

Effects of atorvastatin on biomarkers of acute kidney injury in amikacin recipients: A pilot, randomized, placebo-controlled, clinical trial

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Background: The most common clinical indication of aminoglycosides (AG) is the treatment of serious Gram-negative infections. The aim of this study was to evaluate plausible effects of atorvastatin on the biomarkers of acute kidney injury (AKI) in patients receiving amikacin. **Materials and Methods:** In this double-blinded randomized clinical trial, fifty patients (25 in each group) receiving amikacin (15 mg/kg/day) were randomly assigned to either atorvastatin (40 mg/day) or placebo (40 mg/day) groups for 7 days. Blood urea nitrogen (BUN), serum creatinine (SCr), and urinary neutrophil gelatinase-associated lipocalin (NGAL) levels were measured at days 0, 1, and 7 of amikacin treatment. **Results:** During the study period, 4 (8%) patients including two patients in each atorvastatin and placebo group experienced AKI. Urine NGAL/urine Cr did not change significantly between and within placebo and atorvastatin groups during the study period. Similarly, the mean changes in SCr, BUN, and urine NGAL/urine Cr values did not differ significantly between and within patients with and without AKI. **Conclusion:** Our data suggested that the changing pattern of urine NGAL/urine Cr ratio did not differ significantly between the atorvastatin and placebo groups during the early phase of amikacin treatment.

Key words: Acute kidney injury, amikacin, atorvastatin, biomarkers

How to cite this article: Heydari B, Khalili H, Beigmohammadi MT, Abdollahi A, Karimzadeh I. Effects of atorvastatin on biomarkers of acute kidney injury in amikacin recipients: A pilot, randomized, placebo-controlled, clinical trial. *J Res Med Sci* 2017;22:39.

INTRODUCTION

The most common clinical indication of aminoglycosides (AG) is the treatment of serious Gram-negative infections. However, following the introduction of less toxic agents with comparable efficacy, clinical application of this class of medication is limited, especially as monotherapy.^[1] The main concerns regarding the use of AG are nephrotoxicity and ototoxicity. Acute tubular necrosis is the most common complication of these agents. AG-induced acute kidney injury (AKI) has been reported in 10%–20% of patients. The proximal tubule cells in renal cortex are more

vulnerable to AKI. Following attachment to megalin, AG enters the cells by endocytosis. Mitochondrial dysfunction is the final pathway of AG-induced tubular cells ischemia and necrosis.^[2,3]

Several strategies including once-daily dosing regimen, correction of volume depletion, and electrolytes disturbances before administration of AG and antioxidants (vitamin C, vitamin E, deferoxamine, methimazole, selenium, superoxide dismutase, lipoic acid, dimethyl-sulfoxide, N-acetylcysteine, and melatonin) have been examined for the prevention of AG nephrotoxicity.^[4-9] Statins may prevent drug-induced AKI. Antioxidant, anti-inflammatory, and anti-thrombotic properties and improving endothelial function have

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Received: 27-10-2016; **Revised:** 10-12-2016; **Accepted:** 30-12-2016

been detected for statins.^[10] At least two experimental studies demonstrated that statins including atorvastatin and simvastatin prevented or reduced free radicals-induced proximal tubule cells damage caused by gentamicin.^[11,12]

Considering the limitations of serum creatinine (SCr) as a marker of kidney function in clinical practice and on the other hand, being cumbersome, costly, and not readily available direct measurement methods of Glomerular filtration rate (GFR), several new markers of renal function such as neutrophil gelatinase-associated lipocalin (NGAL) has been studied. NGAL is among the most extensively evaluated novel AKI biomarkers in different clinical settings such as cardiopulmonary bypass, diabetic nephropathy, contrast-induced nephropathy, and cisplatin nephrotoxicity.^[13]

In this randomized clinical trial, the effects of atorvastatin were compared with placebo on urine NGAL in patients received amikacin.

MATERIALS AND METHODS

This study was related to a double-blinded, randomized clinical trial (ID Number: IRCT201301283449N11) that a part of its results has been published recently.^[14] During a 1-year period from June 2013 to July 2014, the study was performed on patients hospitalized in the general Intensive Care Unit (ICU) of Imam Khomeini Hospital, a tertiary teaching hospital affiliated to Tehran University of Medical Sciences, Tehran, Iran. The patients or their responsible first-degree family signed the study consent form and Medical Ethics Committee of the hospital approved the study. Adult patients (16–65-year-old) with documented Gram-negative infection sensitive to amikacin were primarily screened for recruitment. All patients were received 15 mg/kg/day amikacin (DarouPakhsh Pharmaceutical Manufacturing Company, Iran) in two equal divided doses every 12 h as intravenous infusion over 30 min.

Patients with at least one the following characteristics were excluded from this study: (1) documented kidney dysfunction (defined as estimates GFR <60 ml/min), (2) absolute or relative contraindications to statin use including liver dysfunction (defined as serum liver enzymes levels over five times of the upper limit of normal), documented history of atorvastatin hypersensitivity, and documented history of drug-induced myopathy or creatine phosphokinase (CPK) over five times of the upper limit of normal, and (3) concomitant administration of other nephrotoxic agents (e.g., vancomycin, amphotericin b, calcineurin inhibitors) or probable nephroprotective agents (e.g., vitamin C, vitamin E, selenium, N-acetylcysteine, and melatonin).

Sample size of the current study was calculated by considering $\alpha = 0.05$, 80% power ($1-\beta = 0.8$), and data of two relevant experimental studies.^[11,12] Using simple randomization method, recruited patients were assigned to either atorvastatin or placebo groups. Patients in the atorvastatin group received 40 mg/day oral atorvastatin (Sobhan Darou, Iran) for 7 days. Individuals in the placebo group received placebo (Sobhan Darou, Iran) orally for 7 days. A 28-day follow-up period was considered for the included patients.

Required features of the study population (age, sex, concomitant diseases, drug history and cause of hospital admission, and Acute Physiologic and Chronic Health Evaluation (APACHE) score II at time of ICU admission) were extracted from their medical records. The patients' vital signs were monitored daily. In addition, relevant laboratory data including renal and liver function tests, CPK, electrolytes, and cell blood count were registered from the patients' ICU daily charts.

For measuring urinary NGAL, 10 ml of venous blood and urine samples were collected from each patient at baseline, days 1 and 7 of the treatment course. Urine samples were collected at morning from the patients' urine bags containing 12-h urine. Urine and blood samples were centrifuged at 3000 rpm for 10 min and were stored at -80°C until the time of analysis. Urinary NGAL level was measured using commercially available ELISA kit (Donghu Hi-Tech Development, P.R China). Measurement of serum as well as urine creatinine was performed by an auto-analyzer (Biotechnica BT-3000, Italy) using modified Jaffe colorimetric reaction.

AG-induced AKI was defined as a doubling of SCr from the baseline value.^[14]

Statistical analyses

Data were analyzed using the Statistical Package for the Social Sciences software version 14 (SPSS Inc., Chicago, IL, USA). Continuous data were expressed as mean \pm standard deviation (SD). Categorical variables were reported as frequency/percentages. Chi-square or Fisher's exact test (if more than 25% of the categories have expected frequencies <5) was used for comparing categorical variables between the groups. The mean changes in the patients' blood urea nitrogen (BUN), SCr, and urine NGAL at baseline, days 1 and 7 of amikacin treatment were assessed by the repeated measure analysis. $P < 0.05$ was considered as statistically significant for all the above analytical tests.

RESULTS

Initially, 67 patients were screened. Fifty-five patients met inclusion criteria of the study. However, during the

study period, five patients were dropped out because of discharge from the ward ($n = 3$) or death ($n = 2$). Finally, fifty patients (25 patients in each group) completed the study [Figure 1].

The patients' mean \pm SD of age in the atorvastatin and the placebo groups was 59 ± 18 and 54 ± 19 years, respectively ($P = 0.17$). The patients' severity of the diseases based on APACHE score II was not different at the time of admission to the ICU between two groups ($P = 0.68$). The baseline comorbidities and causes of ICU admission were comparable between the groups. In included patients, 50%, 34%, 12%, and 6% of them had respiratory, urinary, blood stream, and abdominal infections, respectively. *Acinetobacter* spp. (42%), *Klebsiella* spp. (28%), *Pseudomonas* spp. (18%), and *Enterobacter* spp. (12%) were isolated microorganisms from the study population. Based on the susceptibility results determined by disc diffusion method, amikacin plus carbapenem (56%), piperacillin-tazobactam (26%), or cefepime (10%) or amikacin alone (8%) were antibiotic

regimens given to the cohort. The type of isolated microorganism and antibiotic regimens was comparable between placebo and atorvastatin groups. Furthermore, no significant difference regarding baseline common laboratory parameters such as BUN and SCr was detected between two groups [Table 1].

Baseline urine NGAL/urine Cr ratio in the atorvastatin and the placebo group was 7.9 ± 4.94 and 6.62 ± 5.4 ng/mg, respectively ($P = 0.21$). Urine NGAL/urine Cr did not change significantly between and within placebo and atorvastatin recipients during the study period [Table 2].

During the study period, 4 (8%) patients including two patients in each atorvastatin and placebo group experienced AKI. As demonstrated in Table 3, the mean changes in SCr, BUN, and urine NGAL/urine Cr values did not differ significantly between and within patients with and without AKI. Considering the low rate of AKI in the study population (two patients in each group), evaluating the

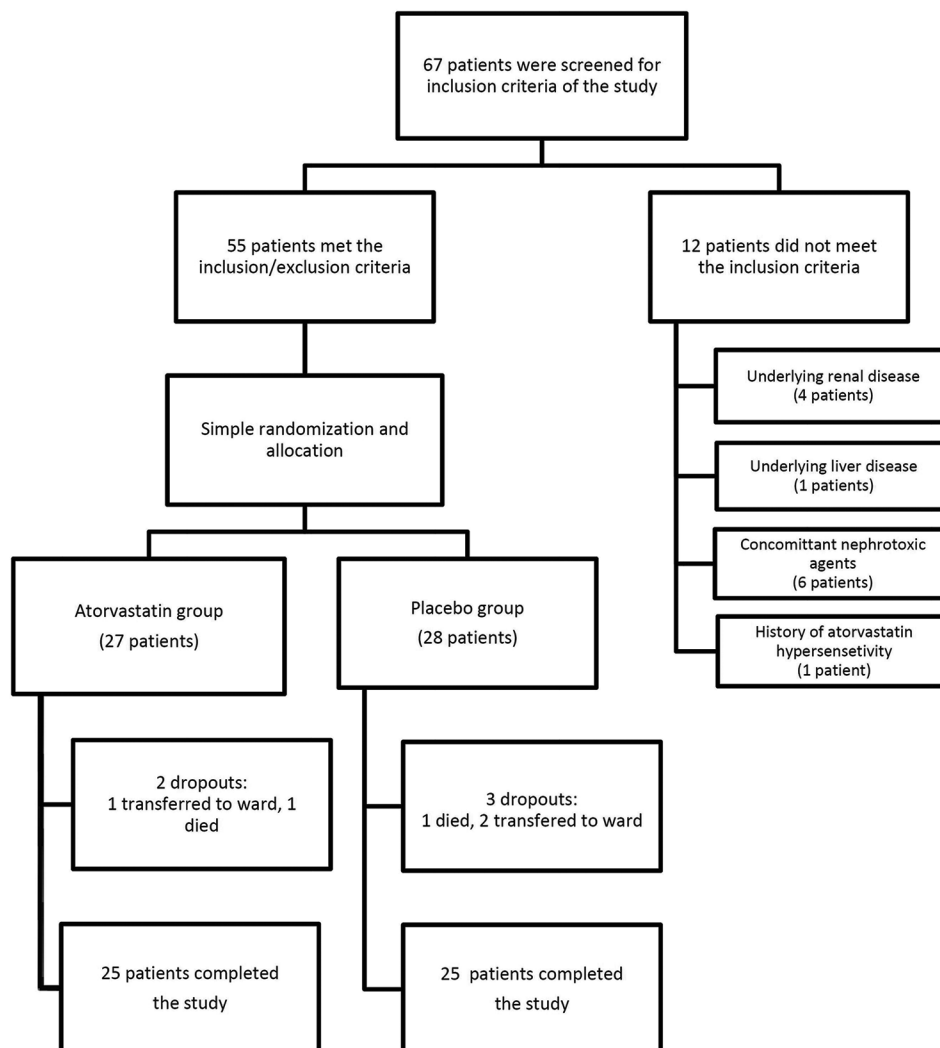


Figure 1: Consort flowchart of the study

Table 1: Demographic, clinical, and paraclinical characteristics of patients in the atorvastatin and placebo groups

Parameter	Atorvastatin group (n=25)	Placebo group (n=25)	P
Age (years), mean±SD	59±19	54±14	0.17 ^a
Gender, n (%)			
Male	15 (60)	14 (56)	0.63 ^b
Female	10 (40)	11 (44)	
APACHE II score (mean±SD)	20.3±5.3	19.7±4.4	0.68 ^a
Baseline diseases, n (%)			
Respiratory diseases	2 (8)	2 (8)	0.08 ^c
Malignancy	10 (40)	11 (44)	
Cardiovascular diseases	5 (20)	5 (20)	
Neurological disorders	1 (4)	2 (8)	
Diabetes mellitus	5 (20)	4 (16)	
Thyroid disorders	2 (8)	1 (4)	
Antibiotic regimens, n (%)			
Amikacin + meropenem	9 (36)	8 (32)	0.46 ^c
Amikacin + imipenem	6 (24)	5 (20)	
Amikacin + piperacillin-tazobactam	6 (24)	7 (28)	
Amikacin + cefepime	2 (8)	3 (12)	
Amikacin	2 (8)	2 (8)	
Concomitant drugs, n (%)			
Proton pump inhibitors	18 (72)	16 (64)	0.34 ^c
H2-receptors antagonists	12 (48)	14 (56)	
Heparin	25 (100)	25 (100)	
Vasopressors	6 (24)	5 (20)	
Inotropes	4 (16)	5 (20)	
Diuretics	2 (8)	3 (12)	
WBC (/mm ³), median (range)	10,500 (8,030-16,600)	11,300 (8,098-14,005)	0.45 ^b
Hemoglobin (g/dl)	9.77±2.45	9.72±1.89	0.92 ^a
Platelet (/mm ³), median (range)	169,600 (105,000-349,000)	179,000 (123,000-298,000)	0.79 ^d
SCr (mg/dl)	0.76±0.21	0.75±0.34	0.93 ^a
BUN (mg/dl)	36.10±15.43	36.43±20.51	0.96 ^a
ALT (IU/l)	37.52±20.83	54.96±48.87	0.12 ^a
AST (IU/l)	40.79±20.00	55.67±40.46	0.15 ^a
ALP (IU/l)	186.22±78.11	145.19±90.50	0.45 ^a
Bilirubin (total, mg/dl)	1.28±0.48	1.55±0.67	0.34 ^a
Albumin (g/dl)	3.46±2.55	3.68±3.23	0.23 ^a
CPK (IU/l)	98.38±9.47	125.18±9.47	0.37 ^a

^aIndependent t-test, ^bChi-square, ^cFisher's exact test, ^dMann-Whitney U-test. APACHE = Acute Physiology and Chronic Health Evaluation; WBC = White blood cell; SD = Standard deviation; ALT = Alanine transaminase; AST = Aspartate aminotransferase; ALP = Alkaline phosphatase; CPK = Creatine phosphokinase; BUN = Blood urea nitrogen; SCr = Serum creatinine

accuracy of studied renal biomarkers (SCr, BUN, and urine NGAL/urine Cr ratio) in detecting AG by the receiver operating characteristic curves were not statistically feasible.

DISCUSSION

AG exhibit potent *in vitro* activity against a wide range of aerobic Gram-negative pathogens, including *Enterobacteriaceae*, *Pseudomonas* spp, and *Acinetobacter* spp. Following emergent of multi-drug resistant Gram-negative infections, clinical use of AG has been increased due to low rates of resistance and limited access to effective less toxic antibiotics. However, nephrotoxicity is the major limiting factor for their clinical use.^[3]

Following hospital admission, AKI was detected in 2%–5% of patients in non-ICU wards. Prerenal azotemia due to dehydration, surgeries and drugs are the most common causes of AKI in these patients. Critically ill patients are more vulnerable to AKI due to older age, severity of baseline diseases, hemodynamic instability, infections, receiving multiple nephrotoxic agents, and mechanical ventilation.^[15] The incidence of AG nephrotoxicity in our study was 8%. Possible disparity in this rate in our survey with that reported from literature (10%–20%) may be due to variation in the definition of AKI, presence of associated risk factors (e.g., underlying renal dysfunction, concomitant nephrotoxic agents), and the type of studied AG (amikacin versus gentamicin).

Table 2: Serum creatinine, blood urea nitrogen, and urine neutrophil gelatinase-associated lipocalin/urine creatinine changes during the study period in patients received atorvastatin or placebo

Variable (mean±SD)	Atorvastatin group	Placebo group	P	
			Within groups	Between groups
BUN (mg/dl)				
Baseline	36.11±15.40	36.41±20.52	0.396	0.916
Day 1	38.13±19.62	37.16±23.12		
Day 7	40.14±26.50	39.24±23.55		
SCr (mg/dl)				
Baseline	0.76±0.22	0.76±0.35	0.879	0.960
Day 1	0.73±0.13	0.75±0.46		
Day 7	0.76±0.21	0.76±0.30		
Urine NGAL/urine creatinine ratio (ng/mg)				
Baseline	7.92±4.97	6.62±5.40	0.594	0.565
Day 1	10.14±6.80	9.64±7.19		
Day 7	11.67±4.71	9.28±6.85		

BUN = Blood urea nitrogen; NGAL = Neutrophil gelatinase-associated lipocalin; SCr = Serum creatinine; SD = Standard deviation

Table 3: Serum creatinine, blood urea nitrogen, and urine neutrophil gelatinase-associated lipocalin/urine creatinine changes during the study period in the patients with and without acute kidney injury

Parameter (mean±SD)	AKI		P	
	Yes	No	Within groups	Between groups
BUN (mg/dl)				
Baseline	36.92±16.91	37.32±18.33	0.43	0.49
Day 1	37.52±14.43	37.92±21.71		
Day 7	41.34±22.61	39.82±19.90		
SCr (mg/dl)				
Baseline	0.73±0.51	0.79±0.60	0.54	0.36
Day 1	0.73±0.36	0.78±0.52		
Day 7	1.30±0.42	0.81±0.25		
Urine NGAL/urine creatinine ratio (ng/mg)				
Baseline	8.41±7.30	10.26±6.77	0.72	0.14
Day 1	6.78±4.57	11.1±7.21		
Day 7	9.7±7.45	10.34±2.63		

AKI = Acute kidney injury; SCr = Serum creatinine; BUN = Blood urea nitrogen; NGAL = Neutrophil gelatinase-associated lipocalin; SD = Standard deviation

Amikacin accumulates in the epithelial cells of renal cortex, especially in the proximal tubule cells. Amikacin enters the cells by endocytosis through known cations transporters; megalin and cubilin.^[16] Intracellular isoprenoid pyrophosphates regulate function of these transporters. Isoprenoid pyrophosphates are metabolites of mevalonate. As it is known, 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase is the main enzyme involved in the synthesis of mevalonate. Multiligand receptor megalin is a GTP-binding protein that mediates endocytosis of AG. By inhibiting HMG-CoA reductase activity and consequently, decrease

in the intracellular isoprenoid pyrophosphates, atorvastatin may limit renal cells accumulation of AG and following their cytotoxicity.^[17]

For the first time in 2009, Ozbek *et al.* reported that the administration of atorvastatin (10 mg/kg/day) along with gentamicin (100 mg/kg/day) prevented increases in BUN and SCr, reduction in calculated creatinine clearance and renal tissue glutathione levels and elevation of kidney malondialdehyde and NO levels in rats. The authors attributed the nephroprotective effects of atorvastatin against gentamicin nephrotoxicity to the inhibition of p38-mitogen-activated protein kinase (MAPK) as well as nuclear factor kappa-β (NF-κβ) signaling pathways and inducible nitric oxide synthase (NOS) expression.^[11] In an *in vitro* study published 1 year later, nontoxic doses of simvastatin (IC₅₀ 1.3 microM), rosuvastatin (IC₅₀ 16.3 microM), and pravastatin (IC₅₀ 38.8 microM) attenuated gentamicin accumulation and cytotoxicity to renal proximal tubule cells through the inhibition of the mevalonate pathway.^[18] Finally, Jabbari *et al.* demonstrated that prophylactic administration of simvastatin (from 2 to 10 mg/kg/day) led to improvement in the histopathology and renal function tests in a dose-dependent manner in rats received low-dose (50 mg/kg/day) and high-dose (80 mg/kg/day) gentamicin probably through its antioxidant effects.^[12] Pharmacokinetic parameters of statins may influence their nephroprotective effects. Lipophilic statins such as simvastatin and atorvastatin are predominantly excreted by liver and do not produce suitable concentrations in the kidney cells. Hydrophilic statins including rosuvastatin and pravastatin may be better options for this goal.^[18]

Several mechanisms have been proposed for the nephroprotective effects of statins against drug-induced AKI. Inhibition of drug accumulation in the proximal tubular cells, anti-inflammatory and antithrombotic effects, upregulation of endothelial NOS, activation of the antioxidant defense enzymes, inhibition of MAPK and NF-κβ signaling pathways, reducing ischemia and angiotensin II-induced AKI, downregulation of angiotensin receptors, and decrease in endothelin synthesis are the main proposed pathways.^[17]

In the present study, no significant difference was detected regarding urinary NGAL/urine Cr ratio between the atorvastatin and placebo groups. Similarly, the mean changes in urine NGAL/urine Cr ratio did not differ significantly in patients with and without AG nephrotoxicity. To eliminate effects of patients' hydration status, measured urine NGAL concentration was adjusted by urine creatinine level. Urinary NGAL as a biomarker of acute renal damage was used for detecting AKI. Urine NGAL increases rapidly due to an upregulated expression and secretion in different sites of the tubule about 6 h after a renal injury.^[19,20]

In accordance to our findings, Shinke *et al.* implicated that urine NGAL to urine creatinine ratio was comparable between patients with and without AKI.^[21] Similarly, the changing pattern of urine NGAL during amphotericin b treatment demonstrated a nonsignificant increase in both patients with and without amphotericin b nephrotoxicity (unpublished data). These results were in contrast to findings of at least three other similar clinical studies in the setting of cisplatin-induced AKI.^[22-24] Shahbazi *et al.* also reported that urine NGAL to urine creatinine ratio increased significantly after cisplatin infusion.^[25] A preliminary clinical trial about the effect of ascorbic acid on colistin-associated nephrotoxicity, urinary excretion of NGAL during and at the end of colistin treatment was significantly higher than baseline values.^[26] Similar findings were observed in the setting of contrast-induced nephropathy.^[27] Finally, Najmeddin *et al.* reported that serum NGAL changes from the baseline were more in the high-dose extended-interval dosage regimen (20 mg/kg every 24 h) in comparison with the moderate-dose nonliberal-interval dosage regimen (12.5 mg/kg every 12 h) at the third ($P = 0.001$) and fifth ($P = 0.002$) day of amikacin treatment.^[28] In most clinical studies discussed above, urine and serum NGAL has increased significantly during the treatment with investigated nephrotoxic agents. Negative results in the current study can be partially justified by the limited measurement frequencies of urine NGAL, inadequate follow-up period, and relatively small sample size of the study. In addition, since the upregulation of NGAL occurs mostly in the thick ascending limb of Henle and the collecting ducts, the real role of NGAL in detection of AKI in proximal tubule, as the most common site of the injury in AKI caused by most medications such as AG, has been questioned.^[29]

The major novelty and strength of our study is determining the role of urine NGAL as a biomarker of renal function in patients receiving amikacin for the first time to the best of our knowledge. Small sample size and inadequate statistical power (due to implementing several inclusion/exclusion criteria and the preference of most intensivists to prescribe antibacterial agents rather than AG), measuring urine NGAL for only three times for each patient (due to financial problems), and considering certain SCr cut points rather than GFR calculated by an exogenous agent or formula can be taken into account as the main drawbacks of this study.

CONCLUSION

Our current data suggested that the changing pattern of urine NGAL/urine Cr ratio did not differ significantly between the atorvastatin and placebo groups during the early phase of amikacin treatment. Similarly, the mean urine NGAL/urine Cr ratio did not differ significantly

in patients with and without amikacin nephrotoxicity. Considering the limitations of this pilot study, performing an investigation with a larger sample size, more frequent and close urine sampling, longer follow-up duration, and exploiting an exogenous agent such as urinary inulin clearance or the plasma 99 mTc-DTPA for calculating GFR is needed to determine the accuracy and clinical applicability of urine NGAL as a biomarker of renal function in patients receiving AG.

Acknowledgments

This study was the result of a PhD student thesis and supported by a Vice-Chancellor for Research of Tehran University of Medical Sciences. We expressed sincere gratitude to all the nursing staff of ICU ward of Imam Khomeini hospital for their kindly supports.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

AUTHORS' CONTRIBUTION

BH contributed in conducting the study, acquisition of data, revising the draft, approval of the final version of the manuscript, and agreed for all aspects of the work. HK contributed in the conception and design of the work, revising the draft, approval of the final version of the manuscript, and agreed for all aspects of the work. MTB contributed in conducting the study, interpretation of data, revising the draft, approval of the final version of the manuscript, and agreed for all aspects of the work. AA contributed in acquisition of data, revising the draft, approval of the final version of the manuscript, and agreed for all aspects of the work. IK contributed in the analysis of data, revising the draft, approval of the final version of the manuscript, and agreed for all aspects of the work.

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