Original Article

Role of Augmenter of Liver Regeneration on Testicular Ischemia and Ischemia/Reperfusion Injury: An Experimental Study

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Abstract

Background: Testicular torsion causes ischemic injury, and torsion causes reperfusion injury. **Aim:** Evaluating the role of augmenter of liver regeneration (ALR) in testicular ischemia and ischemia/reperfusion injury. **Materials and Method(s):** Seventy-eight (78) healthy Wistar albino male rats were randomly divided into four groups; control (C) (n = 6), sham (S) (n = 24), torsion (T) (n = 24), and torsion/detorsion (T/D) (n = 24). S, T, and T/D groups were divided into four subgroups (n = 6) as 1st, 2nd, 3rd, and 4th hours. Blood, tissue ALR, and histology analyses were performed between groups and subgroups. **Results:** The increase in plasma ALR values at the 3rd and 4th hours compared to the 1st hour in the T group were significant ($P < 0.01$, $P < 0.001$, respectively). In the T/D group, a significant increase was observed in plasma ALR values at the 3rd and 4th hours compared to the 1st hour ($P < 0.05$, $P < 0.001$, respectively). Plasma ALR values at the 1st, 2nd, 3rd, and 4th hours were higher in the T and T/D groups than in the C group ($P < 0.001$, $P < 0.05$, respectively). Plasma ALR values were higher in the T group at the 1st, 2nd, 3rd, and 4th hours than in the S group ($P < 0.05$). A significant increase was observed in tissue ALR at the 3rd and 4th hours than at the 1st hour in the T group ($P < 0.05$, $P < 0.001$, respectively). A significant increase was observed in tissue ALR at the 3rd and 4th hours than in the 1st hour in the T/D group ($P < 0.05$, $P < 0.001$, respectively). **Discussion:** ALR in plasma and testicular tissue has a potential role in the early diagnosis of testicular torsion and in predicting the prognosis of T and T/D.

Keywords: *Augmenter of liver regeneration, ischemia–reperfusion injury, testicular torsion*

Received:
15-Oct-2022;
Revision:
01-Jun-2023;
Accepted:
01-Jun-2023;
Published:
03-Aug-2023

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Access this article online

Quick Response Code:

Website: www.njcponline.com

DOI: 10.4103/njcp.njcp_700_22

How to cite this article: Kavak N, Güler İ, Akcan G, Sürel AA, Güngörer B, Abatay K, *et al.* Role of augmenter of liver regeneration on testicular ischemia and ischemia/reperfusion injury: An experimental study. Niger J Clin Pract 2023;26:963-72.

Introduction

Testicular torsion, the rotation of the spermatic cord around itself, results in impaired blood supply to the testis. Impaired blood supply to the testis initiates the process leading to ischemia in the testis. Therefore, it requires urgent intervention.[1,2] Males under 25 years of age have a 1:4000 incidence of testicular torsion.[3] The testis is particularly vulnerable to ischemic injury because the blood supply is terminal, and the tunica albuginea's rigid characteristics prevent the testis from expanding to compensate for stress.[4] The survival of testicular tissue after testicular torsion varies depending on its degree and duration.[2] Testicular viability decreases from 100% to 97% at 6 hour and 0–9% after 24 hour in humans.[5,6]

Early recognition of testicular torsion is very important to save the testicles without ischemia or even atrophy and prevent serious complications such as subfertility, infertility, and testicular atrophy.[2] As long as testicular torsion continues, the testicular tissue remains ischemic, resulting in "ischemic injury." After the detorsion performed to restore blood flow to the testis, a large amount of free oxygen radicals are formed in the tissue by the sudden and excessive blood flow to the ischemic tissue, causing "reperfusion damage."[7,8] In testicular torsion, edema and bleeding occur secondary to the cessation of spermatic cord venous blood flow, and arterial blood flow is also interrupted by the gradual increase in edema, which causes ischemia at a higher rate.[9] Ischemia due to hypoxia affects the oxidative phosphorylation mechanism in the mitochondria, thus damaging adenosine triphosphate-sensitive cell membrane pumps and increasing intracellular Na^+ and Ca^{2+} ion concentrations.[10] Before this irreversible damage occurs, revascularization of the ischemic tissue is vital for the preservation of the testicular tissue. Detorsion should be performed in the early period to preserve the testicular tissue. However, the damage caused by ischemia continues in this period even though there is reperfusion as a result of detorsion. With reperfusion, the amount of reactive oxygen species (ROS) in the tissue increases with a multifactorial mechanism in which neutrophils, granulocytes, cytokines, and other adhesion molecules such as interleukin-10 work together.[7] It is known that ROS in ischemic tissue cause lipid peroxidation.[11] The main source of ROS is polymorphonuclear leukocytes. However, it cannot be ignored that the xanthine oxidase system in parenchymal cells also contributes to the activation.[12]

Under normal physiological conditions, there is a balance between constantly formed ROS and interacting antioxidants. Radicals that increase during ischemia and reperfusion are cleared by the antioxidant system. Exceeding the elimination capacity of antioxidants, ROS causes protein denaturation, lipid membrane peroxidation, protein degeneration, and DNA and cell damage.[13] Additionally, processes including inflammation and ROS stimulate apoptotic pathways, resulting in apoptosis that is exclusive to germ cells.[4]

A definitive diagnosis of testicular torsion in the preliminary period based on the history and physical examination findings is not always possible.[6,14,15] Doppler ultrasonography method cannot provide an effective result in children with immature testicular blood flow images and incomplete torsion.[16] Although studies have stated that several biomarkers could be used in diagnosis, there is still no consensus on a universally accepted biomarker.[9,17,18]

Augmenter of liver regeneration (ALR, hepatic stimulatory substance, hepatopoietin, and growth factor Erv-1-like) first described in regenerating rat livers.[19-21] ALR is also identified as a cytozyme due to its dual activity as a cytokine, implicated in liver regeneration, and an enzyme acting as a flavin-dependent sulfhydryl oxidase.[20] Furthermore, expression of ALR is promoted in numerous tissues of mammalian cells, especially in the liver and testis tissues of humans and rats at high rates.[20,22-24] ALR has recently attracted attention to be studied in experimental study models as an important protein that could be found in different organs. ALR is expressed as two isoforms: short form with a molecular weight of 15 kDa and long form with 23 kDa.[25] The short form, which is present in the nucleus or released into the extracellular media, is crucial for stimulating DNA synthesis and cell growth.[26] In addition, short-form ALR found in the cytosol has anti-apoptotic, anti-oxidative, inflammatory and metabolism regulatory effects.[25] Mitochondrial intermembrane space is the channel that allows cytoplasmic proteins to access the mitochondrial matrix and where the long forms of ALR reside.[21,25,27] This form operates as sulfhydryl oxidase in the mitochondrial disulfide–redox relay system and cytochrome c reductase and instigates Fe/S development of proteins.[23,28,29] The major subcellular target of ischemia/reperfusion damage is thought to be the mitochondrion, which constitutes the core of energy metabolism in each cell.[27] Accelerated adenosine triphosphate decreases, oxidative stress levels elevate, and necrotic and apoptotic cell death occurs as a consequence of mitochondrial ALR deficiency.[30]

Although the function of ALR is still not fully understood today, it is stated to act in a wide variety of important processes such as energy transmission, cell survival, cell regeneration, metabolic homeostasis, cancer biology, immunoregulation, cell respiration, and as defense function against apoptosis and oxidative damage.[21,22,24,30-34]

A corresponding literature search on this topic revealed that our experimental study on the effect of ALR on testicular ischemia and ischemia/reperfusion injury in rats is the first.

Methods

Animals

The Animal Ethics Board of the Technical Universal Verification Ethics Committee (Ankara, Türkiye) approved the study (date: 05.05.2022, number: 0005/2022).

Seventy-eight (78) healthy male Wistar albino rats (8 weeks old, with an average weight of 320 g) were divided randomly into four groups. Each test animal was contained separately in cages with free access to standard pellets and water. Laboratory conditions were 20–23°C temperature and 60–70% humidity with a 12 hour controlled light/dark cycle.

Experimental design and surgical procedure

Control (C) group (n = 6): No experimental procedure was performed in this group. It was used to obtain plasma and tissue ALR baseline values and histopathological parameters.

Sham (S) group (n = 24): A 2-cm incision was made to remove the left testis from the scrotal sac and then restore it. The scrotal incision was sealed with 5–0 silk thread. The S group was tested at 1st, 2nd, 3rd, and 4th hours, where the same six conditioned rats were allocated to be tested at each specific hour.

Torsion (T) group (n = 24): The left testis was made accessible to the spermatic cord and tunica vaginalis after a 2-cm scrotum incision. The removed testis was fastened to the interior of the scrotum using 5–0 silk thread after being turned 720 degrees clockwise. The T group was tested at 1st, 2nd, 3rd, and 4th hours, where the same six conditioned rats were allocated to be tested at each specific hour. Consequently, testicular torsion persisted for 1, 2, 3, and 4 h.

Torsion/detorsion (T/D) group (n = 24): The left testis was made accessible to the spermatic cord and tunica vaginalis after a 2 cm scrotum incision. The removed testis was fastened to the interior of the scrotum using 5–0 silk thread after being turned 720 degrees clockwise. T/D was tested at 1st, 2nd, 3rd and 4th hours, where the same six conditioned rats were allocated to be tested at each specific hour. Consequently, testicular torsion persisted for 1, 2, 3, and 4 hour. Afterward, the testis was exposed again and D procedures were carried out. How many hours we applied T for, we applied D for that much time such as, if T was done for one hour that means D was done for one hour as well [Figure 1].

Under aseptic conditions, intraperitoneal anesthesia was applied to all animals undergoing surgery as scrotal incisions, torsion, and detorsion procedures. Rat groups were sacrificed by decapitation, and their blood samples and left testis were acquired after the study.

Biochemical analysis

Plasma ALR level measurement

The blood sample of the subjects was mixed for 5–10 min using an Ethylenediamine tetraacetic acid (EDTA) or heparin tube and centrifuged for 20 min at 2000–3000 rpm. The plasma obtained after centrifugation was divided into Eppendorf tubes. All reagents were at room temperature before use. In this study, Cat. no E2486Ra, BT LAB, Rat Fad-linked sulfhydryl oxidase ALR and GFER ELISA kit were used in accordance with the recommendations of the manufacturer.

Based on the optical density (OD) values of the standard concentrations of the results, the standard curve regression equation was calculated. To calculate the sample concentrations, the OD data of samples were applied to the regression equation, and the results were obtained. The unit of results is expressed in ng/mL, and the measuring range of the kit was between 20 ng/L and 4500 ng/L. The lower limit of detection of the test (minimum protein concentration whose measurement can be differentiated from zero) was 0 ng/L.

Tissue ALR level measurement

Tissues were rinsed in ice-cold phosphate-buffered saline (PBS, pH 7.4) to thoroughly remove the excess blood. Tissue samples were weighed, divided into small pieces using a scalpel, and were homogenized.

The homogenates were centrifuged for 15 min at 12,000 rpm at 4°C to obtain the supernatant. The resulting supernatant was partitioned into Eppendorf tubes. All remaining procedures were performed in the same way as plasma ALR measurement. The obtained supernatant was read at 450 nm in a Biobase EL-10A model microplate reader. The obtained OD values were calculated by creating a standard curve graph.

Histological analysis

Every step was executed in accordance with institutional regulations. Fixation in 10% buffered formol for at least 48 hours. The portion of each testis tissues was divided into multiple parts and embedded in paraffin and as many parts of the testis tissue as possible were included. Next, 4 μm cuts from sections were fixed onto slides (Dako, Turkey) and deparaffinized with the application of xylenes and different grades of ethanol solutions. Hematoxylin and eosin staining was used on each section to detect spermatid presence, spermatocyte,

spermatogonia, sperm count, germinal epithelium, tubular lumen, and seminiferous tubule diameter.[35]

Testicular mutilation was measured using the histological grading system recommended by Cosentino *et al*.[36] Because all seminiferous tubules in the testis were in various stages, the sections were taken in serial sections until the entire testicular tissue was finished. Testicular tissues were evaluated histologically as well as for spermatogenic potential by adopting the Johnsen scoring system, which has 10 histological characteristics.[37]

Statistical analysis

Descriptive analysis of continuous data included the statistical mean and standard deviation, median, minimum, and maximum values. Kruskal–Wallis analysis of variance was used to compare the differences between the data of C, S, T, and T/D groups. The groups from which the difference originated were analysed using the Kruskal–Wallis analysis of variance multiple comparison test (*post hoc* test). The relationships between plasma and tissue ALR values and Cosentino and Johnsen scores were analysed using Spearman's correlation coefficient. SPSS version 20 (Chicago, IL, USA) program was for evaluations, and $P < 0.05$ was accepted as the statistical significance limit.

Results

The increase in plasma ALR values at the 3rd and 4th hours compared to the 1st hour in the T group observed to be significant ($P < 0.01$, $P < 0.001$, respectively). In the T/D group, a significant increase was observed in plasma ALR values at the 3rd and 4th hours than at the 1st hour ($P < 0.05$, $P < 0.001$, respectively). Plasma ALR values at the 1st, 2nd, 3rd, and 4th hours were higher in the T and T/D groups than in the C group ($P < 0.001$, $P < 0.05$, respectively). In addition, plasma ALR values were found to be higher in the T group at the 1st, 2nd, 3rd, and 4th hours than in the S group ($P < 0.05$) [Table 1] [Figure 2].

The increase in the T group at the 3rd and 4th hours compared to the 1st hour was significant ($P < 0.05$, $P < 0.001$, respectively). The increase in the T/D group at the 3rd and 4th hours compared to the 1st hour was significant ($P < 0.05$, $P < 0.001$, respectively). The 1st hour tissue ALR value was higher in the T group than in the C group ($P < 0.001$). Tissue ALR values in the 2nd hour T and T/D groups were higher than in the C group ($P < 0.001$, $P < 0.05$, respectively). In addition, tissue ALR values were higher in the T group than in the S group ($P < 0.05$). Tissue ALR values in the 3rd hour T and T/D groups were higher than in the C group ($P < 0.001$, $P < 0.01$, respectively). Tissue ALR values were higher in the T group than in the S group ($P < 0.05$). Tissue ALR values in the 4th hour T and T/D groups were higher than the C group ($P < 0.001$, $P < 0.05$, respectively). In addition, tissue ALR values were found to be higher in the T group than in the S group ($P < 0.05$) [Table 2] [Figure 3].

Table 1: Comparison of plasma augmenter of liver regeneration values between groups and 1st, 2nd, 3rd, and 4th hours

Plasma ALR	Mean±SD; Median (Min-Max)				*P*-value	Post hoc test
	1 hour	2 hour	3 hour	4 hour		
Control group[a]	0.04±0.02	0.04±0.02	0.04±0.02	0.04±0.02	-	
	0.14	0.14	0.14	0.14		
	(0.02-0.07)	(0.02-0.07)	(0.02-0.07)	(0.02-0.07)		
Sham group[b]	0.88±0.14	0.93±0.07	0.97±0.13	0.99±0.06	0.382	
	0.93	0.90	0.99	0.99		
	(0.64-1.0)	(0.87-1.05)	(0.78-1.12)	(0.92-1.10)		
Torsion group[c]	2.11±0.18	4.23±4.47	5.97±0.58	6.65±0.33	<0.001	1-2 p=0.849
	2.07	4.32	5.85	6.72		1-3 p=0.009**
	(1.95-2.45)	(3.58-4.75)	(5.18-6.75)	(6.10-6.98)		1-4 p<0.001***
Torsion/detorsion group[d]	1.53±0.24	3.11±0.40	4.44±0.16	4.98±0.21	<0.001	1-2 p=0.846
	1.46	3.20	4.43	5.00		1-3 p=0.019*
	(1.35-1.97)	(2.36-3.54)	(4.24-4.67)	(4.75-5.21)		1-4 p<0.001***
p	<0.001	<0.001	<0.001	<0.001		
Post hoc test	a-b p=0.844	a-b p=0.845	a-b p=0 0.844	a-b p=0.844		
	a-c p<0.001	a-c p<0.001	a-c p<0.001	a-c p<0.001		
	a-d p=0.015	a-d p=0.019	a-d p=0.019	a-d p=0.019		
	b-c p=0.025	b-c p=0.019	b-c p=0.019	b-c p=0.019		
	b-d p=0.720	b-d p=0.845	b-d p=0.844	b-d p=0.844		
	c-d p=1.000	c-d p=0.845	c-d p=0.844	c-d p=0.844		

ALR: Augmenter of Liver Regeneration, *P<0.05; **P<0.01; ***P<0.001

Table 2: Comparison of tissue augmenter of liver regeneration values between groups and 1st, 2nd, 3rd, and 4th hours

Tissue ALR	Mean±SD; Median (Min-Max)				*P*-value	Post hoc test
	1 hour	2 hour	3 hour	4 hour		
Control group[a]	0.13±0.04	0.13±0.04	0.13±0.04	0.13±0.04	-	
	0.13	0.13	0.13	0.13		
	(0.09-0.17)	(0.09-0.17)	(0.09-0.17)	(0.09-0.17)		
Sham group[b]	0.61±0.06	0.66±0.07	0.67±0.05	0.69±0.04	0.106	
	0.61	0.66	0.67	0.70		
	(0.55-0.67)	(0.58-0.78)	(0.62-0.78)	0.63-0.73)		
Torsion group[c]	0.80±0.05	1.35±0.11	2.53±0.27	3.97±0.45	<0.001	1-2 p=0.846
	0.81	1.31	2.62	3.74		1-3 p=0.019*
	(0.72-0.88)	(1.25-1.49)	(1.98-2.69)	(3.54-4.54)		1-4 p<0.001***
Torsion/detorsion group[d]	0.64±0.03	1.22±0.05	2.32±0.25	3.24±0.32	<0.001	1-2 p=0.846
	0.63	1.22	2.36	3.14		1-3 p=0.019*
	(0.61-0.70)	(1.14-1.30)	(1.87-2.61)	(2.98-3.88)		1-4 p<0.001***
p	<0.001	<0.001	<0.001	<0.001		
Post hoc test	a-b p=0.221	a-b p=0.843	a-b p=0.843	a-b p=0.843		
	a-c p<0.001	a-c p<0.001	a-c p<0.001	a-c p<0.001		
	a-d p=0.118	a-d p=0.013	a-d p=0.007	a-d p=0.011		
	b-c p=0.118	b-c p=0.028	b-c p=0.047	b-c p=0.032		
	b-d p=1.000	b-d p=0.662	b-d p=0.470	b-d p=0.609		
	c-d p=0.221	c-d p=1.000	c-d p=1.000	c-d p=1.000		

ALR: Augmenter of Liver Regeneration, *P<0.05; **P<0.01; ***P<0.001

Table 3: Comparison of Cosentino scores between groups and 1st, 2nd, 3rd, and 4th hours

Cosentino score	Mean±SD; Median (Min-Max)				*P*-value	Post hoc test
	1 hour	2 hour	3 hour	4 hour		
Control group[a]	1.08±0.07	1.08±0.07	1.08±0.07	1.08±0.07	-	
	1.08	1.08	1.08	1.08		
	(1-1.17)	(1-1.17)	(1-1.17)	(1-1.17)		
Sham group[b]	1.25±0.07	1.27±0.04	1.40±0.16	1.30±0.09	0.242	
	1.25	1.25	1.47	1.27		
	(1.17-1.33)	(1.25-1.33)	(1.13-1.55)	(1.22-1.43)		
Torsion groupc	2.88±0.30	3.21±0.07	3.32±0.10	3.46±0.21	0.005	1-2 p=1.000
	2.83	3.20	3.33	3.41		1-3 p=0.035*
	(2.58-3.25)	(3.12-3.33)	(3.12-3.42)	(3.25-3.82)		1-4 p=0.009**
Torsion/detorsion group[d]	2.19±0.15	2.42±0.07	2.66±0.29	2.88±0.50	0.003	1-2 p=0.422
	2.25	2.41	2.55	2.77		1-3 p=0.014*
	(2.00-2.33)	(2.30-2.50)	(2.42-3.08)	(2.33-3.60)		1-4 p=0.004**
p	<0.001	<0.001	<0.001	<0.001		
Post hoc test	a-b p=1.000	a-b p=0.839	a-b p=1.000	a-b p=0.847		
	a-c p<0.001	a-c p<0.001	a-c p<0.001	a-c p<0.001		
	a-d p=0.025	a-d p=0.019	a-d p=0.025	a-d p=0.009		
	b-c p=0.014	b-c p=0.019	b-c p=0.015	b-c p=0.042		
	b-d p=0.715	b-d p=0.839	b-d p=0.721	b-d p=0.516		
	c-d p=0.839	c-d p=0.839	c-d p=0.845	c-d p=1.000		

*P<0.05; **P<0.01; ***P<0.001

The increase in the Cosentino scores at the 3rd and 4th hours compared to the 1st hour in the T group was significant ($P < 0.05$, $P < 0.01$). The increase in the Cosentino scores at the 3rd and 4th hour compared to the 1st hour was significant in the T group ($P < 0.05$, $P < 0.01$). Cosentino scores in T and T/D groups at 1st, 2nd, 3rd, and 4th hours were higher than in the C and S groups ($P < 0.001$, $P < 0.05$, $P < 0.05$, respectively) [Table 3] [Figures 4 and 5].

Table 4: Comparison of Johnsen scores between groups and 1st, 2nd, 3rd, and 4th hours

Johnsen score	Mean±SD; Median (Min-Max)				*P*-value	Post hoc test
	1 hour	2 hour	3 hour	4 hour		
Control[a]	9.11±0.08	9.11±0.08	9.11±0.08	9.11±0.08	-	
	9.16	9.16	9.16	9.16		
	(9-9.17)	(9-9.17)	(9-9.17)	(9-9.17)		
Sham[b]	8.94±0.08	9.11±0.34	9.13±0.43	9.16 0.09	0.082	
	9.0	9.2	9.25	9.19		
	(8.83-9.0)	(8.5-9.5)	(8.3-9.5)	(9.0-9.25)		
Torsion group[c]	7.40±0.11	7.13±0.11	6.44±0.50	6.74±0.51	0.002	1-2 p=0.472
	7.39	7.16	6.54	6.75		1-3 p=0.002
	7.25-7.55	(7-7.25)	(5.67-7.15)	(6.17-7.42)		1-4 p=0.029
Torsion/detorsion group[d]	8.33±0.12	7.35±0.19	7.03±0.42	7.55±0.86	0.004	1-2 p=0.070
	8.25	7.37	7.08	7.52		1-3 p=0.003
	(8.25-8.50)	(7.0-7.55)	(6.25-7.50)	(6.30-8.92)		1-4 p=0.409
p	<0.001	<0.001	<0.001	<0.001		
Post hoc test	a-b p=1.000	a-b p=1.000	a-b p=1.000	a-b p=1.000		
	a-c p<0.001	a-c p=0.008	a-c p=0.013	a-c p=0.009		
	a-d p=0.030	a-d p=0.835	a-d p=0.180	a-d p=0.187		
	b-c p=0.010	b-c p<0.001	b-c p=0.001	b-c p<0.001		
	b-d p=0.589	b-d p=0.019	b-d p=0.028	b-d p=0.038		
	c-d p=0.820	c-d p=0.835	c-d p=1.000	c-d p=1.000		

*P<0.05; **P<0.01; ***P<0.001

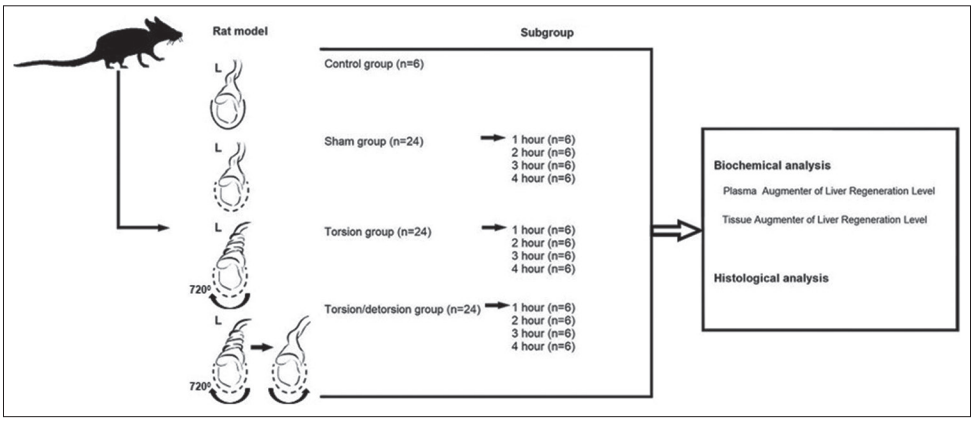

Figure 1: The study method. To obtain baseline values, six mice in the control group were studied. For the other groups, the experimental study was carried out by dividing six rats in each time period (1st, 2nd, 3rd, and 4th hours) into four groups with 24 rats in each group

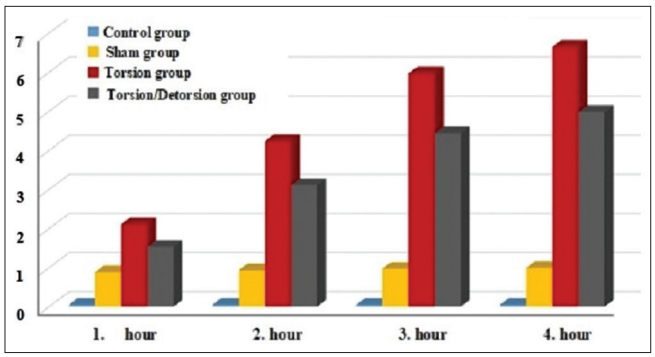

Figure 2: Comparison of plasma augmenter of liver regeneration values between groups and 1st, 2nd, 3rd, and 4th hours

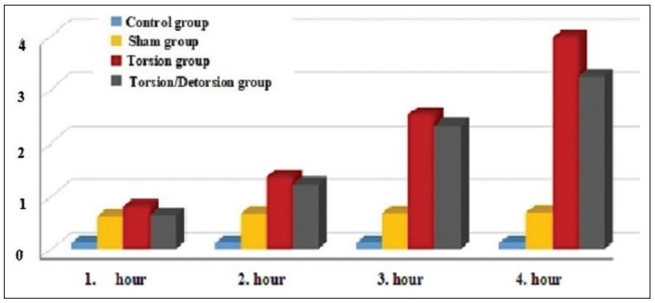

Figure 3: Comparison of tissue augmenter of liver regeneration values between groups and 1st, 2nd, 3rd, and 4th hours

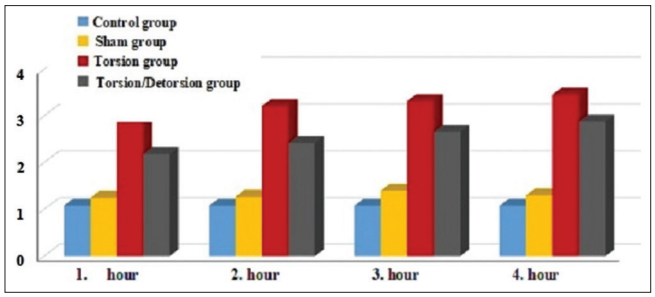

Figure 4: Comparison of Cosentino scores between groups and 1st, 2nd, 3rd, and 4th hours

In the T group, the Johnsen scores at the 3rd and 4th hours decreased significantly compared to those at the 1st hour ($P < 0.01$, $P < 0.05$, respectively). The Johnsen scores at the 3rd hour compared to the 1st hour were significantly lower ($P < 0.01$). The Johnsen scores in the 1st, 2nd, 3rd, and 4th hour T and T/D groups were lower than in the C group ($P < 0.001$, $P < 0.05$, respectively). Johnsen scores at the 1st, 2nd, 3rd, and 4th hours were found to be lower in the T group than in the S group ($P < 0.05$) [Table 4] [Figure 6].

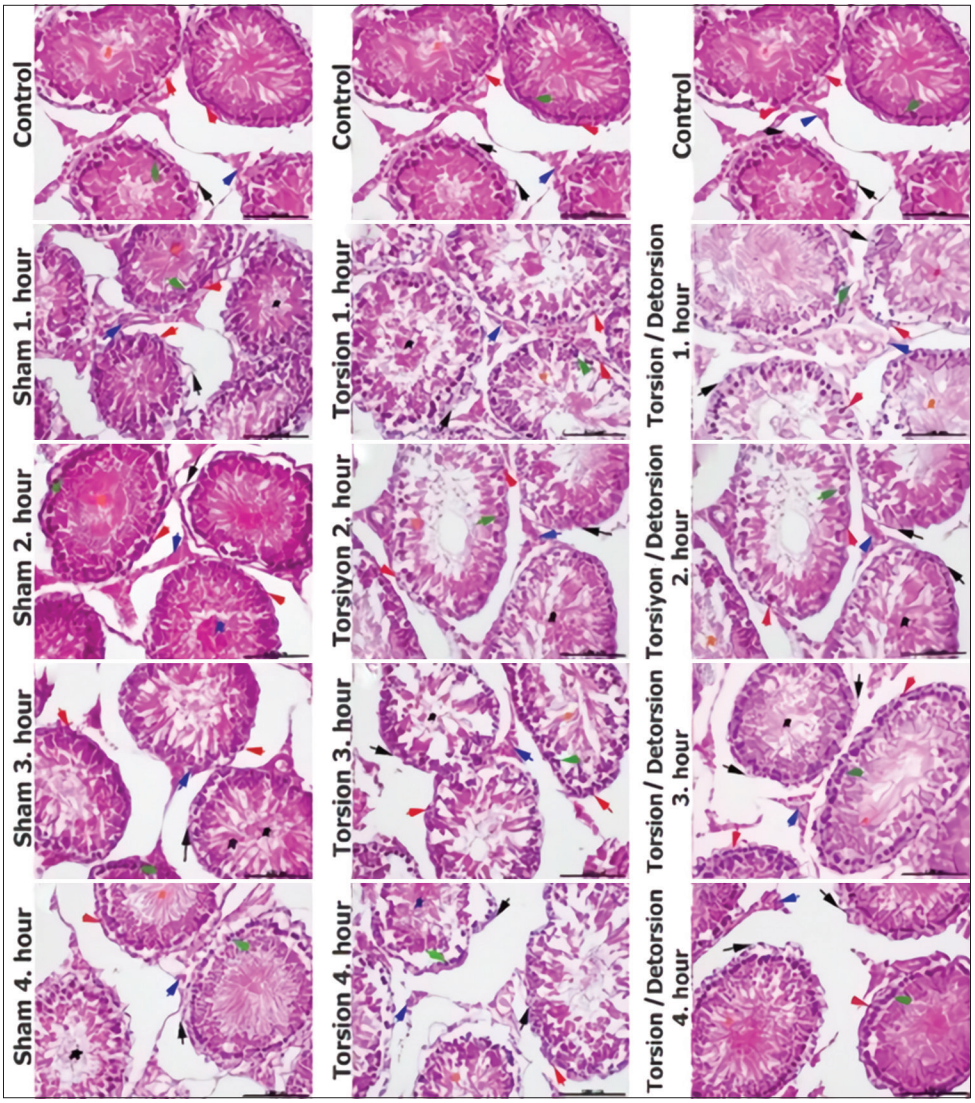

Figure 5: Irregular germinal epithelium (black arrow) and × 400:B: spermatogonia (red arrow), spermatocyte cells (green arrow), sperm (orange star), and coagulation necrosis (black star) and Leydig cells (blue arrow) within the seminiferous tubule shown. In the seminiferous tubules, the germ cells in the regular form (Cosentino Grade 1, control ALR level 0,13 ng/L, sham ALR mean level 0,95 ng/L) H and E, ×400 in control (A, F, K) and sham (B, C, D, and E) groups. In the torsion groups (G, H, I, and J), irregular germinal epithelium, spermatogonial series cells in the seminiferous tubules, decreased sperm count, and signs of coagulation necrosis were observed (Consentino Grade 3, torsion groups ALR mean level 2,63 ng/L) H and E, ×400. In the torsion and detorsion groups (L, M, N, and O), a decrease in the sperm count and evidence of coagulation necrosis were observed in the cells of the spermatogonial series (Consentino Grade 2, torsion and detorsion groups ALR mean level 1,56 ng/L) H and E, ×400

Table 5: Comparison of plasma, tissue and Cosentino and Johnsen scores

			Cosentino Score		Johnsen Score	
			*r**	*P*	*r**	*P*
Control group	1 hour	Plasma ALR	0.000	1.000	0.000	1.000
		Tissue ALR	0.000	1.000	0.000	1.000
	2 hour	Plasma ALR	0.000	1.000	0.000	1.000
		Tissue ALR	0.000	1.000	0.000	1.000
	3 hour	Plasma ALR	0.000	1.000	0.000	1.000
		Tissue ALR	0.000	1.000	0.000	1.000
	4 hour	Plasma ALR	0.000	1.000	0.000	1.000
		Tissue ALR	0.000	1.000	0.000	1.000
Sham group	1 hour	Plasma ALR	0.000	1.000	0.420	0.407
		Tissue ALR	-0.185	0.726	0.533	0.276
	2 hour	Plasma ALR	-0.315	0.543	-0.132	0.803
		Tissue ALR	0.420	0.407	0.176	0.738
	3 hour	Plasma ALR	-0.647	0.165	-0.235	0.653
		Tissue ALR	0.000	1.000	0.000	1.000
	4 hour	Plasma ALR	-0.290	0.577	-0.638	0.173
		Tissue ALR	-0.118	0.824	-0.588	0.219
Torsion group	1 hour	Plasma ALR	0.000	1.000	0.029	0.957
		Tissue ALR	-0.185	0.726	-0.254	0.628
	2 hour	Plasma ALR	-0.471	0.346	-0.359	0.485
		Tissue ALR	-0.455	0.365	0.000	1.000
	3 hour	Plasma ALR	0.093	0.862	0.143	0.787
		Tissue ALR	-0.429	0.396	0.000	1.000
	4 hour	Plasma ALR	0.203	0.700	-0.059	0.912
		Tissue ALR	0.000	1.000	0.045	0.933
Torsion/detorsion group	1 hour	Plasma ALR	-0.739	0.094	-0.853	0.031
		Tissue ALR	0.739	0.094	0.426	0.399
	2 hour	Plasma ALR	0.501	0.311	-0.319	0.538
		Tissue ALR	0.318	0.539	0.206	0.695
	3 hour	Plasma ALR	0.627	0.183	0.448	0.373
		Tissue ALR	-0.806	0.053	-0.313	0.545
	4 hour	Plasma ALR	0.147	0.781	0.265	0.612
		Tissue ALR	0.147	0.781	-0.853	0.031

ALR: Augmenter of Liver Regeneration

A negative correlation was identified between plasma ALR and Johnsen scores at 1st hour in the T/D group ($r = -0.853$, $P < 0.05$). Furthermore, a negative correlation was also identified between tissue ALR and Johnsen scores at the 4th hour in the T/D group ($r = -0.853$, $P < 0.05$) [Table 5].

Discussion

In everyday practice, particularly in the emergency department, testicular torsion is a challenging and time-sensitive diagnosis that must be determined rapidly because it can promote hypoxia and destruction of germ cells across the testicular tissue, which can result in infertility.[2]

In our study, the 720 degree rotation method of the cord in the testis, which is the most commonly used method for experimental testicular torsion, was applied.[38] In addition, a single suture fixation method was used for the fixation process to traumatize the testis less during the torsion procedure. In torsion studies on rats, the critical ischemia time is considered to be 4 hour; so, the experiment was not continued after the 4th hour.[39,40]

This is the first scientific research examining the impact of ALR on testicular ischemia and ischemia/reperfusion damage in rats, based on the conducted English literature review. In our study, it was observed that the plasma ALR level increased as the torsion time increased, and the plasma ALR level in the 3rd and 4th hour torsion groups was found to be higher than in both the S and T/D groups.

The plasma ALR values at the 1st, 2nd, 3rd, and 4th hours were found to be higher in the T and T/D groups than in the C group, and the plasma ALR values at the 1st, 2nd, 3rd, and 4th hours were realized to be higher in the T

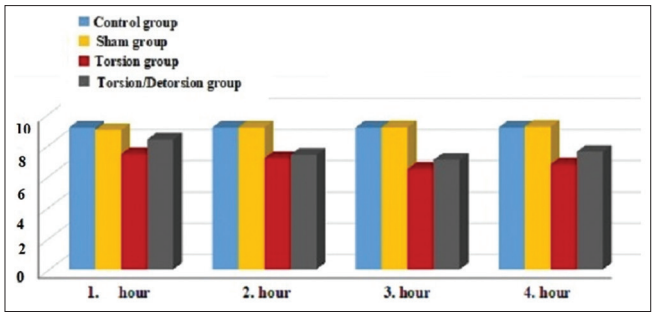

Figure 6: Comparison of Johnsen scores between groups and 1st, 2nd, 3rd, and 4th hours

group than in the S group, and the significant increase in the ALR level between the 1st and 3rd hours, depending on the time, suggests that ALR values have diagnostic potential for testicular torsion.

In our study, blood ALR levels were noticed to be statistically and significantly higher in the 3rd and 4th hour T group compared to the C group without testicular damage. The high amount of ALR found in the inner membrane of the mitochondria in the testis compared to the amount in the cytosol indicates that the isoform of the biomarker released into the blood may differ depending on the development of infarct and necrosis in the cells. More comprehensive studies are required to investigate which isoforms cause the significant heights observed in this study and determine the ALR isoform that should be checked in the 1st hour.

It was determined that the changes started in the 1st hour of torsion in the tissue, and the changes in both torsion and reperfusion damage became significant at the 3rd and 4th hours. It shows that the timing of tissue ALR level changed in both the S, T, and T/D groups and actually continued for hours. The fact that these tissue changes can be followed in the blood with a biomarker between the 1st and 4th hours shows that the early diagnosis of torsion being not fully understood yet, and the early diagnosis of ischemic injury added to each other can contribute to the clinical follow-up processes for its initial treatment.

When the histopathological results were examined, it was observed that the Cosentino scores of the T and T/D groups at the 1st, 2nd, 3rd, and 4th hours were higher than those in the C and S groups. In addition, the high Johnsen scores of the T and T/D groups are thought to be more significant with the onset of histopathological changes in the case of increased ischemia in the 3rd and 4th hours compared to those in the 1st hour. However, parameter correlations were inspected; the negative correlation between tissue ALR and Johnsen scores at the 4th hour in the T/D group indicates that ischemia shows a significant histopathological change in the torsioned testicles at the 4th hour.

Limitations

We would like to point out some limitations of this study;

1. The left testicular tissue was evaluated; however, the other testicular tissue was not examined.
2. Unknown effect of other causes of acute scrotal pain, such as acute epididymitis and orchitis, on plasma and testicular tissue ALR levels.
3. During the reperfusion injury period, evaluation could be made using biochemical parameters.

Conclusion

It is reported for the first time that ALR shows an increase in blood and tissue when testicular T and T/D occurs. ALR in plasma and testicular tissue may have a potential role in the early diagnosis of testicular torsion and in the prognosis prediction of T and T/D. Considering the experimental aspect of the data, in-depth experimental research is required to determine the sensitivity of plasma and tissue ALR in conjunction with other types of evidence, such as follow-up results from clinical cases.

Ethics committee approval

This study was approved by the Animal Ethics Committee of the Technical Universal Verification Ethics Committee (Ankara, Türkiye) (date: 05.05.2022, number: 0005/2022).

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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