Effect of N-Acetyl Cysteine and Vitamin C on Kidney Allograft Function Biomarkers Interleukin-18 and Neutrophil Gelatinase-associated Lipocalin

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Introduction. Delayed graft function (DGF) is a consequence of ischemia-reperfusion injuries in kidney allografts, for which no definite treatment is available. The neutrophil gelatinase-associated lipocalin (NGAL) and interleukin-18 (IL-18) are introduced as the most promising urine biomarkers to detect DGF. N-acetylcysteine (NAC) and vitamin C, well-known potent antioxidants that scavenge free radicals, may alleviate kidney injury. This study investigated the protective effects of NAC alone and in combination with vitamin C on DGF, by measuring IL-18 and NGAL in living donor kidney transplantations.

Materials and Methods. Patients transplanted between January 2011and February 2013 were randomly divided into 3 groups to receive routine anti-rejection medication only (n = 32), NAC plus routine immunosuppressive regimen (NAC group; n = 33), and NAC and vitamin C plus routine regimen (NAC and vitamin C group; n = 19). Urine samples were taken 4 hours and 24 hours after transplantation. Enzyme-linked immunosorbent assay kits were utilized for measuring urine NGAL and IL-18.

Results. There were no significant differences in the DGF prevalence and its duration between the study arms. Although the levels of NGAL and IL-18 decreased in the NAC and NAC and vitamin C groups, these reductions were not significant. Glomerular filtration rate at 30 and 60 days after transplantation were not significantly different between study groups, either.

Conclusions. Our results showed that NAC is a safe drug without significant adverse effects in kidney transplant recipients; however, its potential useful effects on urinary biomarkers of DGF were not illustrated in the present study.

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INTRODUCTION

Kidney transplantation is the best treatment for most patients with end-stage renal disease; however, limited numbers of suitable kidneys are available for transplantation. Therefore, preservation of a kidney allograft is vital in transplant recipients. Delayed graft function (DGF) may predispose the graft to both acute and chronic rejection, leading to prolonged patient hospitalization and increased morbidity rate.[1] Delayed graft function means "the failure of the kidney transplant to function accurately in postgrafting phase due to ischemia-reperfusion and immunological injury.[2]" Older definition of DGF, ie, requirement for dialysis in the first week after kidney transplantation, is not abandoned as it is very subjective depending on

the dialysis criteria variations across hospitals.[2] In recent years, serum creatinine levels, rate of reduction in serum creatinine level, and urine output are used to diagnose DGF in different days following transplantation. According to these criteria, reported rates of DGF are 20% to 40%, of which 4% to 10% are contributed to living donor transplants and 5% to 50% to deceased donor transplants.[1]

Delayed graft function is a consequence of ischemic-reperfusion injuries. Oxygen-free radicals also have a main role in the pathophysiology of DGF, causing acute tubular necrosis.[3-5] The most important management strategy for clinically diagnosed DGF is daily dialysis as well as observation for rejection with serial biopsies. To date, no effective treatment is available for DGF.[6] Measurement of urine biomarkers, as the most new methods used to diagnose DGF, may compensate the delay time in DGF diagnosis and the resultant lack of preventive or therapeutic interventions.[7] Neutrophil gelatinase-associated lipocalin (NGAL) and interleukin-18 (IL-18) are introduced as the most promising urine biomarkers to detect DGF. These biomarkers are precise predictors of the need for dialysis within the first week of kidney transplantation.[8,9]

N-acetylcysteine (NAC), a well-known potent antioxidant that scavenges free radicals, may act as a precursor for glutathione synthesis. N-acetylcysteine inhibits the stimulation of pro-inflamatory cytokines and damages related to free radicals.[10] Animal studies showed that NAC administration alleviates kidney injury In kidneys subjected to ischemia-reperfusion injury, protective effects of NAC have also experimentally confirmed.[11,12] Administration of bolus NAC injection at the time of renal artery declamping resulted in a greater incidence of early graft function during deceased donor kidney transplantation, albeit nonsignificantly.[13] Another study showed that administration of NAC to deceased transplant recipients during the first week posttransplantion, endorses a faster and sustained resurgence of graft function by attenuating oxidative stress .[14,15] Ascorbic acid (vitamin C) is an organic compound with antioxidant properties that acts as a primary defence against free radicals in the blood.[16,17] It has been used in different models of ischemia-reperfusion in human with promising results. Studies on vitamin C in human kidney transplantation are scarce. In a prospective randomized trial, vitamin C decreased, but not significantly, the incidence and the length of DGF.[18] The aim of this study was to investigate the protective effects of NAC alone and in combination with vitamin C on DGF, by measuring early biomarkers of DGF (IL-18 and NGAL) in living donor kidney transplantations.

MATERIAL AND METHODS

Participants

A randomized controlled trial was designed on patients undergoing living donor kidney transplantation at a specialty kidney transplant research center (Urology and Nephrology Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran). All of the patients referred to this center between January 2011and February 2013 were evaluated for inclusion in the study. The immunosuppressive regimen was similar in all patients, consisting of preoperative cyclosporine A, 6 mg/kg/d in 2 divided dosages, and mycophenolate mofetil, 2 g/d in 2 divided dosages, continued postoperatively along with 500 mg of prednisolone after transplant surgery, as well as 250 mg and 100 mg on the second and third days after transplantation, respectively. Patients who developed DGF (with or without the need for dialysis) received a polyclonal antibody (thymoglobulin), and mycophenolate mofetil was discontinued until normalization of serum creatinine.

The study was approved by the ethics committee of Shahid Beheshti University of Medical Sciences and was registered on the Iranian Registry of Clinical Trials (www.irct.ir). A written informed consent was obtained from all study participants.

Inclusion and Exclusion Criteria

All of the patients older than 18 years old receiving a kidney transplant from living donors were enrolled in the study. The cold and warm ischemia times were kept shorter than 1 hour in order to minimize the impact of ischemia time on the results observed in our study. Exclusion criteria were any condition that could interfere with quantifying urine biomarkers (NGAL and IL-18), including active infectious diseases, neoplastic diseases, brain tumor, active inflammatory diseases, Cushing syndrome, sepsis, sickle cell anemia,

meningitis, pregnancy, cardiorenal syndrome, multiple sclerosis, recent acute pancreatitis, long-term use of cimetidine, recent coronary artery bypass grafting, hepatitis C and cirrhosis, Alzheimer disease, recent stroke, hyperoxaluria, and mood disorder or schizophrenia (left untreated).[19-27]

Study Design

Randomization was done by using the RAND function of Microsoft Excel Office Software. Patients were randomly divided into 3 groups of the control group who received only routine antirejection medication based on hospital protocols; NAC plus routine immunosuppressive regimen (NAC group); and NAC and vitamin C plus routine regimen (NAC and vitamin C group). Transplant recipients in the NAC group received 3 doses of NAC (Fluimucil), 600 mg, and effervescent (6 hours before grafting and 12 and 18 hours after transplantation). Patients in the NAC and vitamin C group received a combination of tablets containing 600 mg of NAC and 75 mg of ascorbic acid (ACC®) in the same schedule.

For all of the participants, 100-mL urine samples were taken 4 hours and 24 hours after transplantation. Urine samples were centrifuged at 5000 rpm for 5 minutes to remove particulate matter and cell debris, and stored at -70°C. The enzyme-linked immunosorbent assay kits were utilized for measuring urine NGAL (Antibody Shop, Gentofte, Denmark) and IL-18 (Medical and Biological Laboratories, Nagoya, Japan). Urine biomarkers NGAL and IL-18 were expressed as their total concentrations in urine as ng/mL for NGAL and pg/mL for IL-18. In addition, their levels in the urine were expressed as ng/mg of creatinine for NGAL and pg/mg of creatinine for IL-18 in order to standardize for changes in urine concentration. Daily measurements of urine output and serum creatinine were started on the day of transplantation and continued until discharge from hospital. Routine monitoring of graft function and serum creatinine were done for all of the recipients during the 60 days after transplantation. Demographic and clinical data were collected, as well as laboratory test results and applicable information from the donors.

The primary endpoint of the study was the occurrence of clinical DGF in the first week after kidney transplantation. One of the following criteria was used for clinical definition of DGF: (1) the need for dialysis within the first week after transplantation; and (2) elevated serum creatinine level from baseline that is remained unchanged or decreased by less than 10% per day immediately after surgery. The secondary endpoints were the serum creatinine levels and occurrence of acute kidney allograft rejection on days 30 and 60 after transplantation.

Statistical Analysis

Statistical analysis was performed using the SPSS software (Statistical Package for the Social Sciences, version 19.0, SPSS Inc, Chicago, Ill, USA). The results were expressed as mean values ± standard deviations for continuous variables. Comparisons were performed using the chi-square test or Fisher exact test for categorical data. The 1-way analysis of variance test was applied for normally distributed continuous data. Also, for nonparametric data without a Gaussian distribution, the Kruskal-Wallis test was used.

RESULTS

Of 89 patients who entered the study, 5 patients including, 1 in the standard group, 1 in the NAC group, and 3 in the NAC and vitamin C group dropped out due to missed urine samples. Overall, renal biomarkers were measured for 84 patients; 32 in the standard arm, 33 in the NAC arm and 19 in the NAC and vitamin C arm. Demographic characteristics of the donors and recipients as well as their pretransplant clinical records are presented in Tables 1 to 3. No significant differences were observed between the study groups regarding these characteristics.

Delayed graft function was clinically observed in 14 of the studied patients (16.7%), 4 of whom needed dialysis in the first week after transplantation. There were not any significant differences in the DGF incidence and its duration between study arms (Table 4).

The NGAL level in the patients who had DGF were significantly higher than the patient without DGF, but IL18 level differences were not significant; therefore, NGAL were the appropriate biomarker to follow up for DGF. The concentrations of biomarkers at 4 and 24 hours after transplantation are reported in Table 5. Although the levels of biomarkers decreased in the NAC and NAC and

Table 1. Demographic Characteristics of Kidney Transplant Patients and Their Living Donors*

Characteristic	Study Groups			*P*
	Control	NAC	NAC and Vitamin C	
Recipients				
Sex				
Male	20	16	12	
Female	12	17	7	.44
Age	36.17 ± 17.06	37.23 ± 16.53	36.90 ± 16.71	.97
BMI	22.96 ± 4.53	24.55 ± 4.50	23.96 ± 5.90	.41
Donors				
Sex				
Male	27	29	15	
Female	5	4	4	.65
Age	28.26 ± 4.06	27.32 ± 4.86	28.13 ± 5.33	.68
BMI	24.57 ± 3.94	25.46 ± 4.95	24.94 ± 4.81	.72

*NAC indicates N-acetylcysteine and BMI, body mass index.

Table 2. Clinical Characteristics of Donors and Recipients

Characteristic	Study Groups			*P*
	Control	NAC	NAC and Vitamin C	
Blood Transfusion (%)	27 (84.4)	20 (60.6)	14 (73.7)	.11
Dialysis duration, mo	14.38 ± 18.38	13.17 ± 11.90	10.54 ± 16.52	.30
Retransplantation (%)	2 (6.3)	2 (6.1)	2 (10.5)	> .99
Familial relation (%)	3 (9.4)	3 (9.1)	2 (10.5)	.82
ABO complete match (%)	29 (90.6)	29 (87.9)	17 (89.5)	.68
Sex match (%)	19 (59.4)	18 (54.5)	11 (57.9)	.91
Recipient-donor weight ratio	0.717 ± 0.320	0.894 ± 0.204	0.812 ± 0.235	.43
Underlying disease				
Hypertension	15 (46.9)	13 (39.4)	9 (47.4)	
Diabetes mellitus	6 (18.8)	7 (21.2)	3 (15.8)	
Glomerulonephritis	3 (9.4)	5 (15.2)	2 (10.5)	
Others	8 (25.0)	8 (24.2)	5 (26.3)	.82
Dialysis modality				
Hemodialysis	25 (78.1)	26 (78.8)	15 (78.9)	
Peritoneal dialysis	1 (3.1)	3 (9.1)	1 (5.3)	
No dialysis	6 (18.8)	4 (12.1)	3 (15.8)	.71

Table 3. Clinical Diagnosis of Delayed graft Function (DGF) and Its Duration

Characteristic	Study Groups			*P*
	Control	NAC	NAC and Vitamin C	
DGF	7 (21.9)	4 (12.1)	3 (15.8)	.51
Posttransplant dialysis	2 (6.3)	1 (3.0)	1 (5.3)	> .99
DGF duration	9.16 ± 5.81	8.33 ± 8.38	5.75 ± 3.78	.25

vitamin C groups, these reductions were not significant. Mean changes in urine NGAL and IL18 from the 1st to 2nd postoperative sampling times were not significantly different between the three groups (Table 5).

Patients were followed up for 2 months after transplantation. Their serum creatinine levels and episodes of acute rejections were evaluated. During the first month after transplantation, biopsy-proven acute rejection was diagnosed in 3 patients in the control (9.4%), 1 in the NAC (3.0%), and 1 in the NAC and vitamin C group (5.3%; $P = .46$). During the 60 days posttransplant, 2, 4, and 3 patients in the study arms of control, NAC, and NAC and vitamin C experienced acute rejection, respectively ($P = .54$). None of the patients in the study groups showed a considerable adverse reaction. Also, glomerular filtration rate (GFR) at 30 and 60 days

Table 4. Biomarker Levels in the Three Study Groups*

Characteristic	Study Groups			*P*
	Control	NAC	NAC and Vitamin C	
Four hours				
IL-18, pg/mL	26.65 ± 41.40	26.10 ± 28.01	16.37 ± 8.15	.58
IL-18, pg/mg Ucr	162.68 ± 262.85	119.38 ± 125.11	94.97 ± 76.77	.33
NGAL, ng/mL	2.70 ± 3.41	2.14 ± 1.95	2.24 ± 2.01	.95
NGAL, ng/mg Ucr	13.24 ± 13.54	9.06 ± 7.35	8.85 ± 23.98	.83
Twenty-four hours				
IL-18, pg/mL	26.86 ± 30.86	23.04 ± 20.18	21.04 ± 18.08	.70
IL-18, pg/mg Ucr	169.14 ± 244.25	109.88 ± 69.22	101.39 ± 70.04	.61
NGAL, ng/mL	0.99 ± 1.21	0.72 ± 0.63	0.69 ± 0.82	.49
NGAL, ng/mg Ucr	6.09 ± 7.40	4.51 ± 5.21	5.37 ± 5.82	.77
Differences (4 to 24 hours)				
IL-18, pg/mL	-1.01 ± 37.13	8.39 ± 54.70	9.19 ± 25.31	.70
IL-18, pg/mg Ucr	-1.92 ± 71.97	4.01 ± 168.81	6.19 ± 53.12	.62
NGAL, ng/mL	1.02 ± 1.20	1.49 ± 2.80	1.51 ± 1.89	.99
NGAL, ng/mg Ucr	5.35 ± 7.16	8.81 ± 12.88	11.41 ± 13.93	.60

*IL-18 indicates interleukin-18 and NGAL, neutrophil gelatinase-associated lipocalin.

Table 5. Glomerular Filtration Rate (GFR) at 30 and 60 Days After Transplantation

GFR, mL/min/1.73 m^2	Study Groups			*P*
	Control	NAC	NAC and Vitamin C	
30th day	62.20 ± 4.20	72.21 ± 4.05	68.51 ± 3.71	.73
60th day	64.59 ± 3.20	70.13 ± 3.93	73.69 ± 4.28	.81

after transplantation were not significantly different between the study groups.

DISCUSSION

To our knowledge, our study was the first study that evaluated the preventive effect of a potent antioxidant, NAC alone and in combination with vitamin C, on early biomarkers of DGF in living donor transplant recipients. We observed DGF in 14 patients (16.7%). However, considering the conventional definition of DGF, ie, need for dialysis in the first week after transplantation, DGF rate was 4.8% (4 patients). The incidence of DGF among patients undergoing living donor kidney transplantation is lower than that among patients undergoing deceased donor transplantation. In previous studies evaluating DGF rate among recipients of living donor transplantation, the reported incidence of DGF were 1.6 % to 18.8%.; in recent years, improvement in transplantation protocols has decreased the rate of DGF.[28-31]

Although previous studies utilized urine biomarkers to predict the DGF in deceased kidney transplantations and approved their predictive importance,[8,9] we could not find any value of these biomarkers in patients receiving kidney transplants from living donors. This might be due to the fact that in our study, the level of biomarkers was less than those of reported by studies using deceased transplants.[9,32] The lower level of biomarkers in our study could be a result of significantly shorter cold and warm ischemia times leading to lower ischemia-reperfusion taking place in the renal tissue.

The results of the current investigation indicated that NAC could not significantly reduce renal biomarkers of DGF and its clinical incidence. Although the amount of biomarkers and the need for dialysis in the first week after transplantation (clinical DGF) decreased in the recipients of NAC and NAC with vitamin C, this reduction was not significant. As Table 5 represents, although not statistically significant, there is a larger reduction in urine levels of biomarkers 24 hours posttransplant, when patients received 3 doses of NAC (or NAC plus vitamin C), compared to those of 4 hours postoperative, when patients received only 1 dose of NAC (or NAC plus vitamin C). Moreover, the duration of DGF in the NAC and NAC plus vitamin C groups were shorter than those in the control group.

A study in 2011 appraised the protective effect of NAC on DGF rate in deceased donor kidney

transplantation.[14] In this study, after 7 days of treatment with 600 mg of NAC, twice daily, the study group demonstrated a lower mean serum creatinine and higher mean glomerular filtration rate during the first 90 days and at 1 year after transplantation. Delayed graft function was also less frequent among the NAC group, and these recipients required fewer days of dialysis.[14] In another investigation performed on myocardial infarction patients with contrast-induced renal injury and myocardial reperfusion injury, it was shown that high doses of NAC (1200 mg twice daily for 48 hours) could not provide an additional clinical benefit to placebo.[33] This finding was criticized, expressing that according to experimental studies, an approximate dose of 100 mg/kg body weight of NAC should be used before stress oxidative events.[33] Therefore, the NAC dose used in our study was not large enough to control the oxidative stress in the kidney transplant and to alleviate the DGF biomarkers.

Our results could not prove a significant effect for NAC on kidney function biomarkers and also patient outcomes in 2 months after transplantation. It should be contemplated that our patients as recipients of living donor transplantation had fewer risk factors for DGF. In addition, administration of insufficient doses of antioxidants for a short duration (ie, 3 sporadic doses) could be another reason for the lack of a significant anti-inflammatory effects produced by NAC or vitamin C.

CONCLUSIONS

Our results showed that NAC is a safe medication without significant adverse effects in kidney transplant recipients; however, its potential useful effects on urinary biomarkers of DGF were not illustrated in the present study. A randomized controlled trial with a larger sample size and higher doses of NAC for a prolonged period of time is recommended for future studies in the recipients with ischemic delayed graft function and not immunologic delayed graft function or surgical complications.

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CONFLCIT OF INTEREST

None declared.

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