

Differences of IL-1 Levels and Degree of Histopathological Injury on Contralateral Testicle of the Rat (*Rattus norvegicus* Wistar strain) Undergoing Orchidectomy and Orchidopexy due to Testicular Torsion

Contralateral Testicle of the Rat (*Rattus norvegicus* Wistar strain) Undergoing Orchidectomy and Orchidopexy due to Testicular Torsion

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ABSTRACT

Background: Testicular torsion is a growing urological emergency in the puberty age and early adulthood period. Treatment procedures include surgical detorsion and bilateral orchidopexy through trans-scrotal access, with orchidectomy indicated only for cases with unviable reperfusion after detorsion, or necrosis.

Objective: This study aimed to determine the differences of IL-1 levels and the degree of histopathological injury to the contralateral testicle in rats undergoing orchidectomy and orchidopexy due to testicular torsion.

Methods: Wistar rats were divided into four groups: negative control, testicular ischemia for 6 hours followed by orchidectomy, testicular ischemia for 6 hours without being followed by orchidectomy, reperfusion for 3 hours followed by detorsion and repositioning the testicle in the scrotum. After 24 hours, rats were observed and the effects of each procedure on the contralateral testicle are observed by looking at the IL-1 levels and histopathology of the contralateral testicle. Statistical test are performed using one-way ANOVA and Tukey test for IL-1 and Kruskal-Wallis test and correlation Lambda for histopathological injury.

Results: There were significant differences in the group without orchidectomy compared to control group and the group undergoing orchidectomy, with value of $p < 0.05$.

Conclusion: The testicle that experiencing testicular torsion would affect the damage of contralateral testicular through increased levels of interleukin-1 and increased histopathological injury.

Keywords

Testicular torsion, Orchidectomy, Orchidopexy, Histopathology, IL-1.

Introduction

Testicular torsion is one of the urological emergency that develops in puberty and early adulthood period [1]. The annual incidence

is 1 in 4000 individuals under 25 years. Treatment procedures include surgical detorsion and bilateral orchidopexy through transscrotal access, with orchidectomy indicated only for unviable cases that can not be reperfused after detorsion, or if necrosis present [2]. Various researchers examined testicular torsion from various perspectives, including ischemic effects of the testicular parenchyma, reperfusion ischemia phenomenon, apoptotic

activation and its potential in lesion extension, FAS system involvement in apoptotic induction in germinative cells, damage to the contralateral testis, and steps to avoid or decrease the torsion effect [2-6].

NF-κB is a transcription factor involved in the regulation of various genes that have implications in the inflammatory process [7]. IL-1 is a cytokine inducing the NF-κB activating, in addition to physical stress, oxidative stress, mitogenes, viruses and their products, bacteria and their products and other pathological conditions [8]. Several studies have shown the role of IL-1 as an inflammatory mediator of diseases with acute and chronic onset.

In histological studies, torsion may trigger focal lesions in seminiferous tubules, characterized by apoptosis and germinative cell turnover. This is probably due to humoral and cellular immune responses [9]. Contralateral testicular lesions due to testicular torsion are controversial. Some researchers suggest that unilateral testicular torsion causes contralateral testicular lesions while other researchers suggest otherwise [10-13]. Several proposed theories to explain the mechanism of contralateral testicular damage are autoimmune reactions, subclinical episodes of contralateral testicular torsion, release of acrosome enzymes, vasomotor reflexes, neuroendocrine responses, spermatogenesis defects, and intrinsic gonadal abnormalities [14,15].

Based on the above theory, there is a relationship between ischemia/reperfusion as the main pathophysiological of the testicular torsion with inflammation. Thus, there appears to be an association between inflammation and contralateral testicular damage. Therefore, this study aims to analyze inflammation in the testicular torsion as well as its effect on contralateral testicle.

Methods

This research was conducted by experimental laboratory post-test-only control group design using rat for the experiments. The research was conducted in Pharmacology Laboratory of Faculty of Medicine, Brawijaya University Malang in 2016. This research has been submitted to Research Ethics Committee of Faculty of Medicine Brawijaya University Malang.

Total sample size was 20 Wistar rats. Subjects were divided into four groups (negative control (K0)); group of rats with testicular ischemia for 6hr followed by orchidectomy (K1); group of rats with testicular ischemia for 6h without orchidectomy (K2); and group of rats with reperfusion for 3h followed by detorsion and repositioning of testicle in the scrotum (K 3) randomly. Then observed after 24 hours and compared the effects of each procedure on the contralateral testis by looking at IL-1 levels and histopathology of the contralateral testicle.

Testicular torsion models is made with Davenport modified method [16]. Histological evaluation using microscope is done by the pathologist blindly, randomly, and without any knowledge of the current performed research. Using the grading scale of Cosentino et al. (1986), histological injury can be calculated.

Serum IL-1 analysis was performed with Enzyme Linked Immunosorbent Assay (ELISA) technique using ELISA Kit for Interleukin-1; catalog number K797-100 (Biovision, California, USA), with unit pg/ml.

To determine the differences of IL-1 levels among 4 treatment groups using one-way ANOVA test followed by post-hoc test. The correlation between the degree of histopathological damage to the contralateral testicle and the 4 intervention groups was measured using a Lambda correlation test.

Results

The lowest mean Interleukin-1 levels was found in the negative control group (K0), whereas the highest mean Interleukin-1 levels was obtained in groups of rats with testicular ischemia for 6 hours without followed by Orchidectomy (K2) (Table 1).

Group(s)	Mean IL-1 Levels ($\bar{X} \pm SD$) Pg/ml
K0	442.588 ± 7.768
K1	443.135 ± 13.777
K2	473.399 ± 16.853
K3	450.219 ± 14.284

Table 1: Mean IL-1 Levels Data.

The results of histopathologic examination are shown in Table 2. Using the grading scale of Cosentino et al. [17] can be assessed for the degree of histological injury. Prior to testing using ANOVA, the data obtained for each treatment were analyzed for its homogeneity by using homogeneity of variance test (Levene test). In addition to the homogeneity test, the normality of the data was tested to determine whether the data tested had normal distribution or not by using Kolmogorof-Smirnov test. The tests conclude that the data were homogeneous and normally distributed, thus the test using ANOVA can be continued because both assumptions have been fulfilled.

Rat	Group			
	K0 (grade)	K1 (grade)	K2 (grade)	K3 (grade)
1	1	2	3	3
2	1	1	3	3
3	1	2	3	2
4	1	2	3	2
5	1	2	2	3

Table 2: Histopathological Examination Result of Each Interventions Group.

Analysis of histopathology result using Kruskal-Wallis test then obtained p-value of 0,002 ($p < 0,05$) showed that group difference gave significant differences to histopathology degree. Then followed by Mann-Whitney test, it was found that K0 had a significant difference with K1, K2, and K3 ($p < 0,05$). K1 has significant differences with K2 and K3 ($p < 0,05$), whereas K2 and K3 have an insignificant difference ($p > 0,05$). Lambda correlation test was used to analyze correlation between 2 intervention

groups, and it was found that there was a significant correlation between treatment group and histopathology result ($P=0,007$). The correlation coefficient obtained from the test results is 0.692 which means there is a strong correlation.

Discussion

Testicular torsion is one of the urological emergency that develops in puberty and early adulthood period [1]. The success of the treatment depends on the duration and degree of torsion [18,19]. Treatment procedures include surgical detorsion and bilateral orchidopexy through transscrotal access, with orchidectomy indicated only for unviable cases that can not be reperfused after detorsion, or when necrosis is present [2].

Interleukin-1 is a polypeptide cytokine produced in an inflammatory process with a broad spectrum of immunological activity. Several studies have shown the role of IL-1 as an inflammatory mediator of diseases with acute and chronic onset. IL-1 also plays a role in controlling lymphocytes, whereas the role of IL-1 in the inflammatory process is generally non-specific.

In histological studies, torsion may trigger focal lesions in seminiferous tubules, characterized by apoptosis and germinative cell turnover. This is probably due to humoral and cellular immune responses [9].

This experiment was conducted experimentally by using rats. Subjects were divided into four groups (negative control (K 0), group of rats with testicular ischemia for 6 hours followed by orchidectomy (K1), group of rats with testicular ischemia for 6 hours without orchidectomy (K2), and group of rats with reperfusion for 3 hours followed by the detorsion and repositioning of the testicle in the scrotum (K3) randomly. Then observed after 24 hours and compared the effects of each procedure on the contralateral testicle by looking at IL-1 levels and histopathology of the contralateral testicle.

The data obtained were analyzed statistically to enforce more support on the research results. At this type of research variables, then the comparative analysis test of one way ANOVA were performed.

The ANOVA test results with sig. F value for Interleukin-1 of 0.008. Because of Interleukin-1 parameter has a value of $p<0.05$, it means that there is a significant difference between treatments. To know more about the difference of intervention and mean value of intervention group, Post-Hoc tests analysis is performed. In group 2 (orchidectomy (-)), the highest interleukin -1 levels were obtained. The IL-1 level in group 1 (orchidectomy (+)) is the same as the control group. If the necrotic tissue is removed immediately, it will not caused a bad things, whereas in group 3 to group 1 there was a meaningless differences means the treatment of orchidectomy or orchidopexy yields the same result, in the short term, but still unknown in the long term.

Although levels of Interleukin-1 in group 3 who experienced

ischemic reperfusion injury who performed orchidopexy did not show significant differences with group 0 and group 1, histopathologically in the ischemic reperfusion injury group showed significant damage and almost resembled the neglected 2nd group. Non-orchidectomized testicle will suffer contralateral damage. An orchidectomy will save the contralateral testicle.

Lambda correlation test was used to analyze the correlation between 2 intervention groups, the result of lambda correlation test showed that there was a significant correlation between treatment group and histopathology result ($P = 0,007$). The correlation coefficient obtained from the test results of 0.692 which means there is a strong correlation strength.

The results of this analysis prove that there are differences in levels of IL-1 and the degree of histopathological injury to the contralateral testis in rats that have undergoing orchidectomy and orchidopexy due to testicular torsion. Increased levels of IL-1 in the testicular torsion affect the contralateral testis, and the action of the orchidectomy will immediately save the damage of the contralateral testicle.

So based on this research, the authors can suggest the following things in the future. (1) Urological emergency handling of testicular torsion requires precision for diagnosis and early action to prevent damage to the testicle with torsio and even the contralateral tether, (2) to design experimental model of testicular torsion against long-term contralateral testicular damage, and (3) To create testicular torsion model with other interleukin markers.

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