BJR

Received: 30 June 2016 | Revised: 22 August 2016 | Accepted: 27 September 2016

https://doi.org/10.1259/bjr.20160585

Cite this article as:
Ufuk F, Herek D, Herek Ö, Akbulut M. Role of diffusion weighted magnetic resonance imaging in a rat model of testicular torsion. *Br J Radiol* 2016; **89**: 20160585.

FULL PAPER

Role of diffusion weighted magnetic resonance imaging in a rat model of testicular torsion

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Objective: The purpose of this study was to investigate the usefulness of diffusion-weighted imaging (DWI) in the detection of different degrees of testicular torsion (TT) at the 8th hour and testicular salvageability at the 24th hour of torsion.
Methods: 31 rats were randomly classified into 4 groups. In Group 1 (sham-control group), the left testicle was kept outside and replaced. Left testicles were kept outside and twisted 360° in Group 2, 720° in Group 3 and 1080° in Group 4. Later, DWI was performed at 8th and 24th hours. After DWI, bilateral radical orchiectomy and histopathological examination were performed. Apparent diffusion coefficient (ADC) maps were obtained with *b*-factors of 0 and 800 s mm^{-2}. Comparisons of ADC values and damage in testicles were performed with Kruskal–Wallis test.
Results: Sensitivity of DWI in the diagnosis of TT was 12.5% for 360° torsion, 100% for 720° torsion and 1080° torsion at the 8th hour of torsion. Mean ADC values of the left testicles increased significantly at the 24th hour of torsion in Groups 3 and 4. All testicles in Groups 3 and 4 were observed to be irrecoverable on histopathological examination.
Conclusion: Increased ADC values in the affected testicle may represent irreversible tissue damage. So, immediate surgery is not required at this stage, which may reduce morbidity and mortality caused by immediate surgery and anaesthesia.
Advances in knowledge: TT can be diagnosed easily by DWI without administrating any contrast material. DWI findings in the affected testicle may represent testicular salvageability.

INTRODUCTION

Testicular torsion (TT) is a urologic emergency that causes ischaemia in testicles owing to twisting of the spermatic cord and structures, requiring early diagnosis and treatment. Delay in the diagnosis of TT causes loss of gonad and infertility.[1] Colour Doppler ultrasound (CDUS) is known as the method which provides the best result in the diagnosis of scrotal disorders.[2,3] It is an easily accessible, cheap and non-invasive diagnostic tool, which is used as the first-line method for diagnosis in patients with scrotal pain. It has a high sensitivity and specificity for detecting TT.[4] However, Patriquin et al[5] showed that CDUS could not detect the testicle blood supply in the healthy children aged from 10 weeks to 13 years, owing to the small size of the testicles and insufficient blood flow. So, CDUS can give false-positive results in the diagnosis of TT. In addition, diagnosis by CDUS also depends on user experience and device quality, which are considered as disadvantages especially in the diagnosis of partial torsion and might lead to false-negative results.[6] Contrast-enhanced MRI, which is being used recently for the diagnosis of scrotal pathologies, is known to show high accuracy in the diagnosis of TT.[7,8] However, contrast-enhanced MRI has several disadvantages. In children, sedation may be required and examination time is significantly longer, which makes it inapplicable under emergency conditions. It is expensive and MRI contrast agents can cause nephrogenic systemic fibrosis, renal failure and have the risk of allergic reactions.[7] So, there is still a need for developing new methods which will help in the diagnosis of TT in infants and children.

Diffusion-weighted imaging (DWI) is an MRI sequence that does not require injection of the contrast material.[9] DWI has been shown to be successful in demonstrating ischaemia in rat testicles in the early period (first and second hour) of the testicular artery ligation.[10] However, most patients attend to the hospital in late period[11] and to the best of our knowledge, there is no systematic study

Table 1. Modified Cosentino classification of histopathological damage

Grade 1	Normal testicular germinal cells
Grade 2	Less regular, closely packed seminiferous tubules, interstitial oedema or mild haemorrhage
Grade 3	Irregular pyknotic nucleus, less distinct seminiferous tubule borders and widespread haemorrhage
Grade 4	Closely packed seminiferous tubules with coagulation necrosis of germ cells

examining the mid-period and late period effects of ischaemia in different degrees of TT using DWI.

Once the diagnosis of TT is made, if the testicle can be salvaged, immediate surgery should be performed. However, immediate surgery increases morbidity and mortality in patients who receive anaesthesia in the emergency conditions. When preparing for surgery, complete procedure sets for anaesthesia reduce morbidity and mortality.[12] If the testicle is determined to be irreversibly damaged, it does not require immediate surgery and anaesthesia; so, mortality and morbidity can be reduced. In addition, during surgery, the surgeon subjectively evaluates the testicle intraoperatively for reperfusion. Removal of a salvageable testicle leads to unnecessary organ loss and medicolegal problems. On the contrary, leaving a damaged testicle behind after detorsion may cause contralateral testicular damage and infertility owing to oxygen-free radicals and antisperm antibodies.[13,14] If the testicle is determined to be irrecoverable, orchiectomy indication is also present and prevents antisperm antibodies.

The aim of this study was to determine the role of DW MRI for imaging TT in young rat models and evaluate the changes in apparent diffusion coefficient (ADC) values according to the degree and duration of torsion. We also aimed to show the relationship between the ADC values and testicular damage by comparing the ADC values in the 24th hour of torsion with histopathological findings and evaluate the salvageability of affected testicles.

METHODS AND MATERIALS

Study population and surgical procedures

Our study was approved by the Institutional Animal Care and Use Committee. 31 male Wistar albino rats weighing 250–300 g were randomly selected and a total of 4 groups were formed; there were 7 rats in the first group and 8 rats in each of the other 3 groups. The animals were anaesthetized by intramuscular injection of 50-mg kg^{-1} ketamine (Ketalar; Pfizer Pharma GMBH, Germany) and 10-mg kg^{-1} xylazine hydrochloride (Alfazyne; Alfasan International, Woerden, Netherlands) before surgical procedures and MRI examinations. For all surgical procedures, under standard sterile conditions, a left vertical scrotal incision was performed to access the left testis. In Group 1 (sham-control group), the testicle was kept outside for a minute and replaced. Left testicles were twisted 360° in Group 2, 720° in Group 3 and 1080° in Group 4 in clockwise direction. No surgical intervention was performed to the right testicles. In Groups 2, 3 and 4, left testicles were fixed to the scrotum from their upper and lower poles with 4/0 silk sutures to prevent detorsion. The scrotum was closed with 3/0 catgut plain sutures in all rats. After the operations, the rats were stabilized on a carriage board at 8th and 24th hours and underwent MRI examination.

MRI protocol

All MRI examinations were performed with a 1.5-T MRI system (Ingenia; Philips Medical Systems, Best, Netherlands) with a 16-channel head coil and high-performance gradient (maximum gradient, 45 mT m^{-1}; maximum slew rate, 200 T m^{-1} s). Before DWI, the rats underwent MRI with an axial turbo field echo T_1 weighted sequence (repetition time/echo time, 15/5.2 msec; turbo factor, 42). T_1 weighted images were inspected initially to define section locations for the DW images of the testicles. Then, an axial DWI single-shot echo-planar imaging sequence with sensitivity encoding (repetition time/echo time 4000/90 msec; matrix size, 62×112; slice thickness, 3 mm; interslice gap, 10%; field of view, 49 cm^2; number of signals averaged, 6; acquisition time, 2 min and 50 s) was performed with b-values of 0 and 800 s mm^{-2}.

Image interpretation

We transferred the MRI data electronically to a workstation (Easyvision release 4.4; Philips Medical Systems, Best, Netherlands). One radiologist with 5 years' experience in MRI, who was blinded to surgical procedures, reviewed the images. ADC values of each testicle were measured three times by placing at least three ellipsoid regions of interest (ROIs) on the middle portions of testicles to avoid surgical fixation areas and artefacts in the ADC map images. Ellipsoid ROIs were placed in order to cover whole testicular parenchyma free of artefacts.

Table 2. Modified Cosentino score of the left testicles after orchiectomy

Group	Number of subjects	Modified Cosentino classification				Total
		1	2	3	4	
1	*n*	6 (85.7%)	1 (14.3%)	0 (0%)	0 (0%)	7 (100%)
2	*n*	4 (50%)	3 (37.5%)	1 (12.5%)	0 (0%)	8 (100%)
3	*n*	0 (0%)	0 (0%)	8 (100%)	0 (0%)	8 (100%)
4	*n*	0 (0%)	0 (0%)	3 (37.5%)	6 (62.5%)	8 (100%)
Total	*n*	10 (32.2%)	4 (13%)	12 (38.7%)	5 (16.1%)	31 (100%)

Table 3. Modified Cosentino score of the right testicles after orchiectomy

Group	Number of subjects	Modified Cosentino classification				Total
		1	2	3	4	
1	*n*	7 (100%)	0 (0%)	0 (0%)	0 (0%)	7 (100%)
2	*n*	7 (87.5%)	1 (12.5%)	0 (0%)	0 (0%)	8 (100%)
3	*n*	5 (62.5%)	3 (37.5%)	0 (0%)	0 (0%)	8 (100%)
4	*n*	6 (75%)	2 (25%)	0 (0%)	0 (0%)	8 (100%)
Total	*n*	25 (80.6%)	6 (19.4%)	0 (0%)	0 (0%)	31 (100%)

Histopathological evaluation

After MRI, at the 24th hour of torsion, all rats underwent bilateral radical orchiectomy and testicular tissue samples were sectioned, processed and embedded in paraffin and stained with haematoxylin and eosin. Sections were evaluated under light microscopy by a pathologist with 10 years' experience, who was blinded to surgical procedures. The damage in testicles was evaluated, modifying the classification reported by Cosentino et al.[15] The classification was modified for the presence of haemorrhage and oedema (Table 1). Overall testicular damage was graded as: Grade 1, normal; Grade 2, mild injury; Grade 3, moderate injury; and Grade 4, severe injury. Testicles having Grade 3 and 4 damage were accepted to be irrecoverable. Grading was performed according to the dominant group of cells in sections. For example, when predominantly coagulation necrosis and packed seminiferous tubules were observed, it was regarded as Grade 4.

Statistical analysis

Statistical analyses were performed using SPSS® v. 21 (IBM Corp., New York, NY; formerly SPSS Inc., Chicago, IL). Quantitative variables were expressed as minimum, maximum, mean ± standard deviation and median. Intergroup and intersite comparisons were performed using Kruskal–Wallis test. A p-value <0.05 was considered statistically significant.

RESULTS

During surgery, no TT was observed in the first group. TT was confirmed in second, third and fourth groups. When left testicles were evaluated according to the modified Cosentino classification after orchiectomy, Grade 1 damage in 4 (50%) testicles and Grade 2 damage in 3 (37.5%) testicles were present in the second (360° torsion) group and Grade 3 damage accompanied by interstitial haemorrhage was detected in 1 (12.5%) testicle. In all of the eight cases in Group 3 (720° torsion), Grade 3 accompanied by widespread haemorrhage was found (100%). In the fourth (1080° torsion) group, Grade 3 damage in 3 (37.5%) cases and Grade 4 damage in 5 (62.5%) cases were found (Table 2).

According to the modified Cosentino classification, right testicles had Grade 1 and 2 damage. But, Grade 3 and 4 damage was not observed (Table 3).

Histological evaluation at 24th hour revealed no significant difference in left testicles of Groups 1 and 2 ($p = 0.317$). All the left testicles in the first group (sham-control group) were observed to be recoverable. 7 (87.5%) of 8 left testicles in the second group (360° torsion group) were observed to be recoverable. There was significant damage in left testicles in the third (720° torsion group) ($p = 0.006$) and fourth (1080° torsion group) ($p = 0.0001$) groups compared with the sham-control group. All the left testicles in Groups 3 and 4 were observed to be irrecoverable.

When mean ADC measurements of the left testicles at 8th hour of the torsion were compared with the sham group, significantly

Table 4. Comparison of 8th and 24th hour mean apparent diffusion coefficient (ADC) values

Group	Side	8th hour ADC ± SD (range) ($\times 10^{-3}$ mm^2/sn)	24th hour ADC ± SD (range) ($\times 10^{-3}$ mm^2/sn)	p-value
1	Left	0.744 ± 35 (684–748)	0.752 ± 48 (715–848)	0.917
	Right	0.745 ± 28 (697–782)	0.728 ± 47 (677–816)	0.499
2	Left	0.738 ± 134 (432–850)	0.691 ± 147 (367–796)	0.012
	Right	0.788 ± 30 (745–827)	0.739 ± 56 (669–842)	0.161
3	Left	0.410 ± 33 (369–478)	0.625 ± 165 (377–886)	0.012
	Right	0.732 ± 47 (668–825)	0.753 ± 96 (637–883)	0.327
4	Left	0.421 ± 28 (353–446)	1.086 ± 105 (684–748)	0.012
	Right	0.720 ± 26 (672–752)	0.714 ± 30 (888–1249)	0.327

SD, standard deviation.

low ADC values were found in third ($p = 0.004$) and fourth ($p = 0.048$) groups. No significant difference was detected in the measurements of the second group when compared with the sham group ($p = 0.778$). In 24th hour, mean left testicle ADC values in Group 4 were found to be significantly higher than that in the sham group ($p = 0.025$). Mean ADC values of the left testicles decreased significantly at the 24th hour of torsion compared with the 8th hour of torsion in Group 2 ($p = 0.012$). Mean ADC values of the left testicles were significantly increased at the 24th hour of torsion compared with the 8th hour of torsion in Groups 3 and 4 ($p = 0.012$) (Table 4) (Figures 1–4).

There was no significant difference in mean ADC values of right and left testicles between Groups 1 and 2 at 8th and 24th hours. Mean ADC values of the left testicles in Group 3 significantly decreased at 8th and 24th hours of torsion when compared with right testicles (8th hour $p = 0.0001$; 24th hour $p = 0.027$). In Group 4, there was significantly decrease in mean ADC values of left testicles compared with the right testicles at 8th hour ($p = 0.0001$). However, at the 24th hour of torsion, significant increase was observed in mean ADC values of the left testicles when compared with the right testicles ($p = 0.0001$).

Average ROI value was 84 mm^2 (63–102 mm^2) and there were no significant differences in average ROI values between the groups.

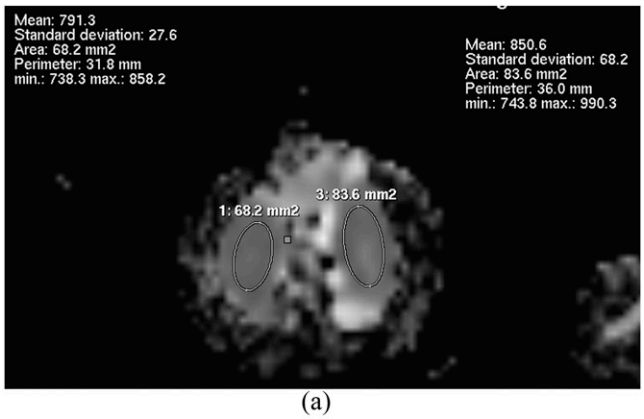
DISCUSSION

Our results showed that in 360° TT, the affected testicles were observed to be 87.5% recoverable and in 720° and 1080° TT, all affected testicles were observed to be irrecoverable at the 24th hour of the torsion. We observed that ADC values of all the twisted testes in 720° and 1080° torsion groups are significantly lower than control group and those of the non-affected testes at the 8th hour. However, the success of DWI for the diagnosis of TT seems to be low in 360° torsion (12.5%) at the 8th hour. So, we can say that DWI sensitivity in the diagnosis of TT is mainly based on twist degree.

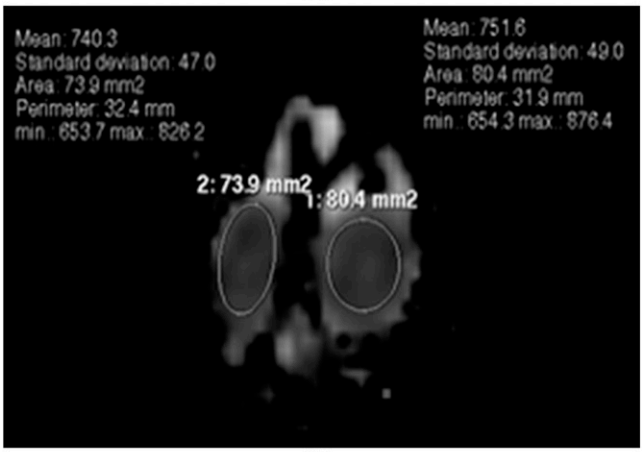
On histopathological examination of the left testicles, we observed Grade 2 testicular damage in one of the rats in the control group and Grade 3 testicular damage in one of the rats in the second group (360° torsion group), which we believe were due to possible injury that occurred during surgery. Table 3 shows histopathological changes in the contralateral (right) testes of the rats. We observed Grade 2 testicular damage in right testicles of 6 (19.4%) out of 31 rats, which we believe was due to the minor trauma during surgery or contralateral testicular damage by oxygen-free radicals and antisperm antibodies (Table 3).

According to our results, owing to increasing ischaemia in the second group (360° torsion group), a significant ($p = 0.012$) reduction of mean ADC values at the 24th hour compared with the 8th hour was detected. In the third and fourth groups, we observed significantly increased ADC values at the 24th hour compared with the 8th hour ($p = 0.012$). We suggest that in the early stages of ischaemia and cell damage, mean ADC values reduced owing to cytotoxic oedema; at the late stages of

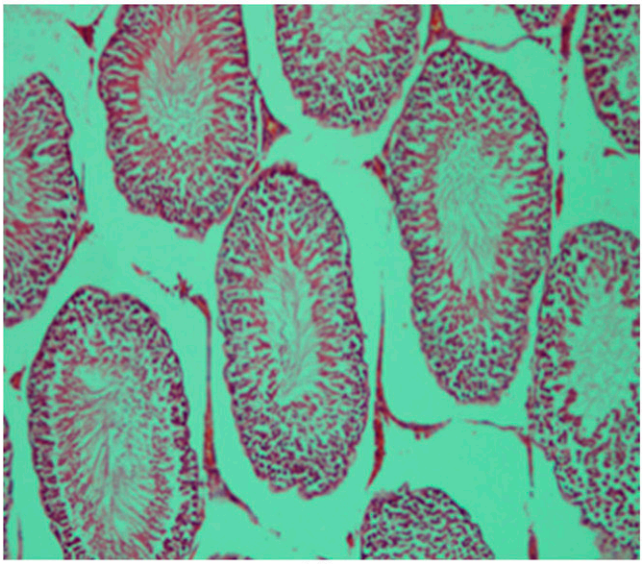
Figure 1. A rat without testicular torsion (sham-control group): (a, b) axial images are showing regions of interest drawn just inside the testicles on apparent diffusion coefficient (ADC) maps at the 8th and 24th hours, respectively; (a) left testicle mean ADC = $791.3 \times 10^{-6}\,mm^2\,s^{-1}$ and right testicle mean ADC = $850.6 \times 10^{-6}\,mm^2\,s^{-1}$, (b) left testicle mean ADC = $740.3 \times 10^{-6}\,mm^2\,s^{-1}$ and right testicle mean ADC = $751.6 \times 10^{-6}\,mm^2\,s^{-1}$. (c) A photomicrograph of the histologic specimen is showing normal testicular germinal cells and seminiferous tubules (haematoxylin and eosin stain, ×40).



(a)

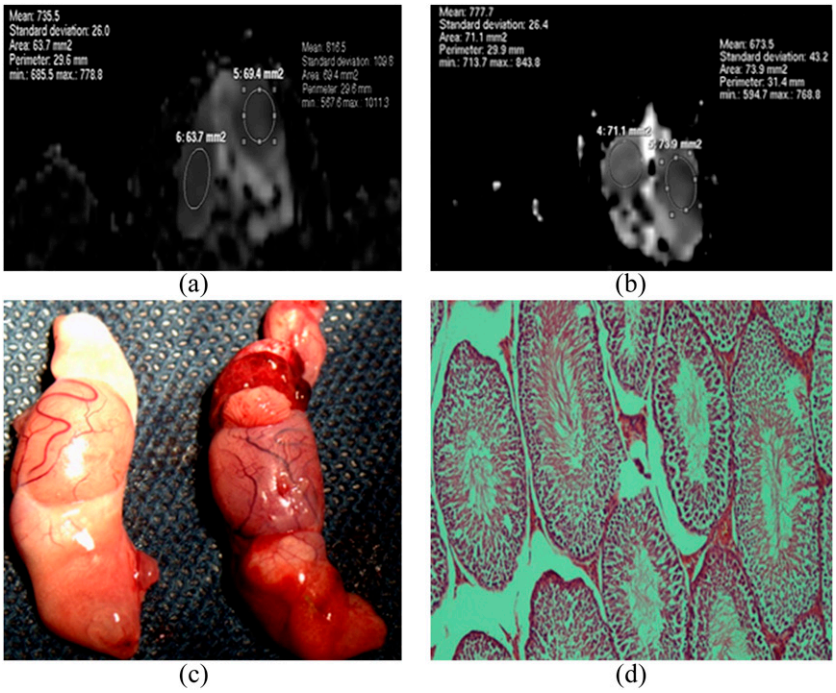


(b)



(c)

Figure 2. A rat with 360° testicular torsion (Group 2): (a, b) axial images are showing regions of interest drawn just inside the testicles on apparent diffusion coefficient (ADC) maps at the 8th and 24th hours, respectively; (a) left testicle mean ADC = $716.5 \times 10^{-6}\,mm^2\,s^{-1}$ and right testicle mean ADC = $735.5 \times 10^{-6}\,mm^2\,s^{-1}$, (b) left testicle mean ADC = $673.5 \times 10^{-6}\,mm^2\,s^{-1}$ and right testicle mean ADC = $777.7 \times 10^{-6}\,mm^2\,s^{-1}$. (c) A macroscopic image of the testicles is showing the reddened left testicle compared with the right. (d) A photomicrograph of the histologic specimen is showing closely packed seminiferous tubules and mild interstitial haemorrhage (haematoxylin and eosin stain, ×40).



ischaemia, ADC values increased owing to necrosis and vasogenic oedema. However, these ADC changes occur at different times in relation to the degree of TT.

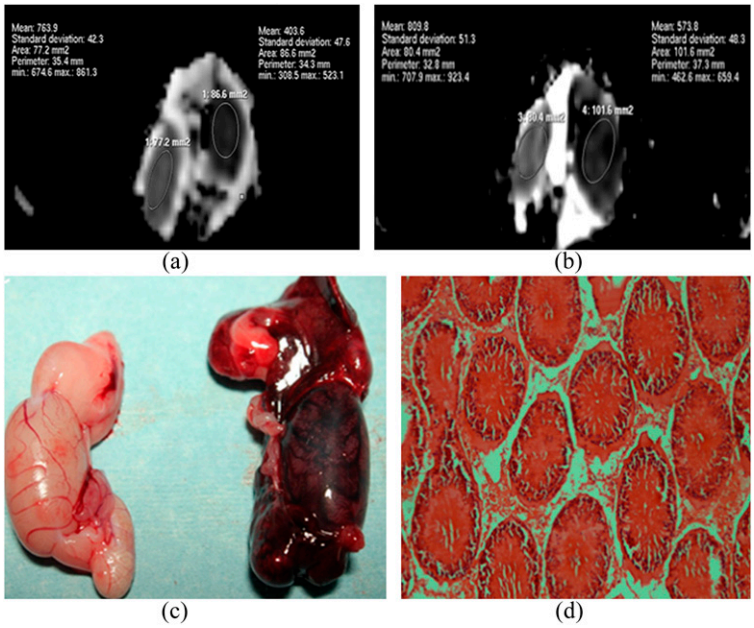
Previous studies showed that haemorrhage can result in reduced ADC values.[16–18] In this study, we observed significantly lower ADC values in left testicles of Group 3 than that of the control group at the 8th and 24th hours. On histopathological examination, widespread haemorrhage was observed in this group. We suggest that low ADC values were due to haemorrhage as well as cytotoxic oedema and haemorrhage may benefit the diagnosis of TT in the early stages owing to reduced ADC values. Our findings are consistent with previous studies.

Previous studies showed that salvageability in TT with widespread haemorrhage (Grade 3) is very low. Even testicular tissue is saved in the early period; testicular atrophy is seen on long-term follow-up in the testicle with Grade 3 damage. Another problem in testicles with Grade 3 damage, which underwent detorsion and orchiopexy, is that it may cause contralateral testicular damage and infertility in the late period owing to oxygen-free radicals and antisperm antibodies.[15,19] However, Lorenzini et al[20] stated that TT does not cause long-term effects on the spermatogenesis of the contralateral testis in pubertal rats. This situation is controversial. In TT with Grade 4 damage, coagulation necrosis is observed and the testis is irrecoverable at this stage,[21,22] as in our study. When we compare ADC values at the 24th hour, in Group 4, ADC values of the left testicles were significantly higher than the control group ($p = 0.025$), which supported irreversible cell damage owing to ischaemia. Based on these data, a reduction in the mean ADC value is seen in the early period of TT on DWI. Over time, the mean ADC value increases and a higher ADC value can be seen compared with the normal side. Our results showed that when we observe evidently higher ADC values in the affected testicle compared with the unaffected side, it supports irreversible damage and we can say that the testicle cannot be recovered. We suggest that when ADC values begin to increase in the testicle, it means critical time is reached for the salvageability of the testicle. To demonstrate the validity of this hypothesis, additional studies are needed with short intervals of ADC measurements performed for TT.

TT is seen bilaterally simultaneously in about 2% of the cases.[23] In cases with bilateral simultaneous torsion or in those that have one testicle, comparison of ADC values between testicles is not possible. So, knowing reference ADC values in the normal testicles is important. We were unable to identify a clear threshold value owing to the small number of subjects. Tsili et al[24] examined 147 healthy testicles with DWI and they stated that reference mean ADC values of testicles were between 1.08×10^{-3} and $1.31 \times 10^{-3}\,mm^2\,s^{-1}$. They used *b*-factors of 0 and $900\,s\,mm^{-2}$.

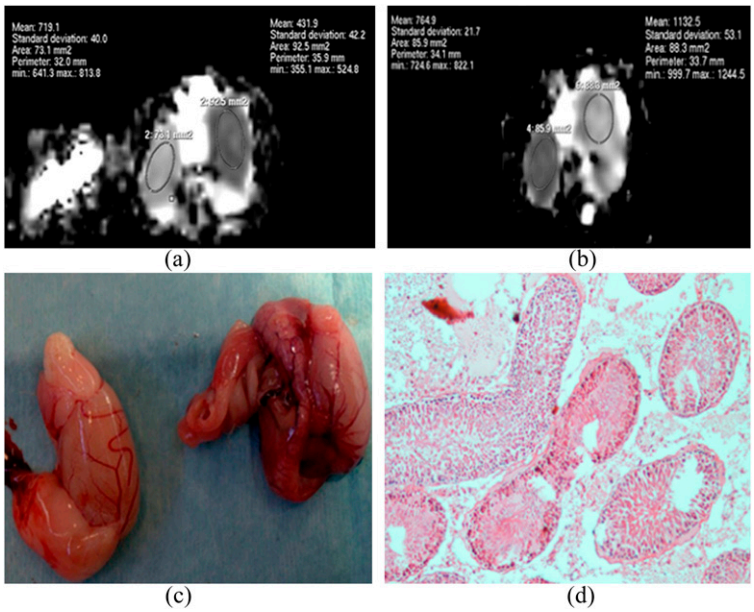
Maki et al[25] showed that DWI of the testicles with ADC measurement can allow for the detection of TT in patients without any use of contrast media. They used *b*-factors of 0 and $800\,s\,mm^{-2}$, as in our study.

Figure 3. A rat with 720° testicular torsion (Group 3): (a, b) axial images are showing regions of interest drawn just inside the testicles on apparent diffusion coefficient (ADC) maps at the 8th and 24th hours, respectively; (a) left testicle mean ADC = $433.6 \times 10^{-6}\,mm^2\,s^{-1}$ and right testicle mean ADC = $763.9 \times 10^{-6}\,mm^2\,s^{-1}$, (b) left testicle mean ADC = $573.8 \times 10^{-6}\,mm^2\,s^{-1}$ and right testicle mean ADC = $839.8 \times 10^{-6}\,mm^2\,s^{-1}$. (c) A macroscopic image of the testicles is showing that the left testicle has turned black in colour. (d) A photomicrograph of the histologic specimen is showing less distinct seminiferous tubule borders and widespread haemorrhage (haematoxylin and eosin stain, ×40).



In cerebral ischaemia, water diffusion is likely to increase after the permeability of the cell membrane completely degrades at late period (7–10 days).[26] Similarly, in our study, we observed increased mean ADC values in the 24th hour compared with the 8th hour, while we found irreversible damage and necrosis on histological examination. The increase in the mean ADC value

Figure 4. A rat with 1080° testicular torsion (Group 4): (a, b) axial images are showing regions of interest drawn just inside the testicles on apparent diffusion coefficient (ADC) maps at the 8th and 24th hours, respectively; (a) left testicle mean ADC = $431.9 \times 10^{-6}\,mm^2\,s^{-1}$ and right testicle mean ADC = $719.1 \times 10^{-6}\,mm^2\,s^{-1}$, (b) left testicle mean ADC = $1132.5 \times 10^{-6}\,mm^2\,s^{-1}$ and right testicle mean ADC = $764.9 \times 10^{-6}\,mm^2\,s^{-1}$. (c) A macroscopic image of the testicles is showing the shrunken and slightly reddened left testicle. (d) A photomicrograph of the histologic specimen is showing closely packed seminiferous tubules with coagulation necrosis of germ cells (haematoxylin and eosin stain, ×40).



in this group was due to irreversible disruption of cell membrane function and necrosis. Testicular cells divide faster than neurons; so, we believe that in testicular cells, disruption of cell membrane function and necrosis occurs faster than in neurons. Therefore, we suggest that the increase of the ADC is determined in testicular tissues in a shorter time.

Previous studies referred to the fact that size and placement of the ROI for ADC measurements cause differences in calculations of tumour ADC values and interobserver variability. An ROI that covers all tumour volume minimizes the interobserver variability and differences between ADC calculations and ensures that the measurements are repeatable.[27,28] Therefore, we used the largest ROI which was not affected by artefacts.

This study had several limitations. First, this is an experimental study, so the reflection of the results should be observed in clinical cases also. Second, owing to the small number of cases, non-parametric tests were used. However, this is an acceptable limitation in experimental studies. Third, pathological evaluation was achieved only at 24th hour. But, measurements should be taken in the same rats; so, it was not possible to evaluate the testes at the 8th hour of torsion. If histopathologic examination had been performed at the 8th hour, the relationship between ADC values and testicular damage would have been demonstrated more clearly. Fourth, we used only two b-values ($b = 0$, 800 s/mm^2). Therefore, we do not know the effect of different b-values on the course. Finally, the results of this experimental study should be established in clinical practice.

In conclusion, only by comparing mean ADC values between the affected and non-affected testicles at the 8th hour in 720° and 1080° of torsion, TT can be diagnosed easily without administrating any contrast material. Increased ADC values in the affected testicle may represent irreversible tissue damage. This finding implies that an urgent surgical intervention is not required, which may reduce morbidity and mortality caused by immediate surgery and anaesthesia. In addition, if irreversible testicular damage is diagnosed, it can guide orchiectomy and prevention of the unaffected side from oxygen-free radicals and antisperm antibodies can also be possible.

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