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Early diagnosis of testicular torsion in rats by measuring plasma d-dimer levels: comparative study with epididymitis

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ABSTRACT

Purpose: To evaluate the differential diagnosis of testicular torsion and acute epididymo-orchitis by measuring the acute increase in plasma D-dimer levels in an experimental rat model.
Methods: Thirty male Wistar rats were randomly divided into 5 groups, 1 – sham operated group (acute term; 4 hours), 2 – early torsion group (acute term; 4 hours), 3 – late torsion group (long-term; 72 hours), 4 – control of epididymitis group (vehicle injected; 0.1 ml physiologic saline injected into the left ductus deferens) (long term; 72 hours), 5 – epididymitis group (0.1 ml *Escherichia coli* injected into the left ductus deferens), ($n = 6$ for each group).
Results: Serum D-dimer levels were significantly higher compared with the sham operated group with early torsion ($p = 0.002$). This elevation remained mildly in the late torsion group compared with the control group ($p < 0.001$), but there was no difference between 4 and 72 hours of the testis torsions ($p = 0.794$). On the other hand, D-dimer levels were significantly higher in the torsion groups compared to the epididymitis group ($p = 0.042$).
Conclusions: The present study demonstrated that testicular damage that occurs following testicular torsion shows a higher increase in D-dimer levels than epididymitis, suggesting that D-dimer level can be used as a diagnostic marker of testicular torsion.

Testicular torsion (TT) is the twisting of the spermatic cord, which cuts off the blood supply to the testicle and surrounding structures within the scrotum [1]. Torsion initially results in obstruction of spermatic cord venous blood flow with secondary edema and hemorrhage [2]. Subsequently, enhanced edema results in arterial obstruction, ischemia, and gonadal necrosis. The testicular damage owing to torsion and detorsion shares similarity with the phenomenon of ischemia–reperfusion injury observed in other tissues [3]. It has been shown that both testis torsion and epididymitis have a potential negative influence on sperm parameters and sperm function [4].

Epididymitis is an inflammation of the epididymis, occasionally accompanied by inflammation of the testis (epididymo-orchitis). The predominant causative organisms vary by patient age, but *Escherichia coli* is a commonly associated pathogen, especially in young children and older men [5].

D-dimer levels have been used as an indicator of intestinal ischemia and venous thromboembolic disorders. Pathophysiology could be expected in TT in which there are firstly venous and later arterial thrombotic vessels in the twisted pedicle and the ischemic testis [6]. The aim of the present study was to evaluate the value of serum D-dimer levels in the differential diagnosis of TT and epididymitis.

1. Materials and methods

1.1. Animals and study design

This study was approved by the ethics committee of the Dişkapi Training and Research Hospital (Approval Number: 2012/5). Wistar rats weighing 250–350 g were used. The animals were housed at room temperature and in a controlled environment of 12-h light–dark periods with free access to water and rat chow. Surgery was conducted under one intramuscular injection of xylazin hydrochloride (10 mg/kg Rompun; Bayer, Leverkusen, Germany), (50 mg/kg) anesthesia. Under adequate anesthesia, a left transverse inguinal incision was performed on all rats.

Male Wistar rats were randomly divided into 5 groups including 6 rats in each group, Group 1: Sham operated group (acute-term; after sham operation to the left testis 4 hours expected). Group 2: Early

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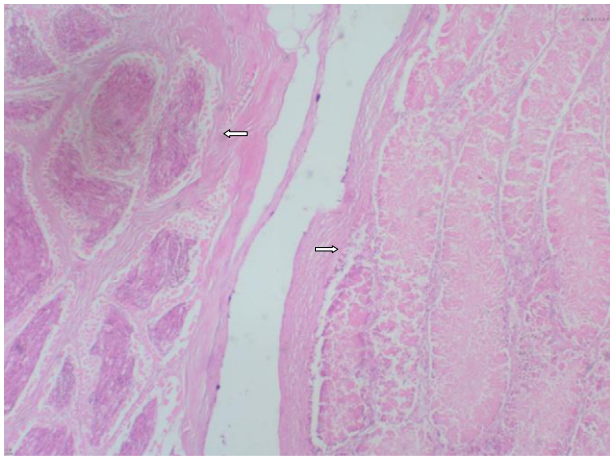

Fig. 1. Testicular torsion, coagulation necrosis, shadow of the seminiferous tubuli and epididymis epithelial necrosis, sloughing (80× HE).

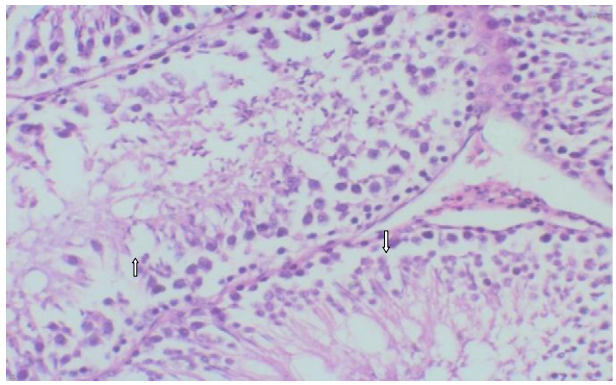

Fig. 3. Testis in epididymitis, mildly damaged seminiferous tubuli (800× HE).

torsion group (left testicular torsion for 4 hours), Group 3: Late torsion group (left testicular torsion for 72 hours), Group 4: Control of epididymitis group (vehicle injected; 0.1 ml physiologic saline was injected into the left ductus deferens), Group 5: Epididymitis group (0,1 ml E. Coli was injected into the left ductus deferens and waited for 72 hours after the procedure). Blood samples were collected before and after the procedure. The rats were then sacrificed and the left epididymis and testis tissue samples were excised.

1.2. Testicular torsion (TT)

Testicular torsion was performed by rotating the testis 720° in a clockwise direction for 4 hours or 72 hours. The torsion was maintained by fixing the testis in the scrotum with a 4–0 silk suture, and the incision was closed. After a 4 hours or 72 hours of torsion period, the incision was entered and the suture was removed. At the end of each procedure, left orchiectomy side by side with excision of the epididymis was performed for histopathological and microbiological examinations of all groups. Histological sections were stained with hematoxylin–eosin (HE). Before and 4 and 72 hours (3 days) after the surgery or sham operation, venous blood was withdrawn for D-dimer analysis.

Orchiectomy materials were placed in buffered formalin solution, dehydrated, cleared and embedded in paraffin. Tissue sections at a thickness of 5 micrometer were cut with a microtome and stained with hematoxylin and eosin (HE). General morphological observations were investigated with a light microscope (Olympus BH-5; Olympus Optical Co., Ltd.; Tokyo, Japan). All microscopic areas were examined as to whether there was coagulation necrosis and inflammation in the testis and epididymis, by a pathologist blinded to sample identity.

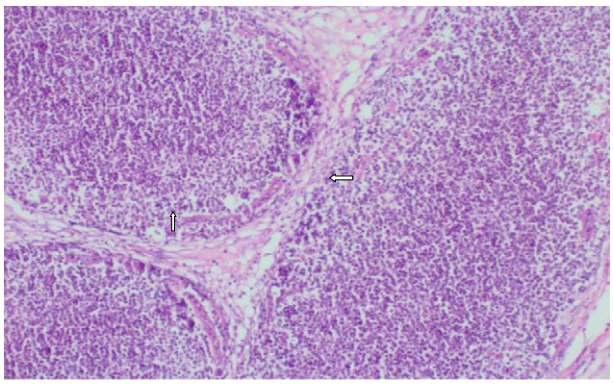

Fig. 2. In the epididymis: extensive inflammation, damaged, sloughing epithelium and exudate in the lumen of the ductus (200× HE).

1.3. Epididymitis

The *E. coli* serotype used was an isolate from the urine of a child patient with a urinary tract infection, which was incubated in 10% bouillon to provide 10^6 cfu/ml. Preparation of stock cultures was as described previously. Before induction of epididymitis, blood samples were taken for D-dimer measurements. Epididymitis was induced in rats (after anesthesia with an intramuscular injection of xylazine) through an inguinal incision and exposure of the right epididymis via the right ductus deferens (by puncture with a 27 G needle 1 cm proximal to the epididymis), followed by injection of 0.1 mL of *E. coli* suspension (10^6 cfu/ml) into the epididymal direction of the vas. After the injection, dissections were closed. No antibiotic was given to the rats for 72 hours. After 72 hours, rats were sacrificed and blood samples were taken for D-dimer measurements. Urine samples were taken by bladder puncture. Epididymis and testis were removed for tissue sections. Epididymitis was verified by histological examination.

Blood samples collected in citrated tubes were centrifuged for 15 min at 1000 rpm at room temperature. Plasma levels of D-dimer in separated samples were quantified using enzyme linked immunosorbent assay kits according to the manufacturer's protocols (CSB-E12984r, ELISA Kit, D-dimer, D2D Donghu Hi-Tech Development Area, Wuhan, Hubei Province 430223, P.R. China). The detection limit of this assay was 7.8 ng/mL-500 ng/mL. Cusabio Rat D-dimer assay was expressed in milligrams per liter, and the intra-assay and inter-assay coefficients of variation were <8% and <10%, respectively.

The Statistical Package Program for the Social Sciences (SPSS 16.0; SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Groups were controlled in terms of conformity to normal distribution by graphical check and Shapiro–Wilk test. The groups were distributed normally and mean and SD parameters were used. Independent sample t-test and ANOVA were used for comparison of two and three independent groups, respectively. Dependent sample t test was used for comparison of dependent groups. P-value of <0.05 was taken as significant.

2. Results

2.1. Microbiological analysis

In epididymitis groups, the infecting organism was identified by urine culture. No bacterial growth was observed in the blood samples but *E. coli* growth was observed in urine and tissue samples. No infection was detected in the control groups.