ORIGINAL RESEARCH

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Thiol-Disulphide Homeostasis in Ovarian Torsion-Detorsion: An Experimental Rat Model

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ABSTRACT Objective: Ovarian torsion is one of the reasons of acute abdomen requiring immediate surgical intervention. There is not any reliable method for its diagnosis. Delay in treatment may result in loss of ovary. In this study, the purpose is to evaluate efficiency of thiol-disulphide homeostasis in early diagnosis of ovarian torsion in experimental rat model and in its follow-up care with biochemical and histopathological findings. **Material and Methods:** In the study, 24 Albino Wistar female rats were divided into three groups. The first group was the sham group. The second group was the torsion group. The last group was the torsion-detorsion group. Histopathological evaluation was carried out in ovaries in all groups, and values of thiol/disulphide homeostasis were measured in serum samples biochemically. **Results:** Values of native thiol and total thiol were lower in the torsion-detorsion group compared to the other groups. However, there was no significant difference between of native thiol and total thiol levels in the sham and torsion groups. Interestingly, the index of native thiol/total thiol was relatively low in the torsion and torsion-detorsion groups compared to the sham group. However, this decrease was not significant. **Conclusion:** These results suggest that native thiol/total thiol values may not be useful for early diagnosis of ovarian torsion but it may be used for follow-up care after detorsion

Keywords: Ovarian torsion; detorsion; thiol-disulphide; rat; ischemia

Ovarian torsion is one of the reasons of acute abdomen requiring immediate surgical intervention.[1] Ovarian torsion is observed in approximately 2.5-7.4% of the cases operated due to pelvic pain.[1,2] Torsion occurs with ovary's total or partial rotation around its own axis. Although it is mostly seen among the women in reproductive period, it can also be observed in all age groups.[3] Early diagnosis and treatment are important for preservation of ovary and reproductive health.[1,2] However, the diagnosis of torsion in early stages is rather difficult, and imaging methods may not always help.[4] It has been reported that while only 38% of cases have been diagnosed correctly before surgical operation, the cases operated immediately for presumed torsion have been misdiagnosed at such a high rate of 56%.[4,5]

After ovarian torsion, venous circulation deteriorates first. Following the venous circulation deterioration, ischemia, oedema and bleeding occur in the ovary. Finally, necrosis in ovary starts with the deterioration of arterial blood flow.[1,3] Free oxygen radicals are produced during the ischemia period.[3] These oxygen radicals have also been shown to be related to pathogenesis of many diseases.[1] The free radicals occurring in case of disease or ischemia can cause oxidation of disulphides and may deteriorate thiol-disulphide homeostasis. Moreover, free radicals can be reduced by thiol groups as thiol groups can take on an antioxidant function. Therefore, thiol-disulphide balance is very important for repressing oxidative stress increasing together with ischemia.

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The purpose of this study is to evaluate the predictive value of thiol-disulphide homeostasis in early diagnosis of ovarian torsion in experimental rat model and in its follow-up care with histopathological findings.

MATERIAL AND METHODS

ANIMALS

This study was conducted in accordance with the Helsinki Declaration and this article was approved by the animal experiments ethics committee of the Van Yüzüncü Yıl University Faculty of Medicine with decision number of 29.08.2019/08. In this study, a total of 24 Albino Wistar female rats weighing 200-300 g and 3-4 months of age were used. The rats in all groups were fed with standard feed during the experiment. The rooms of the rats were heat, humidity-controlled and light-controlled. Before the study, the rats were housed in stainless cages in 12-hour light and 12-hour dark rooms at 22±2°C. The rats were divided into three groups randomly in a way that each group would include 8 rats. All parameters in each group were studied three times.

The sham group: Aproximately 2 cm laparotomy incision in the abdomens of the rats were opened and closed with 5/0 silk sutures after a min. Right oopherectomy was applied three hours later.

The torsion group: Aproximately 2 cm laparotomy incision in the abdomens of the rats were opened and the blood supply of the right ovary was blocked by atraumatic vascular clips from both lower and upper parts. Then, the abdomens were closed with 5/0 silk sutures. The abdomens were re-opened three hours later and right oopherectomy was applied.

The torsion-detorsion group: Aproximately 2 cm laparotomy incision in abdomens of the rats were opened and blood supply of right ovary was blocked by atraumatic vascular clip from both lower and upper parts. Then, the abdomens were closed with 5/0 silk sutures. The abdomens were re-opened three hours later, and after the clips on the right ovary were removed, detorsion was performed. Afterwards, the abdomens were closed with 5/0 silk sutures. After a wait of three hours, the abdomens were re-opened and right oopherectomy was applied.

SURGICAL PROCEDURE

Before the surgical operation, 40 mg/kg ketamine (Ketazol, 10%/10 mL ampoule Richter Pharma, Wels, Austria) and 10 mg/kg Xylazine Hydrochloride (Alfazyne®, Ege Vet, Alfasan International B.V. Holland) were applied to the rats with intraperitoneal injection.

A vertical median incision was made through the midline after the abdomens were cleaned with iodine. A 2 cm incision was made to the sham group, then, the abdomens were closed. In the other groups, after right ovary was found, torsion was achieved by stanching the blood flow to the ovary from both lower and upper parts with the help of atraumatic clips. The detorsion was achieved by opening pre-inserted clips three hours later. The incision line was closed with 5-0 vycril. Lastly, sacrification was performed by taking intracardiac blood from all rats.

SAMPLES AND BIOCHEMICAL ANALYSIS

The parameters showing thiol/disulphide homeostasis in serum samples were measured. The serum samples were obtained from the intracardiac blood samples. The blood specimens were placed into yellow-capped biochemistry tubes. The specimens were centrifuged for 10 min at 3500 *g*. The supernatant was taken to another tube and was kept at -80 °C until being studied.

Thiol and native thiol levels were measured using enzyme linked immunosorbent assay (ELISA) kits which were available commercially (Shanghai YL Biotech Co., Ltd).

Thiol/Disulphide Index: Total thiol content/disulphide rate was calculated by proportioning the obtained results using a method previously published study by Erel and Neselioglu.[6]

Disulphide values: The values were calculated with the following formula published by Erel and Neselioglu (Total thiol-native thiol)/2.[6]

HISTOPATHOLOGY

Histopathological evaluation was carried out on ovaries in all groups. For histopathological evaluation, the ovarian tissue was preserved in 10% formalin solution. After being carried over alcohol series with in-

creased concentration for dehydration, it was made pellucid with xylene. The ovarian tissue was turned into a block by burrying into paraffin. The 4 µm-thick incisions were taken with a microtome. The taken incisions were stained with hematoxylin-eosin after being deparaffinised. They were then evaluated with light microscope (Nikon Y-IM 7551012, Japan).

In histopathological examination, a randomized sampling was done for every animal in all groups and the evaluation was carried out in 15-17 areas. The findings were examined semi-quantitatively. The evaluation was carried out as: normal: - (no lesion), slight: + (1-4 lesions), mild: ++(5-8 lesions) and severe: +++ (9 and over) according to the average number of lesions observed in the areas examined microscopically.

STEREOLOGY

Cavalieri's principle was applied to calculate the total volume of tissue. In the incisions taken randomly, the total volume of each ovary was calculated using point grid. Total volume of each ovary was calculated by multiplying total number of points on the ovary, the area that a point cover, and incision thickness. Thus, the following formula was used:

$$V_{ref} = \sum P \,.\, a(p) \,.\, t$$

$\sum P$: Total number of points on ovary, a(p): The area that a point covers, t: Incision thickness

The error coefficient was determined for sample proficiency test for each animal. In order to apply the sufficiency test for the number of animals in experiment, the coefficient of variation was applied.

STATISTICAL ANALYSIS

The SPSS (version 21) statistical package program was used for calculations. Descriptive statistics were presented as mean and standard deviation. Whether the data were normally distributed or not was checked with Shapiro-Wilk test. For the same parameter, Kruskal-Wallis test was used to determine whether differences between groups were significant or not. Post hoc analysis (Tukey HSD) was carried out to determine which groups the differences stemmed from. A p value of 0.05 or less was considered significant.

RESULTS

BIOCHEMICAL FINDINGS

The mean and standard deviation values of parameters related to thiol-disulphide homeostasis were shown in Table 1 and Figure 1. There was no significant difference between the weights of groups since the rats whose weights were close to each other were used in the study (p=0.066). Especially, native thiol and total thiol values were lower in the torsion-detorsion group compared to the other groups (p=0.001). However, there was not any significant difference between the native thiol and total thiol values of the sham and torsion groups. Moreover, the native thiol/total thiol index in the torsion and torsion-detorsion groups was low in comparison with the sham group. But, this difference did not reach statistical significance (p=0.183). In addition, although the disulphide, disulphide/total thiol and disulphide/native thiol index were high in the torsion and torsion-detorsion groups, the differences were not significant (p=0.186, p=0.151, respectively).

TABLE 1: Mean and standard deviation values of disulphide-thiol homeostasis belonging to serum samples of all groups.

	Sham			Torsion			Torsion-Detorsion			P value
	M±SD	Min.	Max.	M±SD	Min.	Max.	M±SD	Min.	Max.	
Weight (g)	217.50±18.41	196	244	209.75±14.02	194	236	207.50±12.83	192	228	0.066
Native Thiol (pg/mL)	2.29±0.31	1.90	3.01	2.30±0.38	1.66	3.25	1.87±0.24*	1.43	2.31	0.001
Total Thiol (pg/mL)	3.12±0.35	2.56	3.76	3.26±0.47	2.48	4.03	2.78±0.30*	2.27	3.33	0.001
Disulphide (pg/mL)	0.41±0.18	0.15	0.86	0.48±0.26	0.04	1.10	0.46±0.20	0.06	0.79	0.518
Native Thiol/Total Thiol	0.74±0.09	0.53	0.91	0.71±0.13	0.43	0.97	0.68±0.12	0.49	0.95	0.183
Disulphide/Total Thiol	0.13±0.05	0.04	0.24	0.14±0.06	0.01	0.28	0.16±0.06	0.03	0.25	0.186
Disulphide/Native Thiol	0.19±0.09	0.05	0.45	0.22±0.14	0.01	0.66	0.26±0.13	0.03	0.52	0.151

*p: Low compared to sham and torsion groups (p<0.05).

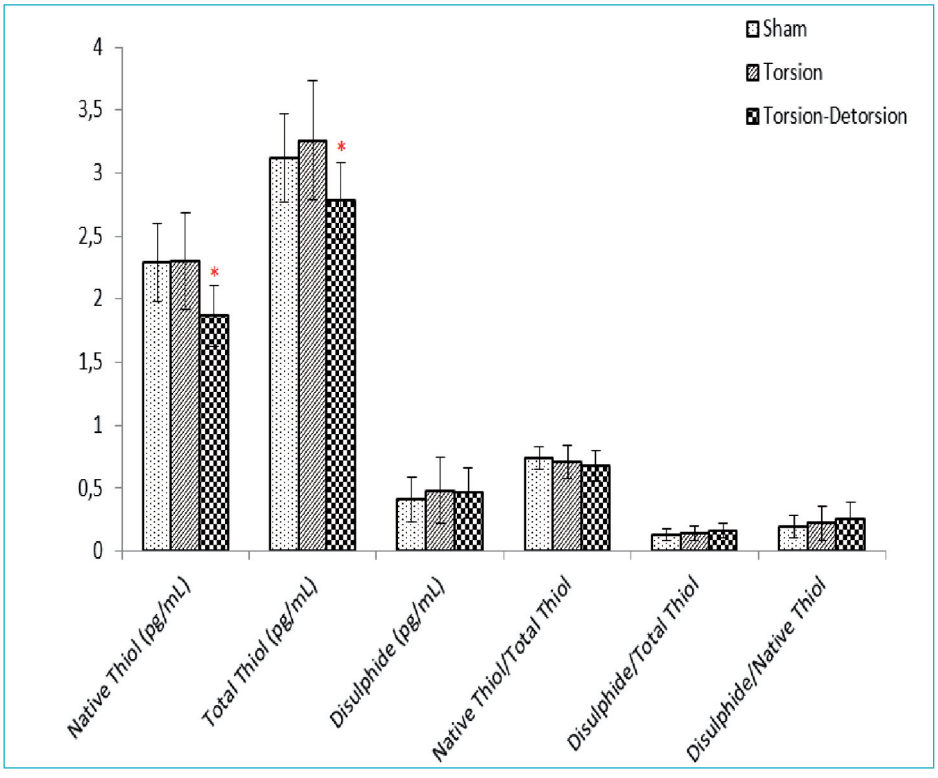

FIGURE 1: Comparsion of the obtained data of thiol-disulphide homeostasis belonging to serum samples of all groups.
*Shows low value compared to sham and torsion groups ($p<0.05$).

HISTOPATHOLOGICAL FINDINGS

In the histopathological evaluation, the ovarian tissue of the rats in the sham group was found to have normal histopathological structure (Figure 2A, B). Severe vascular congestion (hyperaemia) and haemorrhage were observed in torsion group. Mild oedema in the interstitial area was also seen in the same group (Figure 2C, D). However, in the torsion+detorsion group, severe vascular congestion (hyperaemia) and haemorrhage were observed. Severe oedema in the interstitial area was also seen in this group (Figure 2E, F).

STEREOLOGY

An important increase in the total ovarian volume was observed in the torsion and torsion+detorsion groups compared to the sham group (Table 2).

DISCUSSION

It has been suggested that there is not any specific serum marker for early diagnosis of ovarian torsion.[2] The symptoms of ovarian torsion such as abdominal pain, nausea and vomiting are non-specific and, and can be seen in other condiations such as appendicitis, pelvic inflammatory disease and urinary infection. Moreover, the ovarian torsion is uncommon. All these factors may lead to misdiagnosis or delay in diagnosis.[2,7]

There is no specific imaging or laboratory finding which is diagnostic for ovarian torsion.[3,4] Laparoscopy or laparotomy is needed for definitive diagnosis.[3] But, it should be remembered that 56% of laparotomies or laparoscopies performed under emergency conditions are negative.[5] Doppler ultrasonography which is the most frequently used method for diagnosis is not always accurate and could lead to unnecessary surgical interventions or delay in treatment.[2] This delay in diagnosis and treatment may result in ischemia, oedema, bleeding and necrosis on on the ovary.[1-3] Therefore, the early diagnosis of torsion is very important to prevent serious consequences such as infertility, peritonitis and sepsis which may occur as a result of complications after ovarian torsion.[3,8] Thus, we aimed to determine the

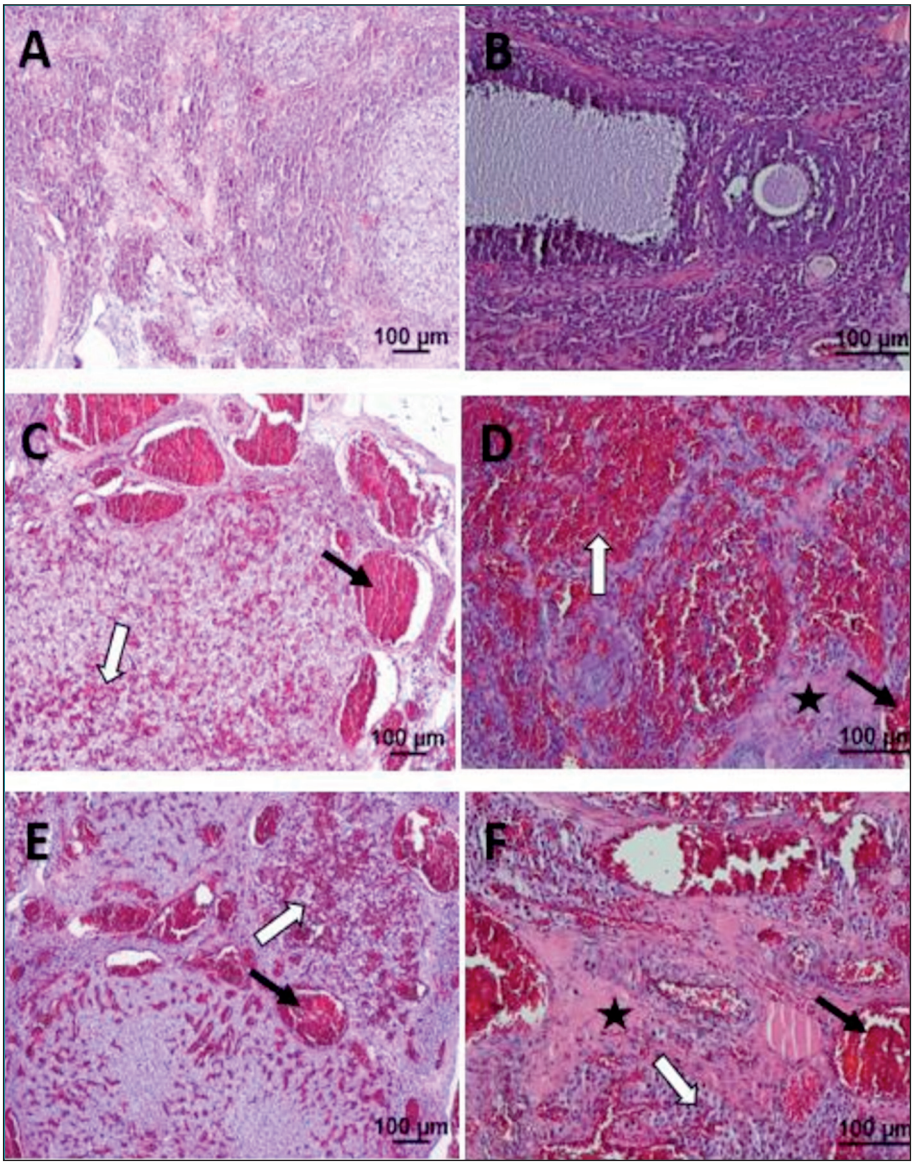

FIGURE 2: Histological images of ovarium tissue A,B; Sham group, C,D; Torsion group, E,F; Torsion+Detorsion group. Black arrow; congestion (Hyperaemia), White arrow; Hemorrhage, Star; Oedema. H-E.

TABLE 2: Ovary volume values of groups.

Groups	N	Median	Mean±SD	Min.	Max	*p value
Sham	8	7.55	7.46±1.14[a]	6.10	9.20	
Torsiyon	8	12.50	12.51±1.70[b]	10.10	15.40	<0.001
Torsiyon-Detorsiyon	8	10.00	10.10±1.19[c]	8.40	12.20	

* P value was determined according to Kruskalwallis test.

[a,b,c] p: They show the difference between groups (according to post-hoc).

role of thiol-disulphide homeostasis in the early diagnosis of ovarian torsion.

Free oxygen radicals are released due to ischemia which occur after ovarian torsion. These increasing free oxygen radicals cause a decrease in the ovarian reserve by damaging follicular units. Primordial follicles do not have regenerative features. Therefore, the risk of infertility and early menopause

increase when the ovarian damage takes place during the reproductive period.[8] All these findings clearly indicate that the ovarian torsion should be diagnosed early and accurately leading to surgical intervention immediately.

Thiols also known as mercaptans comprise an organic compound class. The thiol-disulphide homeostasis is one of the most important check and balance mechanisms preventing oxidative stress in the body. Thiols are a group of organic compounds which have a sulfhydryl group. Sulfhydryl groups can be oxidised to form disulphide bonds, and then, can be degraded into thiol groups.

The thiol/disulphide homeostasis is achieved through these events. Thiols serve as a defense mechanism against reactive oxygen types which cause oxidative stress. Other functions of thiols include apoptosis, detoxification, antioxidant protection, signal transduction, transcription factors, cellular signal mechanisms and regulation of cellular enzymatic activity. Many organs and systems are vulnerable to oxidative stress and redox products. Therefore, deterioration of oxidative balance could cause abnormalities at structural and functional levels.[6,9-15] In the literature, change in the thiol/disulphide homeostasis has been shown in diabetes mellitus, cardiovascular disorders, malignancy, rheumatoid arthritis, adenoid hypertrophy, acute pancreatitis, otitis media, β -thalassemia major, placenta percreta, inflammatory bowel diseases, rosacea and chronic kidney failure.[9-15]

Another study by Atasever et al. also investigated the thiol/disulphide homeostasis in ovarian torsion. However, the results of that study were different than ours. In the study by Atasever, total thiol and native thiol levels have been found to be lower in the torsion and torsion-detorsion groups compared to the sham group. Moreover, it has been stated that although disulphide levels and disulphide/thiol indexes in the same group were found to be higher in comparison with the sham group, these values were not significant.[1] Our study showed slight similarity to the study by Atasever at al. In our study, native thiol and total thiol values were significantly low only in the torsion-detorsion group in comparison with the other groups. Unlike the study carried out by Atasever et al. native thiol and total thiol values in this study were not different in the torsion group in comparison with the sham group. Furthermore, this study showed that the disulphide and disulphide/thiol indexes in the torsion and torsion-detorsion groups were not significantly higher compared to the sham group, which is similar to the study of Atasever et. al. In addition, another difference from the study by Atasever et. al., histopathological and stereological studies were carried out in our study. In the histopathological evaluation, severe vascular congestion and haemorrhage were observed in the ovarian tissue in both torsion and torsion-detorsion groups and in the interstitial area, mild oedema was seen. Moreover, it has been observed that total volume values obtained by using stereological methods increased significantly in the torsion and torsion-detorsion groups.

LIMITATIONS

Our study was carried out in an animal model as ovarian torsion is rarely seen in women. This limits the applicability of findings to women.

CONCLUSION

Early diagnosis and treatment are very important to prevent the development of ischemia and necrosis in the ovarian torsion. Unfortunately, there is no reliable clinical marker for early diagnosis or follow-up care before ischemia or necrosis develops. In this study, we suggest that the thiol-disulphide homeostasis cannot be used for early diagnosis of ovarian torsion at least in a rat model, but low levels of native thiol and total thiol after surgical treatment may be important parameters for patients in follow-up.

Source of Finance

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Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: *Veli Avcı, Zübeyr Huyut, Fikret Altındağ;* ***Design:*** *Veli Avcı, Hamit Hakan Alp, Kemal Ayengin;* ***Control/Supervision****:*

Veli Avcı, Zübeyr Huyut, Fikret Altındağ; ***Data Collection and/or Processing:*** *Veli Avcı, Hamit Hakan Alp, Kemal Ayengin;* ***Analysis and/or Interpretation:*** *Veli Avcı, Zübeyr Huyut, Fikret Altındağ;* ***Literature Review:*** *Veli Avcı, Zübeyr Huyut, Fikret Altındağ;* ***Writing the Article:*** *Veli Avcı, Hamit Hakan Alp, Kemal Ayengin;* ***Critical Review:*** *Veli Avcı, Zübeyr Huyut, Fikret Altındağ;* ***References and Fundings:*** *Veli Avcı, Hamit Hakan Alp, Kemal Ayengin;* ***Materials:*** *Veli Avcı, Zübeyr Huyut, Fikret Altındağ.*

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