

Histological Evidence and Proliferative Potential of the Contralateral Testis and Epididymis in Rats with Unilateral Testicular Torsion

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Abstract

We investigated histological changes in the contralateral testis and epididymis and proliferative potential of constituent cells of the contralateral testis and epididymis by silver staining of argyrophilic nuclear organizer regions (Ag-NORs) after unilateral testicular torsion (UTT). Fifty adult male rats were divided into 5 groups each containing 10 rats. Torsion operations were performed by rotating the left testes 720° in a clockwise direction. Torsion duration was 1, 4, 12 and 24 hrs that was lied in group 1 to group 4, respectively. Then, de-torsion operations were performed and 24 hrs. Later, histopathological changes in the contralateral testes and epididymis were investigated and compared to group 5(control). Control group underwent a sham operation of the left testis. In histologic examination, Johnson's scores of right testes in group 3 and group 4 were different from other groups ($P < 0.001$). In all groups, histological changes in the contralateral testes and epididymis were observed. Also, there was no significant difference in subjective Ag-NOR pattern assessment for constituent cells in the right testes and epididymis between different groups ($P > 0.05$). Our findings demonstrate that UTT results in contralateral testicular and epididymis damage but no the quantitative changes in the rapidity of cell proliferation in cyto-histological preparations.

or approximately 1 in 4,000 per annum [3]. The two most important factors determining testicular damage are the duration and degree of TT and delayed presentation or treatment may result in impaired fertility [4,5]. Controversy also remains on whether unilateral testicular torsion (UTT) followed by detorsion affects the contralateral testis since torsion is sometimes related to injury but it sometimes has no damaging effects [5-10]. Also, a few studies have investigated the effect of UTT on the contralateral epididymis [11]. We need to know the functional changes in testis and epididymis to determine the possible causes of infertility following TT.

Nucleolar organizer regions (NORs) are chromosomal segments, which contain ribosomal genes. NORs also contain a set of acidic, non-histone proteins that bind silver ions [12]. It has been demonstrated that the argyrophilic proteins (Ag-NORs) quantity represents a valuable parameter of cell kinetics, being significantly associated with the rapidity of cell duplication [13].

The present study was designed to evaluate the effect of UTT followed by detorsion on the contralateral testis and epididymis with cytohistopathologic examination.

Material and Methods

Ten-week-old adult male wistar albino rats (200-265 g) were housed in cages of 10 animals. Each animal was kept at 22°C with a 12L: 12D cycle. They had access to water ad libidum and were fed with standard rat chaw. Rats were divided into 5 groups each containing 10 rats. In the beginning, animals were anesthetized using intraperitoneal sodium pentobarbital injection (50 mg/kg) and the scrotum was entered through a midline incision. The tunica vaginalis was opened, and the left testis was delivered to the surgical field. Then, the left testis was rotated 720° in a clockwise direction and maintained in torsion position by fixing the testis to the scrotum. In groups 1 to 4,

Keywords: Torsion; Ag-NOR; Histology; Testis; Epididymis

Introduction

Testicular torsion (TT) is a commonly occurring urological emergency seen most frequently in adolescents and requires immediate surgical intervention [1,2]. Its incidence has been estimated to be as high as 1 in 158 males by the age of 24 years

torsion duration was 1,4,12 and 24 hrs. respectively. After each surgical intervention, the incisions were closed. Then, the spermatic cord was detorsed. After 24 hours of detorsion, re-explorations and contra-lateral epididymo-orchietomies were performed. These groups were designed to study the effects of testicular torsion on the contralateral testis and epididymis. Group 5(control) was designed for histopathologic examination compared to other groups. Animals in this group(Control) underwent a sham operation on the left testis without torsion, and right epididymo-orchietomies were also performed.

Histopathology

Hematoxylin and eosin staining

Testes and epididymides were fixed in Bouin's solution, specimens embedded in paraffin blocks and sections cut and stained using hematoxylin and eosin (H&E). Then spermatogenesis was measured on histologic sections using Johnsen's score. Johnsen's score applies a score of 1 to 10 for each seminiferous tubule cross-section [14]. Also, histologic changes in parenchyma of the contralateral testes and epididymides were examined. The degree of observed edema and congestion was expressed as mild, moderate and severe [15]. The light microscope histologic evaluation was done by an observer in a blind, randomly numbered fashion without any knowledge of which testis had or had not undergone torsion.

Silver staining of NOR's

A modified 1-step silver staining procedure was performed for staining of nucleolar organizer regions [16]. In this method of staining, Ag-NORs appear as brown or black dots within a yellowish background of nucleus. In this study, the counting method was used for quantitative analysis of Ag-NOR proteins [17]. Slides were randomized and analyzed by a single observer as a blind test. In all groups, spermatogonia, spermatocytes (primary or secondary), spermatids (early or late) and sertoli cells in the contralateral testis and epithelial cells in the contralateral epididymis were investigated for each tissue specimen. Then, subjective Ag-NOR pattern assessment (SAPA) was accounted according to the scoring system proposed by [18]. The value of SAPA scoring within each nucleus was recorded and scores were assigned by the estimated number of dots, the size and shape of dots and the clusters and their variation from cell to cell. By using this subjective scoring system, the scores range from 5 to 15.

Statistical analysis

Statistical analysis of the data among different groups was performed using the Kruskal-Wallis analysis of variance test, and results were expressed as mean \pm standard deviations. P Value<0.05 was considered as statistically significant.

Results

Gross examination

In all studied groups, after different periods of torsion no apparent visual changes in the contralateral testes and epididymides were observed as compared to control group.

Histopathological examination

According to histologic grading of spermatogenesis, there was significant histopathologic difference in contralateral testes of groups 3 and 4 as compared to control group (P<0.001)

Table 1: *Lower Johnsen's scores (P Value<0.001). Data are reported as mean \pm SD. In groups 1 to 4, torsion was carried out for 1, 4, 12 and 24 hrs respectively following by detorsion and closing scrotum. Then, 24 hrs later contralateral epididymo-orchietomies were performed. Group 5 was used as normal testes biopsies to compare the tissue specimens in group 1 to 4 (Table 1).

Groups	Johnson's scores
1	10.00 \pm 0.00
2	9.23 \pm 0.36
3	7.87 \pm 1.01*
4	7.87 \pm 0.79*
5	10.00 \pm 0.00

Also, in histologic examination of group 1, interstitial oedema and congestion of the contralateral testes were found and these changes increased in the contralateral testes of groups 2 and 3. Nevertheless in the contralateral testes of group 4 interstitial oedema and congestion were prominent. No evidence of other histological changes in parenchyma of the contralateral testes was detected in all studied groups.

Histological changes were observed in head, body and tail of the contralateral epididymis in different groups. Evidence of slight congestion in the contralateral epididymal head, body and tail of rats was detected only in group 1. In group 2, oedema of the interstice and congestion of the contralateral epididymis were found after 24 hours of de-torsion and these alterations were prominent in the contralateral epididymis of group 3. On the other hand in group 4, oedema of the interstice and congestion significantly decreased in the contralateral epididymis as compared to group 3.

However, in group 3, interstitial fibroblast proliferation and haemorrhage of the contralateral epididymis were detected. Interstitial fibroblast proliferation increased significantly in the contralateral epididymis of group 4 as compared to the group 3, after 24 hours of de-torsion. No haemorrhages were observed in the contralateral epididymis of group 4 after 24 hours of de-torsion. In all groups, no evidence of tubular necrosis was detected in the head, body and tail of the contralateral epididymis. However in group 3, two of the contralateral

epididymis showed nonspecific changes after 24 hours of de-torsion.

Table 2: Subjective Ag-NOR Pattern Assessment scores in different cells of the contralateral testis and epididymis.* *Note: P Value>0.05 in different cells of the contralateral testes and epididymides in group 1 to group 4 as compared to group 5(control). Data are reported as mean \pm SD. Study groups were explained in Table 1.

Cells					Groups
Epithelial cells of the epididymis	Serol cell	Spermatid (early or late)	Spermatocyte (primary or secondary)	Spermatogonia	
10.47 \pm 0.72	9.23 \pm 0.14	9.26 \pm 0.17	11.67 \pm 0.73	12.16 \pm 0.54	1
10.47 \pm 0.67	9.17 \pm 0.08	9.10 \pm 0.13	11.44 \pm 0.63	12.06 \pm 0.74	2
10.61 \pm 0.45	9.17 \pm 0.16	9.10 \pm 0.11	11.70 \pm 0.65	12.13 \pm 0.68	3
10.52 \pm 0.64	9.12 \pm 0.12	9.14 \pm 0.15	11.77 \pm 0.66	12.16 \pm 0.60	4
10.44 \pm 0.74	9.24 \pm 0.14	9.20 \pm 0.18	11.73 \pm 0.60	12.16 \pm 0.61	5

Also, there was no significant difference in the pattern of SAPA per nucleus for different cells of spermatogonia, spermatocytes, spermatids and serol cells in the right testes and epithelial cells in the contralateral epididymis between different groups ($P>0.05$) (Table 2).

Discussion

TT may affect the bilateral testes [5,19] and epididymis [11]. It seems that the main mechanism of testicular injury in the ischemic testis after TT includes damage caused by testicular ischemia and reperfusion [20]. It is also known that 720° but not 360° torsion induces ischemia sufficient to disrupt the seminiferous epithelium [10]. The mechanism of injury to the contralateral testis after UTT was not demonstrated, but it seems that histological changes in the contralateral testis at the time of torsion has been interpreted as a consequence of an adverse effect of the twisted testis [21].

In investigations evaluating contralateral testicular damage after UTT, evaluations were based on histopathological examination and spermatogenesis and for contralateral epididymis damage including histopathological examination. TT can result in irreversible loss of spermatogenesis in the ipsilateral testes despite the return of blood flow, but spermatogenesis in the contralateral testes may remain normal [9,10]. Johnson's score was used to classify the spermatogenesis in testis [14]. This method of histologic analysis gives an accurate measurement of the level of spermatogenesis and also the degree to which spermatozoa maturation is taking place within the seminiferous tubule [18]. In our study, results showed an noxious effect of 12 and 24 hours, 720° UTT at 24 hours after de-torsion on spermatogenesis in the contralateral testis and neither de-torsion of the twisted testes afforded any protection. Also, no significant difference was observed in the adverse effects of 12 and 24 hours, 720° UTT in 24 hours after de-torsion on the contralateral testis. However our finding indicated that following 1 and 4 hours of TT in 24 hours after de-torsion impairment of spermatogenesis in the contralateral testes was not detected, but histologic changes (enema of the interstice

and congestion) occurred in the contralateral testes. The present study indicated that UTT results in the contralateral testicular damage if the torsed testis remains in the scrotum for more than 12 hours after torsion. Ultimately, de-torsion and retention of the de-torsed testis in the scrotum may result in impaired subsequent fertility [22].

In epididymis, sperm undergoes maturation and storage prior to ejaculation. The biochemical changes occur in sperms during their transit to the proximal caudal epididymis that increase the velocities of progressively motile sperm and confer upon them the ability of fertilization. In the present experiment, we tried to infer gross and microscopic histological changes of the contralateral epididymis in response to UTT followed by de-torsion. In our study histologic changes in the head, body and tail of the contralateral epididymis were observed after 1 hour of TT in 24 hours after de-torsion. These changes were significantly increased up to 12 hours after torsion in 24 hours after de-torsion. Interstitial fibrosis was observed in the contralateral epididymis after 12 hours of TT. This change prominently increased after 24 hours of torsion in 24 hours after de-torsion. Interstitial fibrosis in the ipsilateral epididymis is the early emergence that caused by the effect of the vascular obstruction. But different damage mechanisms account for the histological changes of the contralateral epididymis. Obstruction of the epididymis tubules and functional changes of the epididymis caused by inflammatory fibrosis that can result in different squeals (example-infertility) [11].

Ag-NOR staining has been described as a cyto-chemical method in human pathology. It is a criterion to evaluate cell proliferation rates with a prognostic value. This method is based on the specific argyrophilic affinity of some nuclear proteins and is now widely used, especially for the analysis of the nuclear organizer regions (NORs) in cancer cells [23,24]. It has also been proposed that the amount of Ag-NOR proteins is correlated with the duration of the cell cycle [13]. Small amount of Ag-NOR proteins indicates a very low cell proliferation rate, and large amounts a high proliferation rate [25]. In the present study, we have tried to determine the usefulness of the SAPA scoring as well as the various histological parameters. In our investigation,

a total of five groups were studied for the value of Ag-NOR in different cells of the contralateral testis and epididymis. In this study by comparing a healthy contralateral testis and epididymis with a damaged contralateral testis and epididymis in various duration of TT, the SAPA score did not show significant differences. We demonstrated that the SAPA score could not be correlated with the grade of histological changes in the contralateral testis and epididymis and decreased spermatogenesis of the contralateral testis after short times of UTT (1 hour to 24 hours). There was no significant difference in the values of the SAPA score suggesting the same proliferative activity in constituent cells of the contralateral testes and epididymis of the group 1 to group 4 compared with the control group. Further studies for longer term of TT could be contemplated to determine the correlation existing between Ag-NORs and various parameters in TT more exactly. According to AgNOR assay we did not find changes in proliferation activity caused by torsion, however this may be due to low sensitivity of the test.

However, different mechanisms has been suggested to explain the pathway of the contralateral testicular damage after UTT including autoimmune response, decreased testicular blood flow caused by reflex sympathetic response and formation of free oxygen radicals after de-torsion [7,8]. Nevertheless, the most popular mechanism is based on autoimmune response [7]. Cellular immune mechanisms or antibodies that formed testicular antigens may still be involved in the contralateral testicular damage after torsion [1]. Also the main mechanism of epididymis damage following unilateral testicular torsion/de-torsion is unclear but, it might be similar to that of the testis. However, further studies are necessary to elucidate the precise mechanism of this injury. Although the AgNOR is widely used to show the proliferation, to assess proliferative activity more precisely, the more sensitive test such as Ki-67 can be used. The other question that can be answered in future studies is whether these damages can impair the spermatogenesis and sperm function.

Conclusions

The obtained data demonstrated that UTT results in contralateral testicular and epididymis damage. Also, these data indicated that the values of the SAPA score do not correlate with the degree of histological changes in cyto-histological preparations of the contralateral testes and epididymis after short term of UTT. It is necessary to identify the exact mechanism of injury to the contralateral testis and epididymis after UTT for prevention of contralateral testicular and epididymis damage and subsequent infertility. The most important factors influencing this damage and impaired fertility after TT lies with the effective education of parents about paying more attention to acute scrotal pain and swelling in their children and their prompt presentation to the physician or emergency unit. Nevertheless, possible differences between humans and rats in responding to ischemic condition should be noticed. The other point that should perceive is that the testicular damage in patients may occur in shorter or longer time after torsion.

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