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**Research Article**

# The value of multimodal ultrasonography in the evaluation of late presenting testicular torsion in a rat experimental model

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## Abstract

**Objective:** To evaluate the value of multimodal ultrasonography (US) in a rat experimental torsion model after 6 h of torsion with different degrees.

**Methods:** Twenty-one male rats were divided into three groups. Left testes of the rats were twisted around their vascular pedicle 360 degrees in group 1, 720 degrees in group 2, and 1080 degrees in group 3 and intact right testes of the rats were accepted as control group. Grey-scale US, superb microvascular imaging (SMI), colour Doppler ultrasonography (CDUS), strain elastography (SE), and two-dimensional (2-D) shear wave elastography (SWE) examinations were applied 6 h after torsion procedure and testes were removed for pathological evaluation.

**Results:** Short-axis dimensions and volumes of the torsion side were higher than control testes. Lengths of the testes in the 3rd torsion group were smaller than the testes in groups 1 and 2 ($P < 0.002$). SMI was better than CDUS in recognizing blood flow in testicular tissue. Strain ratios were higher in group 1 and decreased with the increasing torsion degree. Emean and standard deviation (SD) measurements increased in the torsion side. Pathologically the mean testicular damage scores were statistically significant between torsion and control testes in all groups.

**Conclusion:** Our results showed that short-axis and volume measurements, SMI, 2D-SWE, and SE are effective in the evaluation and diagnosis of testicular torsion (TT).

**Advances in knowledge:** Evaluation of affected testis and intact testis with multiparametric US in late presenting TT cases is more reliable than being dependent on a single sonographic modality.

**Keywords:** testicular torsion; superb microvascular imaging; colour Doppler ultrasound; strain elastography; two-dimensional shear wave elastography; experimental study.

## Introduction

Acute scrotum is an emergency that requires rapid diagnosis and treatment in both childhood and adulthood. When seen in pre-adolescence it may result in infertility. Overall incidence in the first 25 years of life is reported as 1 per 4000 men. Testicular torsion (TT) is the most emergent condition that must be recognized as early as possible and needs to be treated surgically within 6 h for the salvage of the affected testes and prevention of infertility.[1,2] Ultrasonography (US) is the first-line imaging tool in the emergency evaluation of TT. Besides physical examination and clinical history of the patient, grey scale combined with colour or power Doppler US (CDUS and PDUS) imaging is used as a complementary diagnostic tool. However, CDUS sometimes erroneously causes unintended surgical management in some TT cases or vice versa it may lead to misdiagnosis and may cause missed surgery especially in late presented cases by coding false positive testicular blood flow due to scrotal oedema.[2,3] CDUS settings should optimally be adjusted for slow blood flow with parameters that are set to low wall filter, low pulse repetition frequency (PRF) and 70%-90% colour gain output. But still intratesticular blood flow is being reported incorrectly positive in small boys under 4 years.[4] Novel US techniques as microvascular imaging, strain, and shear wave elastography (SE and SWE) have improved recently for imaging low velocity blood flow and structural changes in tissue.[3,5]

Strain elastography and SWE techniques provide stress over anatomical structures either by manual compression (SE) or by automatically sending shear waves (SWE) to the tissue. Elastography can exhibit the differences in tissue stiffness. Different studies showed tissue stiffness changes in TT, some by using SE and others by using SWE.[1,2,6-9] Tissue stiffness changes according to TT degree and/or torsion time.

Superb microvascular imaging (SMI) is another Doppler ultrasound imaging technique that suppresses the background noise and enhances the visibility of very low-speed flow signals. Thus, detecting microvascular flows in a lesion or anatomical structure is aided. Monochrome (m-SMI) and color-coded SMI (c-SMI) modes are two different modes where background anatomical structures are removed during m-SMI and flow and grey-scale information are obtained during c-SMI. The use of SMI has been defined in many areas, acute scrotum being one of them.[10,11] It is reported that testis is salvageable in the first 6 h of torsion, whereas it

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is not after 24 h of torsion.[1,6,8] So, detecting blood flow in the testis between 6 and 24 h of torsion may change the course of treatment.

According to our review of the literature, the role of novel sonographic methods as SMI, SE, SWE, and traditional methods as grey scale US and CDUS on TT with different torsion degrees has not yet been investigated after 6 h of torsion. In this experimental study, we aimed to demonstrate the usefulness of different ultrasound methods in the evaluation of late presenting TT.

# Methods

## Ethical issues

This study was designed and performed in accordance with the recommendations in the guidelines established by the Guide for the Care and Use of Laboratory Animals from the US National Institutes of Health.[12] Institutional Ethics Committee on Animal Research approved the study.

## Animal model and study design

### Animals

Twenty-one male rats 3-4 months old and each weighing 300-400 g were used for the experiment. All rats were kept in wire mesh cages in the laboratory in a cross-ventilated room (temperature of 22 °C ± 2.0 °C, with a 12-h light/dark cycle). They had free access to rat chow and tap water. Experiments were done in the Animal Research Laboratory and multimodal US evaluations were done in the US section of the radiology department.

### Experimental design

Rats were divided into three groups randomly with being seven rats in each group. Groups were named as group 1, 2, and 3. The left testes of the rats were twisted around their vascular pedicle 360 degrees in group 1 (n: 7), 720 degrees in group 2 (n: 7) and 1080 degrees in group 3 (n: 7). The intact right testes of the rats in each torsion group were accepted as their control testes.

All the experimental surgeries and US evaluations were done under ketamine/xylazine anaesthesia in sterile conditions. The anaesthetic drugs [50 mg/kg ketamin (Ketalar; Pfizer Pharma GmbH, Berlin, Germany) and 10 mg/kg xylazine HCl (Alfazyne; Alfasan International, Woerden, Netherlands)] were administered intramuscularly to the rats in their hind legs. Half of the doses were repeated when needed. After anaesthesia, a 3-cm vertical incision was made into the left scrotal skin. The left testes were taken out and twisted in clockwise direction around their vascular bundle. Testes were twisted once to get 360 degrees torsion in group 1, twice to reach 720 degrees torsion in group 2 and 3 times to get 1080 degrees torsion in group 3. All testes were then placed into the scrotum and fixed from their upper and lower poles to the scrotal wall with 5/0 prolene sutures to prevent detorsion. Scrotal skin incisions were then closed with 5/0 prolene sutures.

### US evaluations

After 6 h of waiting periods, rats were brought to the US section of the radiology department in cages. Anaesthesia was applied before the sonographic examinations. The rats were placed on a wooden tray fixed by their four legs. Ultrasound examinations were done after 6 h of torsion. The evaluation of each torsion group was carried out on different days.

All the sonographic examinations were done by two radiologists (20 and 17 years of experience in ultrasound imaging and radiology) one after other separately. Each radiologist recorded their own findings to the device's hard disc by giving labels to each rat and the multimodal sonography findings were reviewed later.

### Sonographic examination of the testes

All the sonographic evaluations were performed with Aplio i800 (Canon Medical Systems, Tokyo, Japan) ultrasound scanner using a 5-18MHz linear transducer. The transducer was positioned onto the testes so that both right and left testes were seen at the same time in the scanning area. The radiologists evaluated the grey scale, colour Doppler, and SMI findings for each testis. Then SE and two dimensional (2D) SWE (2D-SWE) examinations were done. All calculations for SE and 2D-SWE were repeated three times for each testis of the rats separately. The average of the three measurements were accepted as the final value.

Echotexture properties (echogenicity and homogeneity) of the testes were recorded visually by grey-scale US and size (three dimensions as depth, width, and length) and volumes of the testes (derived automatically by the device software) were calculated. Then Doppler ultrasound imaging was done to evaluate the vascularity in both testes using colour Doppler and SMI modes. SMI was performed both in colour and monochrome modes (cSMI and mSMI). Blood flow in both testes were visually evaluated and classified in three subgroups as "no flow" when no flow was coded, "decreased flow" when a few vascular structures (1-3 vascular structure in the colour frame) were coded and "flow positive" when obvious vascular structures (more than three vascular structure were seen in the frame) were coded by both CDUS and SMI.

Strain elastography was performed by manual compression-decompression manoeuvres using transducer and considering the on-screen strain quality bar. The elastograms of all testes were obtained by setting the colour codes as red being the softest and blue being the hardest tissue parts in a split-screen mode with conventional B-mode image on one side and colour-coded elastogram on the other side on the ultrasound monitor. Then strain ratios (SR) were obtained by using still frames of elastograms that covered the entire testes. The first ROI was placed on the testes and the second was placed on the neighbourhood tissues just near the testes and the SR were recorded on color-coded elastograms.

Two-dimensional shear wave elastography was done by using a standard square sample box placed in the centre of the testes. Colour (speed-mode) and propagation maps were obtained on the monitor, and ROIs were placed between the parallel lines in propagation maps. At least three ROIs were placed, measurements were acquired as mean, median, and standard deviation (SD) values by considering interquartile ranges (IQR) <30%. The SWE values were recorded as Young modulus (kilopascals, kPa) and shear wave velocity (m/s) automatically calculated by the device.

### Pathological evaluation

Right and left testes of each rat were removed surgically after US evaluations. They were kept in 10% formaldehyde solutions and taken to the pathology laboratory. Bilateral orchiectomy specimens (n: 42) from 21 rats were evaluated grossly in the pathology laboratory and sampled by sectioning the specimens along their long axes. Formalin-fixed, paraffin-embedded samples were examined microscopically using haematoxylin-eosin stain and were evaluated by the pathologist.

Histopathological evaluations and pathological grading of all the testes were done according to the description reported by Cosentino et al which is used for evaluation of the histopathological changes in TT in rats (Figure 1).[1,13] According to modified Cosentino testicular pathological damage grading system the normal testicular histology with well-arranged germ cells was graded as "grade 1"; presence of less regular, discohesive germ cells, closely packed seminiferous tubules, oedema, or mild interstitial haemorrhage was graded as "grade 2"; the irregular, pooled germ cells with shrunken, pycnotic nuclei, less well-defined seminiferous tubules, and extensive haemorrhage was graded as "grade 3"; the tightly packed seminiferous tubules with coagulation necrosis in germ cells was graded as "grade 4".

## Statistical analysis

Data analysis was performed on a personal computer using statistical software (SPSS 21 for Windows, Chicago, IL, USA). Descriptive statistics are shown as mean ± SD for continuous variables and as percentages for categorical variables. Chi-square test and Fisher exact test were run for categorical variables, while the One-Way ANOVA and Kruskal-Wallis were preferred to compare the imaging findings. The Mann-Whitney U test was used to compare non-normally distributed continuous variables. Kolmogorov-Smirnov tests were performed to check normal distribution between the groups for continuous variables. The association between the continuous variables was tested by Spearman correlation analysis. A value of $P < .05$ was considered statistically significant. Intraclass correlation coefficient (ICC) scores and their 95% Cl were calculated. Interobserver agreement was categorized as follows: 0-0.20, poor; 0.21-0.40, fair; 0.41-0.60, moderate; 0.61-0.80, substantial; and 0.81-1.00, almost perfect.

### Interobserver agreement

The inter-observer agreement for 2D-SWE measurements and SE ratios was excellent with an ICC score ranging between 0.891 and 0.986 (95% CI).

## Results

The results of parametric values are summarized in Table 1.

Short-axis dimensions of the torsion testes were greater than the intact side ($P < .001$) in all groups. Lengths of the (long-axis dimensions) testes in the 3rd torsion group were significantly smaller than the lengths of the testes in groups 1 and 2 ($P < .002$). Volumes of the left testes on the torsion side were significantly greater than the intact side in all experiment groups ($P < .001$) (Table 1).

Strain ratios of testes were significantly higher on the torsion side of group 1 compared with the contralateral control testes ($P < .05$) (Table 1). SR of testes on the torsion side in groups 2 and 3 decreased with the increasing degree of torsion compared with the torsion side in group 1 (1.18 ± 0.38 in group 1, 0.42 ± 0.20 in group 2, 0.45 ± 0.16 in group 3). This decrease in SR of groups 2 and 3 were statistically

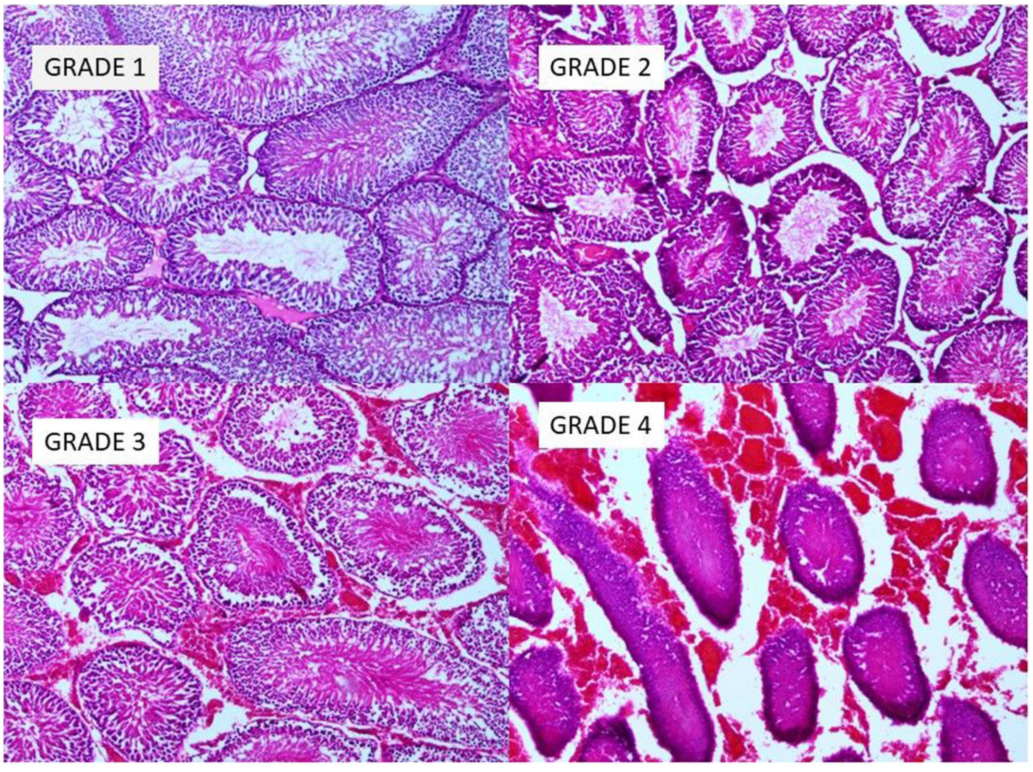


**Figure 1.** Haematoxylin-eosin (H&E) staining of testes on the torsion side for pathological grading (original magnifications x100). Grade 1 (upper left) shows well-arranged germ cells without oedema, haemorrhage or necrosis, grade 2 (upper right) shows less regular and discohesive germ cells, oedema and minimal interstitial haemorrhage, grade 3 (lower left) shows marked haemorrhage and associated seminiferous tubules with indistinct borders and pooled germ cells and grade 4 (lower right) shows seminiferous tubules with remarkable necrosis in germ cells, oedematous haemorrhagic interstitium.

Table 1. Summary of the results of parametric values.

| | Group 1 (360°) | | | Group 2 (720°) | | | Group 3 (1080°) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Right intact testes | Left torsion testes | P | Right intact testes | Left torsion testes | P | Right intact testes | Left torsion testes | P |
| Number of testes | 7 | 7 | | 7 | 7 | | 7 | 7 | |
| Length of the testes (mm) | 13.53 ± 0.94 | 14.25 ± 0.69 | .125 | 14.36 ± 0.80 | 14.91 ± 0.58 | .109 | 13.07 ± 1.62 | 12.94 ± 0.89* | 0.798 |
| Short axis measurements of the testes (mm) | 7.94 ± 0.99 | 10.23 ± 1.40 | .006 | 7.41 ± 0.84 | 9.62 ± 1.15 | .006 | 7.14 ± 0.83 | 11.34 ± 0.77 | .002 |
| Volumes of the testes ($mm^3$) | 453.85 ± 138.13 | 794.42 ± 245.57 | .009 | 417.39 ± 108.28 | 728.38 ± 177.40 | .004 | 351.22 ± 88.59 | 872.09 ± 148.68 | .002 |
| Strain ratio | 0.87 ± 0.18 | 1.18 ± 0.05 | .004 | 0.81 ± 0.38 | 0.42 ± 0.20 † | .02 | 0.30 ± 0.15‡ | 0.45 ± 0.16 † | .110 |
| Shearwave kPa mean (Emean) | 2.30 ± 0.43 | 3,45 ± 0,52 | .02 | 1,97 ± 0,16 | 2.75 ± 0.62† | .007 | 1,91 ± 0,81 | 2,77 ± 0,80† | .04 |
| Shearwave kPa SD (SD) | 0.37 ± 0.25 | 0.58 ± 0.25 | .04 | 0.36 ± 0.10 | 0.57 ± 0.14 | .02 | 0.28 ± 0.16 | 0.50 ± 0.12 | .03 |
| Pathological Score | 1 ± 0 | 2 ± 0 | .001 | 1 ± 0 | 3 ± 0§ | .001 | 1 ± 0 | 3,16 ± 0.87§ | .001 |

The results are presented as mean ± standard deviation.
Abbreviations: kPa = kilopascals.
*P* is the statistical value of comparison of the control and torsion groups in each experimental rat group.
* $P < .01$ compared with group 1 and 2 torsion.
† $P < .01$ compared with group 1 torsion side.
‡ $P < .01$ compared with group 1 and group 2 control side.
§ $P < .01$ compared with group 1 torsion side.

significant when compared with group 1 ($P < .002$) (Table 1). But there was no difference between groups 2 and 3. SR also decreased significantly in the control testes in group 3 compared with groups 1 and 2 ($P < .02$) (Table 1). The examples of SE evaluations for each group were given in Figure 2.

Shear wave elastography mean (Emean) and SD measurements in kPa increased significantly in torsion testes compared with the opposite intact testes in all groups (Table 1). Emean values of the testes on the torsion side decreased significantly in groups 2 and 3 compared with the values in group 1, but no significant change was seen between groups 2 and 3 on the torsion side. Also, SD values increased in all torsion groups but no statistical significancy was seen between groups. The examples of 2D-SWE evaluations for each group were given in Figure 3.

Superb microvascular imaging was found to be significantly better than CDUS in assessing blood flow in the contralateral normal testes in group 3 ($P < .05$) (Table 2). While CDUS detected no flow in all testes on the torsion side, SMI detected decreased blood flow on the torsion side in three rats in group 1 (Table 2), however, these data did not show statistical significance even though the *P* value was calculated very close to the upper limit ($P = .051$). Pathological grades of these three testes were grade 2. While CDUS detected normal blood flow in only one rat on the opposite side (n: 1/7, 14%), SMI detected normal blood flow in six rats (n: 6/7, 86%) ($P < .05$) in control testes of group 3 (Table 2). The examples of CDUS vs SMI evaluations for each group are given in Figure 4.

Histopathological examination results are given in Tables 1-3. The mean testicular damage score was found as 1 ± 0 in all control groups. Mean testicular damage scores on the torsion side were 2 ± 0 in group 1, 3 ± 0 in group 2, and 3.16 ± 0.87 in group 3 (Table 1). There was a statistically significant difference between the torsion and control sides in each group in terms of pathological damage scores ($P < 0.001$). Also, testicular damage scores increased by increasing torsion degrees. There was also a statistical significance between the damage scores of the torsion side in groups 2 and 3 compared with the torsion side in group 1 ($P < 0.01$).

While the testicular damage score in testes with normal echogenicity was found as 1.36 ± 0.67, it was 2.86 ± 0.53 in hypoechogenic testes ($P < .001$) (Table 3). SMI showed better correlation with histopathological findings than CDUS (Table 3). The pathological scores calculated according to CDUS vs SMI were 1 ± 0 vs 1 ± 0 in flow detected testes, 1 ± 0 vs 1.75 ± 0.50 in testes with decreased flow and 2.64 ± 0.65 vs 2.83 ± 0.51 in testes with no blood flow. These calculations were found to be statistically significant (Table 3).

In the correlation test, TT is found to be correlated with short axis dimension of testis, testicular volume, SR values in SE, Emean and SD values in 2D-SWE, CDUS, SMI, and pathology results ($P < .001$).

## Discussion

In this study, we aimed to evaluate late presenting TT with multiparametric US and compare the results with pathology in an experimental torsion model. Scrotal pain is generally neglected by the patients within the first 6 hours of torsion, so they tend to attend emergency room mostly after complete torsion occurs. Testes are 100% salvageable when diagnosed in the first 6 h of torsion and salvage percentage decreases as the duration of torsion increases.[1,14] Although duration time of torsion is important, degree of torsion has also been found to play an important role in the salvage of the testes.[1,15] It is reported that 50% of testes were unsalvageable at 860 degrees of torsion.[15] In the light of current data, we have designed the experiment as sonographic evaluations being done after 6 h of torsion in three different torsion degrees and compare the usefulness of various US techniques in late presenting TT with different torsion degrees.

Diagnosis of TT with CDUS is done by the examination of flow on the affected side and comparison with the contralateral testis. Epididymitis-orchitis is the most common condition causing a diagnostic dilemma. Matteson et al[16] reported that 61% of cases with TT are misdiagnosed as epididymitis.

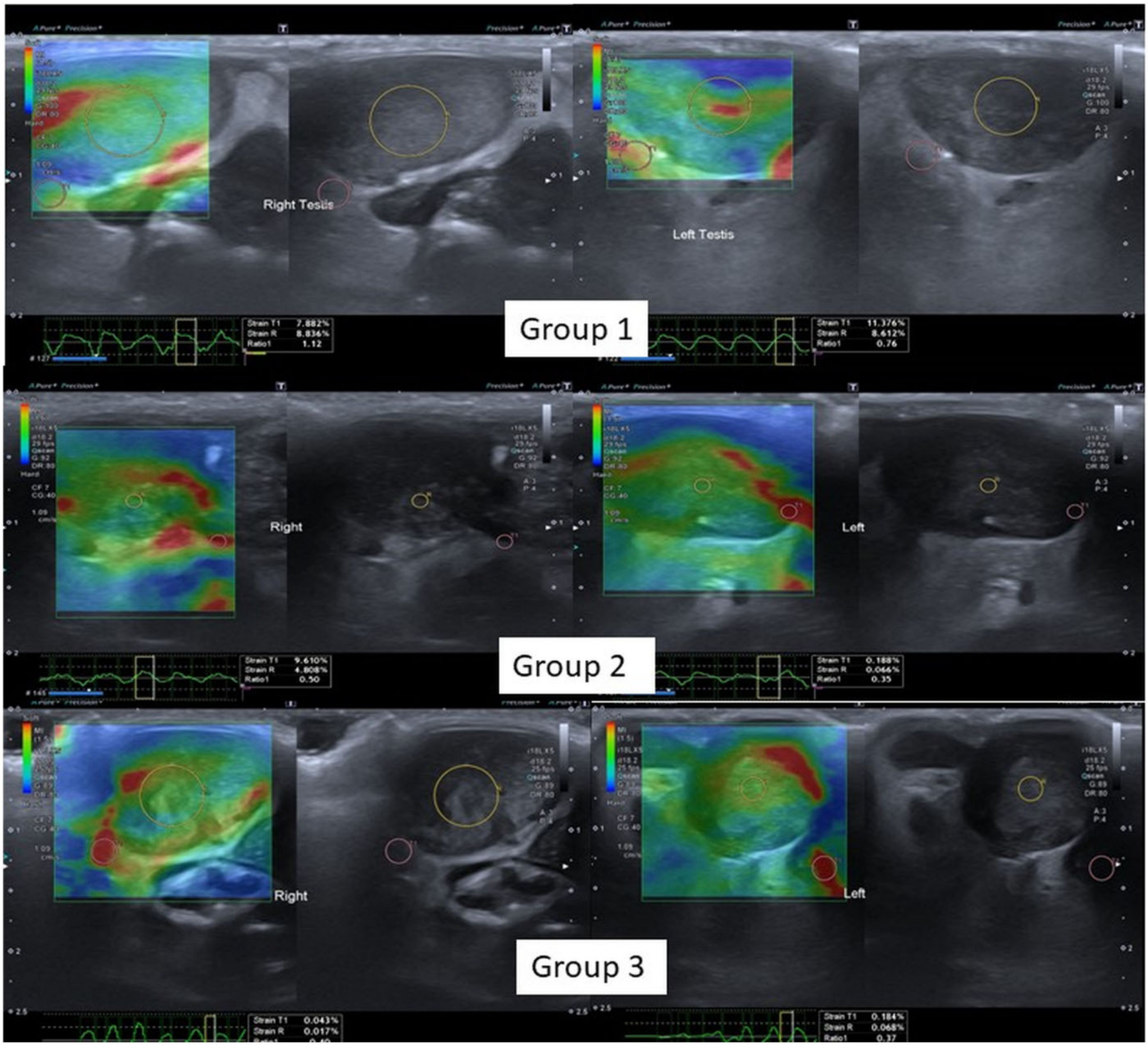


**Figure 2.** Examples of strain elastography images from each experiment group are given. The top row shows right (on the left side of the image) and left (on the right side of the image) testes of a rat in group 1, middle row shows right (on the left side of the image) and left (on the left side of the image) testes of a rat in group 2, and bottom row shows right (on the left side of the image) and left (on the left side of the image) testes of a rat in group 3. Although strain ratios (SR) on the torsion side decreased gradually with the increasing torsion degrees in groups 2 and 3, SR also decreased in the contralateral testis in group 3 (*Colour scale adjusted to show as red representing soft and blue representing hard texture. SR values; Group 1 right: 1.1, left: 0.50, group 2 right: 0.50, left: 0.35, group 3 right: 0.40, left: 0.37*).

However, epididymitis is less common than TT in the pre-adolescent boys who are sexually inactive. For this reason, TT must be the first diagnosis to consider in differential diagnosis of acute scrotum in childhood. However, the examination is more difficult in late presenting cases because of the scrotal oedema. Trying to reach a diagnosis by only evaluating the blood flow in testes may cause misdiagnoses and mistreatments, so establishing various diagnostic parameters for TT seems to be crucial. That is why, in the current study, we have tried to evaluate the efficiency and usefulness of five different ultrasound techniques.

Especially in prepuberty TT can cause infertility by different mechanisms such as decrease in the blood flow of the opposite testis causing damage and production of antisperm antibodies which make early diagnosis and treatment very important for salvage of the testes and fertility. In prepubertal boys with acute scrotal pain and oedema TT must be the first differential diagnosis for early treatment. Grey-scale and CDUS, scintigraphy, and MRI examinations are reported to be used in diagnosis.[17] However, grey-scale US and CDUS are used more than others in clinical practice because they are easily accessible and quickly done.

Spermatic cord whirlpool sign, redundant spermatic cord, and horizontal or altered lie testes are most reliable sonographic findings in the diagnosis.[18] Whirlpool sign is the twisted spermatic cord detected by US and is the most

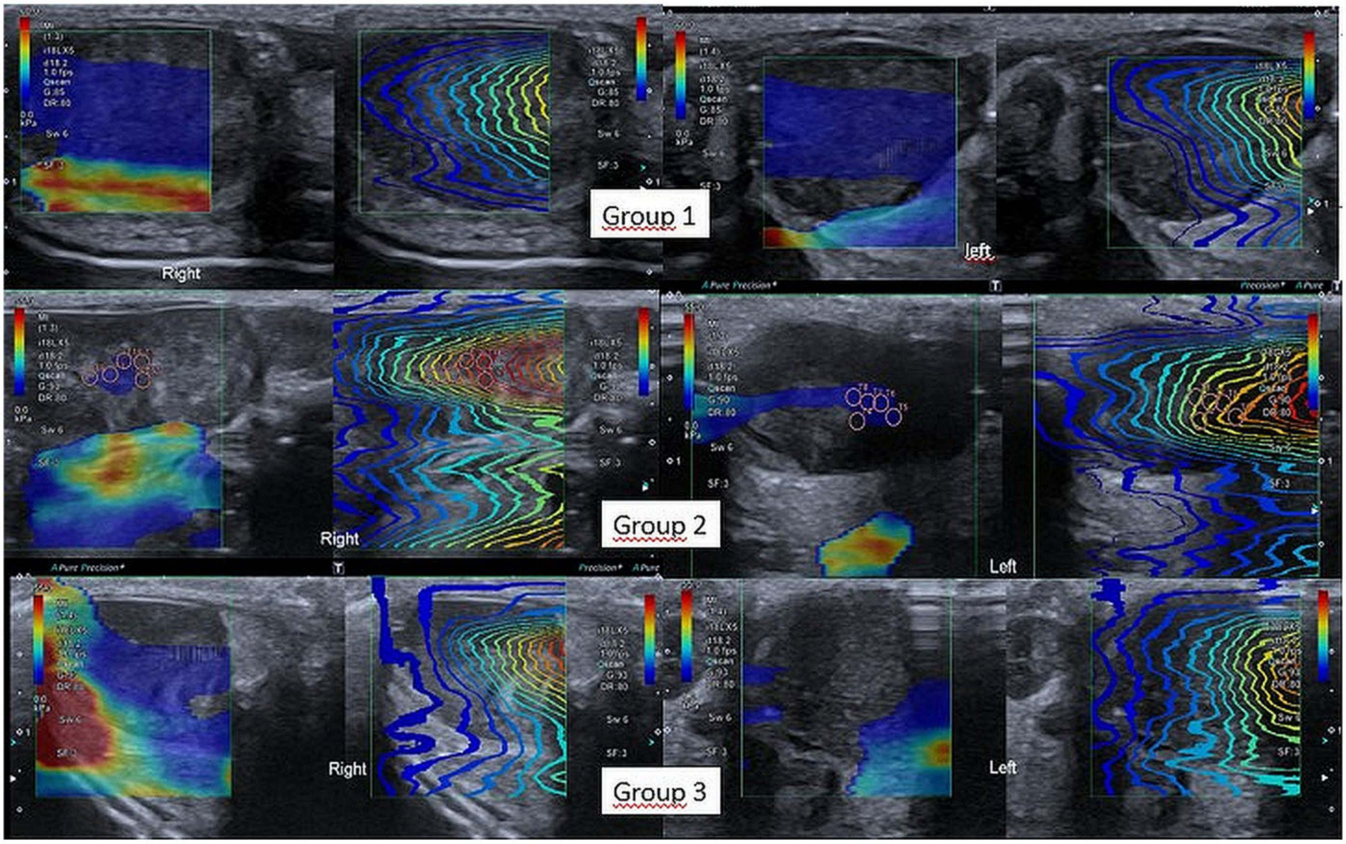


**Figure 3.** Images of 2D-Shear wave elastography with propagation maps from each group are given. The top row shows examples of propagation maps from right (on the left side of the image) and left (on the right side of the image) testes of group 1 (360-degree torsion group), the middle row shows examples of propagation maps from right (on the left side of the image) and left (on the right side of the image) testes of group 2 (720-degree torsion group), and the bottom row shows examples of propagation maps from right (on the left side of the image) and left (on the right side of the image) testes of group 3 (1080-degree torsion group). Note the distortion of the 2D-SWE propagation maps on the torsion side with increasing degree of torsion.

**Table 2.** Colour Doppler ultrasound and superb microvascular imaging results and correlation with pathology.

| | CDUS | | | SMI | | |
|---|---|---|---|---|---|---|
| Groups | Flow+ | Decreased flow | No flow | Flow+ | Decreased flow | No flow |
| Group 1 Torsion<br>*Pathology* | 0 | 0 | 7<br>*Grade 2 n: 7* | 0 | 3<br>*Grade 2 n: 3* | 4<br>*Grade 2 n: 4* |
| Group 1 Control<br>*Pathology* | 7<br>*Grade 1 n: 7* | 0 | 0 | 7<br>*Grade 1 n: 7* | 0 | 0 |
| Group 2 Torsion<br>*Pathology* | 0 | 0 | 7<br>*Grade 3 n: 7* | 0 | 0 | 7<br>*Grade 3 n: 7* |
| Group 2 Control<br>*Pathology* | 7<br>*Grade 1 n: 7* | 0 | 0 | 7<br>*Grade 1 n: 7* | 0 | 0 |
| Group 3 Torsion<br>*Pathology* | 0 | 0 | 7<br>*Grade 3 n: 5*<br>*Grade 4 n: 2* | 0 | 0 | 7<br>*Grade 3 n: 5*<br>*Grade 4 n: 2* |
| Group 3 Control<br>*Pathology* | 1<br>*Grade1 n: 1* | 5<br>*Grade 1 n: 5* | 1<br>*Grade 1 n: 1* | 6#<br>*Grade 1 n: 6* | 1#<br>*Grade 1 n: 1* | 0 |

#$P < .05$ compared with the results of CDUS.

accurate finding of TT. However, this finding is rarely seen. The redundant spermatic cord suggests the presence of "bell clapper deformity" and is the gathering of the redundant spermatic cord on the superior aspect of the testis by twisting. This finding may suggest TT, too. Testes anatomically lie vertically. The alteration of testes position such as horizontal/oblique and transvers lie at the upper portion of the scrotum is an important clinical and sonographic finding in the diagnosis of TT.[18] In our study, we found that the short axis measurements and the volumes of the testes on the torsion side increased significantly compared with the contralateral control testes (Table 1). Increase in short axis measurement in our study might be an indicator of horizontal lie testes because of the twisting spermatic cord pulling the testes horizontally upwards. Although testicular enlargement is seen in other causes of acute scrotum, testicular oedema on the

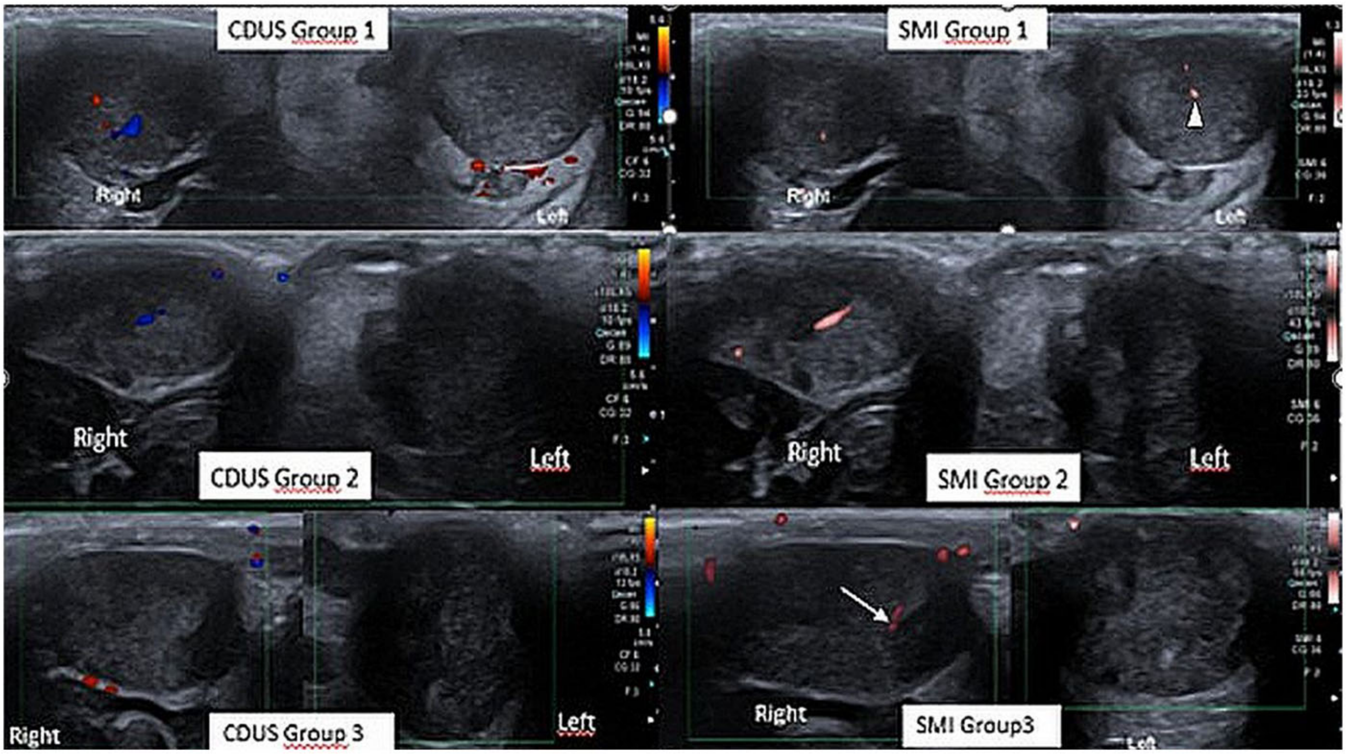


**Figure 4.** CDUS (left column) and SMI (right column) images of right and left testes in the experiment groups. The images belong to the same rats in each group. Although no blood flow is detected with CDUS, SMI shows blood flow in the left testis (torsion side) in group 1 (white arrow) and in the right testis (control side) in group 3 (white arrowhead).

**Table 3.** Correlation of pathological grades with echogenicity, CDUS and SMI.

| | | Pathological grades of all testes n: 42 | | | | | |
|---|---|---|---|---|---|---|---|
| | | Grade 1 | Grade 2 | Grade 3 | Grade 4 | Mean grade | $P$ |
| Echogenicity | Normal | 21 | 4 | 3 | 0 | 1.36 ± 0.67 | |
| | Hypoechogenic | 0 | 3 | 9 | 2 | 2.86 ± 0.53 | <.001 |
| CDUS | Flow + | 15 | 0 | 0 | 0 | 1 ± 0 | |
| | Decreased flow | 5 | 0 | 0 | 0 | 1 ± 0 | |
| | No flow | 1 | 7 | 12 | 2 | 2.64 ± 0.65 | <.005 |
| SMI | Flow + | 20 | 0 | 0 | 0 | 1 ± 0 | |
| | Decreased flow | 1 | 3 | 0 | 0 | 1.75 ± 0.50 | <.001 vs flow + |
| | No flow | 0 | 4 | 12 | 2 | 2.83 ± 0.51 | <.001 vs flow + =.01 vs decreased flow |

torsion side caused increase in testicular volumes. Statistically significant decrease in the lengths (long dimensions) of the testes on the torsion side were found only in group 3 which we think is due to more alteration in testicular position with the increasing degree of torsion. According to our findings, we suggest that detection of increase in the short axis measurements and volumes of the testes should be a strong indicator of TT in grey-scale ultrasound evaluation and an accompanying significant decrease in the lengths of the testis on the affected side should be a warning for higher torsion degrees.

The echotexture of the testis is another important criterion used for the viability of the affected testis. Bandarkar et al[18] reported that significant heterogeneity of the affected testis generally indicates a nonviable testis and clinically late admission to hospital. In this experimental study, we evaluated the testes 6 h after torsion and found similar results. While the mean pathological damage score of the testes those had normal echogenicity was 1.36 ± 0.67, the value was significantly high as 2.86 ± 0.53 in hypoechogenic testes ($P < .001$).

Previously in a study authors investigated and reported the role of strain elastography in the evaluation of TT in a different experimental model.[1] They had found that testicular stiffness increased with TT in early period and in the 24th hour of torsion, stiffness of the contralateral testes and torsion side decreased together with 1080-degree torsion. In the current study we evaluated SWE in addition to SE. We have found almost similar results as SE values in terms of SR increased on the torsion side compared with the opposite control testes in group 1 (1.18 ± 0.05 vs 0.87 ± 0.18, $P = .004$). On the other hand, SR values in SE evaluations decreased significantly by increasing torsion degrees in groups 2 and 3 (0.42 ± 0.20 and 0.45 ± 0.16, $P < .002$ compared with group 1). These findings indicate that testicular damage increases by increasing torsion degrees. SR values of the opposite testes in 1080-degree torsion group (group 3) significantly decreased compared with groups 1 and 2 ($0.87 \pm 0.18$ in group 1, $0.81 \pm 0.38$ in group 2, $0.30 \pm 0.15$ in group 3, $P < .002$). This is important in showing the contralateral testicular damage in TT. In a study, the effect of torsion on the intact testis is reported to be 78%, which may further cause impaired fertility.[19] We recommend using SE in especially the evaluation of the intact testis in late presenting TT cases.

However, use of SE may have some handicaps, especially in children who have severe pain. Manual compression-decompression manoeuvres using a transducer may not be performed easily and appropriately in these patients.[20] This may cause some limitations in the emergency use of this technique. SWE can be applied more practically in emergency technique because it does not require decompression and compression manoeuvres and is little operator dependent.[20] In our current study, we evaluated the role of 2D-SWE on TT in addition to SE. We compared Emean values of the torsion side and intact testes with each other and found higher values on the torsion side meaning increased stiffness due to torsion. The highest Emean value on the torsion side was detected in group 1 (torsion side vs intact side: $2.30 \pm 0.43$ vs $3.45 \pm 0.52$, $p = 0.02$). While little decrease in Emean values was detected on the torsion side in groups 2 and 3 when compared with group 1, similar decrease was seen also in the contralateral intact testes thereby causing a statistically significant increase in stiffness of the testes on the torsion side in all groups. Shear wave elastography SD values also increased significantly in all groups on the torsion side compared with the right intact testes. Our results show that testicular stiffness increased more at lower torsion degrees. We thought that this condition is because venous obstruction occurs first in lower degree torsions or transient torsions, but arterial flow is still present and both venous and arterial obstructions are seen with the increasing torsion degrees. This clinico-pathological condition which causes stiffness in testes supports our finding especially in group 1. In our study, we have found similar results when compared 2D-SWE with SE, besides doing SWE is easier and less time consuming than SE technique.

Colour Doppler ultrasonography is accepted as a reliable tool in the diagnosis of TT. The sensitivity and specificity of CDUS in the preoperative diagnosis was reported as 88.9% and 99.8%, respectively.[21] Thus, CDUS has become the first-choice diagnostic technique for TT. Although CDUS is a reliable technique, it has been reported to cause misdiagnosis and mistreatment, especially in younger patients who have small testes.[17] SMI has many advantages over CDUS as being more reliable in assessment of low-velocity flow within tissues by reducing tissue movement artefacts.[10,11,17,22] Recently, Visalli et al[11] reported that microvascular imaging technique gives more accurate results in child cases admitted with acute scrotum. They especially emphasized that microvascular imaging has more advantage in low-flow state by detection of blood flow in cases where CDUS did not show the vascularization in tissue.[11] We also found that SMI is better in detecting blood flow when torsion occurs with lower torsion degrees and this finding may help early salvage of the affected testes. SMI is also very effective in detecting blood flow on the intact testes when torsion occurs especially with higher degrees. This finding is important because TT also affects opposite testis by decreasing blood flow and causing ischaemia. In our experimental TT model, SMI showed better efficiency than CDUS in the evaluation of the vascularity in the opposite testes, too (Table 2). Our findings supported the clinical results of Visalli et al.[10] In the clinical emergency practice, correct detection of blood flow on the contralateral testes is very important because the diagnosis of TT with Doppler technique is made by comparison of vascularization of the opposite testes with the suspected side. In our study, CDUS detected normal blood flow only in one rat on the contralateral control testes (n: 1/7, 14%) but SMI detected normal flow in six rats (n: 6/7, 86%) ($P < .05$) in group 3 (1080-degrees torsion) (Table 2). This finding also supports the effect of torsion to the contralateral testes with increasing torsion degrees which we also confirmed with our SE findings.

Our study has some limitations. First, this was an experimental TT model, so we cannot predict the outcome of these multimodal ultrasonographic evaluations on normal children and adults. Secondly, since this is a rat experiment, the evaluated testes sizes may only reflect the sizes of the children's testicles and findings in adult testes may be different.

In conclusion, SE, 2D-SWE, and SMI are more reliable techniques than grey-scale US and CDUS in sonographic evaluation of TT and in the control examination of contralateral intact testes. However, we do not completely ignore the diagnostic values of grey-scale US and CDUS as, testicular position difference, increase in short axis and testicular volumes are important parameters supporting the diagnosis of TT. According to our results, we recommend those who have an ultrasound device with these features add SMI, SE and/or SWE to grey-scale US and CDUS when evaluating TT. Further multimodal US studies can be done in human population to confirm our experimental findings in TT.

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None declared.

## Conflicts of interest

The authors declare no conflict of interest.

## Ethics statement

This study was designed and performed in accordance with the recommendations in the guidelines established by the Guide for the Care and Use of Laboratory Animals from the US National Institutes of Health. Institutional Ethics Committee on Animal Research approved the study.

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