Association of Brain-dead Donor's Urine Neutrophil Gelatinase-associated Lipocalin Levels With Kidney Allograft Function

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Introduction. Development of delayed graft function is more prevalent in patients receiving a kidney allograft from brain-dead than living donors. This study aimed to evaluate the association between urine neutrophil gelatinase-associated lipocalin (NGAL) levels in brain-dead donors and subsequent allograft function.
Materials and Methods. Urine NGAL concentration was measured in urine samples obtained from 24 brain-dead kidney allograft donors before organ retrieval. The 24 kidney recipients were followed for 6 months. The immunosuppressive therapy was similar for all of the recipients. Following transplantation, plasma creatinine was recorded daily during the recipient's stay in the hospital and then at 1, 3, and 6 months after transplantation. Delayed graft function was defined as the need for dialysis in the first 7 days after transplantation.
Results. The mean age of the donors was 28.7 ± 11.2 years and 70.8% were men. Their median urine NGAL level was 7.4 ng/ml (range, 2 ng/mL to 45 ng/mL). Urine NGAL levels were only associated with the need for cardiopulmonary resuscitation (P = .007). On the 1st day after transplantation, 16.7% of the recipients developed delayed graft function, which was declined to 12.5% on the 2nd day and to 8.3% during the 3rd day and the following days. No significant association was observed between the donor's urine NGAL levels and graft function (P = .86).
Conclusions. Our results did not show any association between urine NGAL levels and outcome of allograft function obtained from brain-dead donors. Larger studies are required to confirm this finding.

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INTRODUCTION

Transplantation is the first choice of renal replacement therapy for patients with end-stage renal disease. Kidneys for transplantation can be obtained from either living related or unrelated donors or be donated by a brain- or cardiac-death victim. The process of brain death, however, could trigger a complex cascade of molecular and cellular events including the release of various pro-inflammatory mediators. Release of these mediators eventually leads to a pronounced inflammatory state, hemodynamic impairment, endothelial injury, and ultimately, an immunologically activated organ before transplantation.[1,2] Moreover,

vasoconstriction caused by both excessive secretion of catecoleamines and volume reduction increases the risk of hypoperfusion and ischemic injuries to the kidneys in brain-dead donors. On histological examination, these inflammatory and ischemic changes are seen as glomerulitis, periglomerulitis, vacuolization, and necrosis of the proximal and distal tubules, as well as proliferation of arterial intima and glomerular endothelium.[3,4]

Based on the studies, 4% to 10% of live and 5% to 50% of deceased donor kidney transplants are complicated by development of delayed graft function (DGF).[4,5] Patients with kidney transplant who develop DGF are predisposed to acute and chronic allograft nephropathy and graft rejection.[4,6] Chronic allograft nephropathy, known from a pathology perspective as nonspecific interstitial fibrosis and tubular atrophy, is considered as the major cause of long-term allograft loss in kidney recipients.[7] The use of calcineurin inhibitors has produced a major impact on achieving successful organ transplantation; however, chronic allograft nephropathy is still a major problem that manifests by a slow and progressive decrease in allograft function as a result of immunological or nonimmunological injuries.[8,9]

Recently, neutrophil gelatinase-associated lipocalin (NGAL) has emerged as a new sensitive biomarker for both early diagnosis of acute kidney injury (AKI) and prediction of the patient's outcome following kidney injuries.[10,11] Neutrophil gelatinase-associated lipocalin is expressed in cells of the distal nephron. During the course of renal insults, NGAL is excreted into the urine by the thick ascending loop of Henle, the distal tubule, and also the collecting duct.[12] To date, NGAL has been studied in patients with cardiac surgery and liver transplantation,[13,14] patients admitted in intensive care unit (ICU),[15] and in critically ill patients with multiple trauma.[16] However, few studies have been performed on NGAL after kidney transplantation. Among those, a study by Choi and colleagues showed that in patients with early graft function, urine NGAL could predict the development of slow graft function and adverse 1-year outcomes.[17] In another study performed by Parikh and colleagues, urine NGAL collected on day zero of transplantation predicted the trend in levels of serum creatinine after adjustment for age, sex, race, urine output, and cold ischemia time.[18] The authors concluded that urine NGAL could be regarded as a predictive biomarkers of DGF.

Considering that the quality of donor's kidney has an obvious impact on late allograft function and also on survival of the recipients, identifying biomarkers that could give us an accurate estimate of organ status and also could predict the allograft function in ultimate recipients might be helpful in deciding whether to accept or reject the organ donation. Based on the abovementioned observations and also since plasma creatinine level is known to be a poor early detector of AKI, we decided to investigate whether urine NGAL levels of donors could be used as a simple and more accurate biomarker for prediction of kidney graft function in the recipients. To perform this, in a prospective study, we measured the urine levels of NGAL in brain-dead donors and evaluated its association with subsequent development of DGF in kidney transplant recipients.

MATERIALS AND METHODS

Study Design and Patients

This study was performed in the Department of Nephrology of Dr Shariati Hospital, affiliated with Tehran University of Medial Sciences. In this prospective study, we enrolled 24 consecutive brain-dead donors and their 24 adult kidney recipients between August 2012 and December2012. The study protocol was approved by the Ethics Committee of Tehran University of Medical Sciences and written informed consent was obtained from the kidney recipients before enrollment. It should be mentioned that although each donor had donated 2 kidneys, which means 48 recipients could have received the graft, we evaluated function of only 1 kidney and therefore 24 recipients.

The inclusion criteria for the donors were men and women aged less than 60 years. Brain-dead patients who had serum creatinine levels higher than 2 mg/mL were excluded. Donor clinical data including sex, age, cause of brain death, need for cardiopulmonary resuscitation, length of hospital stay before brain death diagnosis, and plasma creatinine levels were obtained from the hospital records. Estimated glomerular filtration rate (GFR) was calculated using the Modification of Diet in Renal Disease equation.[19]

The selection of recipients was made based on standard cross match. Clinical data of the recipients

were obtained from the patients' hospital records. Plasma creatinine concentrations were recorded daily during the recipient's stay in the hospital and then obtained from their records at month 1, month 3, and month 6 after transplantation. Estimated GFR was calculated using the Modification of Diet in Renal Disease equation.[19] The immunosuppressive therapy was similar for all of the patients, consisting of cyclosporine or tacrolimus plus prednisolone and mycophenolate mofetil. All patients received thymoglobulin (1.5 mg/kg daily for 4 days). The primary recipient outcome variable was the onset of graft function after transplantation manifested by a decrease in serum creatinine and an increase in urine volume. Patients with hyperacute kidney rejection were excluded. Delayed graft function was defined as the need for dialysis in the first 7 days posttransplant.[20] Patients who had normal postoperative urine output with gradual decrement of serum creatinine without the need for dialysis were categorized as slow graft function.[20]

Measurement of Neutrophil Gelatinase-associated Lipocalin

A 10-mL urine sample from brain-dead donors was obtained via urinary catheter just before the organ retrieval. The samples were kept on ice, centrifuged for 10 minutes at 2500 rpm at 4°C and then the supernatant was collected and divided into tubes and stored at -80°C until time of analysis. No additives were used. The urine NGAL assays were performed using a commercial enzyme-linked immunosorbent assay.

Statistical Analyses

The SPSS software (Statistical Package for the Social Sciences, version 17.0, SPSS Inc, Chicago, Ill, USA) was used for statistical analyses. All variables were tested for normality of distribution. The Student *t* test and the 1-way analysis of variance were used to compare normally distributed quantitative variables. The chi-square test was used for comparison of categorical variables. The parametric correlations were assessed using the Pearson correlation coefficient. *P* values less than .05 were considered significant.

RESULTS

In this study, 24 brain-dead patients whose kidneys were transplanted to 24 patients were enrolled. Characteristics and clinical data of the donors are reported in Table 1. The mean age of donors was 28.7 ± 11.2 years with 70.8% being male (n = 17). Of the 24 recipients, 3 were the candidates for a second kidney transplant. The mean graft survival in these three patients was 11 years (range, 6 to 11 years), and chronic allograft rejection was the cause of graft failure in 2 of them, while the cause was unknown for 1 patient. Table 2 shows the clinical and laboratory parameters of the 24 kidney transplant recipients.

Urine levels of NGAL in the donors showed an association only with the need for cardiopulmonary

Table 1. Characteristics and Clinical Data of 24 Brain-dead Kidney Donors*

Characteristic	Value
Mean age, y	28.7 ± 11.2
Sex	
Male	17 (70.8)
Female	7 (29.2)
Cause of brain death	
Head trauma	16 (66.7)
Intracranial hemorrhage	3 (12.5)
Others	5 (20.8)
Cardiopulmonary resuscitation	9 (37.5)
Mean hospital days before brain death	2.7 ± 0.5
Mean glomerular filtration rate, mL/min	80 ± 22.8
Mean serum creatinine, mg/dL	1.08 ± 0.27
Median NGAL, ng/mL	7.4 (2 to 45)

*Values are mean ± standard deviation for age and frequency (percentage) for the rest except for the NGAL, which is median (range). NGAL indicates neutrophil gelatinase-associated lipocalin.

Table 2. Characteristics and Clinical Data of 24 Kidney Allograft Recipients*

Characteristic	Value
Mean age, y	40 ± 14
Sex	
Male	54.2 (13)
Female	45.8 (11)
Cause of kidney failure	
Diabetes mellitus	6 (25.0)
Hypertension	3 (12.5)
Systemic lupus erythematosus	2 (8.3)
Polycystic kidney disease	2 (8.3)
Alport syndrome	2 (8.3)
Unknown	4 (16.7)
Treatment before transplantation	
Hemodialysis	22 (91.7)
Peritoneal dialysis	2 (8.3)
Median dialysis time before transplant, mo	17 (2 to 180)

*Values are mean ± standard deviation for age and frequency (percentage) for the rest except for the dialysis time, which is median (range).

resuscitation (P = .007). No association was observed between NGAL and age, duration of hospital admission, serum creatinine levels, or GFR of the donors.

On the 1st day after transplantation, 4 recipients (16.7%) needed hemodialysis (DGF). This figure declined to 12.5% (n = 3) on the 2nd day and to 8.3% (n = 2) on days 3 and 4 after transplantation (Table 3). Two patients continued to have DGF after the 1st week of transplantation, of whom, 1 died on day 18, due to status epilepticus.

The median level of urine NGAL in the brain-dead donors associated with the recipients with DGF was 7.8 ng/mL (range, 4 ng/mL to 37 ng/mL). Donor's median urine NGAL level was 7.5 ng/mL (range, 2 ng/mL to 45 ng/mL) for the recipients with normal graft function and 7.0 ng/mL (range, 2 ng/mL to 35 ng/mL) for those with slow graft function. No significant association was observed between the donor's urine NGAL levels and graft function defined by the need for dialysis (P = .86).

Table 3. Graft Function Parameters in Kidney Recipients*

Characteristic	Value
Mean serum creatinine, mg/dL	
Before transplant	7.75 ± 3.6
1st day	5.4 ± 2.2
2nd day	3.7 ± 2.5
7th day	1.9 ± 1.8
Urine volume after transplant	
1st day	
Anuria	2 (8.3)
≤ 400 mL	2 (8.3)
> 400 mL	20 (83.3)
2nd day	
Anuria	2 (8.3)
≤ 400 mL	0
> 400 mL	22 (91.7)
3rd and 4th days	
Anuria	1 (4.2)
≤ 400 mL	1 (4.2)
> 400 mL	22 (91.7)
5th day and later	
Anuria	1 (4.2)
≤ 400 mL	0
> 400 mL	23 (95.8)
Graft function on the first day	
Delayed graft function	4 (16.7)
Slow graft function	5 (20.8)
Normal	15 (62.5)
Delayed graft function	
1st day	4 (16.7)
2nd day	3 (12.5)
3rd day	2 (8.3)

*Values are mean ± standard deviation for serum creatinine and frequency (percentage) for the rest.

DISCUSSION

Kidney allografts from brain-dead donors are a potential source of kidney transplant, which is though more prone to severe ischemia-reperfusion injury and DGF than live donor transplant. A study that summarized data on kidney transplants from 159 119 deceased donors and 83 471 living donors during the 20-year period between 1988 and 2007 revealed that there was notable difference between survival rates of the recipients from living donors compared to deceased donors.[21] The 15-year graft survival rates were 29% for kidneys from deceased donors and 42% for those from living donors.[21] Brain-dead donors, even those who do not have a history of kidney diseases, experience various kinds of kidney insults before organ recovery. This includes hypoxic-ischemic injury, exposure to nephrotoxic medications, high levels of inflammation caused by cytokine release, disseminated intravascular coagulation, infection, and rhabdomyolysis.[1] All these injuries are likely to make remarkable kidney damage and induce acute tubular necrosis. In this regard, many studies have emphasized the impact of quality of the initial organ on subsequent graft survival.

In practice, creatinine is still the most widely used marker for posttransplant function monitoring; however, some recent studies proposed the measurement of serum and urine NGAL levels in kidney recipients as a more accurate marker than serum creatinine.[10,11] On the other hand, not so many investigations have been done on identification of biomarkers that could accurately, rapidly, and precisely assess the organ quality and subsequently organ outcome in donors.

Neutrophil gelatinase-associated lipocalin is a member of the lipocalin family that is expressed at low levels in several human tissues. It can be measured either in the serum or in the urine. It is believed that circulating NGAL may not originate from the kidney only, and that other tissues are as well responsible for part of serum NGAL concentration. On the other hand, urine NGAL is considered to derive mostly from tubular epithelial cells in response to AKI.[22,23] In the current study, we measured the urine levels of NGAL in

brain-dead kidney allograft donors, to see if there is any association between this biomarker and DGF occurrence in recipients. According to our knowledge, worldwide, there are few studies in which this association has been investigated, and in our region we could not find any study that assessed such a relationship. Given the importance of hospital setting in the success rate of organ transplantation, and also considering the fact that the number of brain-dead individuals whose relatives are willing to donate their organs is increasing in our country, we assume that it would be important to investigate such a relationship. However, our results were insufficient to reveal an association between the donor's urine NGAL levels and graft function.

Hollmen and colleagues[24] evaluated the prediction power of serum and urine NGAL for DGF after transplantation in 99 patients, and showed that in transplantations with high levels of donor's urine NGAL concentrations, prolonged DGF occurred more often than in transplantations with low urine NGAL concentrations. In multivariate analysis, urine NGAL was an independent risk factor for prolonged DGF.[24] We have to mention that in Hollmen and colleagues' study, the association between urine NGAL, as a continuous variable, and DGF was not evaluated. While in our study, we analyzed such an association and did not observe a significant correlation.

Measurement of insulin clearance is regarded as the gold standard for GFR determination. However, in practice, it is impossible to perform this assessment in deceased donors. Therefore, the available tools for donor kidney function assessment are estimated GFR and plasma creatinine levels. Based on serum creatinine levels and estimated GFR, none of our donors showed evidence of AKI and impaired kidney function. In the literature, there is no consensus regarding the cutoff point of urine NGAL that can determine the predisposition to AKI. A study by Makris and colleagues,[25] which performed in multi-trauma adult patients admitted to the ICU of a trauma hospital, showed that a cutoff point greater than 25 ng/mL for NGAL had a sensitivity of 91% and a specificity of 95% in predicting AKI. In our study, the obtained urine NGAL levels were low in general, compared to values that were reported by other studies performed on patients treated in ICU,[15,16,26,27] with only 3 patients having a urine NGAL higher than 25 ng/mL, and only one of them was in a group of recipients who developed DGF. Whether this low concentration of urine NGAL reflects the lower age of our participants compared to other studies or is because of other laboratory or genetic factors needs to be further investigated. It should be noted that the range of urine NGAL levels in our study was wide (2 ng/mL to 45 ng/mL). This wide range could be due to variables such as age, sex, race, and also preexisting kidney diseases which can cause divergence to baseline measurement. This wide range of urine NGAL in our study might be another reason for not seeing a significant association between DGF and urine NGAL levels.

Our study has limitations. First, we did not evaluate the serum levels of NGAL to see if there is an association between these two measures. Second, due to the low number of participants, we could not categorize the outcome of patients according to NGAL levels and so in the subsequent analysis. Third, the number of patients in this study was low and therefore we could not draw a decisive conclusion based on results of this study. Larger studies with a greater number of participants might provide more comprehensive results in this manner.

CONCLUSIONS

This study is the first in our region that tries to evaluate the association between donor urine NGAL levels and outcome of allograft kidney function. Although we failed to establish such an association, due to low number of participants, we cannot strongly reject the existence of this correlation. Performing a study with a larger sample size is sought to confirm our observations.

CONFLICT OF INTEREST

None declared.

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