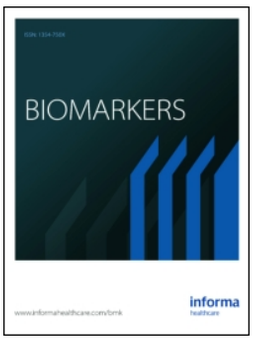

Biomarkers

ISSN: 1354-750X (Print) 1366-5804 (Online) Journal homepage: http://www.tandfonline.com/loi/ibmk20

N-acetylcysteine decreases urinary level of neutrophil gelatinase-associated lipocalin in deceased-donor renal transplant recipients: a randomized clinical trial

Atieh Modarresi, Mohsen Nafar, Zahra Sahraei, Jamshid Salamzadeh, Samira Chaibakhsh, Shadi Ziaie, Mahmoud Parvin, Yunes Panahi & Behzad Einollahi

To cite this article: Atieh Modarresi, Mohsen Nafar, Zahra Sahraei, Jamshid Salamzadeh, Samira Chaibakhsh, Shadi Ziaie, Mahmoud Parvin, Yunes Panahi & Behzad Einollahi (2018): N-acetylcysteine decreases urinary level of neutrophil gelatinase-associated lipocalin in deceased-donor renal transplant recipients: a randomized clinical trial, Biomarkers, DOI: 10.1080/1354750X.2018.1468823

To link to this article: https://doi.org/10.1080/1354750X.2018.1468823

Accepted author version posted online: 23 Apr 2018.
Published online: 04 May 2018.

BIOMARKERS
https://doi.org/10.1080/1354750X.2018.1468823

Taylor & Francis
Taylor & Francis Group

RESEARCH ARTICLE

N-acetylcysteine decreases urinary level of neutrophil gelatinase-associated lipocalin in deceased-donor renal transplant recipients: a randomized clinical trial

Atieh Modarresi[a], Mohsen Nafar[b], Zahra Sahraei[c], Jamshid Salamzadeh[c]#, Samira Chaibakhsh[a,d]#, Shadi Ziaie[c], Mahmoud Parvin[b], Yunes Panahi[e] and Behzad Einollahi[f]

[a]Research Center for Rational Use of Drugs, Tehran University of Medical Sciences, Tehran, Iran; [b]Urology and Nephrology Research Center, Shahid Labbafinejad Medical Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran; [c]Department of Clinical Pharmacy, School of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran; [d]Department of Biostatistics, Shahid Beheshti University of Medical Sciences, Tehran, Iran; [e]Pharmacotherapy Department, Faculty of Pharmacy, Baqiyatollah University of Medical Sciences, Tehran, Iran; [f]Nephrology and Urology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran

ABSTRACT

Context: Acute kidney injury (AKI) is a common complication after kidney transplantation (KT), especially in recipients from deceased donors. Urinary neutrophil gelatinase-associated lipocalin (u-NGAL) is an early and sensitive marker of AKI after transplantation.

Objectives: We assessed the renoprotective effect of N-acetylcysteine (NAC) on u-NGAL levels as an early prognostic marker of graft function immediately after transplantation.

Materials and methods: A double-blind, randomized, placebo-controlled trial was conducted on 70 deceased-donor KT recipients (www.irct.ir, trial registration number: IRCT2014090214693N4). Patients received 600 mg oral NAC or placebo twice daily from day 0 to 5 and urine samples were taken before, and on the first and fifth days after transplantation. U-NGAL and early graft function were compared between the two groups.

Results: NAC significantly reduced u-NGAL levels compared to placebo (p value = 0.02), while improvement in early graft function with NAC did not reach statistical significance.

Conclusions: This study showed that NAC administration in deceased-donor KT recipients can reduce tubular kidney injury, evidenced by u-NGAL measurements. Improvement in early graft function needs a larger sample size to reach a statistical conclusion.

ARTICLE HISTORY
Received 28 August 2017
Revised 19 February 2018
Accepted 20 April 2018

KEYWORDS
Neutrophil gelatinase-associated lipocalin; transplantation; deceased donor; N-acetylcysteine; biomarker; acute kidney injury

Introduction

Acute kidney injury (AKI) is a common complication after kidney transplantation (KT), especially in recipients from deceased donors (Ramirez-Sandoval *et al.* 2014). Reactive oxygen species generated during both phases of ischemia and reperfusion (I/R), play a major role in allograft damage (Nafar *et al.* 2011). The proximal renal tubules are the primary site of I/R injury as they are marginally oxygenated with a high basal metabolic demand (Kumar *et al.* 2016). Delayed graft function (DGF) is a manifestation of AKI caused by I/R injury in the immediate post-transplantation period (Lacquaniti *et al.* 2016). Since both short- and long-term outcomes of KT are influenced by DGF, early diagnosis would help clinicians to optimize postoperative care and avoid nephrotoxic medications (Yarlagadda *et al.* 2009, Siedlecki *et al.* 2011).

Serum creatinine is commonly used to monitor the graft function; however, it has low sensitivity for monitoring graft recovery in the early phase following KT, especially in patients with DGF (Urbschat *et al.* 2011). A variety of urinary and serum proteins have been investigated as the potential early and sensitive markers of DGF. Among these, neutrophil gelatinase associated lipocalin (NGAL) has shown strong clinical evidence in predicting the graft function (Buemi *et al.* 2014, Field *et al.* 2014, Pajek *et al.* 2014). In a meta-analysis involving more than 2500 patients, NGAL measurements showed promising benefit in the early prediction of AKI (Haase et al. 2009). The level of NGAL on the first post-operative day was shown to correlate well with the need for dialysis within the first week of transplantation (Lacquaniti *et al.* 2016).

NGAL production increases in renal tubular cells in response to I/R injury and both blood and urine samples are used to measure the level of the biomarker (Urbschat *et al.* 2011, Buemi *et al.* 2014, Lacquaniti *et al.* 2016). The blood NGAL level may be affected by extrarenal sources, thus urinary NGAL (u-NGAL) can provide more specific evidence of tubular injury (Parikh *et al.* 2006, Hall *et al.* 2010, Nishida

CONTACT Zahra Sahraei zahra.sahraei@yahoo.com Department of Clinical Pharmacy, School of Pharmacy, Shahid Beheshti University of Medical Sciences, Niyayesh cross road, Valiasr st., Tehran, Iran
#Jamshid Salamzadeh and Samira Chaibakhsh are responsible for statistical design/analysis. j.salamzadeh@yahoo.com (J. Salamzadeh); aramesh_sch@yahoo.com (S. Chaibakhsh).

et al. 2010, Lippi *et al.* 2012, Choi *et al.* 2013, Kanter *et al.* 2013, Kohei *et al.* 2013, Hollmen *et al.* 2014, Qurashi *et al.* 2014). NGAL is freely filtered by the glomerulus and is largely reabsorbed in the proximal tubules, and thus, its urinary excretion is increased when there is proximal renal tubular injury (Devarajan 2008).

N-acetylcysteine (NAC) is a potent antioxidant that regenerates glutathione stores and scavenges oxygen-free radicals (Fishbane 2008). Several *in vitro* models have reported the impact of NAC in reducing the I/R injury through reduction of oxidative stress, inflammation and cell apoptosis as well as improving renal perfusion (Sehirli *et al.* 2003, Fuller *et al.* 2004, Nitescu *et al.* 2006, Shimizu *et al.* 2008). NAC has shown clinical benefits in preventing contrast-induced nephropathy (CIN) and increasing renal function after transplantation with no significant adverse effects (Fishbane 2008, Ruiz Fuentes *et al.* 2008, Danilovic *et al.* 2011).

Clinical significance

- Despite many favourable results in animal studies, human data on the clinical benefit of NAC in preventing DGF is limited.
- The measurement of u-NGAL has shown promise as a non-invasive and early diagnostic marker of DGF, but its significance to monitor a renoprotective intervention has not been demonstrated (Barrera-Chimal and Bobadilla 2012).
- The aim of the current study is to assess the effect of NAC on reducing renal tubular injury through measuring u-NGAL in deceased-donor KT recipients. Renal function and the rate of DGF were also assessed.

Materials and methods

Trial setting and design

A prospective, double-blinded, randomized, placebo-controlled trial was conducted on deceased-donor kidney recipients between May 2014 and December 2015 in Shahid Labbafinejad Hospital in Tehran, Iran.

The study was in accordance with the Declaration of Helsinki and was approved by the ethics committee of Urology and Nephrology Research Center, Shahid Labbafinejad Hospital, Tehran, Iran. The research was registered in the Iranian Registry of Clinical Trials (www.irct.ir, registration number: IRCT2014090214693N4). Written informed consents were obtained from all participants prior to enrollment. The consent form described the study, outlined the possible risks, and indicated that an experimental medication or placebo would be administered daily.

Patients were randomly assigned to receive either NAC as 600 mg tablets, or placebo with their immunosuppression protocol. Ten tablets of NAC/placebo were administered: one tablet within 2 hours before operation and nine tablets during five consecutive days post transplantation with twice-daily consumption.

Three urine samples were taken from each patient, one before operation and two in the first and fifth days post-operation.

Inclusion and exclusion criteria

Adult patients aged between 18 to 75 years and recipients of kidney from deceased heart-beating donors were entered into the study. Patients with the following conditions were excluded: (1) history of using NAC within the month prior to operation; (2) history of sensitivity to sulfa drugs; (3) past medical history interfering with the quantification of urine biomarker including recent coronary artery bypass grafting (CABG) or stroke within the 6 months prior to operation (Sahraei *et al.* 2015); (4) less than 2 ml urine samples before transplantation; (5) panel reactive antibody of more than 30%; and (6) history of previous kidney transplantation. Exclusions 5 and 6 were considered because a different induction immunosuppressive regimen (including thymoglobulin) is considered for those patients in the medical centre.

Allocation, randomization and blinding

Prior to randomization, 74 identical boxes were prepared; half containing NAC (ACC long Hexal, Germany) tablets and the other half containing placebo. The placebo tablets were made in the School of Pharmacy, Shahid Beheshti University of Medical Sciences with identical appearance and packaging to those of NAC. Seventy-four six-digit codes were produced by a research assistant using a computerized random number generator and printed in two identical sets. One set of codes was used to label the tablet boxes. The other set of codes was concealed in 74 opaque sealed envelopes each containing a single code. The research assistant and staff responsible for packaging and preparation of the boxes were not further involved in the study. Once a patient admitted to the study, an envelope was taken from the raffle box, assigning a code to the patient. Then, a tablet box with the matching code was administered to that patient. Patients, nurses, physicians who provided care and renal function assessment and the statistician were blinded to patient allocation. The coded data were kept by the study investigator. The statistical analyses were conducted based on patients allocated to two groups, but the statistician was blinded to the identity of the groups, i.e. NAC/placebo. The codes were broken once the statistical analyses were completed.

Data collection

The following data were recorded for each patient: demographic information for recipients and donors, clinical characteristics of deceased donors (i.e. cause of death, serum creatinine, comorbid conditions), clinical characteristics of recipients (i.e. cause of chronic kidney disease, baseline serum creatinine, maintenance dialysis, history of blood transfusion, drug history, preexisting panel reactive antibody) and cold ischemia time.

Sample collection and laboratory measurements

Daily serum creatinine, occurrence of delayed graft function and acute rejection were recorded during the two-week trial. The pre-operative urine sample was collected prior to the administration of immunosuppressant regimen (day 0). The two post-transplant samples were collected in the morning following the surgery (day 1) and on the fifth day (day 5), six hours after the last dose of NAC/placebo consumption.

Urine samples were immediately transferred to laboratory, following which they were centrifuged 5 minutes at 5000 rpm and supernatants were stored at −80 °C. U-NGAL levels were measured using commercially available ELISA kits (KIT 036, BioPorto Diagnostics A/S™, Gentofte, Denmark). The measurements and analysis were carried out in accordance with the instructions given by the manufacturer.

Outcome measures

The primary outcome was the variation of u-NGAL levels measured at baseline and on the first and fifth days after KT. The secondary outcomes included early graft function at the end of the first and second weeks after KT and the occurrence of DGF. The estimated glomerular filtration rate (eGFR) was calculated using the Modification of Diet in Renal Disease (MDRD). DGF was defined as either (a) the need for dialysis in the first week after transplantation; or (b) the serum creatinine level not reducing more than 10% per day in three consecutive days (Boom *et al.* 2000, Daly *et al.* 2005). The decision to undergo dialysis was made by hospital nephrologist and was not influenced by the study investigators.

Transplantation protocol

All patients received the same immunosuppressive therapy based on steroids, mycophenolate mofetil and a calcineurin inhibitor including tacrolimus or cyclosporine. Methylprednisolone 200 mg, I.V. infusion, mycophenolate mofetil 2.0 g, and tacrolimus 0.1 mg/kg or cyclosporine 7 mg/kg/day were administered pre-operatively.

Prednisolone 2 mg/kg (maximum dose120 mg/day), P.O., was started on the first day post-transplantation and tapered to reach a dose of 5–7.5 mg/day during the three months after transplantation. Mycophenolate mofetil was continued 1.0 g twice a day post-operatively and was reduced to 500 mg twice daily in patients receiving tacrolimus as their calcineurin inhibitor. Tacrolimus or cyclosporine was continued through drug level monitoring with the target trough level of 7-10 ng/ml and 150–300 ng/ml, respectively, for the first 3 months after transplantation.

Sample size and statistical analysis

Assuming a standardized difference (difference/standard deviation) of 0.75, significance level of 5% and power of 80%, the sample size was calculated as 28 patients in each group. Then, considering a 30% attrition rate, the final sample size for each group was 37.

The medical data were analysed using SPSS software (Statistical Package for the Social Sciences, version 21.0, SPSS Inc, Chicago, IL). All interval variables were tested for normality of distribution. *T*-test and Mann–Whitney *U*-test were utilized for analysing data with normal and non-normal distribution, respectively. Chi-square and Fisher's exact tests were used for categorical data. Results are presented as mean ± standard deviations (mean ± SD) for normally distributed interval parameters, median (range) for non-normally distributed data, and number of recipients or percent for nominal variables. The generalized estimating equations (GEE) model was used for the analysis of u-NGAL and serum creatinine measurements. A p value <0.05 was considered significant.

Results

Taking into account the inclusion and exclusion criteria, 74 patients entered the study, of which three patients were dropped out due to kidney loss immediately after transplantation caused by technical/surgical issues. In addition, a urine sample for one patient was missed; therefore, a total of 70 patients were considered in data analysis; 36 in the NAC and 34 in the placebo group (Figure 1).

There were no significant statistical differences in demographic and clinical characteristics of deceased donors and recipients between the NAC and placebo groups (Table 1). In addition, the difference in the baseline level of serum creatinine between the two groups was non-significant (7.3 ± 2.4 versus 6.8 ± 2.0 mg/dl, p value = 0.49).

Ten patients (27.7%) in the NAC group and seven patients (20.6%) in the placebo group were dialyzed within 24 hours before transplantation. The mean tacrolimus and cyclosporine trough levels during the immediate post-operative period were within the therapeutic range for both groups. The reported adverse effects including nausea, flatulence, headache and abdominal discomfort were not statistically different between the two groups (p value = 0.40) and did not result in study withdrawal.

The u-NGAL and serum creatinine data had non-normal and correlated distributions, hence the GEE model was utilized to study the treatment effects (Zeger and Liang, 1986). Despite randomization, there was significant difference in the baseline u-NGAL between the two groups, hence covariate adjustment for baseline u-NGAL was used in the GEE model. An unstructured correlation matrix was assumed to generalize the within-subject dependencies. The goodness of fit was used to optimize the correlation matrix and the model parameters.

The interaction between time and study groups showed no statistically significant results, however there was a significant difference across the time points (p value = 0.001) and between the two study groups in u-NGAL levels (p value = 0.02). Considering the adjustment for baseline levels, the mean of u-NGAL in the NAC group was significantly lower than the placebo group ($\beta = 197.8$ pg/ml). The key result is therefore the significant reduction of u-NGAL in the NAC versus placebo group from baseline to day 1 (reduction

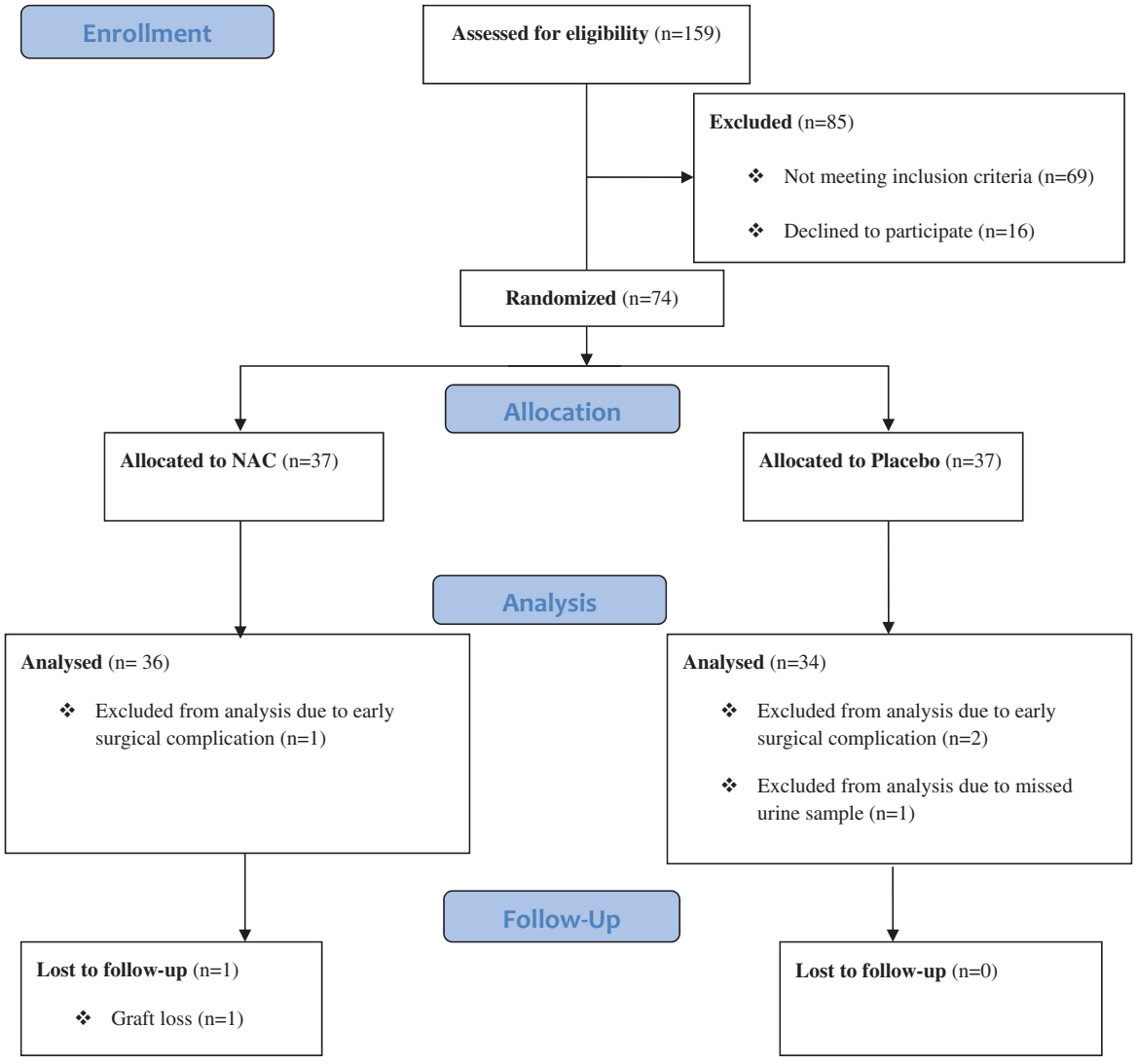

Figure 1. Randomization, treatment, and follow-up of patients.

of 542 ± 352 versus 318 ± 374 pg/ml, p value = 0.01) and from baseline to day 5 (745 ± 489 versus 463 ± 426 pg/ml, p value = 0.01). Table 2 and Figure 2 show the u-NGAL levels for both groups.

The analysis of daily creatinine levels within the first week post-transplantation showed a significant difference across the time points (p value <0.001); however, the difference between the two groups was non-significant (p value = 0.08). Table 2 and Figure 3 show the serum creatinine levels for the NAC and placebo groups.

The average eGFR in the NAC and placebo groups was 50.0 ± 22.5 versus 40.4 ± 20.8 ml/min/1.73 m^2 (p value = 0.07) at the end of the first week, and 54.5 ± 19.3 versus 46.8 ± 20.4 (p value = 0.12) at the end of the second week, respectively.

In addition, the results showed that the occurrence of DGF was less prevalent in the NAC group (13 patients, 36%) than the placebo group (19 patients, 56%), though it was not statistically significant (p value = 0.15). Among the patients with DGF, 3 in the NAC group and 12 in the placebo group did not undergo dialysis within 7 days after transplantation but the rate of creatinine reduction in those patients was less than 10% per day in three consecutive days.

Two cases of biopsy-proven acute antibody rejection in the placebo group and one case of graft loss in the NAC group were observed in the second week after transplantation.

Discussion

To the best of the authors' knowledge, this study is the first randomized controlled trial that evaluates the effect of NAC administration on the u-NGAL level in deceased-donor renal transplant recipients. The majority of previous studies have utilized various markers of oxidative stress to assess the outcomes of NAC administration (Ruiz Fuentes *et al.* 2008, Danilovic *et al.* 2011); however, there are also other biological actions attributed to NAC such as vasodilatory effects. NAC can improve renal perfusion and reduce the I/R injury of kidney graft through stabilizing nitric oxide and reducing angiotensin II, as has been shown in preventing CIN (Fishbane 2008). In the present study, the reduction of u-NGAL level was substantially larger (197.8 pg/ml) with NAC administration than placebo, which confirms the benefit of NAC in

Table 1. Demographic and clinical characteristics of deceased kidney donors and recipients[a].

	NAC group	Placebo group	*p* value
Donors			
Age, (years)	39.7 ± 14.3	42.4 ± 14.7	0.36
Gender, *n* (%)			0.15
Female	11 (30.5)	4 (11.8)	
Male	25 (69.5)	30 (88.2)	
BMI, (kg/m^2)	25.4 ± 4.3	25.5 ± 3.2	0.20
Cause of donor death (%)			0.94
CVA	18 (50)	16 (47)	
Trauma	12 (33.3)	13 (38.3)	
Others	6 (16.7)	5 (14.7)	
eGFR, (ml/min/1.73 m^2)	74.3 ± 27.1	75.5 ± 23.5	0.70
Expanded criteria donor, *n* (%)	15 (41.6)	15 (44.1)	1
Cold ischemia time, (hour)	3.4 ± 1.4	3.3 ± 1.4	0.93
Recipients			
Age, (years)	44.0 ± 12.9	46.6 ± 12.1	0.37
Gender, *n* (%)			0.80
Female	12 (33.3)	12 (35.3)	
Male	24 (66.7)	22 (64.7)	
BMI, (kg/m^2)	24.1 ± 3.6	25.8 ± 4.4	0.09
Underlying disease, *n* (%)			0.54
Hypertension	9 (25)	10 (29.5)	
Diabetes mellitus	8 (22.2)	9 (26.5)	
Glomerulonephritis	5 (13.9)	0	
Others	14 (38.9)	15 (44)	
Patients on maintenance dialysis, *n* (%)	31 (86)	25 (73)	0.24
Pre-transplantation dialysis, *n* (%)			0.42
Preemptive kidney transplantation	5 (14)	9 (26)	
Hemodialysis	30 (83)	24 (71)	
Peritoneal dialysis	1 (3)	1 (3)	
Time on dialysis before transplantation, (month)	14.3 ± 14.5	17.0 ± 10.5	0.14
Patients with history of blood transfusion, *n* (%)	13 (36.1)	13 (38.2)	0.80

[a]BMI: body mass index; Cr: creatinine; CVA: cerebrovascular accident; eGFR: estimated glomerular filtration rate; NAC: N-acetylcysteine; *n*: number of patients.
p values are two-sided.
Plus-minus values are mean ± standard deviation.
The body-mass index is the weight in kilograms divided by the square of the height.
Expanded criteria donor kidney transplantation was defined as: donor older than 50 years; or donor aged 40–49 years with two additional risk factors (hypertension, cerebrovascular accidents, serum creatinine more than 1.5 mg/dl, diabetes without albuminuria).

Table 2. Urinary NGAL levels and serum creatinine in the NAC and placebo groups.

			Statistics			
	NAC group	Placebo group		β	Standard error	*p* value
u-NGAL, (pg/ml)						
u-NGAL0	1188 ± 178	1049 ± 297	Time	−174.4	54.7	0.001
u-NGAL1	646 ± 374	730 ± 423	Group	−197.8	82.8	0.02
u-NGAL5	443 ± 432	586 ± 483	u-NGAL0	0.60	0.13	<0.001
Cr, (mg/dl)						
Cr0	7.3 ± 2.4	6.8 ± 2.0	Time	−0.3	0.04	<0.001
Cr1	4.5 ± 2.0	4.7 ± 1.9	Group	−0.70	0.40	0.08
Cr2	3.3 ± 1.8	3.9 ± 1.9	Cr0	0.08	0.07	0.29
Cr3	3.0 ± 1.9	3.5 ± 1.9				
Cr4	2.7 ± 2.0	3.4 ± 2.0				
Cr5	2.5 ± 2	3.1 ± 2.1				
Cr6	2.3 ± 1.9	2.9 ± 2.0				
Cr7	2.1 ± 1.7	2.7 ± 1.8				

β is the change in response as a result of one unit change in the independent variable. Cr: creatinine; u-NGAL0: urinary levels of NGAL before transplantation; u-NGAL1: urinary levels of NGAL on the first day after transplantation; u-NGAL5: urinary levels of NGAL on the fifth day after transplantation; NAC: N-acetylcysteine.

reducing tubular cell injury and the recovery from I/R damage after KT.

NGAL is an early and robustly induced protein in kidney following ischemic and nephrotoxic insults (Han *et al.* 2012, Magnusson *et al.* 2012) and can be detected in the urine sample as early as two hours following ischemia (Ampatzidou *et al.* 2012). Compared to creatinine which mainly relates to a disturbance in filtration function, the NGAL level can represent tubular injury and DGF (Lacquaniti *et al.* 2016).

The measurement of u-NGAL within the first 24 hours following transplantation is reported to be an early predictor of DGF in deceased-donor renal transplant patients (Parikh *et al.* 2006). Studies have shown that in patients with rapid recovery of graft function, u-NGAL decreased to normal value in the first day post-transplantation while in patients with delayed graft recovery, it remained substantially high during the five days post-transplantation (Eremenko *et al.* 2014).

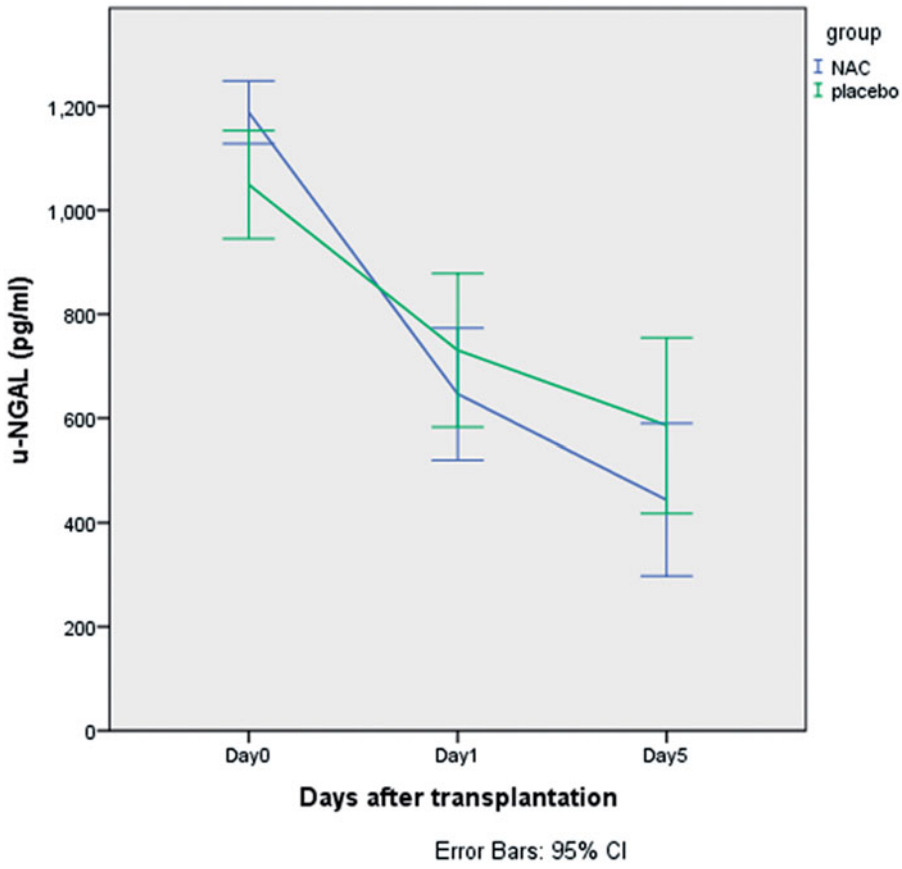

Figure 2. Urine NGAL levels measured at baseline (days 0) and days 1 and 5 after transplantation.

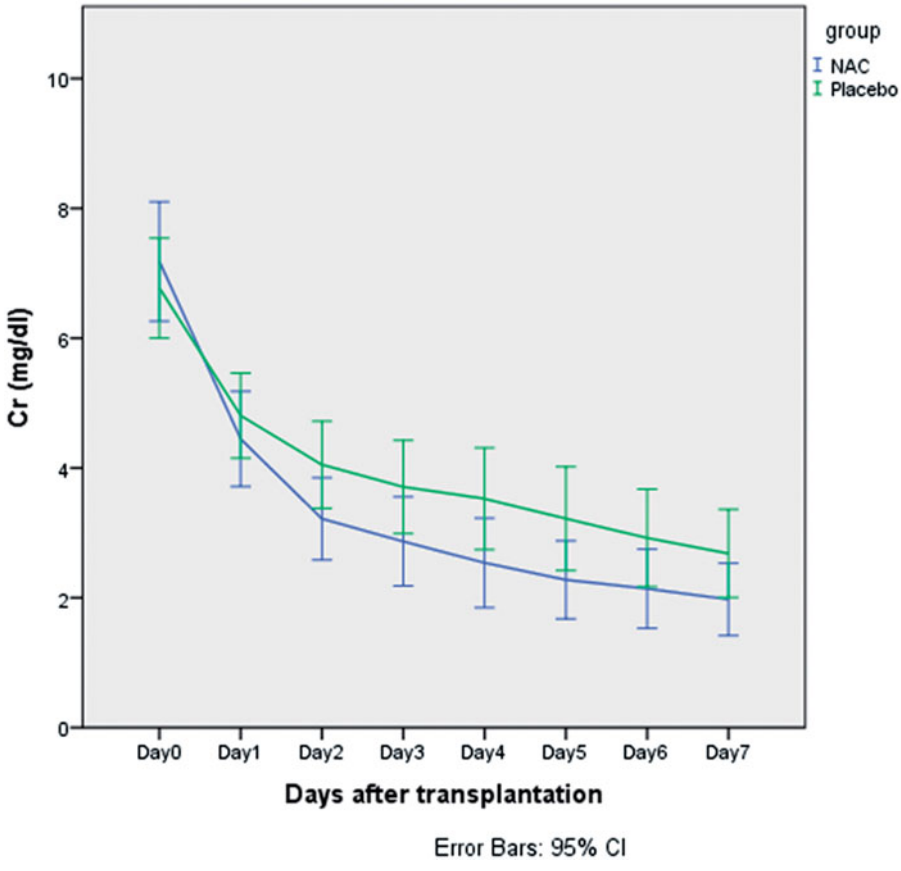

Figure 3. Daily serum creatinine levels measured at baseline (day 0) and on days 1 to 7 after transplantation.

The outcomes from this study showed that 5-day NAC consumption significantly decreased the u-NGAL levels; however, the reduction of serum creatinine levels was not statistically different between the two study groups. This result is in agreement with other studies which reported NGAL as a more suitable biomarker than creatinine for evaluating allograft injury during early phase after transplantation. A change in the creatinine level is usually observed 48–72 hours after the occurrence of AKI, which restricts its utilization to monitor the effects of nephroprotective interventions.

Improvement was observed in the short-term graft function in the NAC group at the end of the first and second weeks after KT. Despite it was not statistically significant (p value = 0.07 and 0.12, respectively), the higher mean of eGFR at the end of the first and second weeks observed in the NAC group is clinically important. The increase of eGFR with NAC consumption is in agreement with results from previous studies (Danilovic *et al.* 2011, Sahraei *et al.* 2015).

The rate of DGF was noticeably lower in the NAC versus placebo group (36% versus 56%), however the result was not statistically significant (p value = 0.15). This is most likely because of the insufficient number of DGF patients in the study to achieve a statistical conclusion. Danilovic et al. reported a similar improvement (55.3% vs. 72.2% reduction in the DGF rate in the NAC versus control group, respectively) in a study where 600 mg NAC was administered to patients twice daily for seven days (Danilovic *et al.* 2011).

In a study by Sahraei et al. on living-donor KT, three doses of 600 mg NAC were administered within 12 hours after KT leading to improvement in the u-NGAL levels, though results were not statistically significant (Sahraei *et al.* 2015). They suggested that longer duration of NAC consumption could result in stronger trends. Indeed, our findings demonstrate that a faster u-NGAL reduction rate was achieved through a 5-day consumption of 600 mg NAC twice daily.

One limitation of the current study was that the preemptive kidney transplantation was not excluded, which is known to have better survival and transplant outcomes. However, this limitation is believed not to have a significant effect on the results because the number of preemptive KT was small and had normal distribution in both study groups. The second limitation was that the sample size was insufficient to come to a statistical conclusion on eGFR and the occurrence of DGF and acute rejection between the two study groups. However, the primary outcome was to assess the biomarker level, which reached statistical significance. Third, in order to standardize the immunosuppressive regimen among the participants, patients with conditions that necessitated the administration of induction polyclonal antibody were excluded. This could limit the generalizability of results to this population of patients. Forth, despite the importance of urinary markers of kidney injury, their use in transplant recipients may be restricted by graft anuria or persistent native kidney diuresis (Fonseca *et al.* 2014). Fifth, only two post-transplantation measurements of u-NGAL were conducted. A more frequent measurement of u-NGAL could result in obtaining a more accurate time-to-effect for NAC.

This paper opens interesting debate on higher doses and longer duration of NAC administration. The collective results from this study and those reported by Sahraei et al. and Danilovic et al. show that longer duration of NAC consumption can lead to faster reduction of u-NGAL and increase in graft function. Since NAC undergoes extensive first-pass hepatic metabolism, which leads to poor oral bioavailability of the drug (Fishbane 2008), future studies can be conducted to assess the effect of higher doses of oral NAC, such as 1200 mg which is commonly used in the prevention of CIN.

Conclusions

The results from this study show that NAC, as a safe, inexpensive and well-tolerated medication, has promising potential in reducing tubular injury. This was shown by a significant reduction in u-NGAL and increase in early-phase eGFR. Future studies may consider larger sample size, higher doses and longer period of NAC consumption in kidney transplant patients.

Acknowledgements

The authors would like to thank the patients, who participated in the study and all the nursing staff of Shahid Labbafinejad Medical Center.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This study was supported by grants from Shahid Labbafinejad Medical Center and Shahid Beheshti University of Medical Sciences, School of Pharmacy [grant number: 313/363].

References

Ampatzidou, F., *et al.*, 2012. Neutrophil gelatinase-associated lipocalin as emerging biomarker of acute renal failure in renal transplant patient after coronary artery bypass surgery. *Journal of cardiovascular surgery (Torino)*, 53, 805–808.

Barrera-Chimal, J. and Bobadilla, N.A., 2012. Are recently reported biomarkers helpful for early and accurate diagnosis of acute kidney injury? *Biomarkers*, 17, 385–393.

Boom, H., *et al.*, 2000. Delayed graft function influences renal function, but not survival. *Kidney international*, 58, 859–866.

Buemi, A., *et al.*, 2014. Is plasma and urine neutrophil gelatinase-associated lipocalin (NGAL) determination in donors and recipients predictive of renal function after kidney transplantation? *Clinical biochemistry*, 47, 68–72.

Choi, H.M., *et al.*, 2013. Urine neutrophil gelatinase-associated lipocalin predicts graft outcome up to 1 year after kidney transplantation. *Transplantation proceedings*, 45, 122–128.

Daly, P.J., *et al.*, 2005. Delayed graft function: a dilemma in renal transplantation. *BJU international*, 96, 498–501.

Danilovic, A., *et al.*, 2011. Protective effect of N-acetylcysteine on early outcomes of deceased renal transplantation. *Transplantation proceedings*, 43, 1443–1449.

Devarajan, P., 2008. Neutrophil gelatinase-associated lipocalin–an emerging troponin for kidney injury. *Nephrology dialysis transplantation*, 23, 3737–3743.

Eremenko, A.A., *et al*., 2014. [Neutrophil gelatinase-associated lipocalin (u-NGAL) in the assessment of renal function in patients after kidney allotransplantation]. *Anesteziologiia reanimatologiia*, 59, 10–15.

Field, M., *et al*., 2014. The use of NGAL and IP-10 in the prediction of early acute rejection in highly sensitized patients following HLA-incompatible renal transplantation. *Transplant international*, 27, 362–370.

Fishbane, S., 2008. N-acetylcysteine in the prevention of contrast-induced nephropathy. *Clinincal journal of the American society of nephrology*, 3, 281–287.

Fonseca, I., *et al*., 2014. Oxidative stress in kidney transplantation: malondialdehyde is an early predictive marker of graft dysfunction. *Transplantation*, 97, 1058–1065.

Fuller, T.F., *et al*., 2004. Influence of donor pretreatment with N-acetylcysteine on ischemia/reperfusion injury in rat kidney grafts. *Journal of urology*, 171, 1296–1300.

Haase, M., *et al*., 2009. Accuracy of neutrophil gelatinase-associated lipocalin (NGAL) in diagnosis and prognosis in acute kidney injury: a systematic review and meta-analysis. *American journal of kidney diseases*, 54, 1012–1024.

Hall, I.E., *et al*., 2010. IL-18 and urinary NGAL predict dialysis and graft recovery after kidney transplantation. *Journal of the American society of nephrology*, 21, 189–197.

Han, M., *et al*., 2012. Renal neutrophil gelatinase associated lipocalin expression in lipopolysaccharide-induced acute kidney injury in the rat. *BMC nephrology*, 13, 25.

Hollmen, M.E., *et al*., 2014. Serum neutrophil gelatinase-associated lipocalin and recovery of kidney graft function after transplantation. *BMC nephrology*, 15, 123.

Kanter, J., *et al*., 2013. Urinary neutrophil gelatinase-associated lipocalin after kidney transplantation: is it a good biomarker to assess delayed graft function? *Transplantation proceedings*, 45, 1368–1370.

Kohei, J., *et al*., 2013. Neutrophil gelatinase-associated lipocalin is a sensitive biomarker for the early diagnosis of acute rejection after living-donor kidney transplantation. *International urology and nephrology*, 45, 1159–1167.

Kumar, A., *et al*., 2016. Induction of IL-8(CXCL8) and MCP-1(CCL2) with oxidative stress and its inhibition with N-acetyl cysteine (NAC) in cell culture model using HK-2 cell. *Transplant immunology*, 35, 40–46.

Lacquaniti, A., *et al*., 2016. Delayed graft function and chronic allograft nephropathy: diagnostic and prognostic role of neutrophil gelatinase-associated lipocalin. *Biomarkers*, 21, 371–378.

Lippi, G., *et al*., 2012. Evaluation of NGAL Test, a fully-automated neutrophil gelatinase-associated lipocalin (NGAL) immunoassay on Beckman Coulter AU 5822. *Clinical chemistry and laboratory medicine*, 50, 1581–1584.

Magnusson, N.E., *et al*., 2012. Plasma neutrophil gelatinase associated lipocalin (NGAL) is associated with kidney function in uraemic patients before and after kidney transplantation. *BMC nephrology*, 13, 8.

Nafar, M., *et al*., 2011. Oxidative stress in kidney transplantation: causes, consequences, and potential treatment. *Iranian journal of kidney diseases*, 5, 357–372.

Nishida, M., *et al*., 2010. Serum and urinary neutrophil gelatinase-associated lipocalin levels in children with chronic renal diseases. *Pediatrics international*, 52, 563–568.

Nitescu, N., *et al*., 2006. N-acetylcysteine attenuates kidney injury in rats subjected to renal ischaemia-reperfusion. *Nephrology dialysis transplantation*, 21, 1240–1247.

Pajek, J., *et al*., 2014. Non-inferiority of creatinine excretion rate to urinary L-FABP and NGAL as predictors of early renal allograft function. *BMC nephrology*, 15, 117.

Parikh, C.R., *et al*., 2006. Urine NGAL and IL-18 are predictive biomarkers for delayed graft function following kidney transplantation. *American journal of transplantation*, 6, 1639–1645.

Qurashi, S., *et al*., 2014. Urinary neutrophil gelatinase-associated lipocalin and the occurrence of delayed graft function after kidney transplant. *Experimental and clinical transplantation*, 12, 396–400.

Ramirez-Sandoval, J.C., *et al*., 2014. Urinary neutrophil gelatinase-associated lipocalin predicts graft loss after acute kidney injury in kidney transplant. *Biomarkers*, 19, 63–69.

Ruiz Fuentes, M.C., *et al*., 2008. Treatment with N-acetylcysteine in stable renal transplantation. *Transplantation proceedings*, 40 (9), 2897.

Sahraei, Z., Salamzadeh, J., and Nafar, M., 2015. Effect of N-acetyl cysteine and vitamin C on kidney allograft function biomarkers interleukin-18 and neutrophil gelatinase-associated lipocalin. *Iranian journal of kidney diseases*, 9, 56–62.

Sehirli, A.O., *et al*., 2003. Protective effect of N-acetylcysteine on renal ischemia/reperfusion injury in the rat. *Journal of nephrology*, 16, 75–80.

Shimizu, M.H., *et al*., 2008. N-acetylcysteine protects against renal injury following bilateral ureteral obstruction. *Nephrology dialysis transplantation*, 23, 3067–3073.

Siedlecki, A., Irish, W., and Brennan, D.C., 2011. Delayed graft function in the kidney transplant. *American journal of transplantation: official journal of the American society of transplantation and the American society of transplant surgeons*, 11, 2279–2296.

Urbschat, A., Obermuller, N., and Haferkamp, A., 2011. Biomarkers of kidney injury. *Biomarkers*, 16 (Suppl 1), S22–S30.

Yarlagadda, S.G., *et al*., 2009. Association between delayed graft function and allograft and patient survival: a systematic review and meta-analysis. *Nephrology dialysis transplantation*, 24, 1039–1047.

Zeger, S.L. and Liang, K.Y., 1986. Longitudinal data analysis for discrete and continuous outcomes. *Biometrics*, 42, 121–130.