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Original Article

The protective effect of tadalafil on IMA (ischemia modified albumin) levels in experimental renal ischemia-reperfusion injury

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Abstract: Introduction: To investigate the effect of the tadalafil in experimental renal I/R injury and to evaluate these changes with IMA (nonspesific early biomarker of ischemia), NO and MDA levels. Materials and methods: Twenty four female Wistar rats were randomly divided into 3 groups (n=8): Group I, sham; Group II, 60 min I/R; Group III, 60 min I/R plus tadalafil. Tadalafil was administered via an orogastric tube (10 mg/kg) 24 h prior to the procedure. After ischemia of the left kidney and 1 h of reperfusion, blood samples were obtained, and the kidney was removed. Results: Statistically significant histopathologic changes were exist between groups, with the most severe injury was determined in group II in comparison to the others (X^2=21,803, P=0.000). Also mean serum IMA levels were higher in group II, but not statistically significant (19.83±7.81 U/ml, 22.26±7.14 U/ml and 19.82±7.77 U/ml, P=0.613). In addition, NO values were lower in I/R groups (P=0.049). There were no differences among the groups in terms of MDA. Conclusions: IMA may be used as a nonselective biomarker for IR injury before the occurrence of necrosis. Decreased IMA levels may indicate the nephroprotective effect of tadalafil in renal IR injury.

Keywords: Tadalafil, ischemia modified albumin, renal ischemia reperfusion injury

Introduction

Human serum albumin has the ability to bind heavy metals such as cobalt and nickel. Because of ischemia, subsequent production of reactive oxygen species disrupts its ability to bind cobalt to the N-terminal sequence of albumin and this new form is called ischemia-modified albumin (IMA) [1]. Increased IMA levels were described previously in patients with transient myocardial ischemia and it has been implicated in the detection of acute ischemia prior to necrosis [2]. IMA is a nonspecific biomarker of ischemia. In this regard, several studies have demonstrated the increased IMA levels under ischemic conditions such as coronary artery disease, peripheral arterial disease, skeletal muscle ischemia, pulmonary embolism, and stroke [3-5]. Several authors have also suggested that the generation of IMA results from contact with reactive oxygen species (ROS) [6]. Furthermore, elevated baseline levels of ROS have been found in individuals with excessive chronic oxidative stress levels, including patients with end-stage renal disease, morbid obesity, diabetes mellitus or hypercholesterolemia [7].

The phosphodiesterase type-5 (PDE5) inhibitor tadalafil has been widely used to treat erectile dysfunction as a result of its ability to inhibit the breakdown of cGMP, the second messenger of nitric oxide (NO). Ischemia reperfusion injury is associated with oxidative stress, which results from the imbalance between the production of reactive oxygen species (ROS) and antioxidants. Increasing levels of NO synthase protect against cellular injury depending on the formation of superoxide-related peroxynitrite [8]. Therefore, PDE5 inhibitors may also possess nephroprotective effects in renal I/R injury. Indeed, some studies have shown that PDE5 inhibitors can

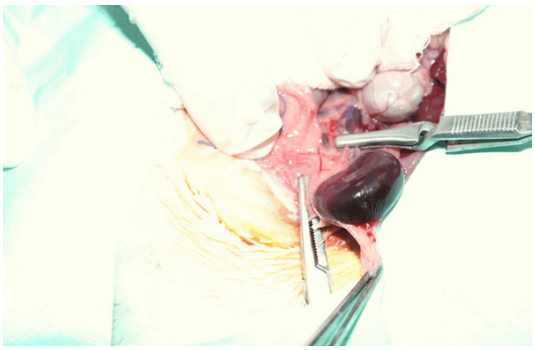

Figure 1. After midline laparotomy and exposing the kidney the renal pedicle was clamped with a bulldog in the ischemic groups (groups II and III).

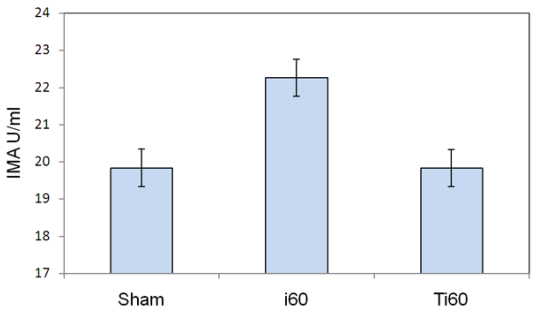

Figure 2. Mean IMA levels according to the groups. Higher IMA levels (22.26±7.14) were detected in group II. Similar values were noted in group I and III (19.83±7.81 and 19.82±7.77). However, there were no significance in Kruskal-Wallis test (P=0.613). (i60=60 minutes of ischemia/reperfusion, Ti60=60 minutes of ischemia/reperfusion + Tadalafil).

exert beneficial effects in an I/R rat model, for example, in the myocardium [9], spinal cord [10], brain [11] and kidney [12, 13].

Our aim in the present study was to evaluate increases in serum IMA levels over time in an experimental rat model of renal ischemia-reperfusion (I/R) injury. Additionally, we compared the histopathological features and IMA levels after tadalafil administration to determine whether tadalafil had an effect on IMA levels and whether it had a protective role in renal ischemia prior to necrosis.

Methods

Animals

Twenty four female Wistar albino rats with a median weight of 248 g (180-360 g) were maintained in conformity of the Guide for the Care and Use of Laboratory Animals. All experiments were performed in accordance with the guidelines for animal research from the National Institutes of Health and approved by the Adnan Menderes University Experimental Animals Ethics Committee. Before the procedure, the animals were given to standard rat food and water. The rats were kept at a constant temperature with a 12-h period of light-dark exposure. Also they were underwent an acclimation period of at least 2 weeks prior to surgery. The subjects were divided into 3 groups with 8 rats in each: Group I, sham control; Group II, 60 min I/R; Group III, 60 min I/R plus tadalafil.

Operative procedures

Preoperatively anesthesia was achieved by 7.5 mg/kg intraperitoneal xylazine (Rhompun, Abdi Ibrahim, Istanbul, Turkey) and intramuscular administration of 10 mg/kg ketamine (Ketalar, Eczacibasi, Istanbul, Turkey). Tadalafil was administered via an orogastric tube (10 mg/kg) 24 h prior to the procedure in group III. Under aseptic conditions, a 3-cm midline incision was performed. The renal pedicle was exposed, and a 3-ml blood sample was taken by cardiac puncture in the sham group. In the ischemic groups, the left renal pedicle was clamped with a bulldog using a 2.5× optical zoom (**Figure 1**). A 1-h reperfusion was performed after one hour of renal ischemia. During reperfusion period, the incision was covered with sterile gauze pads at room temperature. After cardiac blood samples were obtained, the left kidneys were removed surgically prior to sacrifice. The kidneys were stored in 10% formaldehyde for histopathology. The animals were sacrificed by drawing the intracardiac blood followed by decapitation.

Biochemical analysis

After collecting the blood samples, serum specimens were centrifuged at 4000× r.p.m. for 5 min. The supernatants were stored at -80°C in Eppendorf tubes until analysis. ELISA (Cusabio Biotech ELISA kit) was used for assessing the serum IMA levels. The results are reported as units/milliliter (U/ml). Nitric oxide (nitrite + nitrate) was assayed using a modification of the cadmium-reduction method [14]. The samples were analyzed spectrophotometrically using a microplate reader and quantified automatically against a KNO_3 standard curve. The results were expressed as μmol/l.

Table 1. Biochemical parameters of the groups

	Group I (Sham)	Group II (60 min I/R)	Group III (T+60 min I/R)	P
Mean IMA (U/ml)	19.83±7.81	22.26±7.14	19.82±7.77	0.613
Mean MDA (μmol/l)	3.96±1.04	3.98±0.61	4.18±0.54	0.814
Mean NO (μM)	18.76±4.58	12.28±5.71	13.30±4.71	0.049

The MDA (malondialdehyde) production and, hence, lipid peroxidation were assessed in the tissues according to the method described by Ohkowa [15]. MDA forms a colored complex in the presence of thiobarbituric acid, which is detectable by measuring the absorbance at 532 nm. The absorbance was measured using a Shimadzu UV-160 spectrophotometer. Tetraethoxypropane was used as a standard, and the results are expressed as μmol/l.

Histopathology

The specimens were cut into 4-μm-thick serial sections after dehydrated, embedded in paraffin blocks. Hematoxylin and eosin was used for section staining by a pathologist who was blinded to group allocation. The results were scored according to tubule deterioration, cellular vacuolization, necrosis and interstitial inflamation: Gr 0, not damaged; Gr 1, <25%; Gr 2, 25-50%; and Gr 3, >50% [16].

Statistical analysis

The Kolmogorov-Smirnov test was used to evaluate whether the distribution of continuous variables was normal. Comparisons between two groups of non-normally distributed independent variables were analyzed using the Mann-Whitney U-test. The Kruskal-Wallis test was used to compare three groups of independent variables. Descriptive statistics are presented as the mean ± Std. deviation. The *P* values less than 0.05 were considered statistically significant.

Results

The mean serum IMA values were 19.83±7.81 U/ml, 22.26±7.14 U/ml and 19.82±7.77 U/ml, in groups I, II and III, respectively (**Figure 2**). Higher levels were detected in group II, but in Kruskal-Wallis varians analysis there were no statistically significant difference between groups in terms of IMA (P=0.613).

The mean serum NO levels were 18.76±4.58 μM, 12.28±5.71 μM, 13.30±4.71 μM respectively, and were significantly higher in the sham group (Groups I) compared to the other groups (Kruskal-Wallis test, K-W=6.02, P=0.049). Mann-Whitney U test was used for pairwise comparisons and did not show any significant difference between group II and III (M-W U=29, P=0.753). However, comparison of the mean serum MDA levels with Kruskal-Wallis test showed no significant difference between the groups (P=0.814). Also, no correlation was found between variables. The biochemical variables are provided in **Table 1**.

During the experiment, no change in color was observed macroscopically in group I. However, a dark blue to violet color was detected in groups II and III. Additionally, the renal pedicle was more distinct and dilated in the tadalafil group (Group III) using a 2.5× optical zoom. Based on our microscopic assessment, the histopathology scores were significantly higher in group II, in terms of ischemic damage (X^2=21.803, P=0.000). Because of small sample size Monte Carlo method was used in chi square test. Severe tubular dilatation, tubular cell degeneration, necrosis and dilatation of Bowman's capsule have been detected in group II. Whereas tubular dilatation and cell degeneration were minimal in group III (**Figure 3**).

Discussion

Present study is the first in the literature that evaluating nephroprotective effect of tadalafil by IMA and showed that IMA may be a predictive biomarker for ischemic renal injury. Declining of IMA levels by tadalafil may also indicate a protective role of PDE5 inhibitors in renal I/R injury. Degree of ischemic renal injury depends on the duration of ischemia and to date there is no objective predictor biomarker for the irreversible renal injury.

The ischemia modified albumine (IMA) was approved as a biomarker for acute myocardial ischemia by FDA [17]. In the literature, several experimental studies have reported IMA levels under various ischemic conditions. A model of acute mesenteric ischemia in rabbits demon-

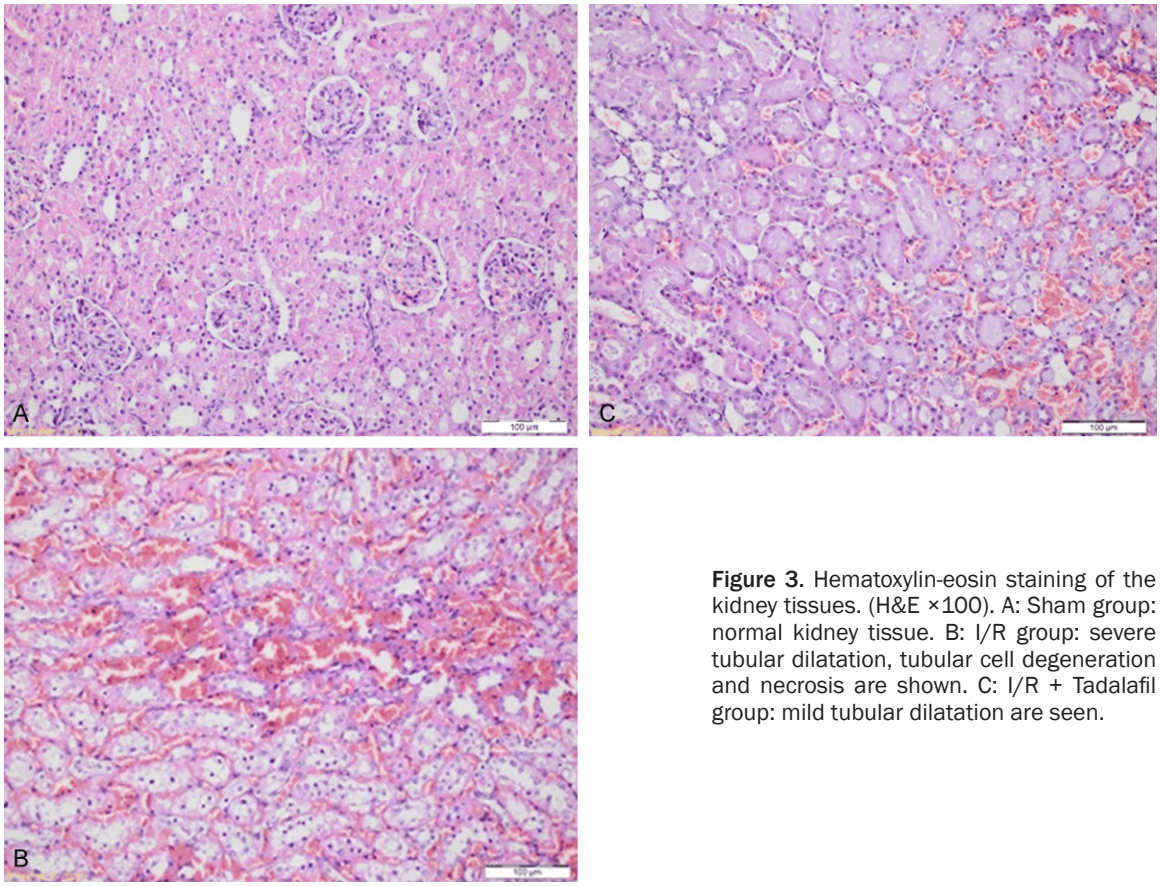

Figure 3. Hematoxylin-eosin staining of the kidney tissues. (H&E ×100). A: Sham group: normal kidney tissue. B: I/R group: severe tubular dilatation, tubular cell degeneration and necrosis are shown. C: I/R + Tadalafil group: mild tubular dilatation are seen.

strated that serum IMA levels were significantly higher after 3 and 6 h of ischemia compared to the control and sham groups [18]. Another study found that IMA levels were significantly higher in a 4-h testicular torsion group than the control groups [19]. The same researchers recently reported that IMA may be a valuable parameter in both the acute and the long-term period to estimate testicular injury in testicular torsion [20]. Increased IMA levels have also been observed in end-stage renal disease. In other supportive data, IMA levels have been strongly correlated with the levels of hemoglobin, creatinine, and lactate in chronic renal disease. Therefore, researchers pointed out the potential value of IMA in chronic renal disease in their study [21]. Furthermore, it has been reported that post-dialysis IMA levels were elevated as a result of the oxidative stress induced by the hemodialysis process [22]. Moreover, Koçan and colleagues proved the correlation of increased duration of ischemia and higher IMA levels in renal ischemia model. However reperfusion injury was not evaluated in their study [23].

It has been well known that NO has a key role in I/R injury pathogenesis [24]. To date, there is no data whether NO is associated with IMA levels in various ischemic conditions. In this regard, it is demonstrated that NO and IMA are positively correlated and higher in hypothyroid patients. The authors of that study claimed that these two oxidative stress markers may aid in clarifying hypothyroid pathogenesis and assessing the disease severity [25]. However, in the present study, we did not discover any correlation between IMA and NO. These contradictory findings can be explained by two different concentration-dependent effects of NO: at high levels, NO is converted into the highly reactive oxidant peroxynitrite; at normal levels, NO may protect cells against DNA damage by stimulating the up-regulation of the p53 gene [26].

There are some supportive data for the nephroprotective effect of PDE5 inhibitors [27-29]. Recently published study designed an experimental renal I/R injury model and found that tadalafil significantly improved kidney function and prevented adverse histological altera-

tions of the ischemic kidney [13]. In contrast, Guzeloglu and colleagues [27] demonstrated beneficial renal effects of tadalafil in an I/R rat model based on histopathology. In another study [28], pretreatment with tadalafil improved only preoperative renal function but not renal damage or kidney dysfunction. Similarly, protective effects of both tadalafil and sildenafil have been detected [29]. However, the same study showed that sildenafil provided a greater benefit for I/R injury in the rat compared to tadalafil.

After the first few minutes of reperfusion, free oxygen radicals are produced and aggravate the acute ischemic injury, leading to renal cells death. The nephroprotective mechanism of PDE5 inhibitors in I/R is not clear. However, it is hypothesized that the inhibition of PDE5, which is localized to the vasculature, mesangial cells, glomeruli, cortical tubules, and inner medullary collecting duct cells, may positively affect the renal hemodynamics by causing vasodilatation and activation of intrarenal vasoconstrictory systems that contribute to vascular congestion in the outer medulla as well as activation of tubuloglomerular feedback [13]. Previously it has shown that low intrarenal NO levels may be indicative of renal I/R injury [30]. In similar to this finding we demonstrated the lower NO levels in I/R groups which was prominent in I/R alone group. In a supporting study reported a decreased eNOS expression in the vascular muscular layer after reperfusion, that may be the leading cause of low NO levels [31]. Furthermore an experimental model described by Choi and colleagues [32] showed that eNOS, which has a protective role in I/R injury, was upregulated following pretreatment with sildenafil. In addition, they demonstrated that sildenafil significantly increased the levels of antiapoptotic protein Bcl-2 and decreased those of the proapoptotic protein Bax following I/R, supporting the antiapoptotic effect of sildenafil in renal I/R injury.

The current study has some limitations such as the restricted duration of ischemia. A longer I/R period might have resulted in distinct adverse histopathological features. Furthermore, tissue IMA, NO, MDA levels were not evaluated which was crucial to explain the nephroprotective mechanism of PDE5 inhibitors. Finally, we observed a reduction of IMA levels in the tadalafil group in comparison to the I/R alone group which was not statistically significant due to small sample size.

Conclusion

Further studies are needed to confirm whether IMA serves as an early biomarker for renal I/R injury. However, the present study demonstrated that, the rising IMA levels might be useful for the prediction of necrosis. Furthermore, the ability of tadalafil to reduce IMA levels may be indicative of a protective role in renal ischemia prior to necrosis.

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Disclosure of conflict of interest

None.

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References

[1] Wu AH, Morris DL, Fletcher DR, Apple FS, Christenson RH, Painter PC. Analysis of the albumin cobalt binding (ACB) test as an adjunct to cardiac troponin I for the early detection of acute myocardial infarction. Cardiovasc Toxicol 2001; 1: 147-151.

[2] Bar-Or D, Winkler JV, Vanbenthuysen K, Harris L, Lau E, Hetzel FW. Reduced albümin-cobalt binding with transient myocardial ischemia after elective percutaneous transluminal coronary angioplasty: A preliminary comparison to creatine kinase-MB, myoglobin, and troponin I. Am Heart J 2001; 141: 985-991.

[3] Cho DK, Choi JO, Kim SH, Choi J, Rhee I, Ki CS, Lee SC, Gwon HC. Ischemia-modified albumin is a highly sensitive serum marker of transient myocardial ischemia induced by coronary vasospasm. Coron Artery Dis 2007; 18: 83-87.

[4] Mentese A, Mentese U, Turedi S, Gunduz A, Karahan SC, Topbas M Turan A, Patan T, Turkmen S, Okur G, Eminagaoglu MS. Effect of deep vein thrombosis on ischaemia-modified albumin levels. Emerg Med J 2008; 25: 811-814.

[5] Turedi S, Patan T, Gunduz A, Mentese A, Tekinbas C, Topbas M Karahan SC, Yulug E,

Turkmen S, Ucar U. Ischemia-modified albumin in the diagnosis of pulmonary embolism: an experimental study. Am J Emerg Med 2009; 27: 635-640.

[6] Falkensammer J, Frech A, Duschek N, Gasteiger S, Stojakovic T, Scharnagl H, Huber K, Fraedrich G, Greiner A. Prognostic relevance of ischemia-modified albumin and NT-proBNP in patients with peripheral arterial occlusive disease. Clin Chim Acta 2014; 438C: 255-260.

[7] Piva SJ, Duarte MM, Da Cruz IB, Coelho AC, Moreira AP, Tonello R, Garcia SC, Moresco RN. Ischemia-modified albumin as an oxidative stress biomarker in obesity. Clin Biochem 2011; 44: 345-347.

[8] Küçük A, Yucel M, Erkasap N, Tosun M, Koken T, Ozkurt M, Erkasap S. The effects of PDE5 inhibitory drugs on renal ischemia/reperfusion injury in rats. Mol Biol Rep 2012; 39: 9775-9782.

[9] Oruc O, Inci K, Aki FT, Zeybek D, Muftuoglu SF, Kilinc K, Ergen A. Sildenafil attenuates renal ischemia reperfusion injury by decreasing leukocyte infiltration. Acta Histochem 2010; 112: 337-344.

[10] Ko IG, Shin MS, Kim BK, Kim SE, Sung YH, Kim TS, Shin MC, Cho HJ, Kim SC, Kim SH, Kim KH, Shin DH, Kim CJ. Tadalafil improves short-term memory by suppressing ischemia-induced apoptosis of hippocampal neuronal cells in gerbils. Pharmacol Biochem Behav 2009; 91: 629-635.

[11] Ockaili R, Salloum F, Hawkins J, Kukreja RC. Sildenafil (Viagra) induces powerful cardioprotective effect via opening of mito-chondrial KATP channels in rabbits. Am J Physiol Heart Circ Physio 2002; 283: H1263-H1269.

[12] Serarslan Y, Yönden Z, Ozgiray E, Oktar S, Güven EO, Söğüt S, Yilmaz N, Yurtseven T. Protective effects of tadalafil on experimental spinal cord injury in rats. J Clin Neurosci 2010; 17: 349-352.

[13] Sohotnik R, Nativ O, Abbasi A, Awad H, Frajewicki V, Bishara B, Sukhotnik I, Armaly Z, Aronson D, Heyman SN, Nativ O, Abassi Z. Phosphodiesterase-5 inhibition attenuates early renal ischemia-reperfusion-induced acute kidney injury: assessment by quantitative measurement of urinary NGAL and KIM-1. Am J Physiol Renal Physiol 2013; 304: F1099-F1104.

[14] Navarro-Gonzálvez JA, García-Benayas C, Arenas J. Semiautomated measurement of nitrate in biological fluids. Clin Chem 1998; 44: 679-681.

[15] Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979; 95: 351-358.

[16] Tabibi A, Nouralizadeh A, Parvin M, Ghoraishian M, Sadeghi P, Nafar M. An established rat model of inducing reversible acute tubular necrosis. Iran J Kidney Dis 2007; 1: 16-20.

[17] Dekker MS, Mosterd A, van't Hof AW, Hoes AW. Novel biochemical markers in suspected acute coronary syndrome: systematic review and critical appraisal. Heart 2010; 96: 1001-1010.

[18] Dundar ZD, Cander B, Gul M, Karabulut KU, Girisgin S. Serum ischemia-modified albumin levels in an experimental acute mesenteric ischemia model. Acad Emerg Med 2010; 17: 1233-1238.

[19] Kutlu O, Mentese A, Turkmen S, Turedi S, Gunduz A, Yulug E, Alver A, Karahan SC. Investigation of the possibility of using ischemia-modified albumin in testicular torsion: an experimental study. Fertil Steril 2011; 95: 1333-1337.

[20] Mentese A, Turkmen S, Karaguzel E, Karaca Y, Tatli O, Sumer AU, Yulug E, Turedi S. The predictive value of ischemia-modified albumin in Long-term results of ischemia-reperfusion injury in an experimental testicular torsion model. Urology 2012; 80: 689-694.

[21] Cichota LC, Moresco RN, Duarte MM, da Silva JE. Evaluation of ischemia-modified albumin in anemia associated to chronic kidney disease. J Clin Lab Anal 2008; 22: 1-5.

[22] Kiyici A, Mehmetoğlu I, Karaoğlan H, Atalay H, Solak Y, Türk S. Ischemia-modified albumin levels in patients with end-stage renal disease patients on hemodialysis: does albumin analysis method affect albumin-adjusted ischemia-modified albumin levels? J Clin Lab Anal 2010; 24: 273-277.

[23] Kocan H, Citgez S, Yucetas U, Yucetas E, Yazici M, Amasyali AS, Unluer E, Tasci AI. Can ischemia-modified albumin be used as an objective biomarker for renal ischemic damage? An experimental study with wistar albino rats. Transplant Proc 2014; 46: 3326-9.

[24] Senbel AM, Omar AG, Abdel-Moneim LM, Mohamed HF, Daabees TT. Evaluation of l-arginine on kidney function and vascular reactivity following ischemic injury in rats: Protective effects and potential interactions. Pharmacol Rep 2014; 66: 976-83.

[25] Dahiya K, Verma M, Dhankhar R, Singh V, Ghalaut PS, Seth S. Alteration of ischemia modified albumin and nitric oxide levels in hypothyroidism. Clin Lab 2014; 60: 969-972.

[26] Amasyali AS, Kucukgergin C, Erdem S, Sanli O, Seckin S, Nane I. Nitric oxide synthase (eNOS4a/b) gene polymorphism is associated with tumor recurrence and progression in superficial bladder cancer cases. J Urol 2012; 188: 2398-2403.

[27] Guzeloglu M, Yalcinkaya F, Atmaca S, Bagriyanik A, Oktar S, Yuksel O, Fansa I, Hazan E. The beneficial effects of tadalafil on renal isch-

emia-reperfusion injury in rats. Urol Int 2011; 86: 197-203.
[28] Faddegon S, Best SL, Olweny EO, Tan YK, Park SK, Mir SA, Cadeddu JA. Tadalafil for prevention of renal dysfunction secondary to renal ischemia. Can J Urol 2012; 19: 6274-6279.
[29] Küçük A, Yucel M, Erkasap N, Tosun M, Koken T, Ozkurt M, Erkasap S. The effects of PDE5 inhibitory drugs on renal ischemia/reperfusion injury in rats. Mol Biol Rep 2012; 39: 9775-9782.
[30] Tofangchiha S, Moazen Jamshidi SM, Emami H, Dormanesh B. Investigating Antithyroid Effects of Propylthiouracil on the Ischemia and Reperfusion Injury in Rat' Kidney and Determining the Role of Nitric Oxide in Mediating this Effect. Iran Red Crescent Med J 2014; 16: e15605.
[31] Miranda LE, Tirapelli LF, Ramos SG, Capellini VK, Celotto AC, Carlotti CG Jr, Evora PR. Nitric oxide synthase in heart and thoracic aorta after liver ischemia and reperfusion injury: an experimental study in rats. Exp Clin Transplant 2012;10: 43-8.
[32] Choi DE, Jeong JY, Lim BJ, Chung S, Chang YK, Lee SJ, Na KR, Kim SY, Shin YT, Lee KW. Pretreatment of sildenafil attenuates ischemia-reperfusion renal injury in rats. Am J Physiol Renal Physiol 2009; 297: F362-F370.