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Pathological evaluation of contralateral testis following various treatment methods for experimental testicular torsion in rats

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Summary: In the research, evaluation of the influences, of the unilateral testicular torsion and different treatment results that is applied to the this side, on contralateral testicle is aimed. For this aim, 40 adult (2.5-3 months old) Sprague Dawley rats were separated randomly (n: 5) into 8 groups. In control group (group 1), the sham operation has been performed on the testicles. In the group 2 and other groups, the left testicle has been made as torsion at the 720 degree counterclockwise and fixed to the scrotum. Then, the operational attempts like detorsion (group 3, after 24 hours) and orchiectomy (group 4, after 24 hours and group 5, after 48 hours) and in some groups, intraperitoneal cortisone in addition to the orchiectomy (group 6, after 24 hours and group 8, after 48 hours) and detorsion (group 7, after 24 hours) have been made to this left testicle. All contralateral testicles were taken by the orchiectomy 4 weeks after the torsion and evaluated histopathologically. There was degeneration at changing intensity in the all contralateral testicles except the control group. The difference between the diameters of seminiferous tubules has been accepted as important except the group 6 and group 7. In these two groups, the contralateral hyperemia, edema and degeneration also were evidently less. At the accompaniment of these findings, when a pubertial children applied to the clinic if maximum 24 hours passed after the unilateral testicular torsion, it has been concluded that the adding of short term immunotherapy to protect the contralateral testicle from the antigenic stimulus is useful, even which treatments that detorsion or orchiectomy were applied.

Key words: Contralateral testicle, histopathology, rat, testicular torsion

Sıçanlarda deneysel testis torsiyonunun çeşitli sağaltım metodları ile tedavisi sonrası kontralateral testisin patolojik değerlendirilmesi

Özet: Çalışmada, unilateral testis torsiyonunun ve bu tarafta uygulanan değişik sağaltım sonuçlarının karşı testise olan etkilerinin değerlendirilmesi amaçlandı. Bu amaçla erişkin (2.5-3 aylık) 40 adet Sprague Dawley sıçan rastlantısal olarak 5'erli 8 gruba ayrıldı. Kontrol grubunda (grup 1) testislere yalancı operasyon (sham) uygulandı. Grup 2 ve diğer gruplarda sol testisler 720 derece saat yelkovanı tersine torsiyone edilip skrotuma tespit edildi. Daha sonra bu testise detorsiyon (grup 3, 24 saat sonra), orşiektomi (grup 4, 24 saat sonra ve grup 5, 48 saat sonra) ve bazı gruplarda orşiektomiye ek olarak intraperitoneal kortizon uygulamaları (grup 6, 24 saat sonra) ve detorsiyon gibi operasyonlar yapıldı. Tüm kontralateral testisler torsiyondan 4 hafta sonra orşiektomi ile alınarak histopatolojik olarak değerlendirildi. Kontrol grubu dışındaki tüm gruplarda kontralateral testislerde değişen şiddette dejenerasyon mevcuttu. Seminifer tubulus çapları arasındaki fark, grup 6 ve grup 7 dışında, önemli bulundu. Bu iki grupta ayrıca, kontralateral hiperemi, ödem ve dejenerasyon da belirgin olarak azdı. Bu bulguların eşliğinde unilateral testis torsiyonu oluşmuş ve üzerinden en fazla 24 saat geçmiş pubertal çocuklar kliniğe başvurduğunda detorsiyon ya da orşiektomiden birisi ile birlikte kontralateral testisi antijenik uyarıdan korumak için kısa süreli immunoterapinin tedaviye eklenmesinin yararlı olacağı sonucuna varıldı.

Anahtar kelimeler: Histopatoloji, kontralateral testis, sıçan, testis torsiyonu

Introduction

The testicular torsion is an acute disease that appears especially in the male children in the adolescent age and it appears in 1/4000 between the males, under 25 years old (15). Although the identifying of the testicular torsion is very easy, the period that is passing between the beginning and the treatment of the disease is very important (7,12). The affection of the unilateral testicular torsion on the contralateral testicle and fertility have been searched by several scientists (1,12,15) but there is no any agreement on the level of these affections and treatment period and type.

There are a controversy between the authors; who believes an affection to the contralateral testis; about the affection mechanisms of contralateral testis after torsion. When some authors suggest that this affection is due to decrease of blood flow as a result of sympathic activation (18), most of the others explain this pathology with immunological mechanisms (9,14).

In this study, examination of the affects, of the unilateral testicular torsion and different treatment results that is applied to this side, on the contralateral testicle is aimed.

Material and Methods

For this aim, 40 adult (2.5-3 months old) Sprague Dawley rats were used. The application was performed on the rats by the permission, no./dated 17/28.11.2000 that was issued by the Ankara University, Faculty of Veterinary Medicine Ethic Commission.

The groups were formed as follows; as the animals were separated randomly into 8 groups, each group has 5 animals.

Group 1 (control): The left testicles of this group's rats were applied the sham operation.

Group 2 (torsion): After the left testicular liberation at the 720 degree counterclockwise and was fixed to the scrotum subcutaneously by nonabsorbable sutures. Then, the scrotum was closed by the absorbable suture materials.

Group 3 (torsion/detorsion): The left testicle was torsioned with the same operational principles of group 2 and the sutures were opened by entering from the same incision after 24 hours and made detorsion and the scrotum was closed by the absorbable suture material.

Group 4 (torsion/orchiectomy after 24 hours): The left testicle was torsioned with the same operational principles of group 2 and the orchiectomy was done by entering from the same incision after 24 hours and the scrotum was closed.

Group 5 (torsion/orchiectomy after 48 hours): The left testicle was torsioned with the same operational principles of group 2 and the orchiectomy was done by entering from the same incision after 48 hours and the scrotum was closed.

Group 6 (torsion/orchiectomy after 24 hours/ cortisone): 2mg/kg/day methyl prednisolone has been given intraperitoneally during 4 weeks to the rats after the same processes in group 4 were applied.

Group 7 (torsion/detorsion/cortisone): 2mg/kg/day methyl prednisolone has been given intraperitoneally during 4 weeks to the rats after the same processes in group 3 were applied.

Group 8 (torsion/orchiectomy after 48 hours/ cortisone): 2mg/kg/day methyl prednisolone has been given intraperitoneally during 4 weeks to the rats after the same processes in group 5 were applied.

The operational processes were done by using pentobarbital sodium anesthesia. The pentobarbital sodium was given by 26 G insulin injector at the 30 mg/kg dosage as intraperitoneally. The drug was effective between 5-15 minutes and its effect was disappeared after 45-90 minutes. After the local cleaning by povidone iodine under the anesthesia, the left testicles of the rats were explored by the incision. In the control group, after scrotal incision and liberation of the testis, scrotum was closed by the absorbable sutures (sham). All rats were put into the separate cages in the first 24 hours after the operational process and then they were united with their groups.

The animals were kept in the cages that was clear, in 27x17x42 cm dimensions, rectangular in shape and its over was suitable for placing the special water cup and feedbox and with wire cover in the special lighting conditions and room temperature and they were fed by the special rat feed. At the end of the 4 weeks, the orchiectomy was applied on the right testicles of all rats and the euthanasia has been applied by over dose anesthesia.

The right testicles that were taken by orchiectomy were fixed in Bouin's solution. The fixed tissue samples were blocked in paraffin vax by applying the routine methods. The sections in 4-6 µm thickness that were taken from these blocks, were stained with hematoxylin-eosin (HE) and evaluated in light microscope. Each rat's body weight and the group averages of the data that belongs to the testicle weight were calculated. Ten seminiferous tubules diameters in each animal were measured by helping of ocular micrometer in the light microscope and their averages were taken. These averages were accepted as data of each rat. Thus, the average body weight, testicle weight and data of the seminiferous tubules diameters of 5 rats in each group have been formed. Kruskall-Wallis variance analysis for the evaluation of data, and Whitney-U test for the differention among the group or groups were used.

Results

Histopathological findings

In contralateral testicles, the histopathological changes in every case were presented in Table 1, and the average testicle weight, body weight and seminiferous tubules diameters were presented in Table 2. The histological changes in all groups were evaluated together. In all groups except the controls, it was fixed that seminiferous tubules diameters were getting diminishing and they were showing a distribution randomly as various dimensions.

In all testicles that belong to the group 1, it was observed that the seminiferous tubules have a regular structure and normal spermatogenesis (Figure 1).

In group 2, in all testicles except one case, the hyperemia in vessels and interstitiel edema in a light intensity were observed. There is a vacuolar and hydropic degeneration in changing intensity in germinal epithelium that covers the seminiferous tubules. In some tubules, the

Table 1. The evaluation of histological findings. + : Light, ++: Medium, +++: Intensive.

Group	Animal no.	Hyperemia	Edema	Degeneration	Spermatogenesis
1	1	-	-	-	++
	2	-	-	-	+++
	3	-	-	-	+++
	4	-	-	-	++
	5	-	-	-	++
2	1	+	+	++	-
	2	-	-	++	-
	3	+	+	++	-
	4	+	+	++	+
	5	+	+	+++	+
3	1	++	++	+	+
	2	+	+	+	+
	3	+	+	+	+
	4	+	+	++	+
	5	+	+	+	+
4	1	++	+	+	+
	2	++	++	+	-
	3	++	+++	++	-
	4	++	+	+	-
	5	+	+	+++	-
5	1	+	+	+	+
	2	++	++	+++	
	3	+	++	+	
	4	+	+	+	+
	5	+	+	++	-
6	1	+	+	+	+
	2	+	+	+	+
	3	-	-	+	+
	4	-	-	+	-
	5	-	-	+	-
7	1	-	-	+	+
	2	+	+	+	+
	3	-	-	+	+
	4	+	+	++	-
	5	+	+	+	++
8	1	-	-	+	++
	2	++	+++	+++	
	3	+	+	+++	-
	4	+	+	+	+
	5	-	-	+	++

Table 2. The average seminiferous tubules diameters, average body weight and average testicle weight in the groups.

Group code	Average seminiferous tubules diameter (micron)	Average body weight (gram)	Average testicle weight (gram)
1	197,0 ± 6,670832	229,14 ± 18,9196	2,588 ± 0,115
2	*178,8 ± 10,76754	220,00 ± 16,7827	2,490 ± 0,117
3	*177,4 ± 6,516134	255,60 ± 08,7784	2,882 ± 0,219
4	*160,8 ± 7,638063	275,60 ± 16,0144	2,998 ± 0,111
5	*160,2 ± 2,817801	263,80 ± 08,5288	3,122 ± 0,103
6	+ 192,2 ± 4,973932	237,40 ± 07,8269	2,624 ± 0,084
7	+ 193,4 ± 9,102747	268,20 ± 18,9196	2,898 ± 0,235
8	*173,8 ± 9,340236	268,80 ± 14,5410	2,420 ± 0,216

*p<0.05 important (while compared with control group).
+p>0.05 not important (while compared with control group).

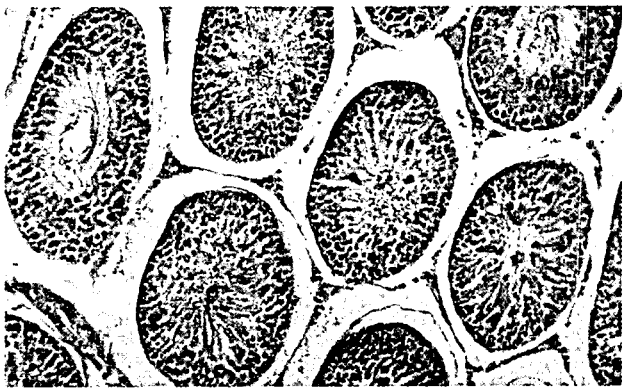

Figure 1. Normal testicular tissue and spermatogenesis. Group 1. HE x 90.

Figure 3. Normal spermatogenesis together with degeneration in light intensity. Group 3. HE x 380.

Figure 2. The degeneration in the germinal epithelium and interstitial edema. Group 2. HE x 90.

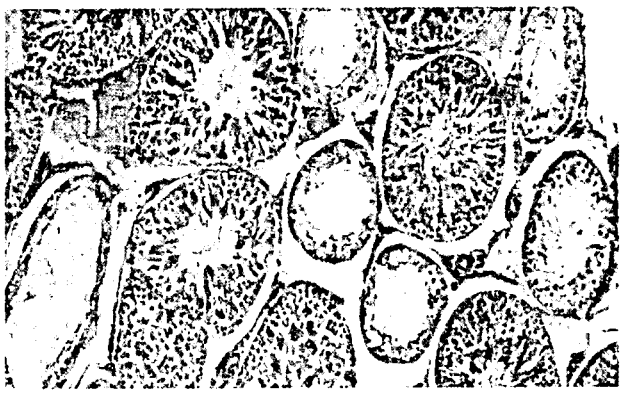

Figure 4. The untidy appearance in the seminiferous tubules diameters, the germinal epithelium that covers some tubules are completely removed. Group 5. HE x 90.

germinal epithelium was completely removed and basement membranes were in a waved appearance (Figure 2). In only one case, the spermatogenetic activity was observed in a few tubules.

In group 3, there was a vacuolar and hydropic degeneration in changing intensity in germinal epithelium that covers the seminiferous tubules and interstitial edema in a light intensity and the vessels were hyperemic in the testicles. In all case, the spermatogenesis was observed (Figure 3).

The vessels of testicles belong to the group 4, were hyperemic in an advanced level and the interstitial tissue was edematous in intensive in one case, and light intensive in other cases. The basement membranes were in waved appearance. The germinal epithelium was removed completely in some seminiferous tubules. In addition to some degenerative changes, together with spermatogenetic activitiy were found in a few tubules in one case.

The vessels were hyperemic and interstitial tissue was edematous in appearance in group 5. The degenerative changes, changing intensity, in the germinal epithelium was observed. In some parts, the tubules diameters were quite diminished and the basement membranes were in waved appearance (Figure 4). Both in two cases, there was spermatogenesis in less proportion.

The vessels were hyperemic in a light intensity and interstitial tissue was edematous in two cases in group 6. In all seminiferous tubules, there were degenerative changes in a light intensity. There was spermatogenesis in a less proportion in all cases except two cases.

The vessels were hyperemic in a light intensity and interstitial tissue was edematous and the germinal epithelium had degenerative changes in changing intensity in three cases in group 7. A few multinuclear giant cells in the degenerated epithelia were noticed in one case. Changing proportions spermatogenesis was observed in all cases except one.

The vessels were hyperemic in changing intensity and interstitial tissue was edematous in three cases in group 8. The degenerative changing as in changes intensity in were observed all cases but in two cases these were intensive (Figure 5). The germinal epithelium was completely removed in some tubules, and there were large amount of multinuclear giant cells in intensive cases

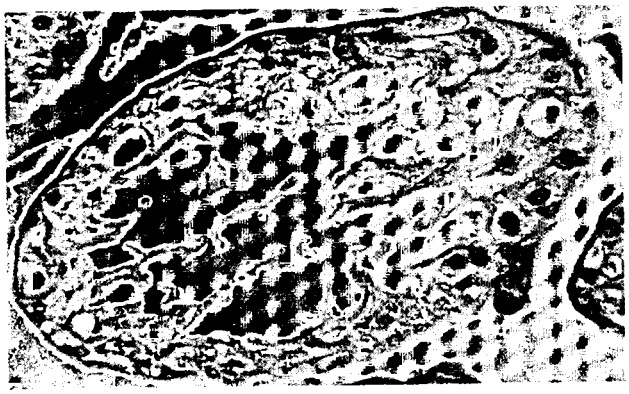

Figure 5. The intensive degeneration in the germinal epithelium, close appearance. Group 8. HE x 380.

Figure 6. The degeneration in the germinal epithelium, multinuclear giant cells and intersititial edema. Group 8. HE x 90.

(Figure 6). The spermatogenesis was in changing proportion except these two intensive cases.

Statistical findings

The difference between the average body weight and testicle weights in the groups were not important in the statistical evaluation using with the Kruskall-Wallis variance analysis. However, the difference between seminiferous tubules diameters were important.

Whitney-U test was made for the determination of the differences between groups. The diminishing in the seminiferous tubules diameters were found statistically important according to control group except the 6th and 7th groups.

Discussion and Conclusion

In the unilateral testicle torsion, it was showed statistically and histopathologically that the contralateral testicle was effected in changing intensity by the applied treatment methods. The testicle torsion is an acute disease especially seen in the children who are in the puberty. It is accepted that the unilateral torsion affects the contralateral testicle and fertility (3,10), but there is no any agreement on the subject, when this affection starts the forming after the torsion and how it is and which treatment can be used.

It is recorded that the 4-6 hours torsion period is enough to affect the contralateral testicle (2). In addition, the torsion degree also is important. In 360 degree torsion, there are medium level pathological changes in torsioned testicle and the full infarct appears after 4 hours torsion in 720 degree (19).

Nagler and White (14) declared that contralateral testicular atrophy formed by the torsion is prevented by orchiectomy, and it is unsuccessful with the detorsion. In the testicles were applied detorsion, the immunologic reaction starts by the blood-testicle barrier makes the sperms known were perished as a result of ischaemia and it causes a deformation in contralateral testicle and contrary to this, it is declared that there is less changing on the contralateral testicle, because the source is removed in them that the orchiectomy has been applied, so there will not be any immune response (6). It is reported that when the immunosuppressive treatment is given as anti-lymphocyte globulin (ALP), it decreases the contralateral testicular damage but can not remove completely, whereas ALP + splenectomy prevents completely (14). In addition, it is suggested that following detorsion, an adjuvant immunotherapy (hydro-cortisone) in 5 days prevents the antigenic stimulation (11).

Wallace et al. (20) pointed out that there is an impairment in blood-testicle barrier of contralateral testicle after 7 days of unilateral testicle torsion and while IgM is determined, the morphological changes at this level is in a small level and in the 28th day, IgG took place instead of IgM and the morphologic impairment became clear. Koşar et al. (9) in a similar study has determined that, the anti-rat IgG antibodies are contrary to spermatozoa antigen in contralateral testicles tissue 1 month after of detorsion.

In addition, Nagler et al. (13) has showed that the unilateral testicular torsion in immature rats did not make any testicular damage and did not effect the contralateral testicle when they are reached to the puberty. The following is its comment: Pre-pubertial testicular torsion does not show the same affect as of postpubertial torsion, so why immunologic stimulate that depends on possibly sperm antigens and makes changes in the testicle does not exist before puberty. Cosentino et al. (5) has also worked with prepubertial rats and found that while they reached to the puberty, there is a decreased contralateral spermatogenesis as secondary to the torsion.

In the literature, there are also some publications that point out the opposite results. In the similar experi-

mental researches, it is not shown the existence of contralateral testicle damage followed by unilateral testicular torsion (8,17) and it could not found a decrease in semen parameters (16). In some publications, the immunological damage in the contralateral testicle after the unilateral torsion, are also not supported (4,8,17).

Our results were indicated that while the contralateral testicle was compared with the control group, the unilateral testicle torsion affected the average seminiferous tubules diameters by decreasing meaningfully. The detorsion after 24 hours, orchiectomy after 24 hours, orchiectomy after 48 hours and orchiectomy+cortisone after 48 hours treatments of late period treatments that were applied, aiming to be able to decrease this affect were not affective and also in these groups, the contralateral average seminiferous tubules diameters decreased statistically. But, in the groups (6 and 7) that detorsion or orchiectomy applied after 24 hours and adding cortisone treatment during 1 month, the average seminiferous tubules diameters were close to the control group. We suppose that cortisone decreases the immunologic affect in the treatment. In addition, contralateral testicular hyperemia and edema were clearly less in these groups while it was compared with other groups and the degeneration also was less. The spermatogenesis was observed in all groups but decreased clearly when it was compared with the control group.

As a result, when a pubertial children applied to the clinic with a unilateral testicle torsion which was formed maximum 24 hours ago, we proposed that a short term immunotherapy will be useful by adding to the treatment even which treatments were applied as detorsion+fixation or orchiectomy to protect the contralateral testicle from the antigenic stimulus.

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