantral follicle, large antral follicle, and corpora lutea counts between the control group and the metformin and detorsion group (Table 4). There was also no significant difference in the counts of primordial, small antral, and atretic follicles between the study groups. There was a statistically significant difference in the preoperative vs. postoperative anti-Müllerian hormone (AMH) level changes between the control, detorsion only, and

| Follicle cell degeneration | Control Group (n,%) | Detorsion Only Group (n,%) | Metformin and detorsion Group (n,%) | P-value |
|---------------------------|---------------------|---------------------------|--------------------------------------|---------|
| None                      | 1 7.14%             | 2 14.29%                  | 2 14.29%                             | 0.048   |
| Mild                      | 8 57.14%            | 5 35.71%                  | 7 50.00%                             |
| Moderate                  | 5 35.71%            | 2 14.29%                  | 5 35.71%                             |
| Severe                    | 0 0.00%             | 5 35.71%                  | 0 0.00%                              |
| Vascular congestion       | None                | 1 7.14%                  | 0 0.00%                              | 0.069   |
| Mild                      | 9 64.29%            | 5 35.71%                  | 5 35.71%                             |
| Moderate                  | 4 28.57%            | 6 42.86%                  | 9 64.29%                             |
| Severe                    | 0 0.00%             | 3 21.43%                  | 0 0.00%                              |
| Haemorrhage               | None                | 2 14.29%                  | 4 28.57%                             | 0.075   |
| Mild                      | 10 71.43%           | 3 21.43%                  | 10 71.43%                            |
| Moderate                  | 2 14.29%            | 3 21.43%                  | 1 7.14%                              |
| Severe                    | 0 0.00%             | 4 28.57%                  | 1 7.14%                              |
| Inflammation              | None                | 12 85.71%                 | 4 28.57%                             | 0.002   |
| Mild                      | 2 14.29%            | 2 14.29%                  | 3 21.43%                             |
| Moderate                  | 0 0.00%             | 6 42.86%                  | 0 0.00%                              |
| Severe                    | 0 0.00%             | 2 14.29%                  | 0 0.00%                              |

*Total damage score 3.64 ± 1.34 6.5 ± 3.32 4.14 ± 1.46 0.004

Chi-square test, *One Way ANOVA
(0: none, 1: mild, 2: moderate, 3: severe).
P-value of <0.05 was considered to be statistically significant.
metformin and detorsion groups (P = 0.0001) (Table 5). The detorsion only group showed negatively statistically different changes in this parameter compared with the other groups (P = 0.001) (Table 6). However, there was no statistically significant difference for this parameter between the control group and the metformin and detorsion group.

4. Discussion
In present study, our aim was to evaluate the efficacy of metformin considering its antioxidant and antiinflammatory benefits in addition to detorsion for preserving ovarian reserve and ovarian structure and we revealed that Metformin and detorsion treatment may be effective in protecting the ovarian reserve after ovarian torsion.

Excision of the adnexa is the traditional approach for treating ovarian torsion. However, recent studies do not recommend this treatment approach, considering the importance of the ovary for women of reproductive age [21,22]. Moreover, there is still some concern about leaving necrotic tissue in situ and complications such as infection,
increased risk of malignancy, and systemic problems such as pulmonary embolism or thrombosis in other organs [23]. In our study, our main goal was to determine the ovarian reserve after ovarian detorsion and we did not observe any signs of pelvic infection.

Ischemia-reperfusion injury is generally explained by the hypothesis that there is an accumulation of neutrophils and thrombocytes due to the activated complement and other inflammatory components around the inflammation site. This aggregation of inflammatory cells enhances the production of ROS. In addition, glycolysis, increased lactic acid concentration, and intracellular Ca accumulation result in decreased intracellular pH and acidosis. This results in increased intracytoplasmic lysozyme enzymes causing damage to proteins and the cell membrane [24]. Enzymes such as glutathione peroxidase and catalase play a protective role against cellular ROS. Moreover, cysteine, glutathione, ceruloplasmin, and vitamins A/C/E also act as intracellular and extracellular antioxidants and protect the cell structure from ROS [25]. The balance between oxidants and antioxidants is lost in ischemia-reperfusion injury. In this case, enzymes such as lipid peroxidase, superoxide dismutase, inducible nitric oxide synthase, and myeloperoxidase levels increase [26,27]. In a study by Bostanci et al. [28], granulocyte colony-stimulating factor (G-CSF), a glycoprotein commonly used to treat neutropenia by mobilizing bone marrow-derived hematopoietic cells into peripheral blood, was used in an experimental model of ischemia-reperfusion injury. The authors administered intraperitoneal injections of G-CSF (100 IU/kg) and evaluated the mean total oxidant status (TOS), oxidative stress index (OSI), and the total histopathological scores of rats with ischemia-reperfusion injury. G-CSF administration decreased the mean TOS and OSI levels significantly when compared with the controls. Moreover, there was a decrease in total histopathological scores for rats conservatively treated with G-CSF compared with the control groups. Bakacak et al. [29] used platelet-rich plasma, which is clinically used to promote wound healing, in an experimental ischemia-reperfusion injury model. They found that the TOS, OSI, and total ovarian histopathological scores were higher in the nontreated group than in the group treated with 0.5 mL platelet-rich plasma. Halici et al. [27] evaluated the long-acting calcium channel blocker amlodipine in an experimental model of ischemia-reperfusion injury. They administered 3 and 5 mg/kg doses of amlodipine, and concluded that amlodipine is effective in preventing ovarian damage. Kuntepe et al. [30] studied the angiotensin 2 type 1 receptor blocker telmisartan, which is used as an antihypertensive agent in daily practice, in an experimental ischemia-reperfusion model. Telmisartan, at doses of 10 and 20 mg/kg,
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