ORIGINAL ARTICLE

Urotensin-II and endothelin-I levels after contrast media administration in patients undergoing percutaneous coronary interventions

Turgay Ulas, Hakan Buyukhatipoglu[1], Mehmet S. Dal[2], Idris Kirhan, Zekeriya Kaya[3], Mehmet E. Demir[4], Irfan Tursun[5], Mehmet A. Eren, Timucin Aydogan, Yusuf Sezen[3], Nurten Aksoy[6]

Department of Internal Medicine, Harran University, Faculty of Medicine, Sanliurfa, [1]Division of Medical Oncology, Faculty of Medicine, Gaziantep University, Gaziantep, [2]Department of Internal Medicine, Faculty of Medicine, Dicle University, Diyarbakir, [3]Cardiology, Faculty of Medicine, [4]Medicine, Division of Nephrology, Faculty of Medicine, [5]Department of Internal Medicine, Igdir Training and Research Hospital, Igdir, Turkey, [6]Biochemistry, Faculty of Medicine, Harran University, Sanliurfa, Urfa

Background: Contrast induced kidney injury is an acute renal dysfunction that is secondary to the administration of radio contrast media. The purpose of this study was to evaluate the levels of urotensin-II (UT-II) and endothelin-I (ET-I) after contrast media administration in patients undergoing percutaneous coronary interventions. **Materials and Methods:** In this prospective cohort study, we evaluated 78 patients with coronary artery disease who were scheduled for and ultimately underwent percutaneous coronary interventions. As a contrast material, nonionic contrast media was used in various amounts (70-480 mL). Blood and urine samples were obtained to measure U-II, ET-I just before and at the twenty-fourth hour of percutaneous coronary interventions. **Results:** Compared to baseline, twenty-fourth hour creatinine levels were significantly increased ($P < 0.001$). The twenty-fourth hour serum and urine levels of both UT-II and ET-I were also significantly increased compared to baseline ($P < 0.001$ for all) and 24th hour serum and urine UT-II ($r = 0.322$, $P = 0.004$; $r = 0.302$, $P = 0.007$ respectively), ET-I ($r = 0.511$, $P < 0.001$; $r = 0.266$, $P = 0.019$ respectively) levels were significantly correlated with the amount of contrast media. **Conclusion:** Our study indicates that; increased UT-II and ET-I levels seem to be a consequence of hazardous effects of contrast media on blood vessels and the kidney.

Key words: Acute kidney injury, contrast media, coronary angiography, endothelin-I, urotensin-II

INTRODUCTION

Renal hemodynamics changes due to the effects of contrast media (CM) depending on the action of many mediators, and the mediators are not still clearly known. Dopamine-I, adenosine, angiotensin II, nitric oxide, and endothelin are accused of the process.[1-8] It is known that urotensin-II (UT-II) and endothelin-I (ET-I) are highly expressed in the kidney, which may be the principal site of UT-II and ET-I synthesis in humans, and also expressed from all endothelial cells.[9-12] CM injection related endothelial damage based on histopathological endpoints; leads to apoptosis, cell death of endothelial and tubular cells and may be initiated by cell membrane damage.[13] Mechanical shear stress besides physicochemical properties such as osmolality or viscosity cause endothelial damage.[14]

A reduction in renal perfusion caused by a direct effect of CM on the kidney and toxic effects on the tubular cells are generally regarded as the main factors. However, the pathophysiologic relevance of direct effects of CM on tubular cells is contentious.[15,16] Although based upon these relationships between CM, UT-II and ET-I mentioned above, no clinical research has been performed yet to investigate these mediators on kidney injury. By this way, we aimed in our study to investigate both the serum and urine levels of UT-II, ET-I after CM administration in patients undergoing percutaneous coronary intervention.

MATERIALS AND METHODS

Study design and patients

This prospective cohort study was conducted at the Harran University School of Medicine, Sanliurfa, Turkey. Prior to subject recruitment, the study protocol was reviewed and approved by the local ethics committee, in accordance with the ethical principles for human investigations (Ethical approval number: B.30-2-HRÜ.0.20.05.00.050.0 1.04-0101), as outlined by the Second Declaration of Helsinki and written informed consents were obtained from all the patients. From January-2011 to July-2011 consecutively 78 patients with coronary artery disease who were scheduled for and ultimately underwent PCI (according to prior coronary angiography results) and who had no exclusion criteria were recruited to the study.

Address for correspondence: Dr. Turgay Ulas, Department of Internal Medicine, Harran University School of Medicine, Yenisehir Campus, Sanliurfa, Turkey. E-mail: turgayulas@yahoo.com
Received: 03-08-2012; **Revised:** 09-09-2012; **Accepted:** 25-09-2012

The exclusion criteria that would influence UT-II, ET-I and renal functions were as follows: intravascular administration of iodinated CM within 7 days before study entry or a history of serious reaction to intravascular iodinated CM; the administration of theophylline, N-acetylcysteine, or mannitol within 7 days before or after contrast administration; the initiation, discontinuation, or change in dose of any of the following angiotensin-converting enzyme inhibitor, or angiotensin receptor blocker-within 72 h before study entry; initiation of nephrotoxic agents, or non-steroidal anti-inflammatory drugs within 72 h of study entry; acute coronary syndromes; any coexisting cardiac disease; any evidence of liver, kidney, or respiratory disease; diabetes mellitus; malignancy; any infectious, inflammatory, or infiltrative disorders; unregulated hypertension; reduced left ventricular ejection fraction, or any findings or history of congestive heart failure; pregnancy; lactation. Just before the PCI, blood and urine samples were obtained to measure baseline UT-II, ET-I.

As a contrast material, nonionic CM was used in various quantities (70-480 mL) depending on the clinical indications (Xenetix 300; Guerbet, Roissy, France, contains Iobitridol in 300 mg iodine/mL concentration). Adequate hydration was ensured before the procedure by advising all patients to drink at least 1500 ml of water during the preceding 24 h. In addition, just before the procedure, each patient was given 500 mL isotonic saline. Patients were also hydrated to ensure at least 2000 cc urine output after the procedure. Blood and urine samples were obtained again to measure UT-II, ET-I at 24 h.

Baseline definitions and measurements

Height and weight were measured according to standardized protocols. Body mass index was calculated as the weight in kilograms divided by the height in meters squared (kg/m^2). Blood pressure was measured using an aneroid sphygmomanometer. The average of three BP measurements was calculated after 15 min of comfortably sitting in each subject.

Biochemical analysis

All blood samples were drawn from a large antecubital vein without interruption of venous flow, using a 19-gauge butterfly needle connected to a plastic syringe. Twenty milliliters of blood were drawn, with the first few milliliters discarded. Ten milliliters were used for baseline routine laboratory tests. The residual content of the syringe was transferred immediately to polypropylene tubes, which were then centrifuged at 3000 rpm for 10 min at 10 to 18°C. Supernatant plasma samples were stored in plastic tubes at -80°C until assayed.

Measurement of UT-II and ET-I

UT-II and ET-I levels were measured by new fluorescent enzyme immunoassay (EIA) kits (Phoenix Pharmaceuticals, Burlingame, CA, USA). For the UT-II immunoreactivity assay, the cross-reactivity with human UT-II was 100%. No cross-reactivity was found with human ET-I, angiotensin II, bradykinin, neurotensin or brain natriuretic peptide. For the ET-I immunoreactivity assay, cross-reactivity with human ET-1 was 100%. No cross-reactivity was found with human angiotensin II and [Arg^8]-Vasopressin. The intra- and inter-assay coefficients of variation for both UT-II and ET-I were <10%.

Other variables

Serum urea, creatinine, fasting blood glucose, aspartate aminotransferase, alanine aminotransferase, triglycerides, total cholesterol, high-density and low-density lipoprotein cholesterol levels were determined using the commercially-available assay kits (Abbott®, Abbott Park, North Chicago, Illinois, USA) with an auto-analyzer (Abbott®, Abbott Park, North Chicago, Illinois, USA).

Statistical analysis

All statistical analyses were performed using SPSS for Windows version 17.0 (SPSS, Chicago, IL, USA). *Kolmogorov-Smirnov* test was used to test the normality of data distribution. The data were expressed as arithmetic means and standard deviations. *Paired T-Test* and *Wilcoxon signed-rank* tests were used to analyze changes within each group. *Pearson's correlation* analysis was used to examine the association of demographic and biochemical variables. A linear regression analysis was performed to identify the independent predictors of UT-II and ET-I levels. A two-sided P value < 0.05 was considered statistically significant.

RESULTS

Clinical, laboratory and demographic characteristics of all subjects were presented on Table 1. Compared to baseline, twenty-fourth hour creatinine levels were significantly increased ($P < 0.001$). The twenty-fourth hour serum and urine levels of both UT-II and ET-I were also significantly increased compared to baseline ($P < 0.001$ for all) [Table 2].

In bivariate analysis, twenty-fourth hour serum and urine UT-II (r = 0.322, $P = 0.004$; r = 0.302, $P = 0.007$ respectively), ET-I (r = 0.511, $P < 0.001$; r = 0.266, $P = 0.019$ respectively) levels were significantly correlated with the amount of CM [Table 3, Figure 1]. In a linear regression model with UT-II as a dependent variables, and the other continuous variables as an independent factors; no effect on UT-II levels were observed (r = 0.453, adjusted $r^2 = 0.205$, $P = 0.567$). In another model, in which ET-I level as a dependent variable the only CM was found to affect ET-I levels (r = 0.634, adjusted $r^2 = 0.232$, $P = 0.001$).

DISCUSSION

The present study yielded intriguing results, and the main findings were that; (i) twenty-fourth hour levels both in serum and urine samples of UT-II and ET-I were significantly increased compared to baseline, (ii) and were significantly correlated with the amount of CM.

The exact pathogenesis of contrast agent induced injury is still unclear, which is considered to arise from interactions of several major pathogenetic mechanisms. Researchers found evidence for direct renal tubular cell toxic effects of CM.[17-19] The CM induces renal vasoconstriction and subsequently causes renal medullary ischemia leading to tubular injury or even necrosis and eventually reduces the glomerular filtration rate.[20,21] This reduction may have direct cytotoxic effects due to high tissue osmolality on the renal tubules that undergo vacuolization and apoptosis and increase the local release of vasoconstrictive mediators such as ET-I, adenosine, free oxygen radicals, and calcium ions after CM administration.[8,16]

Changes of UT-II levels in the plasma and urine in patients with renal dysfunction imply a role of UT-II in renal diseases.[9,12] Plasma and urinary concentrations of UT-II are increased in essential hypertension; plasma UT-II is also increased in patients with renal dysfunction and in type II diabetics with renal nephropathy.[22,23] Nothaker *et al*. suggested that the kidney was the principal site of UT-II synthesis in humans,[24] while Matsushita *et al*. proposed that the human UT-II measured in urine was mainly derived from a renal source.[25]

Several previous reports showed that ET-I has a pivotal role in the pathogenesis of acute renal failure of various etiologies, including ischemia, CM, glycerol injection, and obstruction.[26] Namely, it has been reported that renal injury

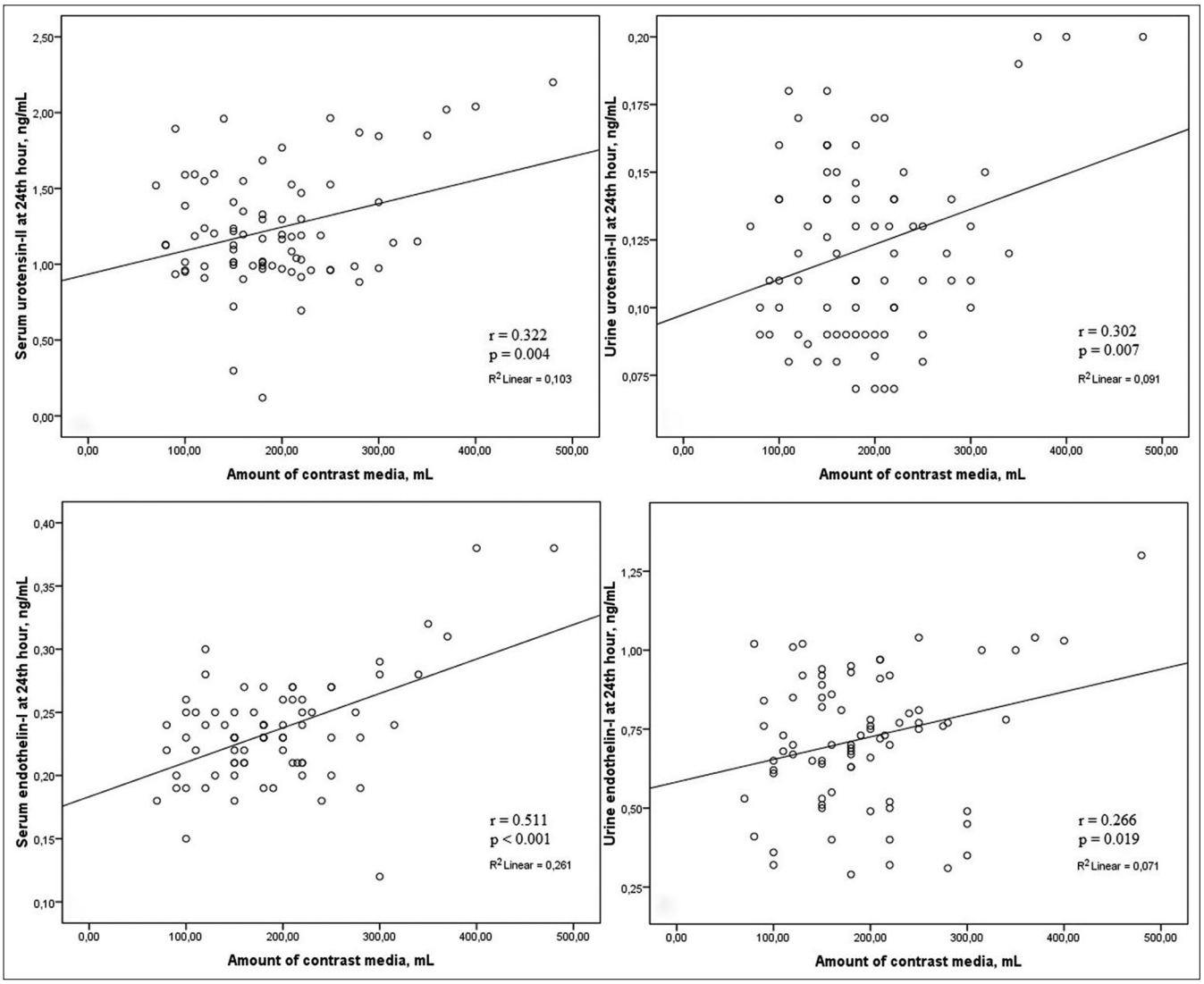

Figure 1: Relationship between twenty-fourth hour serum and urine urotensin-II, endothelin-I levels and the amount of CM

Table 1: Demographic, laboratory and clinical characteristics of the patients

Patient characteristics	Patients (*n*=78)
Gender, male/female	52/26
Age, years	60.03±10.45
BMI, kg/m^2	27.64±4.47
Systolic BP, mmHg	129.36±17.80
Diastolic BP, mmHg	78.97±10.73
Fasting blood glucose, mg/dL	102.14±19.27
Urea, mg/dL	35.87±13.29
Creatinine, mg/dL	0.76±0.12
AST, U/mL	26.06±16.66
ALT, U/mL	29.03±19.58
Total cholesterol, mg/dL	180.53±40.51
LDL cholesterol, mg/dL	110.74±34.84
Triglyceride, mg/dL	179.06±110.34
Sodium, mEq/L	139.87±3.29
Potassium, mEq/L	4.21±0.50
Calcium, mg/dL	9.51±0.89
Amount of CM, mL	191.86±78.33

All measurable values were given with mean±standard deviation; BP=blood pressure; BMI=body mass index; AST=aspartate aminotransferase; ALT=alanine aminotransferase; LDL=low density lipoprotein; CM=Contrast media

Table 2: Baseline and twenty-fourth hour comparisons of creatinine, urotensin-II and endothelin-I levels

Patients (*n*=78)	Baseline	At 24th hour	*P*
Serum UT-II, mg/mL	0.57±0.16	1.23±0.37	<0.001[a]
Serum ET-I, mg/mL	0.14±0.02	0.24±0.04	<0.001[b]
Urine UT-II, mg/mL	0.11±0.04	0.13±0.03	0.018[a]
Urine ET-I, mg/mL	0.36±0.12	0.72±0.21	<0.001[b]
Creatinine, mg/dL	0.76±0.12	0.85±0.15	<0.001[a]

All measurable values were given with mean±standard deviation; UT-II=urotensin-II; ET-I=Endothelin-I; Paired sample T[a] and Wilcoxon signed-rank[b] tests were used

Table 3: The correlation analysis of twenty-fourth hour of serum UT-II and ET-I levels

Patients (*n*=78)	Urotensin-II		Endothelin-I	
	r	*P*	r	*P*
Age, years	0.024	0.836	0.035	0.762
BMI, kg/m^2	0.055	0.631	–0.071	0.538
Systolic BP, mmHg	0.077	0.501	0.220	0.052
Diastolic BP, mmHg	0.172	0.131	0.195	0.087
Fasting blood glucose, mg/dL	–0.032	0.782	–0.054	0.636
Creatinine, mg/dL	0.071	0.539	–0.123	0.283
AST, U/mL	–0.031	0.787	–0.073	0.528
ALT, U/mL	0.050	0.667	0.068	0.553
Total cholesterol, mg/dL	–0.137	0.230	0.053	0.645
LDL cholesterol, mg/dL	–0.154	0.179	0.100	0.383
Triglyceride, mg/dL	–0.035	0.760	–0.118	0.302
Sodium, mEq/L	0.065	0.570	–0.147	0.200
Potassium, mEq/L	0.038	0.748	0.025	0.825
Calcium, mg/dL	–0.154	0.178	–0.055	0.632
Amount of CM, mL	0.322	0.004	0.511	<0.001

BP=blood pressure; BMI=body mass index; AST=aspartate aminotransferase; ALT=alanine aminotransferase; LDL=low density lipoprotein; CM=Contrast media

induces synthesis of endogenous ET-I, which then leads to continuation of its own production after the cessation of initial injury.[27] It is noteworthy that renal medullary ET-I synthesis is higher than any other body tissue and renal vasculature shows greater sensitivity to ET-I than other vascular beds in the systemic circulation.[10] An involvement of endothelin in contrast induced nephropathy appears likely due to the enhanced endothelin levels in plasma and urine, which is observed after radio contrast application.[15] In addition, the transcription and release of endothelin from endothelial cells is enhanced by CM. Moreover, in patients suffering of impaired renal function, the increase in endothelin after giving radio contrast is exaggerated.[15] Abassi ZA *et al*. reported that large amounts of ET are found in the urine compared with the small amounts present in blood and proposed that degradation of ET in the proximal tubule which filtered ET from plasma by neutral endopeptidases and that urinary ET is probably renal origin[28] would be the mechanisms for the inconsistency of serum and urine with regard to ET levels. Also Tsau YK *et al*. suggested that renal production, rather than clearance from the circulation by glomerular filtration, may be the source of urinary ET-I.[29]

However, it is important to note that only a very limited number of studies have been performed to investigate both UT-II and ET-I levels. Chai SB *et al*. suggested that increased plasma levels of UT-II and ET-I due to the injured endothelium following percutaneous transluminal coronary angioplasty.[11] In this study, baseline and twenty-fourth hour of both UT-II and ET-I levels were found to be increased compared to healthy subjects. At third day ET-I levels were found to be increased than baseline, however ET-I levels were similar with baseline levels. The UT-II and ET-I levels were found to be decreased at seventh day after the percutaneous transluminal coronary angioplasty. However, the authors have not declared the CM amount and have not correlated the amount of CM with UT-II and ET-I levels.[11] Hirose T. *et al*. investigated possible changes of the UT-II expression in cardiovascular tissues with hypertension; they examined and compared the gene expression of UT-II with ET-I, in heart, aorta and kidney of hypertensive rats in comparison with control rats and expression of UT-II gene was significantly increased in the aorta, similarly to those in the kidney in contrast to significantly decreased expression of ET-I gene.[30] In our study, we found an increased UT-II and ET-I levels both in the serum and urine after twenty-fourth hour of CM administration compared to baseline. These findings raised the possibility that, CM injures both endothelial and tubular cells, and causes increased expressions of these levels.

Certain limitations of the present study should be considered. Firstly, it is a single center study and the sample size was relatively small. Secondly, more detailed information would be gained by assessing UT-II and ET-1 levels in consecutive days the investigation would perhaps

provide deeper insight to the pathogenesis of the kidney injury and might add to the value of our manuscript.

CONCLUSION

Both UT-II and ET-I were found to be increased after the CM administration -which would be a consequence of the hazardous effects of CM on endothelial and tubular cells- and increased UT-II and ET-I might be the biochemical markers of renal injury after CM. Future large-scale prospective cohort studies are needed to confirm/exclude the findings of the present study and to elucidate the pathophysiological mechanisms of increased UT-II and ET-I levels after CM.

REFERENCES

1. Sadeghi MM, Gharipour M, Nilforoush P, Shamsolkotabi H, Sadeghi HM, Kiani A, *et al*. Influence of the timing of cardiac catheterization and amount of contrast media on acute renal failure after cardiac surgery. J Res Med Sci 2011;16:502-8.
2. Budhiraja P, Chen Z, Popovtzer M. Sodium bicarbonate versus normal saline for protection against contrast nephropathy. Ren Fail 2009;31:118-23.
3. Buyukhatipoglu H, Sezen Y, Yildiz A, Bas M, Kirhan I, Ulas T, *et al*. N-acetylcysteine fails to prevent renal dysfunction and oxidative stress after noniodine contrast media administration during percutaneous coronary interventions. Pol Arch Med Wewn 2010;120:383-9.
4. Russo D, Minutolo R, Cianciaruso B, Memoli B, Conte G, De Nicola L. Early effects of contrast media on renal hemodynamics and tubular function in chronic renal failure. J Am Soc Nephrol 1995;6:1451-8.
5. Bakris GL, Lass NA, Glock D. Renal hemodynamics in radiocontrast medium-induced renal dysfunction: A role for dopamine-1 receptors. Kidney Int 1999;56:206-10.
6. Arend LJ, Bakris GL, Burnett JC Jr, Megerian C, Spielman WS. Role for intrarenal adenosine in the renal hemodynamic response to contrast media. J Lab Clin Med 1987;110:406-11.
7. Arakawa K, Suzuki H, Naitoh M, Matsumoto A, Hayashi K, Matsuda H, *et al*. Role of adenosine in the renal responses to contrast medium. Kidney Int 1996;49:1199-206.
8. Murphy SW, Barrett BJ, Parfrey PS. Contrast nephropathy. J Am Soc Nephrol 2000;11:177-82.
9. Mori N, Hirose T, Nakayama T, Ito O, Kanazawa M, Imai Y, *et al*. Increased expression of urotensin II-related peptide and its receptor in kidney with hypertension or renal failure. Peptides 2009;30:400-8.
10. Longaretti L, Benigni A. Endothelin receptor selectivity in chronic renal failure. Eur J Clin Invest 2009;39 Suppl 2:32-7.
11. Chai SB, Li XM, Pang YZ, Qi YF, Tang CS. Increased plasma levels of endothelin-1 and urotensin-II in patients with coronary heart disease. Heart Vessels 2010;25:138-43.
12. Shimizu T, Kuroda T, Ikeda M, Hata S, Fujimoto M. Potential contribution of endothelin to renal abnormalities in glycerol-induced acute renal failure in rats. J Pharmacol Exp Ther 1998;286:977-83.
13. Sendeski MM. Pathophysiology of renal tissue damage by iodinated contrast media. Clin Exp Pharmacol Physiol 2011;38:292-9.
14. Tsuda N. *In situ* quantification of endothelial cell damage caused by iodinated contrast media using a rat vena cava model. Eur J Radiol 2012;81:879-84.
15. Persson PB, Hansell P, Liss P. Pathophysiology of contrast medium-induced nephropathy. Kidney Int 2005;68:14-22.
16. Thomsen HS, Morcos SK. Contrast media and the kidney: European Society of Urogenital Radiology (ESUR) guidelines. Br J Radiol 2003;76:513-8.
17. La Manna G, Pancaldi LG, Capecchi A, Maska E, Comai G, Cappuccilli ML, *et al*. Risk for contrast nephropathy in patients undergoing coronarography. Artif Organs 2010;34:E193-9.
18. Talner LB, Davidson AJ. Effect of contrast media on renal extraction of PAH. Invest Radiol 1968;3:301-9.
19. Humes HD, Hunt DA, White MD. Direct toxic effect of the radiocontrast agent diatrizoate on renal proximal tubule cells. Am J Physiol 1987;252:F246-55.
20. Barrett BJ. Contrast nephrotoxicity. J Am Soc Nephrol 1994;5:125-37.
21. Fung JW, Szeto CC, Yu CM. Preventing contrast nephropathy in catheter laboratory. Heart 2007;93:654-5.
22. Cheung BM, Leung R, Man YB, Wong LY. Plasma concentration of urotensin II is raised in hypertension. J Hypertens 2004;22:1341-4.
23. Totsune K, Takahashi K, Arihara Z, Sone M, Murakami O, Ito S, *et al*. Elevated plasma levels of immunoreactive urotensin II and its increased urinary excretion in patients with Type 2 diabetes mellitus: Association with progress of diabetic nephropathy. Peptides 2004;25:1809-14.
24. Nothacker HP, Wang Z, McNeill AM, Saito Y, Merten S, O'Dowd B, *et al*. Identification of the natural ligand of an orphan G-protein-coupled receptor involved in the regulation of vasoconstriction. Nat Cell Biol 1999;1:383-5.
25. Matsushita M, Shichiri M, Imai T, Iwashina M, Tanaka H, Takasu N, *et al*. Co-expression of urotensin II and its receptor (GPR14) in human cardiovascular and renal tissues. J Hypertens 2001;19:2185-90.
26. Chen CL, Fang HC, Chou KJ, Lee JC, Lee PT, Chung HM, *et al*. Increased endothelin 1 expression in adult-onset minimal change nephropathy with acute renal failure. Am J Kidney Dis 2005;45:818-25.
27. Jerkić M, Miloradović Z, Jovović D, Mihailović-Stanojević N, Elena JV, Nastić-Mirić D, *et al*. Relative roles of endothelin-1 and angiotensin II in experimental post-ischaemic acute renal failure. Nephrol Dial Transplant 2004;19:83-94.
28. Abassi ZA, Klein H, Golomb E, Keiser HR. Urinary endothelin: A possible biological marker of renal damage. Am J Hypertens 1993;6:1046-54.
29. Tsau YK, Tsai WS, Chen CH. Urinary endothelin-1 in children with acute renal failure of tubular origin. J Formos Med Assoc 1998;97:387-91.
30. Hirose T, Takahashi K, Mori N, Nakayama T, Kikuya M, Ohkubo T, *et al*. Increased expression of urotensin II, urotensin II related peptide and urotensin II receptor mRNAs in the cardiovascular organs of hypertensive rats: Comparison with endothelin-I. Peptides 2009;30:1124-9.

How to cite this article: Ulas T, Buyukhatipoglu H, Dal MS, Kirhan I, Kaya Z, Demir ME, Tursun I, *et al*. Urotensin-II and endothelin-I levels after contrast media administration in patients undergoing percutaneous coronary interventions. J Res Med Sci 2013;18:205-9.

Source of Support: Nil, **Conflict of Interest:** None declared.