Is procalcitonin valuable in the differential diagnosis of testicular torsion and epididymo-orchitis

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ABSTRACT

الأهداف: تقييم فعالية عقار بروكالسيتونين في التشخيص المختلف لانفتال الخصية والتهاب الخصية.

الطريقة: أجريت هذه التجربة في مختبر الأبحاث جامعة دايكل – كلية الطب – دياربكر – تركيا خلال الفترة ما بين مارس ويونيو 2008م. اشتملت هذه الدراسة عدد 24 ذكر جرذ وتم تقسيمهم عشوائياً إلى 3 مجموعات، مجموعة سليمة، ومجموعة مصابة بالتهاب الخصية، ومجموعة مصابة بانفتال الخصية. تم الحصول على عينات الدم من جميع الجرذان في بداية الدراسة. بعد الانفتال ظهر الالتهاب في عينات الدم الجديدة التي تم الحصول عليها لقياسات عقار بروكالسيتونين PCT. بعد ذلك تم تقييم جميع الفحوصات النسيجية المرضية. تم استعمال فحص ويلسكوكسون لتقييم الإحصائي.

النتائج: تمت مقارنة مستويات بروكالسيتونين PCT قبل وبعد الفحوصات وكانت مستويات عقار بروكالسيتونين PCT عالية بشكل ملحوظ لدى المجموعة المصابة بالتهاب الخصية.

خاتمة: قد يكون عقار بروكالسيتونين مؤشر سريع وآمن وسهل الاستخدام للتشخيص المختلف لانفتال الخصية والتهابها.

Objectives: To evaluate the efficacy of procalcitonin (PCT) in the differential diagnosis of testicular torsion and epididymo-orchitis.

Methods: This experimental study was performed in the research laboratory of Dicle University, School of Medicine, Diyarbakir, Turkey between March and June 2008. The study included 24 male rats randomized equally in 3 groups: sham, epididymo-orchitis, and torsion groups. Blood samples were obtained from all rats at the beginning of the study. After torsion and infection occurred in the testes, new blood samples were obtained for PCT measurement. Then, all the right testes of the rats were excised for histopathological evaluation. The Wilcoxon signed test was used for statistical evaluation.

Results: Pre- and post PCT levels were statically compared, and PCT levels were significantly higher in the epididymo-orchitis group.

Conclusion: Procalcitonin could be an easy, fast, and safe marker for use in the differential diagnosis of testicular torsion and epididymo-orchitis.

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Findings in the acute scrotum generally include severe pain, a mass, and tenderness. Various pathologies might be responsible for this urologic emergency. In some cases, immediate surgery is the only method of treatment for rescuing the testis. As such, the differential diagnosis of acute scrotum should be performed expeditiously. Testicular torsion is the most urgent cause of acute scrotum. The incidence of torsion in cord structures in patients ≤25 years of age is 1/160, and in the testes is 1/4000. The peak incidence of torsion is seen during the pubertal period.[1] Primarily, torsion alters venous flow in the testis, which is followed by hemorrhage and arterial obstruction. Decreasing blood flow promotes hypoxia in testicular tissue. It was shown that the most sensitive cells to hypoxia are the germ cells, most commonly the spermatocytes and spermatogonials. Reperfusion is necessary to maintain the vitality of ischemic tissues. Detorsion should be performed promptly, and the prognosis is best if detorsion is performed within 6 hours. The most confusing pathology in the differential diagnosis is epididymo-orchitis. Physicians must differentiate these 2 pathologies precisely and

promptly. Any misdiagnosis might cause loss of the testis. Misdiagnosed cases are usually seen in patients ≤35 years of age, as each pathology could be seen relatively frequently. Anamnesis, physical examination, scrotal color Doppler ultrasonography, and testicular scintigraphy is the main diagnostic method. The most accurate diagnostic method is testicular scintigraphy, although performing it in a timely fashion is not always possible. Scrotal color Doppler ultrasonography is another effective method, but is operator dependent. In case of doubt, scrotal exploration is advised as the best method for diagnosis. Procalcitonin (PCT) increases during bacterial inflammation, but remains stable during other types of inflammation. A literature search did not reveal any studies on the use of PCT in the differential diagnosis of torsion and epididymo-orchitis; therefore, the present study was planned to examine the utility of PCT in such cases.

Methods. This experimental study was performed in the research laboratory of Dicle University, School of Medicine, Diyarbakir, Turkey, between March and June 2008. The study began after obtaining the approval of the Dicle University School of Medicine Ethics Committee with 24 adult male Wistar-Albino rats weighing between 250-300 g. The experimental animals were housed at 22 ± 10°C under a 12 h light/12 h dark cycle, with free access to a standard rat pellet diet and tap water. The 24 rats were randomly divided into 3 equal groups: group 1 (n=8): sham group; group 2 (n=8): testicular torsion group; and group 3 (n=8): epididymo-orchitis group. Under sterile conditions, intraperitoneal anesthesia (100 mg kg^{-1} ketamine) was administered to all rats after an Angiocath was inserted into their caudal veins for obtaining 1-ml blood samples for studying PCT levels. The right tunica vaginalis was opened through a midline scrotal incision and closed without any additional procedure in group 1.The right testis was freed through a midline scrotal incision, twisted 720° clockwise, and fixed to the scrotum for 4 hours with 4/0 silk sutures in group 2.The right testis was freed through a midline scrotal incision, and the tunica vaginalis opened and the right vas deferens was isolated one cm apart from the epididymis in group 3. Then, 0.1 ml of 10^6 cfu mL^{-1} *Escherichia coli* (ATCC 25922 *E. coli* standard suspension 10^6 colony forming units mL^{-1}) suspension was injected retrogradely with a 27G needle into each group 3 rat and the scrotum was closed in layers. After 4 hours the rats in groups 1 and 2 were anesthetized with 100 mg kg^{-1} of ketamine, 1-mL blood samples were obtained for PCT measurement and then all the rats were sacrificed by intracardiac aspiration. After scarification the right testis of each rat was removed and placed in Bouin's solution for histopathologic examination. All blood samples were centrifuged for 10 minutes at 3000 rpm, and the sera were stored at -70°C. The rats in group 3 were observed for the symptoms of epididymo-orchitis and all developed epididymo-orchitis within 48 hours. The left scrotum was normal, without any symptoms. The same procedures were performed after 48 hours in group 3.

Histopathological methods. All right testes were fixed in 10% neutral formalin for 24 hours, washed with water, and then fixed in Bouin's solution for 24 hours and washed with 70% alcohol. Afterwards, tissues passed through a 70%, 80%, 90%, 96%, and 100% ethanol series, and were then dehydrated. All tissue samples were cleared in xylene for 15 minutes. This procedure was repeated 3 times. Then, tissue samples were kept for one hour in 2 different paraffin baths in a -58°C incubator and fresh paraffin blocks were embedded. Five-µm thick sections were obtained from paraffin blocks. The paraffin sections were placed on slides and kept for 24 hours in a 37°C incubator. Afterwards, sections were passed through a xylene and ethanol series, and were stained with Hematoxylin-Eosin (H&E) and Periodic Acid Schiff (PAS). The prepared slides were examined with a BH-2 Olympus light microscope and microphotographs were obtained.

Statistical analysis. The mean and standard deviation (± SD) for continuous variables was calculated. The normality of the variables was analyzed and confirmed using the Kolmogorov-Smirnov test. The ANOVA test was used to test the means of baseline, torsion and detorsion in group 2 and group 3 separately, and Dunnett's post hoc test was used when ANOVA results were significant. Two-sided p-values were considered statistically significant at $p<0.05$. Statistical analyses were performed using SPSS v.15.0 for Windows (SPSS, Inc., Chicago, IL, USA).

Results. Macroscopically, all testicles in group one were histologically normal before orchiectomy (**Figures 1a & 1b**). Macroscopically, torsion developed in all rats in group 2, and on histologic examination of sections of right testis, torsion findings like coagulative necrosis, disseminated hemorrhagia, completely emptied seminiferous tubules, and locally accumulated germinal epithelium cells were observed (**Figures 2a & 2b**). Also, following *E. coli* injection epididymo-orchitis developed in all rats within 48 hours after the injection. The histopathologic findings of infected testis in sections of right testis like disseminated degeneration in germinal epithelium, hemorrhagia in interstitial area, and congestion with infiltration of leucocytes with polymorph nucleus and neutrophils were observed (**Figures 3a & 3b**). The PCT levels in each group before procedures are shown in **Table 1**. There was no difference

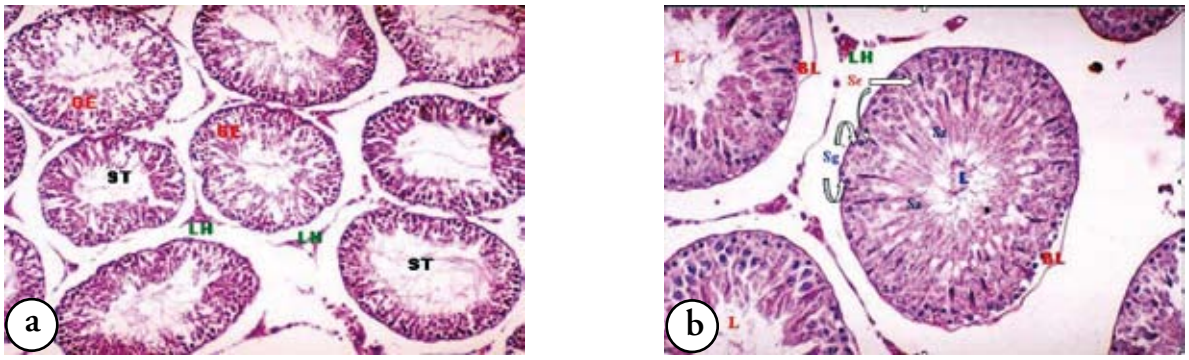

Figure 1 - Section of right testis of sham group showing **a)** histologically normal seminiferous tubules with normal germinal epithelial cells and Leydig cells. **b)** Histologically normal seminiferous tubules with all cell series of spermatogenesis. GE - germinal epithelium, ST - seminiferous tubule, LH - Leydig cell, BL - basal lamina, L - seminiferous tubule lumen, Sc - primer spermatocyte, Sg - spermatogonia, Sz - spermatozoa (Periodic Acid Schiff x 82).

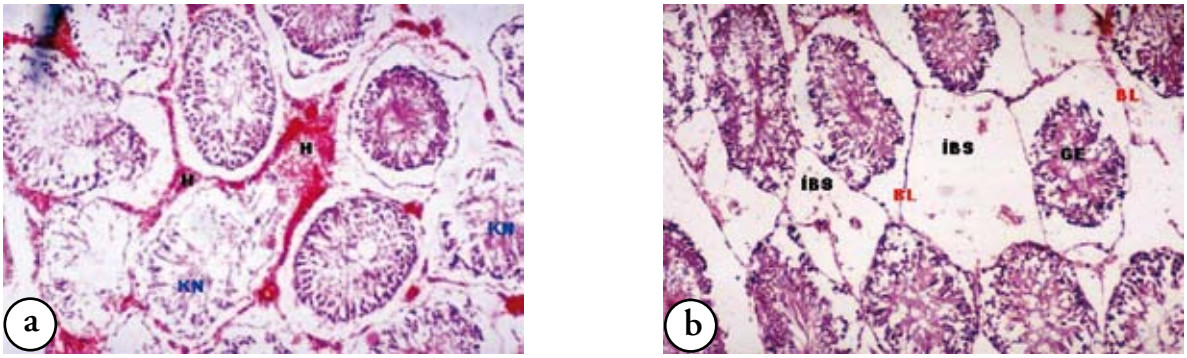

Figure 2 - Right testis sections of torsion group showing **a)** disseminated coagulative necrosis hemorrhagia (Hematoxylin & Eosin x 41), and **b)** completely emptied seminiferous tubules and locally accumulated germinal epithelium cells in lumen (Periodic Acid Schiff x 41). KN - coagulative necrosis, H – hemorrhagia, IBS - emptied seminiferous tubules, GE - germinal epithelium, BL - basal lamina.

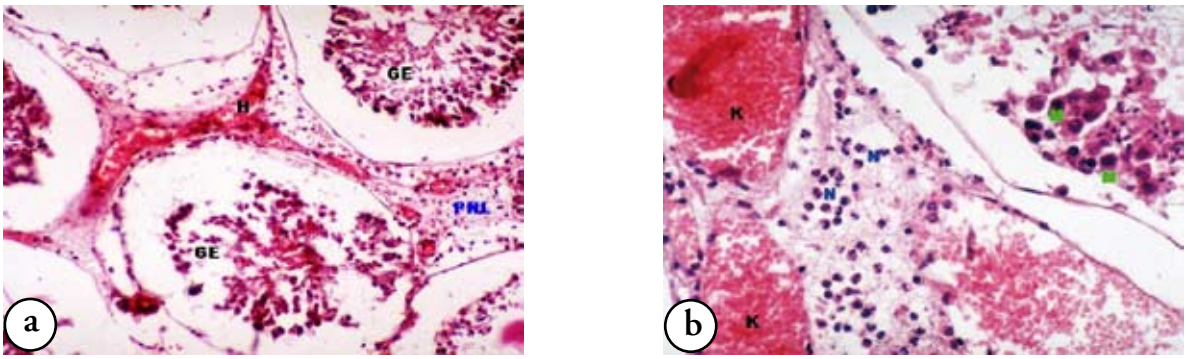

Figure 3 - Right testis sections of epididymo-orchitis group showing **a)** disseminated degeneration of germinal epithelium, hemorrhagia in interstitial area and leucocytes with polymorph nucleus (Hematoxylin & Eosin x 82), and **b)** disseminated neutrophile infiltration with macrophages and congestion (Periodic Acid Schiff x 164). GE - germinal epithelium, H - hemorrhagia, PNL - leucocytes with polymorph nucleus, N - neutrophile, K - congestion, M – macrophage.

Table 1 - Procalcitonin levels of the groups before procedures.

Group	n	Mean	± SD	95% CI for means		*P*-value
				Lower limit	Upper limit	
Sham	8	0.0204	0.00817	0.0136	0.0273	
Torsion	8	0.0271	0.02197	0.0087	0.0454	0.337
Epididymo-orchitis	8	0.0169	0.00310	0.0143	0.0195	
Total	**24**	**0.0215**	**0.01374**	**0.0157**	**0.0273**	

CI - confidence interval

Table 2 - Procalcitonin levels of the groups after procedures.

Group	n	Mean	± SD	95% CI for means		*P*-value
				Lower limit	Upper limit	
Sham	8	0.0191	0.0042	0.0156	0.0226	
Torsion	8	0.0233	0.0046	0.0195	0.0271	0.040
Epididymo-orchitis	8	0.0306	0.0138	0.0191	0.0421	
Total	24	0.0243	0.0097	0.0203	0.0284	

CI - confidence interval

in the PCT levels of all groups and this was statistically insignificant according to ANOVA test (p=0.337). The PCT levels in each group after procedures are shown in **Table 2**. The PCT levels in group 3 were higher than groups 1 and 2, and the difference was statistically significant using ANOVA test (p=0.04).

Discussion. Testicular torsion must be diagnosed quickly and accurately. Diagnostic delay risks loss of testicular viability, whereas over-diagnosis subjects patients to unnecessary surgery. Testicular torsion might be seen in all age groups, but its incidence peaks during the first years of life and during the pubertal period. It has been reported that 65% of all cases occur during the pubertal period, most often in 13-year-olds. In 2% of cases torsion was bilateral, otherwise, it usually affected the left testes. The most confusing pathology in the differential diagnosis is epididymo-orchitis. In this study, we aimed to evaluate the efficacy of PCT in the differential diagnosis of testicular torsion and epididymo-orchitis.

It is widely known that epididymo-orchitis is not very common in children; McAndrew[2] reported that its incidence in children is 10%, which is similar to the incidence of testis torsion. A recent study reported a mean age of patients with epididymo-orchitis of 28 years.[3] Nonetheless, clinicians should keep in mind that epididymo-orchitis can be seen in all age groups (4 months-76 years). Epididymo-orchitis is seen with similar frequency in the right and left testes. Bilateral cases are rare, with a reported incidence of 9%.[4] As testis torsion and epididymo-orchitis are most often seen in patients ≤35 years, misdiagnosed cases were also seen during this period.

Scrotal color Doppler ultrasonography is the most frequently used method for the differential diagnosis of testis torsion and epididymo-orchitis. The user dependence of this method is its most important disadvantage. The possibility of measuring the blood flow in testis smaller than 1 cm is 69%. This difficulty measuring normal blood flow with scrotal color Doppler ultrasonography in prepubertal testes might increase the frequency of misdiagnosis. The diagnostic sensitivity of this method is reported as 76-88%. Increased blood flow is expected in epididymo-orchitis cases, but increased blood flow can also be seen in some cases of appendix testis and testis torsion.[5] Some researchers suggest scrotal exploration instead of radiological methods to prevent misdiagnosis.[6] The other method used for differential diagnosis is testicular scintigraphy, which takes approximately 30 minutes to perform, and has an accuracy of 95%. In some institutions, it is not possible to prepare a scintigraphy system and the necessary radioisotopes in an appropriate amount of time, which could delay diagnosis. As rapid diagnosis is not always possible with scintigraphy, this method may not be the optimal one. Blask et al[4] studied color Doppler ultrasonography and testicular scintigraphy together in cases with acute scrotum. Sonography correctly diagnosed 11 of 14 surgical and 31 of 32 nonsurgical conditions. There was one indeterminate and 3 false negative examination results. The sensitivity

of ultrasonography was 78.6%, and specificity was 96.9%. Scintigraphy correctly diagnosed 11 of 14 surgical, and 29 of 32 nonsurgical conditions. There were 2 indeterminate scintigrams, and 2 false positive and 2 false negative examination results. The sensitivity of scintigraphy was 78.6%, and specificity was 90.6%. They concluded that color Doppler sonography and scintigraphy are similarly sensitive and that a small number of false negative cases can occur with either modality. This study highlights the need for new diagnostic methods.

The biochemical assay used to measure PCT is quick, non-invasive, and easy. There are many studies on the diagnostic efficacy of PCT for many infectious diseases, but not for epididymo-orchitis. The literature contains a few experimental studies on PCT assays in rats. In one of these Yücel et al[7] studied the efficacy of white blood cells, rectal fever, C-reactive protein, and PCT in Wistar-Albino rats for monitoring the effect of normobaric oxygen treatment in peritonitis. The PCT and white blood cell levels were reported to be superior to the other markers. Reports of the efficacy of PCT in monitoring various infections other than epididymo-orchitis encouraged us to study PCT. The mean basal level of PCT in our study was 0.0215 ng mL^{-1} (range: 0.005-0.0804 ng mL^{-1}). The PCT levels were higher in the epididymo-orchitis group than in the other 2 groups, and the difference was statistically significant. The PCT level in healthy humans was reported as 0.1 ng ml^{-1}. During infections it rises to over 0.5 ng ml^{-1}. Chua et al[8] reported that bacterial, fungal, and parasitic infections increase the PCT level, but that viral infections or infections without systemic symptoms do not or minimally increase the level of PCT. Post procedure high PCT levels in group 3 could help us in the differential diagnosis. The increase in the epididymo-orchitis group could help us to differentiate the epididymo-orchitis from testicular torsion, and we think that patients with high levels of PCT should be accepted as epididymo-orchitis and treated by suitable antibiotics primarily.

In conclusion, the mean PCT level in the epididymo-orchitis group was higher than in the sham and torsion groups, and the difference was statistically significant. We think that PCT is a fast, non-invasive, inexpensive, easy, and useful diagnostic marker for use in the differential diagnosis of torsion and epididymo-orchitis. Future studies in humans are needed to determine more precisely the value of PCT in clinical cases.

References

1. Schneck FX, Bellinger MF. Acute scrotum. In: Walsh PC, Retik AB, Darracott Vaughn E Jr. Campbell's Urology. 8th ed (Turkish edition). Istanbul (Turkey): Gunes Kitapevi; 2005. p. 2353-2384.
2. McAndrew HF, Pemberton R, Kikiros CS, Gollow I. The incidence and investigation of acute scrotal problems in children. ***Pediatr Surg Int*** 2002; 18: 435-437.
3. Luzzi GA, O'Brien TS. Acute epididymitis. ***BJU Int*** 2001; 87: 747-755. Review.
4. Nussbaum Blask AR, Bulas D, Shalaby-Rana E, Rushton G, Shao C, Majd M. Color Doppler sonography and scintigraphy of the testis: a prospective, comparative analysis in children with acute scrotal pain. ***Pediatr Emerg Care*** 2002; 18: 67-71.
5. Baldisserotto M. Scrotal emergencies. ***Pediatr Radiol*** 2009; 39: 516-521.
6. Asgari SA, Mokhtari G, Falahatkar S, Mansour-Ghanaei M, Roshani A, Zare A, et al. Diagnostic accuracy of C-reactive protein and erythrocyte sedimentation rate in patients with acute scrotum. ***Urol J*** 2006; 3: 104-108
7. Yücel T, Gönüllü D, Güçlü S, Sit M, Adaleti R, Tetikkurt S, et al. [The therapeutic effect of normobaric oxygen in experimental peritonitis and the efficiency of rectal fever, WBC, CRP and procalcitonin in monitoring response of the therapy]. ***Ulus Travma Acil Cerrahi Derg*** 2008; 14: 14-20. Turkish
8. Chua AP, Lee KH. Procalcitonin in severe acute respiratory syndrome (SARS). ***J Infect*** 2004; 48: 303-306.