Ischemia Modified Albumin and D-dimer in the Diagnosis of Testicular Torsion: An Experimental Model

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Purpose: We aimed to investigate the potential early diagnostic value of ischemia modified albumin (IMA) and D-dimer in testicular torsion.

Material and Methods: A total of 42 prepubertal Wistar-Hannover rats (26-30 days old, weighing 75-125 grams) were used in the study. They were randomly divided into 2 groups as torsion (21 rats) and control (21 rats). Both torsion and control groups were subdivided into three subgroups as 30th, 120th and 240th minutes. Intraperitoneal injection of 70 mg/kg ketamine (Ketalar, Pfizer, Istanbul, Turkey) plus 10 mg/kg of xylazine (Rompun, Bayer, Istanbul, Turkey) were used for general anesthesia. In the control group, scrotal incision was made and the left testis gently extracted. Then, intracardiac blood and testicular tissue were obtained at 30th, 120th and 240th minutes. In torsion group, testicular ischemia was achieved by rotating left testis 720° clockwise and maintained by fixing the testis. Blood and testicular samples were obtained at 30th, 120th and 240th minutes. All animals were sacrificed after completion of the study.

Results: There was a statistically significant difference between the IMA and D-dimer levels at 30th, 120th and 240th minutes of torsion group when compared with the control group (p = .001). When compared in terms of pathological changes at 30th, 120th and 240th minutes, significant difference was found for all 3 periods (p = 0.039, p = 0.014, p = 0.03, respectively). The D-dimer and IMA estimated torsion with reasonable accuracy [Area under the curve (AUC)= 0.771 (p = 0.003, 95% confidental interval: 0.620-0.922) and AUC = 0.706 (95% confidental interval: 0.549-0.863, p = 0.022), respectively.

Conclusion: The elevated D-dimer and IMA serum levels observed in the experimental testicular torsion model seem to have a potential role as a serum marker in the early diagnosis of testicular torsion.

Keywords: D-dimer; ischemia modified albumin; testicular torsion

INTRODUCTION

Testicular torsion (TT) occurs due to the loss of blood flow to the testis and surrounding tissues as a result of spermatic cord rotation.[1] Testicular recovery is likely if intervention is performed within the first 6 hours after the onset of symptoms.[2-4] Testicular torsion causes ischemic injury; detorsion causes reperfusion damage and they both cause structural and biochemical changes in the testis.[5-7] In case of ischemia, cellular stress factors such as hypoxia, acidosis, free radical damage and deterioration of membrane integrity change the structure of the albumin molecule. At the N-terminal end of the albumin, some changes that reduce the binding capacity of transitional metals such as copper, cobalt and nickel occur. This newly-formed damaged albumin is called "ischemia modified albumin" (IMA).[8-10] D-dimer is a degradation product of fibrin. Local fibrin formation and lysis are part of the inflammatory response and fibrin degradation products such as D-dimer, regulate the acute phase response and the production of systemic inflammatory mediators. Both markers are mainly elevated in ischemic-hypoxic and thromboembolic conditions[11-16].

Examination of testicular blood flow by color doppler ultrasonography (USG) or scintigraphy are the main diagnostic methods in the diagnosis of TT. However, these methods may not be easily accessible in every case . So, there is a need for fast laboratory tests which are practical, easily accessible and have a high diagnostic value. Some limited animal studies have shown that IMA and D-dimer can have a significant value in the diagnosis of TT.

In this experimental study, we aimed to investigate the role of serum levels of IMA and D-dimer in the early diagnosis of TT in prepubertal rats. Since TT is mostly

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Received November 2018 & Accepted April 2019

Table1. Summary of population characteristics of the rats

	Torsion group	Control group
n	21	21
Mean age days, (range)	27.9 (26-30)	28.1 (27-29)
Mean weight days, (range)	101.2 (75-125)	103,4 (75-125)

seen in pediatric age group and young adults, we preferred to use prepubertal rats.

MATERIAL AND METHODS

Study design

The present animal stud y was approved by Bezmialem Vakif University (BVU) Local Ethics Committee of the Animal Experiments (IRB number: 2018/18) and was carried out in the BVU Experimental Animal Research Laboratory. Animals used in the experiment were kept in steel cages at a room temperature of 22°C and were fed with normal water and standard food until the day of the study. Water-only diet was provided for the last 12 hours before the induction of the study. A total of 42 experimentally naïve and drug-naïve male prepubertal Wistar-Hannover rats were used in the study. They were randomly divided into 2 groups as torsion (21 rats) and control (21 rats) group. Both torsion and control groups were subdivided into three groups as 30th, 120th and 240th minutes. Intraperitoneal injection of 70 mg/kg ketamine (Ketalar, Pfizer, Istanbul, Turkey) plus 10 mg/kg of xylazine (Rompun, Bayer, Istanbul, Turkey) were used for general anesthesia. In control group, scrotal incision was made and the left testis gently extracted. Then intracardiac blood and testicular tissue were obtained at 30th, 120th and 240th minutes. In torsion group, testicular ischemia was achieved by rotating left testis 720° clockwise and maintained by fixing the testis. Blood and testicular samples were obtained at 30th, 120th and 240th minutes. All animals were sacrificed after completion of the study.

Biochemical investigations

To measure serum IMA and D-dimer levels, rat IMA ELISA kit (Catalog No. CK-E91024, Eastbiopharm., Hangzhou Eastbiopharm Co. Ltd.) and rat D-dimer (D2D) ELISA kit (Catalog No. CK-E91432, Eastbiopharm., Hangzhou Eastbiopharm Co. Ltd.) were used, respectively. Specimen absorbances were determined on a Biotek ELX800 (Biotek, Winooski, VT, USA) microplate reader at a wavelength of 450 nm. The IMA results were expressed in IU/mL and the minimum detectable level was 1 IU/L. The d-dimer results were expressed in ng/mL and the minimum detectable level was 5 ng/L.

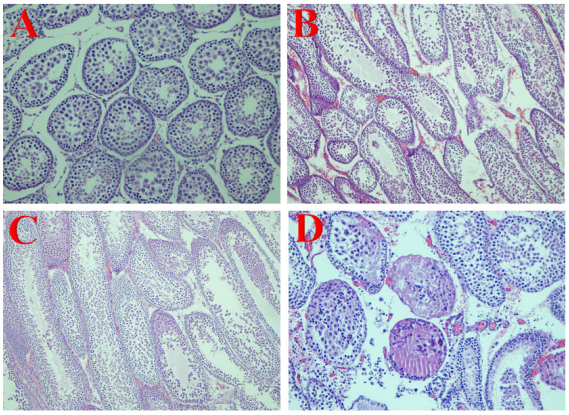


Figure 1. Histopathological findings of each stage **A.** normal testicular tissue (stage 1) **B.** less regular germ cells, irregular convergent seminiferous tubules (stage 2) **C.** irregular germ cells, diminished pycnotic nuclei and destructed bounded seminiferous tubules (stage 3) **D.** seminiferous tubules filled with irregular germ cells which have coagulation necrosis (stage 4)

Histopathological examinations

Testicular tissues were fixed in 10% formaldehyde solution and they were embedded into paraffin for follow-up procedures. Standard sections of four microns were prepared and they were stained with hematoxylin and eosin (H&E). The slides were evaluated by using a light microscope and classified according to the classification system which was designed by Cosentino et al.17:

Stage 1: Normal testicular tissue (**Figure 1.A**)

Stage 2: Less regular germ cells, irregular convergent seminiferous tubules (**Figure 1.B**)

Stage 3: Irregular germ cells, diminished pycnotic nuclei and destructed bounded seminiferous tubules (**Figure1.C**)

Stage 4: Seminiferous tubules filled with irregular germ

Table 2. Comparison of serum IMA and D-dimer levels at 30, 120 and 240 minutes between torsion and control groups

	D-Dimer			IMA		
	Control group Median (IQR) (min-max)	Torsion group Median (IQR) (min-max)	*P*[a]	Control group Median (IQR) (min-max)	Torsion groupc Median (IQR) (min-max)	*P*[a]
30 min	118.2 (34.1) (105.5-171.6)	127.7 (23.7) (113.1-160.6)	.001	32.0 (17.5) (24.7-48.0)	36.3 (6.0) (32.1-41.6)[d, e]	.001
120 min	110.1 (14.4) (94.0-234.3)	142.6 (25.7) (126.4-208.4)	.001	35.6 (12.3) (25.5-46.4)	37.2 (10.3) 9(30.3-54.5)[f]	.001
240 min	116.0 (18.3) (86.5-187.9)	149.3 (54.7) (119.6-258.5)	.001	35.5 (16.4) (22.5-47.3)	61.9 (22.4) (43.4-82.9)	.001
P[b]	.428	.967		.174	.02	

Abbreviations: IMA: Ischemia modified albümin; IQR: Interquartile range; min-max: minimum-maximum

[a] Wilcoxon test

[b] Kruskal-Wallis test

[c]Mann-Whitney *U* test was performed to test the significance of pairwise differences using Bonferroni correction (p = .05/3=.017) to adjust for multible comparisons; [d]p = .565 (comparison of 30 min and 120 min); [e] p = .002 (comparison of 30 min and 240 min); fp = .006 (comparison of 120 min and 240 min).

Table 3. The predictive characteristics of D-dimer at different cut-off values

D-Dimer (mg/dL)	Sensitivity	Specifity	PPV	NPV
118.9	90.5%	61.9%	70.4%	86.7%
123.3	81%	66.7%	70.8%	77.8%
131.4	66.7%	81%	77.8%	70.8%

Table 4. The predictive characteristics of IMA at different cut-off values

IMA (mg/dL)	Sensitivity	Specifity	PPV	NPV
35.5	81%	52.4%	63%	73.3%
35.63	76.2%	57.1%	64%	70.6%
36.84	66.7%	66.7%	66.75%	66.7%

Abbreviations: PPV: positive predictive value; NPV: negative predictive value

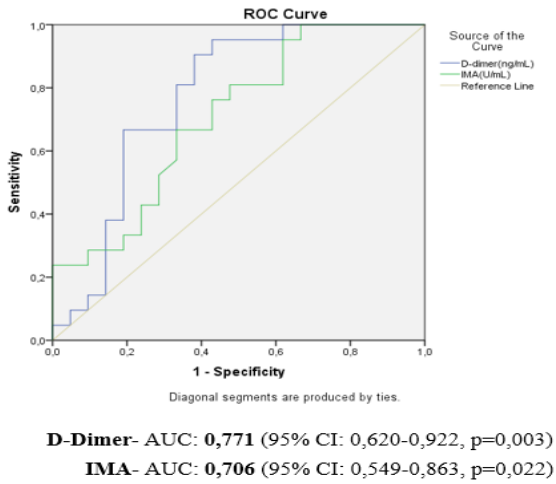
cells which have coagulation necrosis (**Figure 1.D**)

Statistical analysis

Data were analyzed by using Statistical Package for the Social Sciences software package version 16 (SPSS Inc., Chicago, IL, USA). Descriptive analyses were presented using median, interquartile range (IQR), minimum and maximum for non-normally distributed variables. The Wilcoxon test was used to compare torsion group with it's control group. More than two group comparisons were made by Kruskal Wallis test; if there was a significiant difference, Mann-Whitney U test was performed to test the significance of pairwise differences using Bonferroni correction (p = .05/30 = .017) to adjust for multiple comparisons. The comparison of torsion group with its control group in terms of pathological staging was performed with the chi-square test. Cut-off point value was determined by ROC analysis. Statistical significance was accepted as p <.05.

RESULTS

The characteristic of the rats is shown in **Table1**. Also, serum IMA and D-dimer levels of torsion and control groups are summarized in **Table 2**. There was a statistically significant difference between the IMA and D-dimer levels at 30th, 120th and 240th minutesin the torsion group when compared tothe control group (p = .001). There was a significant difference in terms of IMA levels between subgroups of the torsion group (30th vs 240th minutes and 120th vs 240th minutes, p = .002 and p = .006, respectively). However, no significant difference was detected in terms of D-dimer values (p = .174).



D-Dimer- AUC: **0,771** (95% CI: 0,620-0,922, p=0,003)
IMA- AUC: **0,706** (95% CI: 0,549-0,863, p=0,022)

Figure 2. The receiver operating characteristics (ROC) curves of D-dimer and IMA

When torsion and control groups were compared in terms of pathological changes at 30, 120 and 240 minute according to the Cosentino classification, significant difference was found for all 3 periods (p = .039, p = .014, p = .03, respectively). In the torsion group, the mean Cosentino stage was 2.6, 3.3 and 3.4 at 30, 120 and 240 minute, respectively. However, these values were between 1.1 and 2.1 in the control group.

The receiver operating characteristics (ROC) curves of both markers are shown in **Figure 2**. The D-dimer and IMA estimated torsion with reasonable accuracy [Area under the curve (AUC) = .771 (p = .003, 95% confidental interval: 0.620-0.922) and AUC = 0.706 (p = .022, 95% confidental interval: 0.549-0.863), respectively.

Sensitivity, specificity and predictive values of D-dimer and IMA are shown in detail in **Table 3 and Table 4**. At a cut-off point of 118.9 mg/dL, the D-dimer has a sensitivity of 90.5%, specificity of 61.9%, PPV of 70.4% and NPV of 86.7%. The IMA was 81% sensitive and 52.4% specific in the diagnosis of TT at a cut-off point of 35.5 mg/dL.

DISCUSSION

Viability and preservation of testis in TT is dependent on the degree and the duration oftorsion. It has been shown that 360 degrees of TT does not have an effect on fertiliy, whereas, 720 degrees and above has negative impacts on fertiliy. It has been stated that chances of testicular preservation in 6, 12 and 24 hours of TT is 90%, 50% and 10%, respectively.[6] Therefore, immediate diagnosis and treatment of TT is required in order to preserve testis and fertility.

Sensitive and specific laboratory parameters which may aid in the early diagnosis of TT are limited. Suspicion of TT generally ends up with surgical exploration of the testis. Sensitive, fast and practical biochemical markers are of importance as they would serve as adjunct to diagnosis and increase efficiency of TT management. The D-dimer and IMA assays are fast and practical laboratory tests that are routinely available in an outpatient setting via quantitative assays. Therefore, in the present animal model, we studied the D-dimer and IMA markers. Both markers are mainly elevated in ischemic-hypoxic and thromboembolic conditions. Because of fact that torsion is an ischemic condition and it creates thrombotic formations in arterial and venous vasculature, it is expected that the D-dimer and IMA levels increase in ovarian and testicular torsion.

IMA measurement has recently been proposed as a sensitive marker for the diagnosis of myocardial ischaemia. Clinical usage of IMA in pathological conditions has grown in number, with additional application in deep venous thrombosis, pulmonary thromboembolism, lower limb ischemia, cerebrovascular events and

disseminated intravascular coagulation. Also, IMA is regarded as a marker of oxidative stress related to ischaemia reperfusion in any organ, because it is found elevated in various clinical entities associated with oxidative stress such as systemic sclerosis, type-2 diabetes and polycystic ovary syndrome.[17]

In an animal torsion model study by Mentese et al., detorsion was performed 4 hours later and testicular tissues were histopathologically examined 2 hours and 2 weeks after. IMA values were found to be elevated in early and late stages. The authors stated that IMA values were valuable in evaluation of acute and long-term testicular injury and evaluation of fertility capacity. [11] In contrary to our study, the samples were obtained after TT, thus, the effect of reperfusion on histopathological results wasinevitable. In our study, we have investigated markers which can aid in early diagnosis of TT. Ischemia was performed but detorsion was not applied and effects of reperfusion was not investigated. In an experimental ovarian ischemia/reperfusion (I/R) model, IMA values were found to be higher when compared with the control group and also, positive correlation between IMA values and histopathologic results were detected in the I/R group.[12]

In an experimental testicular torsion, it was shown that an increase of D-dimer level could be detected in the blood of rats within 4 hours.[13] Other experimental studies showed that D-dimer started to increase in minutes after the onset of ischemia and reached its highest value in 6-12 hours.[14-15] All these results suggest that D-dimer can be a potential valuable marker in the early diagnosis of TT. In the present study, the predictive characteristics of the both markers (D-dimer and IMA) were satisfactory (AUC = 0.771 and AUC = 0.706, respectively; these results can be interpreted as reasonable accuracy). In the patients who had ovarian torsion, D-dimer sensitivity was detected to be 71.4% and specifity was detected to be 85% in ROC curve analysis. [14] Other than IMA and D-Dimer, some new biomarkers have been proposed in early diagnosis of TT. In a randomized, controlled, experimental study, Turedi et al studied plasma SCUBE1 (a novel marker of platelet activation) protein and they proposed that its measurement may have diagnostic, therapeutic or prognostic value in TT. (7) In a clinical study, Gunes et al investigated some hematological parameters (neutrophil / lymphocyte ratio; NLR, platelet/lymphocyte ratio (PLR), mean platelet volume (MPV), and platelet) and they claimed that NLR may be used as a predictive factor for testicular viability following TT. [3] Peretti et al. proposed that lower MPV value in "early-presenting" patients with TT playsa role in predicting testis viability.[18] Gul et al. reported that caspase-3 immunoreactivity increases in the torsion group and that melatonin and melatonin plus pulsed magnetic field (PMF) treatment reduces the rate of immuno-reactivity.[19] Despite these promising results, there is a need for further studies to routinely use these markers in clinical practice.

Conducted studies have generally focused on the ischemia/reperfusion injury and approaches to treatment. [20] Postpubertal rats were used in almost all of them. We investigated the ischemia markers on pre-pubertal rats. However, our study hadsome limitations. The major limitation was the relatively small sample size; thus, large-scale randomized experimental and clinical trials are encouraged to be designed, so that the above conclusions can be verified with an increased statistical power. Other biochemical markers were not studied in our study and this can be cited as another limitation.

CONCLUSIONS

On the basis of the findings ofthis experimental study, serum D-dimer and IMA levels are significantly higher in rats with TT compared to the control group. The elevated serum D-dimer and IMA levels seem to have a potential role as a serum marker in the early diagnosis of TT. Future investigations about biomarkers for the early diagnosis of TT should be the focus of further clinical studies.

ACKNOWLEDGEMENT

We are thankful to the Academic Studies Commitee of Haseki Training and Research Hospital for providing financial support for the expenses (including IMA ELISA kit and D-Dimer ELISA kit) associated with this study.

CONFLICTS OF INTEREST

The authors report no conflict of interest.

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