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pH and antioxidant measurements in rats with testicular torsion and their correlation with viability

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ABSTRACT

Purpose: Testicular torsion (TT) is a urologic emergency and to make an instant decision about the fate of the torsioned testis based just on the macroscopic features is a dilemma. A quantitative method to evaluate the viability is needed. Since ischemia results in acidosis, pHs of testes might provide objective information about viability. The present study aimed to assess the relationship between tissue pH, histopathologic and biochemical changes in a rat model of TT via a pH sensor.
Methods: The study included 40 Wistar albino rats divided into four equal groups. Group 1 was the control group. The others (group 2, 3, 4) underwent 1, 8, 24 h of TT and detorsion, respectively. After detorsion, the pH measurements, histopathologic and biochemical analysis were made.
Results: The pH levels of control group and group-24t were statistically different. As the ischemia prolonged, the mean testicular injury scores decreased and the morphological grades worsened.
Conclusion: The pH sensor presented herein, can be used intraoperatively to detect pH changes and to predict viability. A pH value of less than 6 appears to be incompatible with viability and may provide decision of orchiectomy.

Testicular torsion (TT), properly the torsion of the spermatic cord, is one of the most important urologic emergencies which may lead to testicular injury and impaired fertility. The main pathophysiology is the ischemia/reperfusion injury of the testis and overgeneration of reactive oxygen species.

The tissue injury in TT is directly related to the duration and the degree of torsion. Testicular viability is affected within 8 h. Torsions more than 360 degrees and longer than 24 h result in atrophy of the testis [1–3].

In the literature, there are many studies explaining the mechanism of TT, showing in both biochemical and histopathological aspects that testicular torsion and detorsion cause some kind of ischemia–reperfusion (I/R) injury where cytokines are produced and apoptosis is augmented and aiming to prevent the formation of damage [4–10].

During the operation, after detorsioning, the surgeon evaluates the testis for any sign of reperfusion, looks at the color and size of the testis and solidity on palpation. But his evaluation is qualitative and subjective. Eventually, non-reperfusing, infarcted testis is excised where as reperfusing one is kept in place. It's a dilemma for the surgeon to make an instant decision about the fate of the torsioned testis, whether to excise or preserve it, based on the macroscopic features. There is obviously a need for a quantitative method to evaluate the tissue injury and viability.

The pathological examination says the last word for the excised testis. Therefore, any method helping on excising or preserving the injured testis during the operation would probably prevent many unnecessary orchiectomies.

Herein this study, the pH levels of testes with I/R injury were measured by a pH probe developed by Işıldak et al. and the correlation between the pH levels and histopathological findings in the necrotic tissue was assessed [11] (Fig. 1.1). A quantitative method to evaluate the viability of the detorsioned testis was aimed to be defined.

Early detorsion conserves fertility in TT but as the duration of the torsion lengthens, and if the necrotic testis is mistakenly preserved, fertility of both testes is affected. In unilateral TT, depletion of the contralateral bloodflow and tissue injury is related to the overproduction of free oxygen radicals [4,13].

As described in the experimental testicular torsion models in the literature, testes should be torsioned for 720 degrees (two tours) to create total ischemia and injury occurs after at least one hour. Testes should be torsioned 2-tours for more than 4 h to obtain a serious ischemia/reperfusion injury [1,12,13,14,15].

1. Material and methods

This experimental study was performed during 2010–2011 at the Ondokuz Mayis University Experimental Animals Research Center

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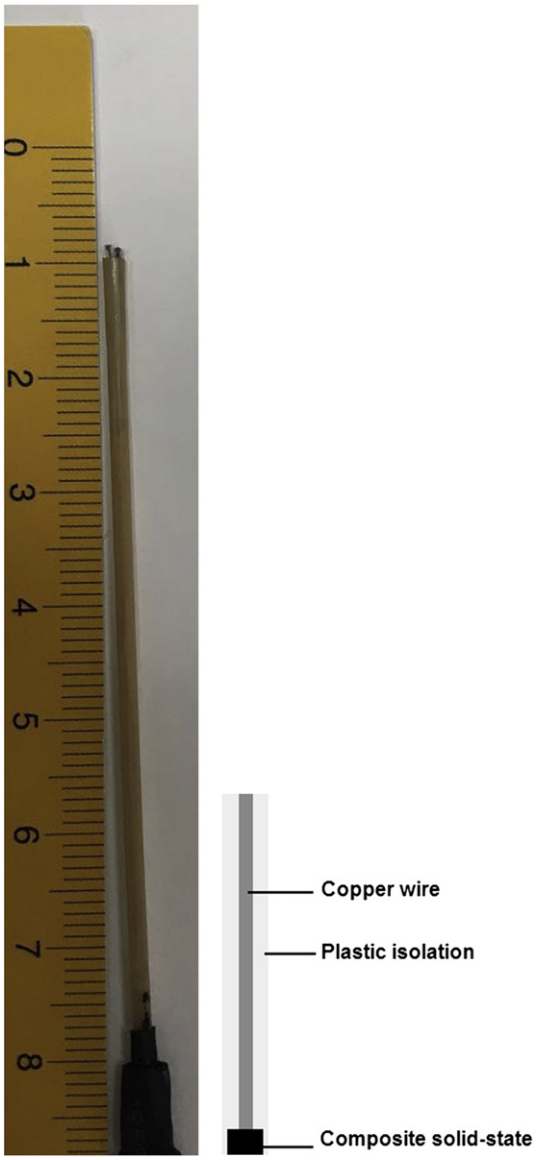

Fig. 1. Composite, ion-selective pH sensors (1.1&1.2).

with the participation of Pediatric Surgery, Chemistry, Biochemistry and Pathology. Fourty, 30–35-day old, prepubertal Wistar albino rats weighing 200–250 g were used. All animals were followed up by a protocol approved by the Ethics Committee.

1.1. Study groups

To be used within the study, micro-sized potentiometric pH sensors of new technology were prepared from composite material. (Patent pending/Turkish Patent Institute application number: PT2015–01,208). These composite sensors did not require inner reference electrode or reference solution. They were prepared by mixing graphite, epoxy resins, hardeners, plasticizers and some polymers with active material in proper proportions. The parameters related to their potentiometric performances such as selectivity, linear working range, response time and service life, were tested static and dinamically by a computer-controlled measurement system.

These sensors contained 10.81% quinhydrone, 21.62% graphite, 21.62% polimethylmetacrylate (PMM) and 43.24% acrylic liquor and 2.70% PVC-COOH. They showed Nerst behavior between the pHs 2 and 12 and had a high potential of repeatability between pHs 4 and 7. They had linear behavior at the calibration graphic between the pHs 2 and 12. Their response time was 25 s from 2 to 12 and 30 s from 12 to 2. All the criteria above supported that these sensors were suitable for measurements at biological tissues.

The rats were divided into four equal groups in a random manner. They were kept in single cages, at normal room temperature and humidity throughout the study. 50 mg/kg intramuscular ampicillin-sulbactam prophylaxis was administered. All surgical procedures were performed under ketamine (50 mg/kg) and xylazine HCl (8 mg/kg) anesthesia. Access to left testes was through left scrotal incisions in all. (Table 1).

In the control group (Group C), left testis was taken out from the left scrotal incision, some tissue was spared for histopathologic and biochemical analysis, tissue pH was measured and the remaining testis tissue was repaired, put back in and fixed via 5-0 vicryl®.

In the 1t, 8t and 24t groups, testes were taken out, 720 degrees of torsions were performed for 1, 8 and 24 h, respectively. Testes were fixated to the scrotum by 5-0 vicryl® to avoid spontaneous detorsion. Scrotal incisions were closed. After completion of torsion periods, detorsions were made. After warm compress, testis pHs were measured. Tissues were spared aside for histopathologic and biochemical analysis, macroscopic features were noted, remaining testis tissues were repaired. The animals were not sacrificed. (Fig. 2). (See Fig. 3.)

1.2. Histopathological analysis

All testicular tissues were fixated in Bouin solution and embedded in paraffin blocks. Tissue sections were stained with H&E (hematoxylin-eosin). A double-blind histologic analysis under light-microscopy was performed by a pathology specialist.

The morphological damage was staged using both the 4-Graded System defined by Cosentino et al. and also by Modified Johnsen Scoring System [16–18]. For the mean testis biopsy evaluation, 20 seminiferous tubules were counted from different areas of the same slice, scored according to Modified Johnsen Scoring and mean values were calculated.

1.3. Chemical analysis

After detorsioning and warm compress application to the testes, these sensors were dipped into the testicular tissues and pHs were noted.

1.4. Biochemical analysis

Following detorsions, some testis tissue was excised to measure lipid peroxidase, total and reducted glutathione levels. Excised testicular tissues were homogenized by liquid nitrogen manually and were put into buffer solution (K buffer, 100 mM, pH 7.4). After one-minute of sonication (ultrasonic cleaner, 220 V, METU electromechanic S.N: 30,607, Germany), kept at −70 °C until the day of the evaluation. On the experiment day, after dissolving at room temperature, the samples were centrifuged for 5000*g*, 10 min, the supernatants were spared for bichemical measurements and LP, GSH, GSSG and protein levels were measured. Total glutathione (TGSH) was calculated by summation of GSH and GSSH.

GSH is oxidated easily and forms the disulfide dimer GSSG. GSSG is the product of reduction of hydroxyperoxidases by glutathione peroxidase, which is further reducted into GSH by glutathione reductase. GSH is the main form found in biological systems.

Table 1
Study groups.

Group C	Control group
Group 1t	1 h torsion + detorsion
Group 8t	8 h torsion + detorsion
Group 24t	24 h torsion + detorsion