

The Effect of Tunica Albuginea Incision on Testicular Tissue After Detorsion in the Experimental Model of Testicular Torsion

Aynur Gultekin¹, Halil Ibrahim Tanriverdi¹, Sevinc Inan², Omer Yilmaz¹, Cuneyt Gunsar¹, Aydin Sencan^{1*}

Purpose: Testis torsion is a surgical emergency, and sometimes we cannot sufficiently prevent injury even surgical detorsion of the testis is performed in the appropriate time period due to some reasons such as tissue edema. In this experimental study, we investigated the effect of tunica albuginea incision (TAI) on testicular torsion-detorsion model (TDM).

Materials and Methods: Twenty four male rats were used. The rats were randomly divided into three groups. In Group I, testicular torsion (TT) of 720° was created. After 4 hours of torsion period, the testis was detorsioned. Then three longitudinal incisions were made on tunica albuginea of the testis. In Group II, torsion and detorsion was created by the same way as in Group I, but TAI was not added to the procedure. Group III was Sham group. At the end of the first week, rats in the experimental groups were sacrificed and the testes were harvested for histological, immunohistological examinations and for the assessment of apoptotic activity.

Results: In Group I, the procedures led to partial improvement in color of the testes. Modified Johnsen Scores in Groups I, II and III were detected as 7.8, 4.3 and 9.6 respectively ($P = .001$). In Group I, immunoreactivity of anti-APAF-1 was moderate in 7 rats, and strong in 1 rat. Immunoreactivity of anti-cytochrome C and anti-caspase 3 were moderate in 6 rats, and strong in 2 rats. Immunoreactivity of anti-caspase 8 and 9 were moderate in 5 rats, and strong in 3 rats. The differences of immunoreactivity between the groups were statistically significant. TUNEL percentages were detected as 40, 62% in Group I, 60% in Group II and 11,75% in Group III respectively ($P = .001$).

Conclusion: As a result, multiple incisions made on tunica albuginea after detorsion in the TDM in rats, decrease the amount of ischemia- reperfusion injury. This effect might be related with the decrease in testicular edema and free oxygen radicals together with increase in tissue perfusion. Moreover, the decreased apoptotic activity seems to play a role in the decrease in inflammatory response and preservation of tissue parenchyma consequently.

Keywords: apoptosis; incision; rat; testicular torsion; tunica albuginea.

INTRODUCTION

T (testicular torsion) is one of the important and emergency action demanding conditions in childhood. Despite many experimental studies aiming to prevent or keep minimum the damage due to torsion, there has been no method to be used in clinical practice yet. The limited number of studies about tunica albuginea incision (TAI) produced conflicting results⁽¹⁻⁴⁾. In addition, association of histological changes as a result of TAIs made on testis with apoptosis and apoptotic pathways involved in this association have not been studied so far.

In this study, decreasing the compartment pressure with TAI was aimed in the rat testicular torsion-detorsion model (TDM) and it was evaluated as histological and immunochemical that how ischemic-reperfusion damage due to lowered tissue pressure after TAI affects testis histology and the role of apoptosis. We hypothesized that multiple incisions made on tunica albuginea after detorsion decrease the ischemia-reperfusion damage in testicular parenchyma.

MATERIALS and METHODS

The study was carried out in Celal Bayar University Experimental Animals Laboratory after approval of study protocol by ethic committee (Protocol No: 77637435-005).

Twenty-four young adult Wistar albino male rats with body weights ranging from 230 to 280 g were used. They were hosted at Celal Bayar University Animal Laboratory. Three groups were established, each containing eight rats.

Group I: Torsion + Detorsion + Incision Group (Treatment Group)

Group II: Torsion + Detorsion Group (Control Group)

Group III: Sham Control Group (Sham Group)

Surgical Procedure: All surgical procedures were carried out under intraperitoneal anesthesia (ketamine 75 mg/kg, xylazine 10 mg/kg) and sterile conditions where skin cleanliness was realized through use of povidone iodine. After the operation, rats were allowed to feed orally. Rats were allowed to live for a week and then sacrificed under anesthesia, and testes were excised.

Group I (Treatment Group): Testis was exposed through a vertical incision over left groin. Left testis

¹Celal Bayar University Medical School, Department of Pediatric Surgery, Manisa, Turkey .

²Celal Bayar University Medical School, Department of Histology, Manisa, Turkey.

*Correspondence: Celal Bayar University, Medical School, Department of Pediatric Surgery, 45030, Uncuboz-koy, Manisa, Turkey.

Tel: +90 236 233 85 86. Fax: +90 236 233 14 66. E-mail: aydin.sencan@yahoo.com.tr.

Received December 2016 & Accepted August 2017

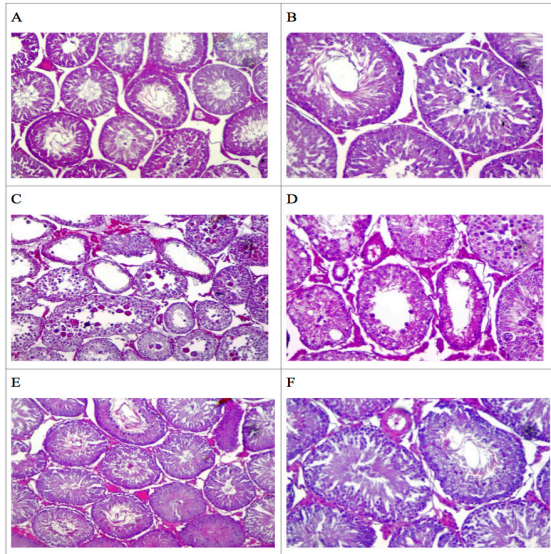


Figure 1. Normal testis histological appearance is observed in Group III (E,F). Seminiferous tubule structures and order of spermatogenic cells were disrupted, multinucleated granulation cells were observed within tubules, no spermatogenic cells were found within some seminiferous tubules, vascular stasis and Leydig cells were impaired in interstitial area in Group II (C,D). In dissections from Group I in which incision was made to tunica albuginea after torsion, histopathological findings were diminished compared to Group II and a closer to normal appearance was observed (A,B). H&E x100 (A, C, E), H&Ex 200 (B, D, F).

together with spermatic cord were taken out of incision area. Torsion was carried out through twisting the testis and spermatic cord 720° clockwise. In order to maintain the torsion, testis was fixed from its tunica albuginea to scrotum via two silk sutures. After the procedure, the incision was closed and torsion was maintained for four hours. After four hours, the incision was opened and sutures fixing testis to scrotum were removed. Testis was twisted counterclockwise. Then, three longitudinal incisions were made over the tunica albuginea of testis and protrusion of testicular tissue in incised areas was realized. Testis was put back into scrotum and scrotum skin was closed.

Group II (Control Group): Torsion and detorsion was carried out in eight rats of this group using the same method described for Group I. However, unlike Group I, no incisions were realized in tunica albuginea.

Group III (Sham Control Group): The rats of this group had only left inguinal scrotal incision. Nothing was made with testes and skin incision was closed back.

Tissue sampling and paraffin tissue monitoring: All testes were fixed in bouin solution. Using microtome, 5 µm thick dissections were taken from paraffin blocks onto polylysine coated slides, stained with hematoxylin

eosin and evaluated histologically. Additional dissections were evaluated under a light microscope using TUNEL method and indirect immunohisto-chemical methods.

Modified Johnsen Testicular Biopsy Score: In hematoxylin and eosin (H&E) stained testicular tissue samples taken from each rat, appearance of spermatogenic cells in seminiferous tubules was evaluated and scored based on modified Johnsen Testicular Biopsy Score⁽⁵⁾. Ten transversal dissections were evaluated in each dissection and a score of 1 to 10 was assigned to each rat. **Indirect immunohistochemical evaluation via Avidin-Biotin Peroxidase Method:** Additional dissections taken from paraffin blocks of groups were prepared for indirect immuno-histochemical staining. Primary antibodies for anti-APAF (apoptotic protease activating factor 1, sc-65890, Santa Cruz Biotechnology Inc.), anti-cytochrome-C (sc-13156, Santa Cruz Biotechnology Inc.), anti-caspase 3 (sc-56046, Santa Cruz Biotechnology Inc.), anti-caspase 8 (sc-52063, Santa Cruz Biotechnology Inc.) and anti-caspase 9 (Novus-NB 100-92022) were used in 1:100 dilutions. Avidin-biotin-peroxidase system (Invitrogen®-Histostain Plus Bulk Kit, Invitrogen® 2nd Generation LAB-SA Detection System, Cat no: 85-9043, Broad spectrum, Camarillo, CA) was used as secondary kit. Primary antibody based immunoreactivity staining intensity of testis samples from groups were scored as slight (+), moderate (++) and strong (+++).

TUNEL (Terminal deoxynucleotidyl transferase dUTP nick end labeling): Apoptotic activity was evaluated using TUNEL method. Samples were evaluated under a light microscope using TUNEL method. Apoptotic activity percentages detected in subjects of different groups.

Statistical Evaluation: Parameters were evaluated statistically among groups using three different methods. Modified Johnsen test was evaluated using ANOVA (one-way) test, while TUNEL test was evaluated using Mann-Whitney test, and primary antibody based immunoreactivity strength was evaluated using Chi-square test. $p < 0.05$ was considered significant. Statistical analyses were performed with SPSS Statistics 24 (IBM, 2016).

RESULTS

1. Findings based on macroscopic observation during the operation: Testes were light pink colored when taken out of incision area. However, their color changed rapidly from light pink to purple after torsion. Four hours after torsion, edema developed in addition to color change. After detorsion at the end of four hours, color was restored partly but edema continued.

2. **Histochemical Findings:** Ten samples were taken from testicular tissue of each subject, stained by hematoxylin-eosin method and scored under a light micro-

Table 1. Evaluation of testis based on Johnsen scores after modified hematoxylin-eosin staining. Comparison of three groups $p = 0.001$ (ANOVA posthoc: Benferroni alpha test) ($p = 0.001$ Group I and Group II, $p = 0.001$ Group II and Group III).

Groups	Number of samples	Mean	Standard deviation		Standard error	95% confidence interval
			Lowest	Highest		
Group I	80	7,8875	0,74619	0,08343	7,7214	8,0536
Group II	80	4,3250	0,77582	0,08674	4,1523	4,4977
Group III	80	9,6000	0,54191	0,06059	9,4794	9,7206
Total	240	7,2708	0,14900	0,14900	6,9773	7,5643

Table 2. Staining intensities of groups after indirect immunohistological staining using APAF-1 (Apoptotic protease activating factor-1) primary antibody. Group I and Group II $p = 0.02$, and Group II and Group III $p = 0.02$ (Chi-square test).

Anti-APAF 1*	Slight	Moderate	Strong	Total
Group I		7	1	8
Group II		4	4	8
Group III	7	1		8
Total	7	12	5	24

scope using modified Johnsen scoring criteria. Average scores were given in **Table 1**.

Group I (Treatment Group): In groups for which incision was made in tunica albuginea after four hours torsion period, seminiferous tubules maintained their close to normal appearance, multi-nuclei granulation cells were observed among spermatogenic cells of some tubules, slight edema and capillary dilatation were observed in interstitial area (**Figure 1A,B**).

Group II (Control Group): In the group which had four hours of torsion and detorsion, lumen diameters of seminiferous tubules changed, order of spermatogenic cells was disrupted, some tubules lacked spermatogenic cells, multinucleated cells were observed within seminiferous tubules, capillary dilatation, stasis, increased inflammatory cells and disordered Leydig cells and intense edema in interstitial area were observed (**Figure 1C,D**).

Group III (Sham Control Group): Seminiferous tubules and interstitial connective tissue had normal histological appearance in testis dissections. Of the serial spermatogenic cells located on seminiferous tubule wall, basally located spermatogonia, primary and secondary spermatocytes located in the middle area and round spermatids located close to lumen as well as sperms in lumen were considered normal. Sertoli cells lied from basal to apical, oval in shape and had evident nucleus. Capillary vessels and Leydig cells in interstitial connective tissue retained their normal histological structures

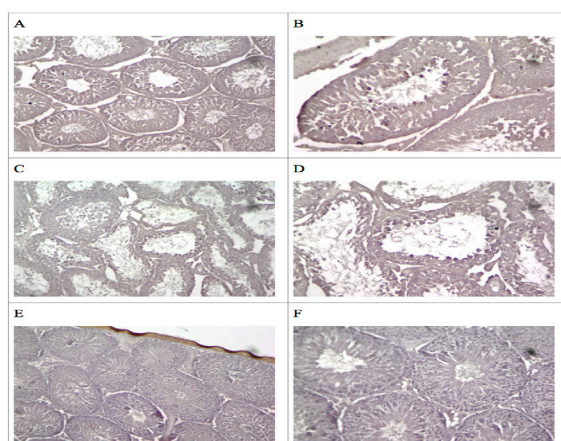


Figure 2. Light microscope images of testis dissections of groups after indirect immunohistochemical staining using anti-APAF-1 primary antibody. In Group III, slight immunoreactivity was observed in spermatogenic cells in seminiferous tubules of testis (**E,F**), while moderate/strong immunoreactivity was observed in Group II (**C,D**) and moderate immunoreactivity was observed in Group I (**A,B**).

x100 (A, C, E), x200 (B, D, F).

Table 3. Staining intensities of groups after indirect immunohistological staining using primary anti-cytochrome C antibody. Group I and Group II $p = 0.038$, and Group II and Group III $p = 0.038$ (Chi-square test).

Anti-cytochrome C	Slight	Moderate	Strong	Total
Group I		6	2	8
Group II		1	7	8
Group III	4	4		8
Total	4	11	9	24

and did not have capillary stasis and inflammatory cell infiltration (**Figure 1E,F**).

3. Immunohistochemical Findings

Anti-APAF 1: Staining intensities of different groups based on indirect immunohistochemical staining using anti-APAF 1 primary antibody were shown in **Table 2**. In Sham control group, slight (+) immunoreactivity was observed in spermatogenic cells in seminiferous tubules of testis (**Figure 2E,F**). Moderate/strong (++) immunoreactivity was observed in control group (**Figure 2C,D**) while moderate (++) immunoreactivity was observed in treatment group (**Figure 2A,B**). Differences between the groups were statistically significant ($P = .02$).

Anti-cytochrome C: Results of indirect immunohistochemical staining using primary anti-cytochrome C antibody were summarized in **Table 3**. Slight/moderate (++) immunoreactivity was observed in spermatogenic cells located in seminiferous tubules of testis in sham control group (**Figure 3E,F**). Immunoreactivity was strong (+++) in affected spermatogenic cells in control group (**Figure 3C,D**) and moderate (++) in treatment group (**Figure 3A,B**).

Anti-caspase 3: Results of indirect immunohistochemical staining using anti-caspase 3 primary antibody were summarized in **Table 4**. Slight (+) immunoreactivity was observed in spermatogenic cells located in seminiferous tubules of testis in sham control group (**Figure 4E,F**). On the other hand, moderate/strong (++) immunoreactivity was observed in affected spermatogenic cells in control group (**Figure 4C,D**) and moderate (++) in treatment group (**Figure 4A,B**).

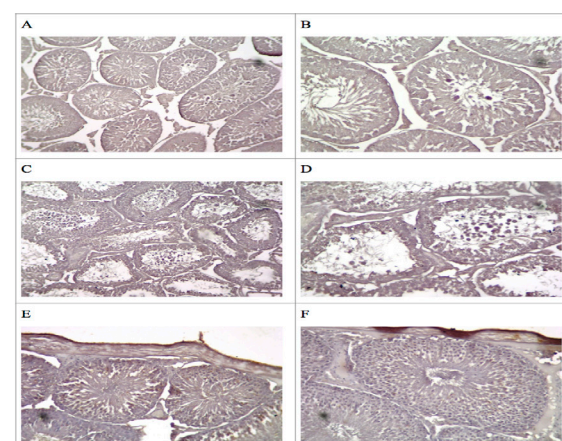


Figure 3. Light microscope images of testis dissections of groups after indirect immunohistochemical staining using anti-cytochrome-C primary antibody. Slight/moderate immunoreactivity was observed in spermatogenic cells located in seminiferous tubules of testis in Group III (**E,F**), whereas strong immunoreactivity was observed in affected spermatogenic cells in Group II (**C,D**) and moderate immunoreactivity was observed in Group I (**A,B**).

x100 (A, C, E), x200 (B, D, F).

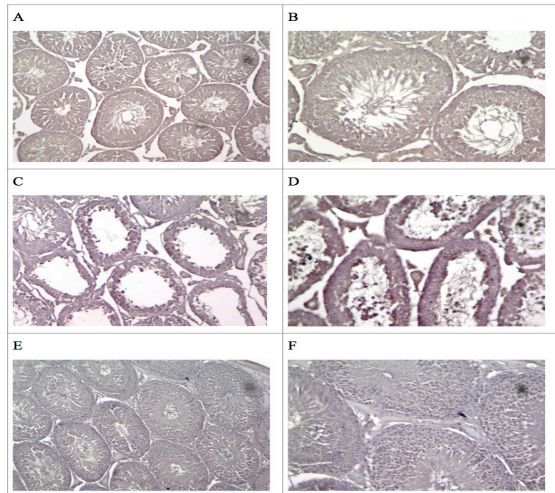


Figure 4. Light microscope images of testis dissections of groups after indirect immunohisto-chemical staining using anti-caspase-3 primary antibody. Slight immunoreactivity was observed in spermatogenic cells located in seminiferous tubules of testis in Group III (E,F), while strong immunoreactivity was observed in Group II (C,D) and moderate immunoreactivity was observed in Group I (A,B).

x100 (A, C, E), X200 (B, D, F).

immunoreactivity was observed in affected spermatogenic cells in control group (Figure 4C,D) and moderate immunoreactivity (++) was observed in treatment group (Figure 4A,B).

Anti-caspase 8: Results of indirect immunohistochemical staining using anti-caspase 8 primary antibody was given in Table 5. Moderate (++) immunoreactivity was observed in spermatogenic cells located in seminiferous tubules of testis in sham control group (Figure 5E,F). Reactivity was moderate/strong (+/++) in control group (Figure 5C,D) and moderate (++) in treatment group (Figure 5A,B).

Anti-caspase 9: Results of indirect immunohistochemical staining using primary anti-caspase 9 antibody was given in Table 6. Moderate (++) immunoreactivity was observed in spermatogenic cells located in seminiferous tubules of testis in sham control group (Figure 6E,F). Control group showed strong immunoreactivity (+++) (Figure 6C,D) while treatment group (Figure 6A,B) showed moderate/strong (+/++) immunoreactivity.

4. TUNEL (Terminal deoxynucleotidyl transferase dUTP nick end labeling) Results

Apoptotic activity percentages detected in subjects of different groups were summarized in Table 7. Lower number of TUNEL positive spermatogenic cells (mean $11.75\% \pm 1.75$) were observed in seminiferous tubules of testis in sham control group (Figure 7E,F). On the

Table 3. Staining intensities of groups after indirect immunohistological staining using primary anti-cytochrome C antibody. Group I and Group II $p = 0.038$, and Group II and Group III $p = 0.038$ (Chi-square test).

Anti-cytochrome C	Slight	Moderate	Strong	Total
Group I		6	2	8
Group II		1	7	8
Group III	4	4		8
Total	4	11	9	24

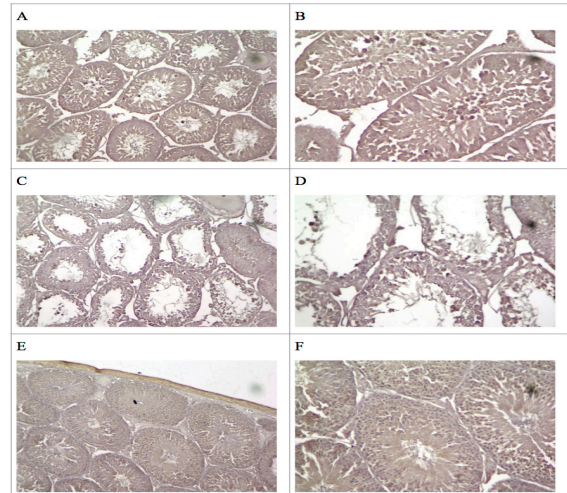


Figure 5. Light microscope images of testis dissections of groups after indirect immunohistochemical staining using anti-caspase-8 primary antibody. Slight/moderate immunoreactivity was observed in spermatogenic cells located in seminiferous tubules of testis in Group III (E,F), while moderate/strong immunoreactivity was observed in Group II (C,D) and moderate/strong immunoreactivity was observed in Group I (A,B).

X100 (A, C, E), X200 (B, D, F).

other hand, higher number of TUNEL positive cells (mean $60\% \pm 5.34$) were observed in control group (Figure 7C,D) while number of TUNEL positive cells (mean $40.625\% \pm 3.37$) were moderate in treatment group (Figure 7A,B). Differences between Group I and Group II ($p = 0.001$) and between Group II and Group III ($P = .001$) were statistically significant (Figure 8).

DISCUSSION

TT is still one of the important problems in Pediatric Urology. Although many experimental studies have been conducted to prevent or keep minimum the damage in testis after torsion, no results to be applied in clinical practices have been obtained so far. Tunica TAI studies which have been conducted recently produced only inconsistent results. Besides, association of histological changes arising from TAIs with apoptosis have not been investigated so far. In the present study, we aimed to decrease tissue compartment pressure through incisions in tunica albuginea. Thus, how the ischemia-reperfusion damage in testis due to decreased tissue pressure affected testis histology and the role of apoptosis during the process were investigated using histological and immunohistochemical methods.

Tunica albuginea, the outer cover of testicular tissue, is a very strong and non-elastic layer. Testicular tissue

Table 4. Staining intensities of groups after indirect immunohistological staining using primary anti-caspase 3 antibody. Group I and Group II $p = 0.04$, and Group II and Group III $p = 0.004$ (Chi-square test).

Anti-Caspase 3	Slight	Moderate	Strong	Total
Group I		6	2	8
Group II		3	5	8
Group III	7	1		8
Total	7	10	7	24

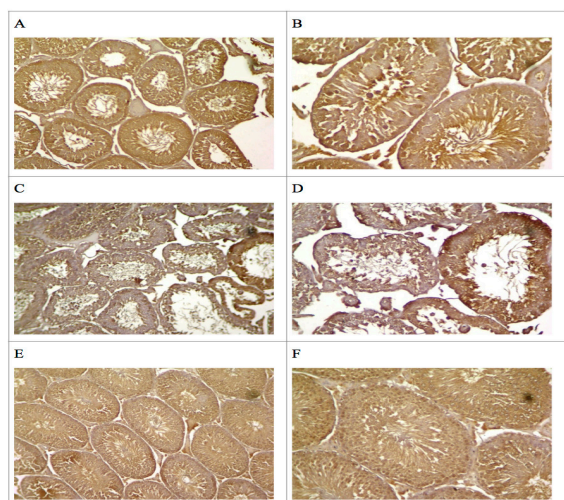


Figure 6. Light microscope images of testis dissections of groups after indirect immunohistochemical staining using anti-caspase-9 primary antibody. Moderate immunoreactivity was observed in spermatogenic cells located in seminiferous tubules of testis in Group III (E,F), while strong immunoreactivity was observed in Group II (C,D) and moderate/strong immunoreactivity was observed in Group I (A,B).
x100 (A, C, E), x200 (B, D, F).

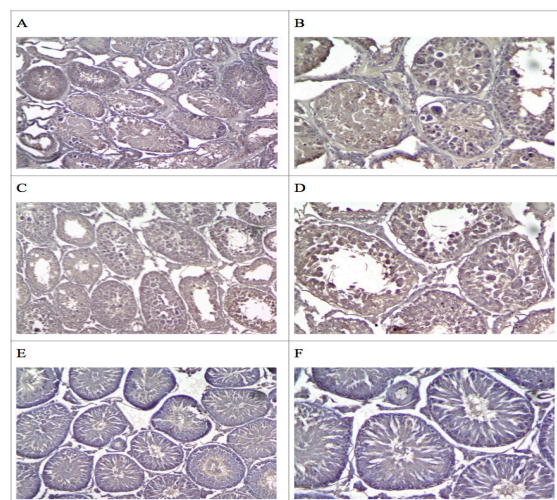


Figure 7. Light microscope images of testis dissections of groups after staining with TUNEL method. Lower number of TUNEL positive spermatogenic cells were observed in seminiferous tubules of testis in Group III (E,F), while higher number of TUNEL positive cells were observed in Group II (C,D) and moderate number of TUNEL positive cells were observed in Group I (A,B).
x100 (A, C, E), x200 (B, D, F).

develops edema in TT either due to venous occlusion at the beginning or due to ischemia-reperfusion damage developing after detorsion. This situation lowers perfusion pressure while increasing tissue pressure^(6, 7). In addition, the fact that reactive oxygen metabolites and abnormal neutrophil activation increase due to reperfusion also increases edema in testicular tissue^(7, 8). Since the outer cover of testis, tunica albuginea, is not elastic enough, it cannot offset the increasing pressure in testis, and compartment syndrome may develop. Acute compartment syndrome may lead to tissue ischemia and necrosis.

Recent studies revealed that exposure to chemical events, androgen withdrawal and cryptorchidism can trigger apoptosis in germ cells⁽⁹⁻¹¹⁾. Germ cell apoptosis is stimulated by increasing intratesticular oxidative damage and diapedesis of leucocytes⁽¹²⁻¹⁴⁾. Higher concentrations of reactive oxygen species lead to cell death, while lower concentrations trigger apoptosis⁽¹⁵⁾. Although the ischemia due to reperfusion is recovered after testicular detorsion, loss of spermatogenesis continues. This loss has been considered to be associated with specific germ cell apoptosis⁽¹²⁾. Excess amount of free oxygen radicals in the environment can also function as a mediator in apoptotic cycle^(16,17). In the present study, apoptotic activity in the group which had TAI was significantly less (19.4%) compared to the group

which had only torsion + detorsion. This decrease could be associated with lower edema arising from TAIs and, consequently, lower inflammatory response and lower formation of reactive oxygen species. It has been known that especially acute compartment syndrome leads to tissue ischemia and disrupts microcirculation. Apoptotic activity was significantly higher in control group and TAI group compared to sham group in the present study. It is highly probable that this higher apoptotic activity was a result of ischemia reperfusion damage due to torsion-detorsion.

Caspases belong to cysteine-aspartic acid protease family and they break the peptide bond after aspartic acid. They are known as the effectors that break down cells. Caspase activation takes place either by the activation of cell surface death receptors or by induction of APAF-1, caspase-9 binding protein. Induction of APAF-1, is mediated by release of cytochrome-C from mitochondria. Induced APAF-1 activates caspase-9 (intrinsic activation), which in turn activates caspase-3. On the other hand, caspase-8 is activated by death ligands (such as Fas ligands) which induces extracellular apoptosis and by activity arousing agents (extrinsic activation). Caspase-3, is known as the common pathway of apoptotic cell death activated by both intrinsic (mitochondrial) and extrinsic (death ligands) pathways. Expression levels of Caspase-1,2,3 and BAX increase in

Table 5. Staining intensities of groups after indirect immunohistological staining using primary anti-caspase 8 antibody. Group I and Group II $p = 0.023$, and Group II and Group III $p = 0.023$ (Chi-square test).

Anti Caspase 8	Slight	Moderate	Strong	Total
Group I		5	3	8
Group II		3	5	8
Group III		3	5	8
Total	3	13	8	24

Table 6. Staining intensities of groups after indirect immunohistological staining using primary anti-caspase 9 antibody. Group I and Group II $p = 0.008$, and Group II and Group III $p = 0.008$ (Chi-square test).

Anti-Caspase 9	Slight	Moderate	Strong	Total
Group I		5	3	8
Group II	1	7	8	
Group III	1	7		8
Total	1	13	10	24

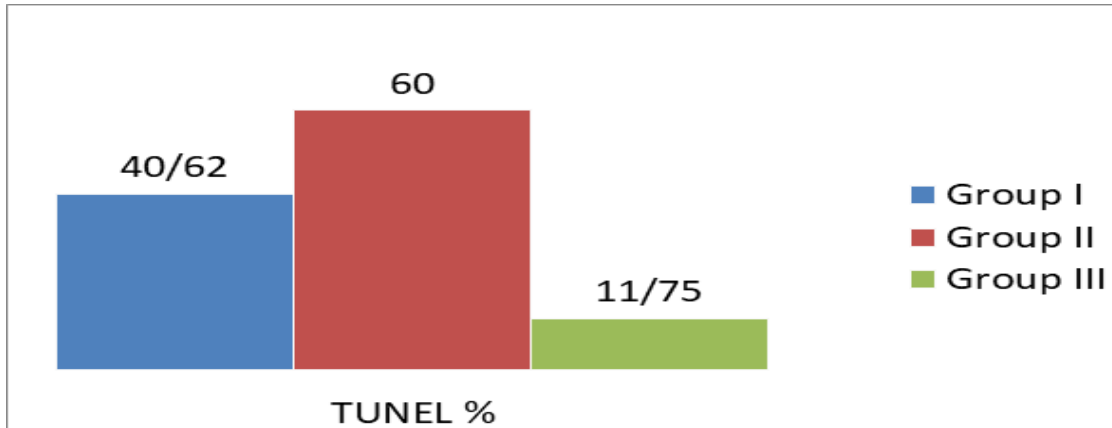


Figure 8. Average TUNEL percentages of groups. Apoptotic activity percentages of subjects based on TUNEL method. Group I and Group II, $p = 0.001$; and Group II and Group III, $p = 0.001$ (Mann-Whitney test).

ischemia-reperfusion damages⁽¹²⁾. Increased expression of proapoptotic Bax molecule suggests that an apoptotic pathway starting in mitochondria is induced in torsion process⁽¹⁸⁾. It's an important point for starting the apoptotic activity that cytochrome-C which is releasing from mitochondria and which accompanies the increase of Bax mRNA after torsion-detorsion. Therefore, we evaluated caspase-3, caspase-8, caspase-9, cytochrome-C and APAF-1 immunohistochemically to shed light on pathways by which apoptotic activity are induced.

Immunohistochemical stainings carried out using some molecules associated with apoptotic pathway (anti-APAF-1, cytochrome-C, caspase-3-8-9) showed significantly less immunoreactivity in group which had TAI compared to the one which had torsion and detorsion only. In other words, activation of apoptotic cascade after torsion-detorsion enhanced immunoreactivity. TAI, on the other hand, led to decreases in apoptotic activity after torsion-detorsion, and consequently, to decreases in the strength of immunoreactivity. Stronger staining in torsion-detorsion group with both caspase-8 and caspase-9 pointed the activation of both extrinsic (caspase-8) and intrinsic (caspase-9) pathways. Activation of both pathways is reversible until a given point. However, in torsion-detorsion, spermatogenic cells were observed to mostly enter irreversible process through activation of caspase-3, while in the group which had TAI number of spermatogenic cells entering irreversible process were less. Thus, experimentally established torsion-detorsion of testes causes histological damage in testicular parenchyma. As well as reactive oxygen metabolites in the ischemia-reperfusion damage environment, triggering of apoptosis could also play a

role in this damage. On the other hand, decreased immunoreactivity staining intensity and significantly less apoptotic cell percentage in group with TAI compared to control group suggested the benefit of the procedure. This effect could very probably be due to the fact that TAIs diminished acute compartment syndrome, decreased reactive oxygen metabolite release into the environment and prevented apoptotic pathway from entering irreversible cycle. Therefore, it seems that TAIs are effective in protecting testis from ischemia-reperfusion damage developing as a result of torsion-detorsion process.

Effects of TAIs on testicular tissue and spermatogenesis function in TT have been investigated in a very limited number of experimental studies. In limited number of case series, tunica albuginea decompression after detorsion has been shown to contribute positively to testis perfusion, however, this result has not been entirely confirmed by some other experimental studies^(1,2). However, recent clinical data predominantly indicates that TAI or fasciotomy decompression is beneficial in post-detorsion compartment syndrome.

Using an experimental torsion model, Kolbe et al. was the first to suggest that tunical capsulotomy could lower testicular pressure and hence prevent testis injury⁽¹⁾. Nevertheless, the authors observed that capsulotomy after detorsion did not prove beneficial histologically for testicular tissue. In the present study, histological changes in testicular tissue was first evaluated using modified Johnsen scoring, and significantly less cell injury was detected in treatment group which had tunical incision after torsion-detorsion compared to control groups. In addition, less apoptosis percentage in testes post TAI in the present study also points to less testis

Table 7. Apoptotic activity percentages of subjects based on TUNEL method. Group I and Group II, $p = 0.001$; Group II and Group III $p = 0.001$ (Mann-Whitney test).

Subject No	Group I TUNEL%	Group II TUNEL%	Group III TUNEL%
1	40	60	10
2	45	55	12
3	40	65	13
4	38	50	12
5	35	60	10
6	42	65	15
7	45	60	12
8	40	65	10

injury. However, duration of torsion and testis evaluation methods in the present study are different from the study by Kolbe et al. In addition, observations in Kolbe et al. belong to late period (35 days) while the results reported in the present study represent early period results (7 days).

The first clinical observation about TAIs post torsion-detorsion of testis was reported by Kutikov et al. in 2008⁽²⁾. In their case series involving three patients of 11-16 years of age with TT, the authors reported that testes subjected to prolonged ischemia were congested and in blackish color, but macroscopic color of testis rapidly turned to normal after TAI. The authors measured testicular tissue pressure in a case with TT using Stryker intra-compartmental pressure monitor and found that pressure in the twisted section after detorsion was 34 mmHg, while the pressure of TAIs decreased as low as 5 mmHg. They concluded that TAI lowered intratesticular compartment pressure and made a positive contribution to blood supply to testicular tissue.

In a clinical study published in 2012, it was reported that of 59 patients operated and followed for TT, 31 of these had macroscopic testis appearance which returned to normal after detorsion and orchiopexy but 28 of these had no evidence of improvement after detorsion⁽³⁾. The authors reported that an orchietomy rate before TAI and flap application was 35.9%, which decreased significantly to about 15% after TAI. This clinical finding supports the results of the present study.

Moritoki et al. recently studied the association between intratesticular pressure and testicular function using a TDM⁽¹⁹⁾. They observed that tissue pressure increased in twisted testes but decreased after detorsion. In addition, the authors found strong correlations between lower testicular pressure and testis weight, and between epididymal sperm number and seminiferous tubule diameter. Similar to our results, these findings suggest that TAIs are effective in TT.

Oktar et al. studied how TAIs affected testicular pressures before and after torsion using a Stryker monitor in an experimental TDM and evaluated the pressure values they measured with testicular viability and functions⁽⁴⁾. The authors revealed that average testicular pressure after TAI was significantly lower compared to no incision group. Despite the lower pressure in TAI group compared to no incision group, the authors failed to find any significant change in modified Johnsen score, average seminiferous tubule diameter and testis weight. This finding is interesting when physiopathology of ischemia-reperfusion damage is considered. In the present study, however, modified Johnsen scores of TAI group improved significantly compared to torsion-detorsion group. In other words, in the present study, lowering the pressure which leads to acute compartment syndrome decreased ischemia-reperfusion damage in testis.

There were some limiting factors in the present study. First, histopathological data represented the results from early period. It is not known how testicular viability and function is affected in long term post TAI. Another limiting factor was that histological changes were considered to be associated with lower intratesticular compartment pressure, but pressure measurements were not conducted. Therefore, direct relationship between histology and pressure changes were not shown. Apoptotic activity was evaluated using TUNEL method in the present study. While identifying apoptotic cells,

TUNEL method can also stain cells that undergo necrosis. Therefore, it is possible that reported apoptotic activity percentage might not represent actual apoptotic cell percentage.

CONCLUSIONS

In conclusion, in our testicular TDM, multiple incisions made on tunica albuginea after detorsion decreased ischemia-reperfusion damage in testicular parenchyma. This effect could be due to decreased edema in testis as a result of lowered tissue pressure, improved tissue perfusion and decreased amount of free oxygen radicals. In addition, lowered apoptotic activity seems to contribute to decreased inflammatory response and, consequently, to protection of parenchyma.

CONFLICT OF INTEREST

The authors report no conflict on interest.

REFERENCES

1. Kolbe A, Sun CCJ, Hill JL. Unpredictability of capsulotomy in testicular torsion. *J Pediatr Surg.* 1987;22:1105-09.
2. Kutikov A, Casale P, White MA, Meyer WA, Chang A, Gasalbez R, et al. Testicular compartment syndrome: A new approach to conceptualizing and managing testicular torsion. *Urology.* 2008;72:786-89.
3. Figueroa V, Salle JLP, Braga LHP, Romao BR, Koyle MA, Bagli DJ, et al. Comparative analysis of detorsion alone versus detorsion and tunica albuginea decompression (fasciotomy) with tunica vaginalis flap coverage in the surgical management of prolonged testicular ischemia. *J Urol.* 2012;188:1417-23.
4. Oktar T, Küçükdurmaz F, Erdem S, Kılıçaslan I, Ceylan C, Sanli O, et al. Tunica albuginea decompression fails to alter the injury of prolonged arterial occlusion during testicular torsion. *J Urol.* 2013;190:239-43.
5. Johnsen SG. Testicular biopsy score count—a method for registration of spermatogenesis in human testes: Normal values and results in 335 hypogonadal males. *Hormones.* 1970;1:2.
6. Anaya-Prado R, Toledo-Pereyra LH, Lentsch AB, Ward PA. Ischemia/reperfusion injury. *J Surg Res.* 2012;105:48-58.
7. Mars M, Hadley GP. Raised intracompartmental pressure and compartment syndromes. *Injury.* 1998;29:403-11.
8. Balogh ZJ, Butcher NE. Compartment syndromes from head to toe. *Crit Care Med.* 2010;38:445-51.
9. Nandi S, Banerjee PP, Zirkin BR. Germ cell apoptosis in the testes of Sprague Dawley rats following testosterone withdrawal by ethane 1,2-dimethanesulfonate administration: relationship in Fas? *Biol Reprod.* 1999;61:70-5.
10. Dunkel L, Taskinen S, Hovatta O, Tilly JL, Wikstrom S. Germ cell apoptosis after treatment of cryptorchidism with human

- chorionic gonadotropin is associated with impaired reproductive function in the adult. *J Clin Invest.* 1997;100:2341-46.
11. Lee J, Richburg JH, Younkin SC, Boekelheide K. The Fas system is a key regulator of germ cell apoptosis in the testis. *Endocrinology.* 1997;138:2081-88.
 12. Turner TT, Tung KSK, Tomomasa H, Wilson LW. Acute testicular ischemia results in germ cell-specific apoptosis in the rat. *Biol Reprod.* 1997;57:1267-74.
 13. Thompson CB. Apoptosis in the pathogenesis and treatment of disease. *Science.* 1995;267:1456-62.
 14. Johnson TM, Yu XZ, Ferans VJ, Lowenstein RA, Finkel T. Reactive oxygen species are downstream mediators of p53-dependent apoptosis. *Proc Natl Acad Sci.* 1996;93:11848-52.
 15. Packham G, Ashmun RA, Cleveland JL. Cytokines suppress apoptosis independent of increases in reactive oxygen species. *J Immunol.* 1996;156:2792-800.
 16. Payabvash S, Kiumehi S, Tavangar SM, Deppour AR. Ethyl pyruvate reduces germ cell-specific apoptosis and oxidative stress in rat model of testicular torsion/detorsion. *J Pediatr Surg.* 2008;43:705-12.
 17. Guimaraes SB, Aragao AA, Santos JM, Kimura OS, Barbosa PH, Vasconcelos PR. Oxidative stress induced by torsion of the spermatic cord in young rats. *Acta Cir Bras.* 2007;22:30-3.
 18. Lysiak JJ, Turner SD, Turner TT. Molecular pathway of germ cell apoptosis following ischemia/reperfusion of the rat testis. *Biol Reprod.* 2000;63:1465-72.
 19. Moritoki Y, Kojima Y, Mizuno K, Kamisawa H, Kohri K, Hayashi Y. Intratesticular pressure after testicular torsion as a predictor of subsequent spermatogenesis: a rat model. *BJU Int.* 2012;109:466-70.