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SURGICAL MEDICAL SCIENCES / CERRAHİ TIP BİLİMLERİ

Determination of Pentraxin 3 Levels in Diagnosis of Testicular Torsion in Experimental Rat Model

Deneysel Sıçan Modelinde Testis Torsiyonu Tanısında Pentraxin 3 Düzeylerinin Belirlenmesi

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Abstract

Objectives: Testicular torsion is a common surgical emergency in pediatric surgery and urology practice. The timing of surgery and the degree of torsion are the factors affecting testicular salvage. The aim of this study is to investigate the efficiency of Pentraxin 3 (PTX 3) levels as a diagnostic marker in an experimental rat testicular torsion model.

Materials and Methods: A total of 17 Wistar albino male rats were randomly divided into two groups: seven rats in Sham group (Group S), ten rats in testicular torsion group (Group TT). Blood was taken at the 4th and 4th, 24th and 72nd hours following testicular torsion for group S and group TT, respectively. Histopathological evaluation was performed. PTX 3 levels were measured for each rat.

Results: The median plasma levels of PTX 3 were 1,409 ng/mL at the 4th hour in group S and 1,393 ng/mL, 1,283 ng/mL, 1,094 ng/mL at the 4th, 24th and 72nd hours, respectively in group TT. There was a statistically significant difference between the 72nd-hour plasma levels and group S (p=0.004).

Conclusion: PTX 3 levels decreased at the 72nd hour in the experimental rat testicular torsion model. But the diagnosis should be made within 6 hours from the beginning of the symptoms to protect the testes. PTX 3 levels do not seem to be helpful in clinical practice as a useful marker for early diagnosis of testicular torsion.

Key Words: Biochemical Marker, Pentraxin 3, Rat Model, Testis Torsion

Öz

Amaç: Testis torsiyonu, çocuk cerrahisi ve çocuk üroloji pratiğinde yaygın görülen cerrahi acildir. Ameliyatın zamanlaması ve torsiyon derecesi testis kurtarılmasını etkileyen faktörlerdir. Bu çalışmanın amacı, deneysel bir sıçan testis torsiyon modelinde Pentraxin 3 (PTX 3) düzeylerinin tanısal bir belirteç olarak etkinliğini araştırmaktır.

Gereç ve Yöntem: Toplam 17 Wistar albino erkek sıçan rastgele olarak yedisi Sham grubu (Grup S), onu testiküler torsiyon grubu (Grup TT) olmak üzere 2 gruba ayrıldı. Grup S'de 4. saatte, Grup TT'de sırasıyla 4., 24. ve 72. saatlerde kan alındı. Histopatolojik değerlendirme yapıldı. PTX 3 seviyeleri her sıçan için ölçüldü.

Bulgular: Grup S'nin PTX 3 medyan plazma seviyesi 1.409 ng/mL ve grup TT'nin 4., 24. ve 72. saatlerindeki PTX 3 medyan plazma seviyeleri sırasıyla 1.393 ng/mL, 1.283 ng/mL, 1.094 ng/mL olarak bulundu. Grup TT'nin 72. saat ölçümleri ile Grup S'nin ölçümleri arasında istatistiksel olarak anlamlı fark vardı (p=0,004).

Sonuç: Deneysel sıçan testis tosiyon modelinde 72. saatte PTX 3 seviyeleri düştüğü gözlendi. Ancak testislerin korunması için tanı, semptomların başlamasından itibaren 6 saat içinde konulmalıdır. PTX 3 seviyeleri, testis torsiyonunun erken teşhisi için yararlı bir belirteç olarak görülmemektedir.

Anahtar Kelimeler: Biyokimyasal Marker, Pentraxin 3, Sıçan Modeli, Testis Torsiyonu

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Introduction

Testicular torsion is a common surgical emergency in both pediatric surgery and urology practices (1,2). Twisting or torsion of the testis results in the occlusion of the gonadal blood supply resulting in edema and hemorrhage. Testicular loss due to gonadal necrosis is the main concern. Thus, early diagnosis and intervention are necessary for these patients (3,4).

The timing of surgery and the degree of torsion are the factors affecting testicular salvage (1). Following the sixth hour of torsion, ischemic changes become irreversible and testicular loss becomes inevitable in delayed surgical detorsion. Thus early diagnosis and intervention is crucial for testicular torsion (1,3,5).

Although testicular torsion is usually diagnosed by Doppler ultrasound; medical history, physical examination and blood parameters are also important to support the diagnosis of testicular torsion. Loss of vascularity of the affected testicle by Doppler ultrasound can confirm the diagnosis of torsion. Preoperative blood parameters such as white blood cells and C-reactive protein (CRP) are non-specific. These inflammatory markers can be elevated in many other acute scrotum conditions (3). Any confirmative blood parameter for testicular torsion diagnosis has not yet been established.

CRP and Pentraxin 3 (PTX 3) are members of an acute-phase protein family named Petraxins (6). The pentraxin protein family has two subgroups: short and long pentraxin groups. CRP is a member of the short pentraxin group, on the other hand PTX 3 is a member of the long pentraxin group (7). PTX 3 is synthesized by cells such as monocytes, macrophages, polymorphonuclear cells, endothelial cells. PTX 3 is stored in the granules of polymorphonuclear cells and are released to increase more rapidly in cases of inflammation and infection (8,9). Akman et al. (9) showed that plasma PTX 3 levels were increased in ovarian torsion in an experimental rat model. In accordance with our review of literature, it was hypothesized that PTX 3 levels would increase in testicular torsion of rat model.

According to these data, we aimed to investigate the role of PTX 3 levels in cases of testicular torsion and its efficacy in early diagnosis in a rat model.

Materials and Methods

Animals

Seventeen male, Wistar albino rats (10-12 weeks of age) weighing 250-300 gr were used. Seven rats were included in the Sham group (group S) and ten rats in the testicular torsion group (Group TT). All procedures involving the rats were performed in accordance with Ankara University Faculty of Medicine, Medical Laboratory of Animal Sciences. This study was conducted after being approved by the Ankara University Animal Experiments Local Ethics Committee (approval no: 2017-14-119, date: 05.07.2017). The rats were maintained in a temperature-controlled room (23±2 °C) with a 12-hour light-dark period and were given free access to standard food and water.

Group S: Sham Group

Group TT: Testis torsion.

Study Design and Surgical Procedure

The operation was performed under general anesthesia by intraperitoneal injection of ketamine 90 mg/kg and xylazine HCl 10 mg/kg. To create testicular torsion, the testicle was taken out by a scrotal incision and rotated 720 degrees clockwise then fixed to the scrotum with 4/0 silk sutures. The incisions were closed with 4/0 silk sutures, after the completion of surgery. In Group S, scrotal incision was performed, the testicle was taken out and then the incision was closed with 4/0 silk sutures without rotation of the testicle. While blood was taken at the 4th hour in group S, and at the 4th, 24th and 72nd hours in group TT. 1 mL of blood was taken through intracardiac puncture and stored at -80 °C. During the blood sampling, one rat in the testicular torsion group died in the postoperative 1st day. After all blood samples were taken, PTX 3 levels were measured for each rat. Scrotal incisions were reopened at the 72nd hour of operation in both groups and orchiectomy was performed for histopathological examinations. Scrotal incisions were closed after the orchiectomy. The rats were sacrificed. The procedures followed were in accordance with animal rights.

Biochemical Analysis

Following venipuncture venous blood samples were centrifuged within 30 minutes, at 1000×g for 15 min. Then they were stored at -80 °C until the assays were performed. Serum PTX-3 levels were measured by Elabscience PTX 3/TSG-14 rat ELISA Kit. The analytic coefficient of variation was <10%.

Histological Examination

For light microscopic evaluation, tissue samples were fixed in 10% neutral buffered formalin solution for 72 hours at room temperature and routine histological procedures were applied. They were dehydrated in a graded series of ethanol, rinsed in xylene and embedded in paraffin. Then 5 mm thick serial sections were obtained with rotary microtome (Leica RM 2125 RT Bensheim, Germany) and were stained with Hematoxylin-Eosin (HE). Stained sections were investigated and photographed under Carl Zeiss AxioScope, A1 microscope (Oberkochen, Germany).

Statistical Analysis

The data was analyzed using the SPSS software for Windows (SPSS 11.5) and p-value less than 0.05 was considered

statistically significant. Results were calculated as mean± standard deviation. The data was compared using non-parametric statistical analysis Kruskall-Wallis test, Friedman test for categorical variables.

Results

The median plasma levels of PTX 3 were 1,409 ng/mL at the 4th hour in group S and 1,393 ng/mL, 1,283 ng/mL, 1,094 ng/mL at the 4th, 24th and 72nd hours in group TT, respectively. Plasma PTX 3 levels seemed to be decreased after torsion, even though the decrease was not statistically significant. The results of group TT in the 4th and 24th hours, compared to group S in the 4th hour were not statistically significant (p=1.000, p=0.833). On the other hand, there was a statistically significant difference between the 72nd hour results of group TT and the 4th hour results of group S (p=0.004) (Table 1, Figure 1). Testes specimens of group S were observed to be normal in histological examination. In the testicular sections of group TT, inflammation of the tunica vascular layer, an increased number of capillaries and hemorrhage were noted. Degeneration, edema, hemorrhage and mononuclear cell infiltration were observed in the germ cells lining the seminiferous tubules' walls (Figure 2).

Discussion

Testicular torsion, which leads to testicular ischemia, is an immediate indication for surgery because of the risk of diminished fertility. The reported incidence of testicular torsion is 3.8/100,000 in males who are younger than 18 years old (1,10). Diagnosis of testicular torsion is often difficult and non-specific because the tests do not have 100% specificity. Positive signs in physical examination and history may help the diagnosis, but these are not certain or objective (2). The gold standard preoperative diagnostic tool is Doppler ultrasonography with 88.9% sensitivity and 98.8% specificity (10). PTX 3 is a member of an acute phase protein family (6). In this study, PTX 3 was studied as a possible marker for testicular torsion. We aimed to observe the plasma PTX 3 level changes and to determine its place in early diagnosis in an experimental rat model of testicular torsion.

PTX 3 is an inflammatory marker with plasma levels ≤2 ng/mL in healthy people. Plasma levels of PTX 3 increase 3-5 times higher than its basal value and peak at 6-8 hours in inflammatory situations such as appendicitis and cholecystitis (6,7,11). Akman et al. (9) showed that plasma PTX 3 levels increased in an

Table 1: Plasma PTX 3 levels

	n	Median levels	Standard deviation	Minimum	Maximum	p^0	p^1	p^2
Group S	7	1,409	±0,207	1,147	1,725			
Group TT (4th hours)	10	1,393	±0,110	1,189	1,610	1.000		
Group TT (24th hours)	9	1,283	±0,086	1,136	1,410	0.833	0.102	
Group TT (72nd hours)	9	1,094	±0,107	0,968	1,294	0.004	0.001	0.472

p^0: Group S- 4th hours- 24th hours - 72nd hours
p^1: 4th hours- 24th hours - 72nd hours
p^2: 24th hours - 72nd hours
PTX 3: Pentraxin 3, Group S: Sham group, Group TT: Testicular torsion group

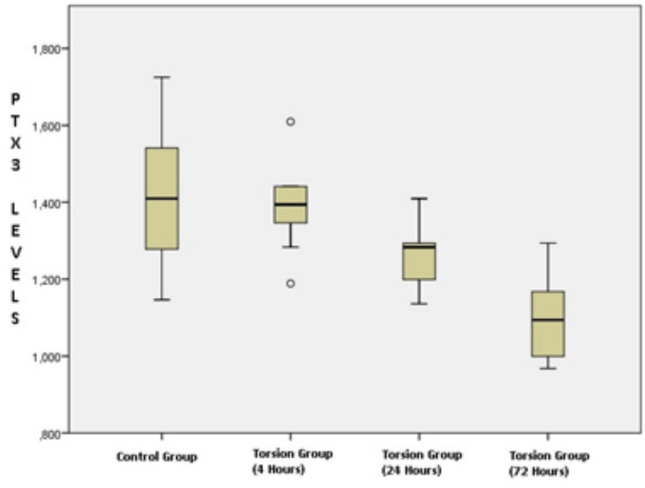

Figure 1: Kruskal-Wallis analysis of variance: PTX 3 levels all groups
PTX 3: Pentraxin 3

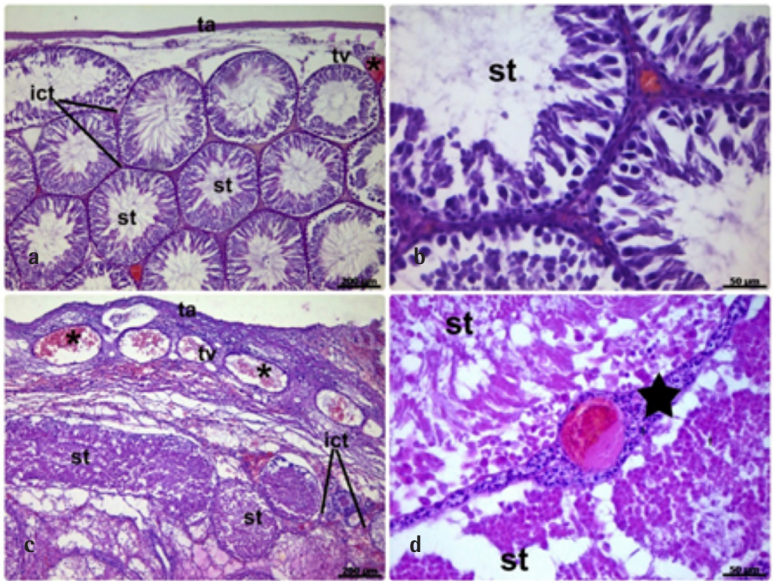

Figure 2: Representative light micrographs of testicular sections from group S **(a,b)** and Group TT **(c,d)** rats.ta; Tunica albuginea, tv; Tuniva vasculosa, ict; interstitial connective tissue, st; seminiferous tubule, *; capillary, star; inflamatory cells. Magnification, a,c; x 10, b,d; x 40. Stain, Hematoxylin-Eosin

Group S: Sham group, Group TT: Testicular torsion group

experimental rat model of ovarian torsion. Testicular torsion was supported by histopathological injury in our study. The PTX 3 levels show a decrease within the normal range. Even though this decrease was not statistically significant except for the 72nd hour samples, this decrease may be explained by the non-inflammatory nature of testicular torsion. PTX 3 levels may be increased in epididymoorchitis because of inflammation. Thus, unnecessary surgery in children who do not have testicular torsion may be avoided.

Testicular torsion is the most common non-inflammatory scrotal pathology and epididymoorchitis is the most common inflammatory scrotal pathology (12,13). Blood parameters, doppler ultrasound and radionuclide imaging such as Tc-99 pertechnetate are used in the differentiation of acute testicular torsion from epididymitis and orchitis (3,10,14). Acute phase proteins such as CRP, fibrinogen and others, increase during inflammation, which may help the diagnostic process (12). In literature, epididymitis has higher CRP values than testicular torsion (3,12,13). Doehn et al. (12) showed that CRP did not increase in the testicular torsion group except for one patient while CRP increased in the epydidymoorchitis group.

Early differential diagnosis of acute testicular torsion from epididymoorchitis is often difficult due to reasons discussed previously. Current Doppler ultrasonography is both subjective and not available in all hospitals which causes a loss of time that is crucial for these patients (3). For this reason, a specific blood test that gives objective and faster results may be helpful for early diagnosis and treatment to reduce complications in these patients.

Study Limitations

Our limitation in this study is that there was no epdidiymoorchitis group to compare the testicular torsion group. Future animal studies that compare alterations of PTX 3 levels in epididymoorchitis versus testicular torsion may help the diagnosis in children.

Conclusion

Acute phase proteins such as CRP and fibrinogen are used for helping acute scrotum diagnosis. In this study, PTX 3 levels were significantly reduced in the 72nd hour in group TT, as expected for negative acute phase proteins. However, early diagnosis is crucial for testicular torsion and it should be made within 6 hours of the onset of symptoms. Thus, PTX 3 levels are not helpful for early diagnosis of testicular torsion.

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Ethics

Ethics Committee Approval: This study was conducted after being approved by the Ankara University Animal Experiments Local Ethics Committee (approval no: 2017-14-119, date: 05.07.2017).

Informed Consent: Animal experiment study.

Peer-reviewed: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: K.B., U.A., G.G., G.E., Concept: K.B., U.A., E.E., M.D., M.Ç., Design: K.B., B.İ.E., M.Ç., Data Collection and Processing: E.E., G.G., M.D., B.İ.E., Analysis or Interpretation: G.G., D.B., Literature Search: K.B., U.A., E.E., G.E., M.Ç., Writing: K.B., U.A., E.E., G.E., D.B., M.Ç.

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