ORIGINAL RESEARCH

Protective Effects of L-Carnitine Against Delayed Graft Function in Kidney Transplant Recipients: A Pilot, Randomized, Double-Blinded, Placebo-Controlled Clinical Trial

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Objective: Delayed graft function (DGF) is an early complication after deceased donor kidney transplantation with significant adverse effects on graft outcomes. Ischemia-reperfusion injury during transplantation is a major cause of DGF. Tissue concentrations of carnitine, an antioxidant and regulator of cellular energy supply, decrease in the kidney following ischemia-reperfusion insult. Based on promising animal data, this study evaluated the possible protective effect of L-carnitine against DGF.

Design: This study is a pilot, randomized, double-blind, placebo-controlled clinical trial that was conducted on kidney transplantation patients in kidney transplant ward of Imam Khomeini hospital complex affiliated to Tehran University of Medical Sciences, Tehran, Iran.

Subjects: Patients older than 14 years old undergoing their first kidney transplantation from a deceased donor were evaluated for eligibility to take part in this study. Fifty-six patients were randomly assigned to L-carnitine or placebo groups.

Intervention: During this trial, 3 g of oral L-carnitine or placebo was administered in 3 divided doses each day for 4 consecutive days starting the day before kidney transplantation (i.e., days −1, 0, 1, and 2).

Main Outcome Measure: The need for dialysis within the first week after transplantation, serum creatinine and urine output were assessed daily. After hospital discharge, patients were followed for 3 months regarding organ function.

Results: DGF incidence did not differ between the L-carnitine and placebo groups (18.51% vs. 23.8%, respectively; $P = .68$). Total allograft failure within 3 months after kidney transplantation happened in 6 patients in the placebo and 1 patient in the L-carnitine group ($P = .05$).

Conclusion: This study showed no protective effects of oral L-carnitine supplementation against DGF occurrence recipients; however, 3-month graft loss was lower in the L-carnitine supplemented group.

Introduction

DELAYED GRAFT FUNCTION (DGF) with the frequency of 5-50% in deceased donor kidney transplants[1] is a considerable early complication that has significant negative impact on graft outcomes.[1-3]

Although clinical definitions of DGF differ among consultants,[4] it is generally defined by the need for at least one dialysis treatment within the first week after transplantation.[5] Definitions used thus far are not ideal for diagnosis of DGF immediately after transplantation.[5] Measurements of urine or blood biomarkers are promising newer methods for early diagnosis of DGF. Neutrophil gelatinase-associated lipocalin (NGAL) has been introduced as a new biomarker of proximal renal tubular injury that might be correlated with DGF following kidney transplantation.[6-8]

Many experimental studies and clinical trials have proposed strategies to reduce DGF risk factors and occurrence.[9,10] Ischemia-reperfusion (I/R) injury and consequent acute tubular necrosis are major causes of DGF. Strategies to decrease I/R injuries play key roles in the prevention of acute tubular necrosis.[5] Oxidative stress and free radicals, hypoxia, switching to anaerobic

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metabolism pathway, decreased adenosine triphosphate synthesis, radical mediated oxidation of lipids, and proteins are major mediators in the pathophysiology of I/R injuries.[11-13] Compared with healthy subjects, kidney transplant recipients have higher oxidative status and decreased activities of antioxidant enzymes.[14] Although in recent years, improvement in transplantation protocols has reduced the rate of DGF, the role of several antioxidants on kidney function after transplantation has been evaluated.[11,15-19]

Carnitine is endogenously biosynthesized from the amino acid lysine and methionine in the kidney, liver, heart, muscle, and brain. Nevertheless, most of the body's carnitine pool is exogenously acquired mainly from animal sources.[20] Carnitine is an essential cofactor required for transportation of long chain acyl forms of fatty acids from the cytosol into mitochondria, where fatty acid β-oxidation generates energy via converting acylcarnitine to acetyl-CoA.[20] It also exerts substantial antioxidant,[21] anti-apoptotic,[21,22] and anti-inflammatory properties.[23]

In chronic kidney disease patients, plasma levels of free and total carnitine are unchanged, but serum acylcarnitine rises in inverse relation to the decline in the glomerular filtration rate and the ratio of acylcarnitine to free carnitine markedly increases.[24] This pattern is slightly different in hemodialysis patients. In hemodialysis patients, plasma total carnitine concentration is normal or elevated; the free carnitine concentration significantly reduces; the acylcarnitine concentration and the ratio of acyl to free carnitine markedly increase.[25] Chronic kidney disease results in abnormal renal handling of carnitine, leading to dyslipidemia, lethargy, muscular weakness, hypertension, cardiac dysfunction and arrhythmias, and recurrent cramps.[26] Nutritional supplementation of L-carnitine is beneficial in chronic kidney disease and particularly hemodialysis patients in particular for anemia, insulin sensitivity, and protein catabolism, cellular defense against chronic inflammation, and oxidative stress.[27-29]

The study on stable kidney transplant recipients without carnitine supplementation showed deficiency of free carnitine (in serum and urine) in 8.6% of kidney transplant recipients. Serum concentrations of total and free carnitine showed negative correlation with graft function, and glomerular filtration rate had a significant effect on plasma concentrations of total carnitine and free carnitine.[30] One study on kidney transplant patients with 3 different categories of graft function during 1-70 months following the kidney transplantation revealed no significant differences in the urinary excretion of free carnitine and short-chain acylcarnitine. Plasma levels of carnitine fractions in patients with functioning kidney transplants were completely normalized. Authors suggested that the reduced ratio of acylcarnitine to free carnitine following successful kidney transplantation might reveal a better availability of free carnitine and thereby an enhanced fatty acid oxidation.[31] Wanner and Horl[32] also reported normalization of the pattern of carnitine fractions after kidney transplantation in patients with plasma creatinine levels of less than or equal to 120 μmol/L.

Because carnitine has antioxidant effects and regulates energy supply across cell membranes, several researchers have assessed its effect in preventing kidney damage induced by I/R injury.[33-39] These findings suggest that L-carnitine use in human transplantation is worth testing. This study was designed to evaluate the protective effects of L-carnitine against DGF by measuring plasma NGAL, as an early biomarker of DGF, in deceased donor kidney transplantation.

Material and Methods

This pilot, prospective, double-blinded, randomized, placebo-control clinical trial was conducted in kidney transplant ward of Imam Khomeini hospital complex affiliated to Tehran University of Medical Sciences, Tehran, Iran, from February 2014 to the end of January 2015. An average 100 kidney transplantation surgeries have been annually performed in this center.

Inclusion and Exclusion Criteria

Patients older than 14 years old undergoing their first kidney transplantation from a deceased donor were evaluated for eligibility to take part in this study. Patients with the history of using L-carnitine derivatives during the last month, history of hypersensitivity to carnitine, or at risk for or had a history of seizure were excluded from the study. Other exclusion criteria were the presence of any condition that could increase plasma levels of NGAL including acute and severe infectious diseases, acute inflammatory diseases, and sickle cell anemia. All patients with hepatic, cardiac, or pulmonary instability immediately after transplantation were also excluded.

Intervention

Patients were randomly assigned to either L-carnitine or placebo groups (Fig. 1) using the block randomization procedure. The randomization was done by a computer-generated sequence in block sizes of 4, prepared by an investigator with no clinical involvement in the trial, allocating patients in the L-carnitine or placebo group at a ratio of 1:1. Allocations were contained in opaque, sequentially numbered, sealed envelopes concealed from assessors during the study. Patients and investigators allocated to the groups were kept blinded to the allocation arm, and the packaging of L-carnitine and placebo was identical. Transplant recipients in the L-carnitine group received a daily dose of 3-g L-carnitine oral syrup (Alborz Daru, Iran) in 3 divided doses each day for 4 consecutive days starting the day before kidney transplantation (i.e., days -1, 0, 1, and 2). In day -1, L-carnitine started about 12 hours before grafting and patients were educated to space these 3 doses throughout the day (i.e., every 3-4 hours) before transplantation. Patients in the placebo group were administered

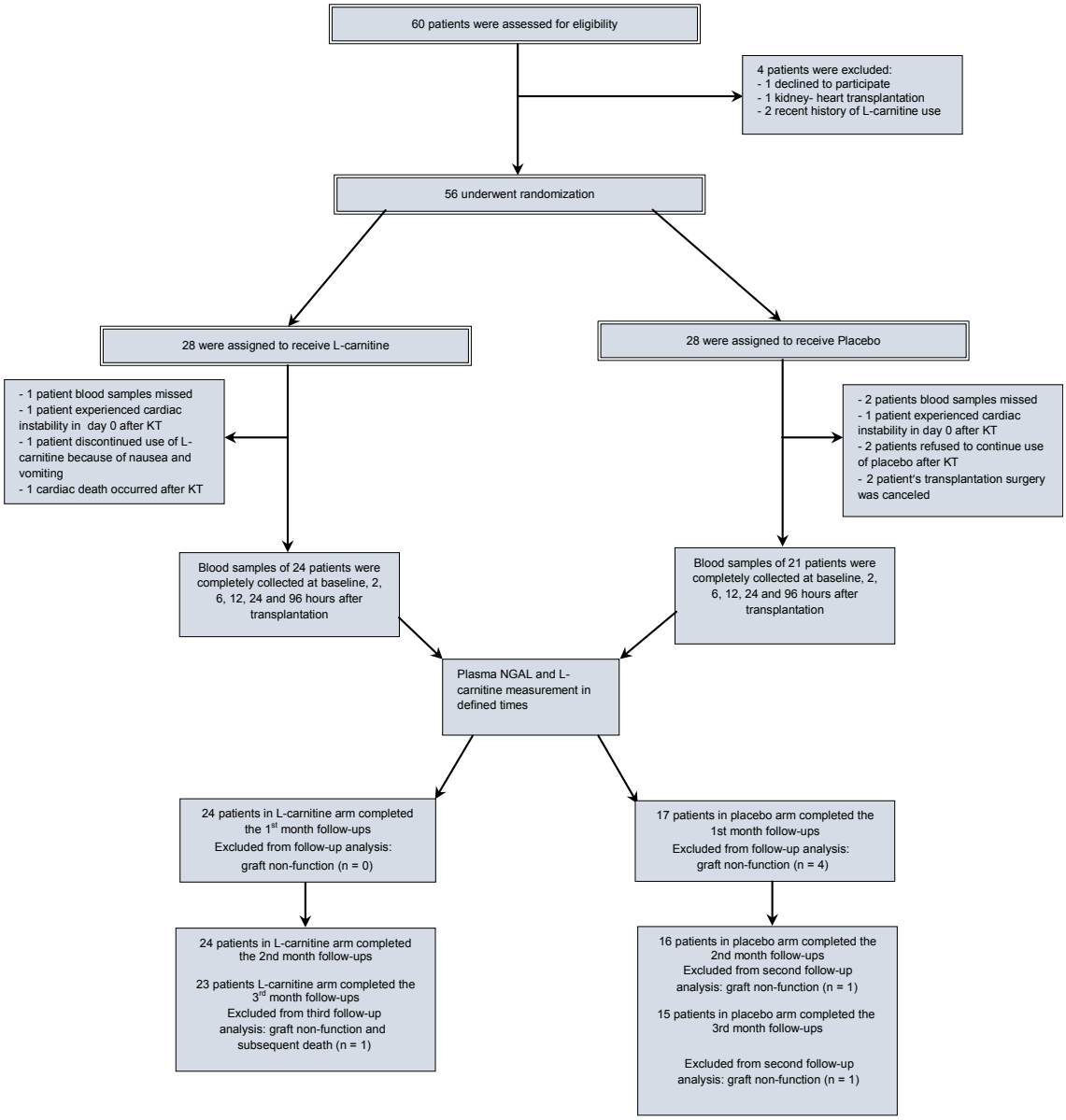

Figure 1. Patients' inclusion flowchart.

simple syrup as a vehicle without any effective ingredient as the same prescribed schedule for L-carnitine group. We administered each dose of L-carnitine oral solution preferably during or after meals to decrease GI distress. Our patients were ordered to be on "PO" (by mouth diet as tolerated) 4-5 hours after the end of transplantation surgery. To reduce the possible gastrointestinal distress induced by syrup in first hours after transplantation, we explained to patients to consume each dose slowly and in multiple small amounts.

Clinical Management

Kidney organs from deceased donors were preserved in Belzer University of Wisconsin cold storage solution before implantation. All subjects who fulfilled the inclusion criteria and received kidney transplantation from deceased donors were managed with a similar immunosuppressive regimen according to this center's protocol. This includes preoperative administration of 1-g mycophenolate mofetil, rabbit thymoglobulin (rATG) 1 mg/kg intravenous infusion started 1 hour before surgery along with 500 mg intravenous methylprednisolone immediately before transplant surgery. ATG 1 mg/kg was continued in the days after surgery with dose adjustment based on white blood cells and platelet counts according to the local protocol. The total prescribed dose of rATG was up to a minimum cumulative dose of 6 mg/kg as a usual immunosuppressive protocol in this center. Methylprednisolone 250-mg single dose on the first day and 125-mg single dose on the second day after transplantation were administered. On the third day after

transplantation, oral prednisolone 1 mg/kg/day was started and rapidly tapered down to 5 mg/kg/day at the end of month 1 after transplantation and continued at this does thereafter. Mycophenolate mofetil along with 1 member of calcineurin inhibitors (CNIs; cyclosporine or tacrolimus) and oral prednisolone were prescribed as standard maintenance immunosuppressive regimen according to local practice. We administered CNIs twice daily and adjusted its dose to maintain trough and/or 2-hour post-morning dose blood levels within defined ranges in the local immunosuppressive protocol of the center. All patients received prophylaxis for *pneumocystis jirovecii* (Trimethoprim/sulfamethoxazole), cytomegalovirus (ganciclovir or valganciclovir), and candidiasis (clotrimazole troche) according to defined duration of prophylaxis in the center protocol.

Sampling and Measurements

A pretransplant blood sample was taken upon arrival to the transplant unit before starting oral L-carnitine or placebo. For all of the participants, 5-mL blood samples were taken in ethylenediaminetetraacetic acid-containing tubes at 2, 6, 12, 24, and 96 hours after transplantation. Blood samples were immediately centrifuged at 2,500 RPM for 20 minutes to collect plasma and stored at −70°C. The double antibody sandwich enzyme-linked immunosorbent assay kits were utilized for measuring plasma NGAL (Bioassay Technology Laboratory, Shanghai, China) and total (free and esterified) L-carnitine (Bioassay Technology Laboratory, Shanghai, China) concentrations.

Plasma NGAL concentrations were assessed before transplantation and at 2, 6, 12, 24, and 96 hours after transplantation. Plasma L-carnitine concentrations were measured before and at day 4 after transplantation. Carnitine deficiency was defined as the total serum carnitine value of less than 40 nmol/mL.[40,41] We tried to determine average daily intake of carnitine in the past 3 months up to the time of the transplant with the food frequency questionnaire (FFQ) method. The dietary content of carnitine in the participants' daily meals was evaluated according to a prepared list of carnitine-rich containing foods such as beefsteak, ground beef, milk, chicken breast, ice cream, and whole wheat bread (Supplement 1). Participants were asked to determine how often and how much in a day, they consume each of the foods. Then, the average daily carnitine intake was estimated for each participant based on his/her food carnitine content.[42]

Daily measurements of urine output and serum creatinine levels were started before transplantation and continued until discharge from the hospital. All patients were routinely monitored during first 3 months after transplantation regarding their serum creatinine concentration, possible episodes of acute allograft rejection, infections, or adverse drug reactions. Also, all demographic data and considerable information before transplantation from the donor and recipient were gathered. We also evaluated donors regarding criteria for donors.[43]

Study End Points

The major end point of the study was the occurrence of DGF between the 2 groups. DGF in this study was defined as the need for dialysis within the first week after transplantation or less than 10% per day decrease in serum creatinine concentration during 3 consecutive days in the first week after transplantation.[4,44] The hemodialysis requirement was based on clinical diagnosis by a nephrologist. The major criterion for hemodialysis after transplantation was fluid overload. Within the first week after transplantation, when dialysis to treat hyperacute rejection, hyperkalemia, or vascular and urinary tract complications is excluded, DGF is primarily a consequence of I/R.[5]

After DGF diagnosis, the related medical group of the transplantation center was responsible for its treatment. DGF duration was defined as the number of days from the transplantation to the last session of hemodialysis or the day that serum creatinine levels started to decrease more than 10% per day. Secondary end points were creatinine clearance at the end of days 30, 60, and 90 after transplantation using Cockcroft-Gault formula as well as incidence of acute kidney allograft rejection episodes. During hospitalization and follow-up period, each episode of increased serum creatinine level was considered as acute kidney rejection episode, whenever proposed differential diagnosis such as viral infection, bacterial pyelonephritis, urinary leak, and obstruction were ruled out and the decrease in serum creatinine level was observed with acute cellular or antibody-mediated rejection treatments (such as glucocorticoid pulse, rATG, IVIG, plasmapheresis, rituximab). Also, patients who had a renal biopsy with histology report of acute cellular or antibody-mediated rejection were considered as patients who experienced acute rejection episode in this study. All patients were assessed during hospitalization for possible adverse drug reactions induced by L-carnitine such as skin rash, hypersensitivity, seizure, abdominal cramps, diarrhea, and nausea or vomiting. All patients were under daily observation of health care professionals involved in the study for monitoring of patients compliance with L-carnitine or placebo.

Ethical Considerations

The study protocol was approved by the local ethics committee of Tehran University of Medical Sciences and was registered in the Iranian Registry of Clinical Trials (IRCT 201312233043N9). All patients provided written informed consent forms prior to randomization. The authors state that they have obtained appropriate institutional review board approval and followed the principles outlined in the Declaration of Helsinki for all patients.

Statistical Analysis

Statistical analyses were performed using the SPSS software (Statistical Package for the Social Sciences, version 19.0; SPSS Inc., Chicago, Illinois). The results are expressed as mean ± standard deviations or median (interquartile range). The Kolmogorov–Smirnov test was used to assess the normal distribution of variables. Comparisons were performed using the unpaired Student's *t*-test and Mann–Whitney U-test for variables with normal and skewed distribution, respectively. Chi-square and Fisher's exact tests were employed in analyses of nominal variables. *P* values of less than .05 were considered as statistically significant.

Results

Characteristics of the Patients

From 60 kidney transplantation patients, 56 patients who met the inclusion criteria were enrolled in our study. Of this total, 3 (5.4%) patients were excluded from the trial due to cardiac death after transplantation or canceled transplantation surgery exactly before the entrance of patient into the surgery room. Therefore, the intention-to-treat statistical analysis was performed on 53 patients: 27 (51%) subjects in the L-carnitine group and 26 (49%) in the placebo arm.

Forty-five patients included in the study completed intervention regimen, and their blood samples were collected at all defined time points of the study. Of them, 38 patients (15 in the placebo arm and 23 in L-carnitine arm) completed the 3-month follow-up period (Fig. 1). Table 1 shows demographic and clinical characteristics of the recipients and donors. No statistically significant differences were observed between the 2 groups regarding demographic data and clinical characteristics when the intention-to-treat analysis was conducted. Of 53 recipient patients, the most common cause of end-stage renal disease (ESRD) was hypertension (30.2%). This was true for both L-carnitine and placebo groups (33.3% and 26.9%, respectively). The panel reactive antibody scores for all included

Table 1. Demographic and Clinical Characteristics of Kidney Transplant Recipients and Donors

Characteristic	L-Carnitine Group (n = 27)	Placebo Group (n = 26)	*P* Value
Recipients			
Age (y)	41.07 ± 13.87	42.08 ± 14.05	.795
Sex (male) n (%)	16 (59.3)	16 (61.5)	.865
BMI (kg/m^2)	23.17 ± 4.1	23.36 ± 3.8	.859
History of blood transfusion, n (%)	16 (59.3)	13 (50)	.498
Mode of dialysis, n (%)			
Hemodialysis	24 (88.9)	24 (92.3)	>.99
Peritoneal dialysis	0 (0)	0 (0)	
No dialysis	3 (11.1)	2 (7.7)	
Time on dialysis before transplantation, mo*	31.5 ± 17	22.92 ± 17.87	.095
Primary disease, n (%)			
Hypertension	9 (33.3)	7 (26.9)	.444
Diabetes mellitus	7 (25.9)	6 (23.1)	
Glomerulonephritis	0 (0)	3 (11.5)	
ADPKD	2 (7.4)	3 (11.5)	
Renal stone	2 (7.4)	0 (0)	
Bladder reflux	3 (11.1)	2 (7.7)	
Unknown	4 (14.8)	5 (19.2)	
Blood group complete match (%)	25 (92.6)	26 (100)	.491
Sex match (%)	17 (63)	19 (73.1)	.43
Recipient-donor weight ratio	0.85 ± 0.24	0.85 ± 0.22	.937
Panel reactive antibody (%)	0	0	
Donors			
Age (y)	35.56 ± 12.93	38.96 ± 14.39	.369
Sex (male)	16 (59.3)	16 (61.5)	.967
BMI (kg/m^2)	26.3 ± 4.6	25.35 ± 2.56	.358
Donor SCr (mg/dL)	1.27 ± 0.3	1.2 ± 0.29	.443
SCD, n (%)	25 (92.6)	22 (84.6)	.42
ECD, n (%)	2 (7.4)	4 (15.4)	

ADPKD, autosomal dominant, polycystic kidney disease; BMI, body mass index; ECD, expanded criteria donor (deceased donor who, aged ≥ 60 years old or aged 50-59 years old and has 2 of the following 3 criteria: (1) cause of death is cerebrovascular accident; (2) preexisting history of systemic hypertension; and (3) terminal serum creatinine of >1.5 mg/dL); SCD, standard criteria donor (all deceased donors who do not meet any of the criteria for an ECD and from whom donation occurred after brain death are considered as an SCD); SCr, serum creatinine concentration; SD, standard deviation.

Data have been presented as mean ± SD or number (%) as indicated.

*In average, all patients received hemodialysis 3 times per week for 4 hours in each session.

Table 2. Clinical Occurrence of DGF, DGF Duration, Length of Hospital Stay, and Percentage of Acute Rejection During Hospitalization

Characteristics	L-Carnitine Group (n = 27)	Placebo Group (n = 26)	*P* Value
DGF	5 (18.51)	6 (23.1)	.68
Dialysis needs during the first week after transplantation due to DGF	3 (11.1)*	0 (0)	.242
Less than 10% per day decrease in serum creatinine concentration during 3 consecutive days in the first week after transplantation without dialysis needs	2 (7.4)	6 (23.1)	.142
DGF duration (d)	10.8 ± 10.8 [8 (5-19)] range: 5-30	6.5 ± 1.76 [6 (5.5-8.25)] range: 4-9	.426
Length of hospital stay	19.11 ± 12.37 d [15 (10-21)]	16.58 ± 12.37 d [13 (10.5-17.25)]	.381
Acute rejection during hospitalization	8 (29.62)	8 (30.76)	.928

DGF, delayed graft function; SD, standard deviation.

Data have been presented as mean ± SD [median (interquartile range)] or number (%) as indicated.

*The number of dialysis sessions needed for each of these 3 patients were 2, 7, and 11 session. Average time of each session of dialysis was 2-4 hours.

kidney transplant recipients were 0% before kidney transplantation.

There were no significant differences among donors regarding the cause of death and baseline risk factors between the study arms. No significant differences were found between the 2 groups regarding drug history in kidney transplant recipients. Of 53 donors, 88.67% were standard criteria donor, and 11.32% were expanded criteria donors. There was no significant difference between the 2 groups of the study regarding the type of donor criteria (Table 1). Also, the frequency of standard criteria donors and expanded criteria donors was not different between patients who experienced and who did not experience DGF ($P = .529$).

DGF Occurrence and Duration

We performed an intention-to-treat analysis of everyone who received at least one dose of L-carnitine or placebo for the primary end point of the study, the incidence of DGF.

Of 7 patients who needed hemodialysis within the first week after transplantation, 4 patients returned to maintenance dialysis due to primary graft failure and 3 of them needed dialysis due to DGF. DGF was clinically observed in 11 of the 53 studied patients (20.8%). There were no significant differences in the DGF incidence and its duration between the L-carnitine and placebo groups (Table 2). One member of L-carnitine group had a prolonged DGF period (30 days) that caused more prolonged DGF duration in the statistical analysis of the L-carnitine group.

Plasma NGAL Measurements

Although the plasma NGAL levels within the first day after transplantation were lower in the L-carnitine group, these differences did not reach statistical significance. Mean changes in plasma NGAL from the 1st to 2nd, 2nd to 3rd, 3rd to 4th, 4th to 5th postoperative sampling times were not significantly different between the 2 groups of the study (Table 3). Figures S1 and S2 (available online) show plasma NGAL levels for each patient in the placebo and the L-carnitine groups over time.

Figure 2 compares plasma NGAL concentrations between patients with and without DGF. This analysis was done with the exclusion of those 4 patients that returned to maintenance hemodialysis from the first week because of primary transplantation failure. Plasma levels of NGAL in DGF-experienced patients decreased sooner in patients

Table 3. Plasma NGAL Levels (ng/mL) in 2 Groups of the Study

Time (h)	L-Carnitine Group (n = 24) Mean ± SD [Median (Interquartile Range)]	Placebo Group (n = 21) Mean ± SD [Median (Interquartile Range)]	*P* Value
0 (baseline)	307.11 ± 270.11 [158.3 (107.02-516.73)]	334.70 ± 419.47 [149.2 (115.73-365.19)]	.964
2	302.47 ± 355.24 [154.5 (106.13-340.99)]	378.72 ± 387.47 [137.1 (117.13-723.97)]	.838
6	294.38 ± 323.20 [159.02 (116.64-381.8)]	384.52 ± 454.78 [154.6 (110.64-568.73)]	.873
12	277.88 ± 284.8 [151.5 (115.23-329.86)]	409.448 ± 491.86 [145.36 (111.85-706.5)]	.802
24	283.82 ± 246.4 [170.54 (120.11-409.8)]	364.92 ± 374.63 [213.93 (108.03-562.05)]	.891
96	299.87 ± 276.85 [148.5 (120.84-417.8)]	275.55 ± 295.99 [146.47 (117.56-343.74)]	.750
Differences 2-6 hours	−8.1 ± 159.84	5.792 ± 218.98	.439
Differences 6-12 hours	−16.5 ± 142.4	24.932 ± 111.17	.133
Differences 12-24 hours	5.94 ± 156.62	−44.52 ± 269.23	.203
Differences 24-96 hours	16.05 ± 163.21	−89.37 ± 210.26	.165

NGAL, neutrophil gelatinase-associated lipocalin; SD, standard deviation.

who received L-carnitine (2 hours after transplantation) compared with patients who received placebo (after 12 hours after transplantation), although the difference did not reach statistical significance (Fig. 3).

Daily L-Carnitine Intake

There was no significant difference in dietary intake of carnitine before transplantation between the L-carnitine and placebo groups (27.94 ± 14.59 vs. 26.67 ± 13.28 mg/day; $P = .742$), although the mean of daily dietary intake of carnitine in patients who experienced DGF was lower than no DGF patients (20.55 ± 12.83 vs. 29.09 ± 13.69 mg/day), but the difference was not significant ($P = .068$).

Plasma L-Carnitine Measurement

Plasma L-carnitine levels did not differ between patients in the L-carnitine and placebo groups neither at baseline (116.3 ± 61.85 and 107.9 ± 48.21 nmol/mL, respectively; $P = .63$) nor 4 days after kidney transplantation (113.7 ± 58.63 and 102.19 ± 60.21 nmol/mL, respectively; $P = .519$).

Carnitine deficiency was detected in 1 patient (4.76%) in the placebo group and 3 (12.5%) patients in the L-carnitine group ($P = .363$) at the initiation of the study. The 4 carnitine deficient patients had more time on maintenance dialysis before transplantation compared with patients with carnitine sufficiency (34.5 ± 15.78 vs. 28.28 ± 18.78 months; $P = .529$).

Baseline plasma carnitine concentration was significantly lower in patients who showed DGF compared to patients without DGF (73.74 ± 47.39 vs. 122.97 ± 54.157 nmol/mL respectively; $P = .017$).

Patient Follow-Up

The length of hospital stay after transplantation procedure did not differ between L-carnitine and placebo groups (Table 2). Total allograft failure during hospitalization or within 3 months after kidney transplantation happened in 6 patients in the placebo and 1 patient in the L-carnitine group ($P = .05$). There was no significant difference in possible acute rejection during hospitalization (Table 2) and 3 months after transplantation (Table 4) between the 2 groups. Serum creatinine concentrations and creatinine clearances were similar between the 2 groups of the study during 3 months after transplantation (Table 4). As seen, during 3 months of follow-up, serum creatinine levels were lower, and creatinine clearances were higher in the L-carnitine group. Although these differences did not reach statistical significance, however, the trend was toward significance. During hospitalization after kidney transplantation, the study groups showed no statistically significant differences in the incidence of urinary tract infection, cytomegalovirus, and polyomavirus infections or other post-transplant complications (data are not shown).

There were some adjustments of medications due to recognized side effects of medications involved in post-transplantation treatments. However, these did not affect L-carnitine compliance. Oral L-carnitine administration was well tolerated during this study. One patient reported nausea and vomiting within the first day

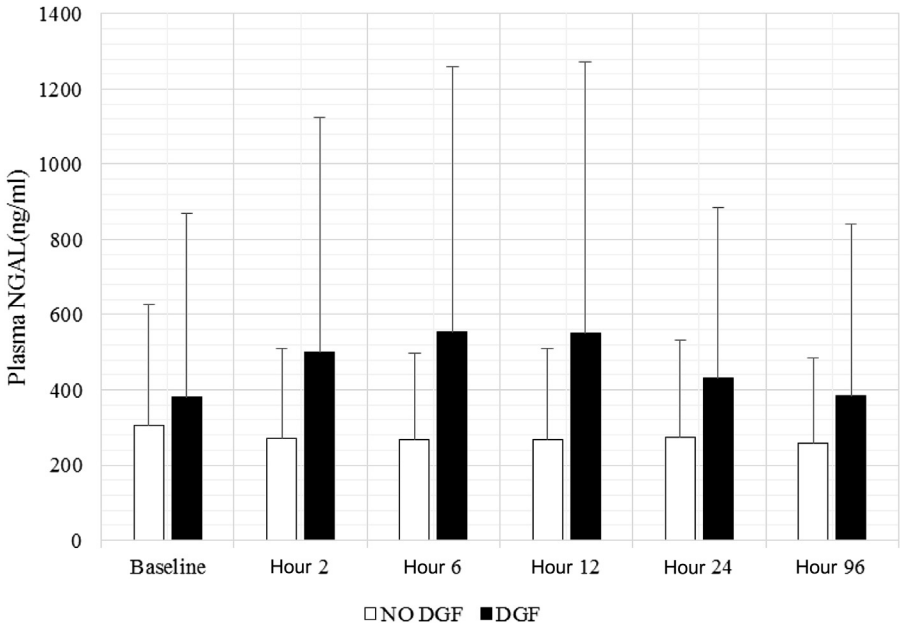

Figure 2. Levels of plasma NGAL with time in DGF (n = 9) and no DGF (n = 32) patients (mean + SD). This analysis was done with the exclusion of those 4 patients that returned to maintenance hemodialysis from the first week because of primary transplantation failure. Samples of 2 patients in DGF group was missed. DGF, delayed graft function; NGAL, neutrophil gelatinase-associated lipocalin; SD, standard deviation.

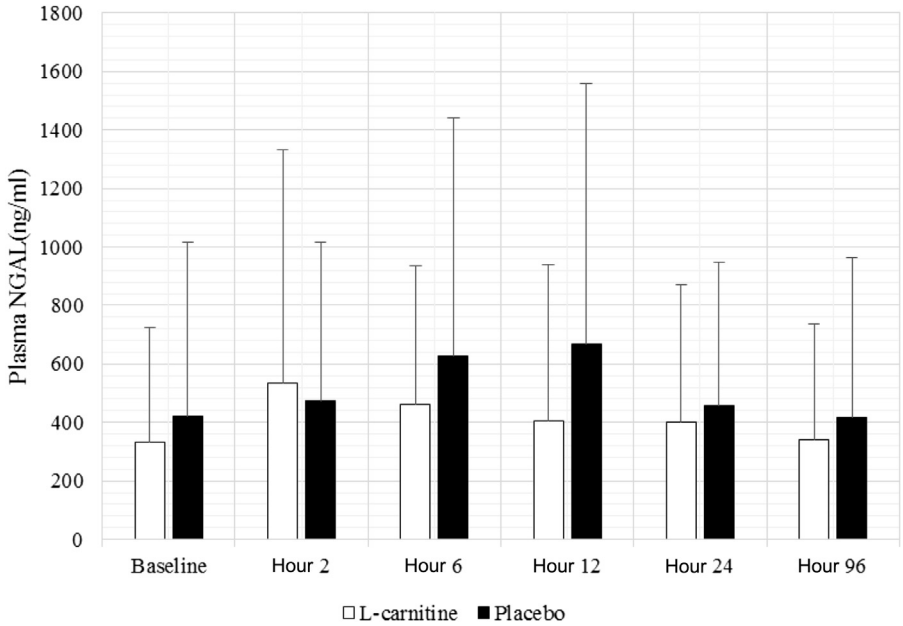

Figure 3. Levels of plasma NGAL (mean + SD) with time in DGF-experienced patients who received L-carnitine (n = 4) or placebo (n = 5). Samples of 2 patients in DGF group was missed. DGF, delayed graft function; NGAL, neutrophil gelatinase-associated lipocalin; SD, standard deviation.

after transplantation and discontinued L-carnitine administration. There was no significant difference between 2 arms in terms of adverse drug reactions during hospitalization.

Concerning the compliance of L-carnitine or placebo use, in the placebo group, 2 patients were not cooperative for continuing use of syrup and were excluded from the study because of nonadherence. In the L-carnitine group, 1 patient refused to continue regimen on day 0 after transplantation because of nausea and vomiting after L-carnitine administration, although we wanted the patient to use it during meals, but patient refused continuing participation in this study (Fig. 1).

We also evaluated that whether L-carnitine supplementation alters the immunosuppression. There were no significant differences in the number of CNIs dosing changes during the first week after CNI initiation ($P = .648$) between the 2 groups. There was only one report of CNI toxicity in placebo group within days of hospitalization after transplantation which was not significant in comparison with L-carnitine group ($P = .491$). There was no report of CNIs toxicities during 3-month follow-up after transplantation in both groups of patients. Unfortunately, we missed recording the CNI blood levels for all patients during the first week after transplantation to evaluate effects of L-carnitine on changes of CNI levels.

There were no significant differences between the 2 groups regarding any adverse drug reactions during the 3-month follow-up. During 3 months after kidney transplantation, no statistically significant differences were observed in the incidence of urinary tract infection (8 patients in the L-carnitine group vs. 7 patients in placebo group), cytomegalovirus infection (2 patients in the placebo group), and polyomavirus infection (1 patient in the placebo group) or other complication that patients in both arms were possible to be encountered. During follow-up period, 1 patient in the L-carnitine group died at month 3 after transplantation because of brain abscess. The brain abscess event in this patient was unlikely to be related to taking L-carnitine supplement at the time of transplant.

Discussion

This is the first clinical study that assessed the effect of L-carnitine on the function of transplanted kidneys from deceased donors and DGF incidence. The frequency of DGF varies from 4 to 10% in living donor transplants and 5 to 50% in deceased donor kidney transplants.[1] Therefore, DGF occurrence in our study (20.8%) was similar to other studies. The results revealed that perioperative oral administration of 3 g/day of L-carnitine did not significantly reduce the incidence of DGF and its duration in comparison with the placebo group. Patients who suffered DGF showed lower serum carnitine levels before transplantation compared with non-DGF patients. All patients in both groups of the study received similar induction immunosuppressive therapy with potent polyclonal agent thymoglobulin. Nevertheless, 3-month graft loss was significantly higher in the placebo group compared with L-carnitine group. Higher incidence of graft loss in the placebo group despite administration of exactly the same protocol of induction immunosuppressive therapy highlights the positive

Table 4. Serum Creatinine and the Creatinine Clearance at Days 0 to 7, 30, 60, and 90 After Transplantation, After Excluding Graft Failures, and Percentage of Acute Rejection During 3 Months After Transplantation

Daily Serum Creatinine (mg/dL)	L-Carnitine Group (n = 27)	Placebo Group (n = 26)	*P* Value
Baseline before transplantation	7.85 ± 3.57	8.38 ± 2.42	.533
Day 1	5.76 ± 2.47	5.66 ± 2.09	.881
Day 2	4.18 ± 2.32	4.31 ± 2.35	.855
Day 3	3.23 ± 2.21	3.87 ± 2.35	.314
Day 4	2.79 ± 2.09	3.6 ± 2.29	.181
Day 5	2.55 ± 2.13	3.36 ± 2.36	.197
Day 6	2.32 ± 1.98	3.13 ± 2.37	.185
Day 7	2.25 ± 2.15	2.95 ± 2.39	.263
First Month Follow-Up	**L-Carnitine Group (n = 27)**	**Placebo Group (n = 22)**	
Serum creatinine (mg/dL)	1.48 ± 0.83 1.29 (1.02-1.7)	1.59 ± 0.95 1.35 (1.14-1.75)	.438
Percentage of change in serum creatinine (%)	−13.42 ± 24.92	−11.62 ± 23.78	.799
CrCl (mL/min)	61.31 ± 21.97	53.42 ± 15.39	.162
Acute rejection n (%)	3 (11.1)	3 (13.6)	.789
Second Month Follow-Up	**L-Carnitine Group (n = 27)**	**Placebo Group (n = 21)**	
Serum creatinine (mg/dL)	1.39 ± 0.5	1.48 ± .65	.572
Percentage of change in serum creatinine (%)	−5.75 ± 30.82	0.1824 ± 21.056	.454
CrCl (mL/min)	61.26 ± 20.71	55.19 ± 14.75	.262
Acute rejection n (%)	4 (14.8)	3 (14.2)	.959
Third Month Follow-Up	**L-Carnitine Group (n = 26)**	**Placebo Group (n = 20)**	
Serum creatinine (mg/dL)	1.18 ± 0.31	1.32 ± 0.63	.345
Percentage of change in serum creatinine (%)	−13.66 ± 19.74	−8.75 ± 17.88	.371
CrCl (mL/min)	69.72 ± 18.58	62.58 ± 17.17	.189
Acute rejection n (%)	0 (0)	2 (10)	.186

CrCl, creatinine clearance (mL/min) estimate by Cockcroft-Gault equation; SD, standard deviation.
Data have been presented as mean ± SD. If the distribution of variable was not normal, data are shown as median (interquartile range).

impact of L-carnitine. Besides, we did not observe that L-carnitine causes a difference in the number of CNI dosing change during the first week after CNI initiation or CNI toxicity during 3-month follow-up period in comparison with the placebo group. Based on these findings, L-carnitine could be a promising agent in future studies for evaluation in kidney transplant centers that use less potent immunosuppressive induction drugs.

Some previous studies suggested plasma NGAL concentrations as a sensitive marker to predict the DGF in deceased kidney transplant recipients.[45,46] In the present study although plasma levels of NGAL were higher at any assessed time in patients with DGF compared with those without DGF, however, the difference did not reach statistical significance. This finding is somewhat consistent with the finding of Lee et al.[45] and Lebkowska et al.[47] Lebkowska et al. observed significant fall in serum NGAL level, as early as 1 day following kidney transplantation from deceased donors. In their study, serum NGAL was significantly higher among patients with DGF compared with those without DGF.[47] In agreement with Kusaka et al.[8] findings, in our study, plasma NGAL did not fall into the high normal range (53 ± 30 ng/mL) 4 days after transplantation.

Various studies reported the removal of NGAL with hemodialysis. The significance of serum NGAL to reflect severity and prognosis of acute kidney injury may be lost in patients receiving renal replacement therapy because intensified convection or substantial adsorption (or both) on currently used dialysis membranes enhances plasma clearance of this biomarker.[48] In this study, 3 of subjects who experienced DGF required dialysis within the first few days after transplantation. Although we did not find any significant differences in NGAL levels in various measuring time between DGF patients with and without need of HD, it should be kept in mind that dialysis in days of plasma NGAL level measurement can affect on plasma NGAL concentrations of these patients.[48]

Some studies evaluated the influence of carnitine supplementation on the intravenous iron-induced oxidative stress in CKD patients by measuring blood NGAL levels. Administration of carnitine decreased plasma levels of NGAL and advanced oxidative protein products in these patients possibly due to the anti-inflammatory and antioxidative properties of carnitine.[49] In another study, using carnitine in the rats with intra-abdominal sepsis model decreased the serum cytokine levels, renal damage, and apoptosis via suppressing the oxidative stress and inflammation both in blood and tissue. The NGAL as a marker of renal tissue damage decreased after intraperitoneal administration of carnitine in this sepsis model.[50] In our study, L-carnitine supplementation could not significantly reduce

plasma NGAL, but lower plasma NGAL levels at different time points after transplantation were observed in the L-carnitine group compared with placebo group (without a significant difference) (Table 3). Moreover, a decrease in plasma NGAL level was seen sooner in the L-carnitine group compared with placebo group. The same results were observed in those 9 patients who encountered DGF and received placebo or L-carnitine (Fig. 3). This finding shows that L-carnitine supplementation may accelerate the decrease in plasma NGAL level.

Several attempts have been made to evaluate the nephroprotective effect of L-carnitine against I/R injury. In the human proximal tubule epithelial cell line, L-carnitine showed its protective role against H_2O_2-induced injury through the inhibition of oxidative damage, mitochondrial dysfunction, and inhibition of cell apoptosis.[21] L-carnitine also partially prevented oxidative stress in an animal model of I/R injury induced by operations of the infrarenal abdominal aorta.[38]

An animal study proposed that pretreatment with L-carnitine in solid organ transplantation induces protective effect against reperfusion injury of the kidney via preventing cell membrane damages due to lipid peroxidation in the process of oxidative stress.[35]

An ex vivo study also revealed that pre-exposure of isolated kidneys to propionyl-L-carnitine, before establishing the ischemia reduced lipid peroxidation and free radical generation. They also found that when kidneys are preserved in cold Belzer University of Wisconsin solution containing propionyl-L-carnitine and then transplanted to binephrectomized recipients, renal function was largely preserved compared to untreated ischemic grafts. Propionyl-L-carnitine almost completely prevented infiltration of polymorphonuclear cells to the graft and reduced posttransplant tubular injury.[33] Intraperitoneal administration of L-carnitine 15 minutes before the renal ischemia insult in a rat model of I/R injury also decreased lipid peroxidation, neutrophil function, and nitric oxide metabolism in the kidney tissues.[36] Azzollini et al. conducted another study in allogeneic transplant model that supports findings of all studies mentioned above. They reported that storage of grafts in Belzer University of Wisconsin solution supplemented with propionyl-L-carnitine during the cold ischemia period improved the 3-month graft survival compared to rats receiving untreated kidneys with equal cold ischemia times.[51] These results suggest that L-carnitine derivatives may prevent I/R injury via inhibition of inflammation, apoptosis, neutrophil infiltration, and lipid peroxidation.

Tissue carnitine levels decrease in I/R injured kidneys after reperfusion.[37,39] This carnitine deficiency may affect utilization of fatty acid (major energy source in the kidneys) for energy metabolism because carnitine is required as a carrier for fatty acid transport to mitochondria by carnitine palmitoyltransferase I (CPT1). CPT1 activity decreases in kidney tissue after I/R injury.[39] These data supported our rationale to replenish carnitine for treating renal I/R injury.

More chronically administration of L-carnitine before I/R insult also exerted a protective effect that is mediated by its antioxidant and anti-inflammatory effects or by increased intracellular carnitine content, with a consequent improvement in mitochondrial oxidative phosphorylation and energy production.[37]

Besides the prophylactic use of L-carnitine in I/R injury in studies mentioned above, an animal study designed to evaluate its effect as a treatment after the occurrence of I/R injury.[39] Intravenous administration of carnitine after reperfusion at the early stage of developing I/R injury insignificantly increased adenosine triphosphate level and CPT1 activity. The administration of carnitine and a CPT1 activator together at the reperfusion stage significantly reduced renal damage after I/R.[39] Another research suggested that longer duration of intraperitoneal administration of L-carnitine (30 minutes prior to renal ischemia and during the reperfusion period for 7 days) in a long-term I/R injury rat model attenuated I/R-induced histological alteration, lipid peroxidation, and oxidative stress.[34]

All of these results from animal studies support the clinical applicability of carnitine as additive to organ storage solution or administration to organ recipients with the assumption that it might be useful for prevention of DGF induced by I/R injury. According to these experimental studies, L-carnitine should be used before stress oxidative events in I/R injury. Therefore, we started administration of L-carnitine several hours before transplantation surgery. It was not possible for us to provide L-carnitine to subjects prior to the immediate peritransplant period.

Since this was the first human study, we chose the daily oral dose of L-carnitine according to other clinical use of this agent in human such as carnitine deficiency,[52] peripheral neuropathy,[53] prevention of chemotherapy-induced cardiac toxicity,[54] and ESRD patients.[55] Oral L-carnitine in ESRD patients was administered in a ranging from 1 to 3 daily doses, from 10 mg/kg body weight per day to 3 g per day.[55] Some patents have been approved for use of L-carnitine and its derivatives for the prevention or treatment of nephrotoxicity of CNIs. It does not seem that L-carnitine causes any serious adverse event in patients after transplantation.[56,57]

Most animal studies used L-carnitine. However, some used propyonl-L-carnitine.[33,51] We used L-carnitine because it is the available formulation in our country. Also, L-carnitine has a greater maximum plasma concentration and longer half-life compared with its analogs (acetyl-L-carnitine and propionyl-L-carnitine) following oral administration.[58] Oral absorption of L-carnitine has a dose-dependent manner because of saturation of its transporters in the small intestine with L-carnitine doses of larger than 2 g[59-62] that necessitate dividing its administration when higher doses of this agent are needed.

Thus, L-carnitine was prescribed in 3 divided doses in this study.

Another concern for oral administration of L-carnitine is the accumulation of trimethylamine, 1 metabolite of L-carnitine which is generated by the enterobacterial flora.[63] Trimethylamine and trimethylamine-N-oxide possibly contribute in neurotoxicity and "uremic breath" in patients with ESRD,[64,65] but they are efficiently removed during a single hemodialysis session.[66] The increase in the plasma concentrations of trimethylamine-N-oxide may occur after administration of more than 2-g L-carnitine 3 times a day for 7 days.[67] Furthermore, in dialysis patients who receive oral L-carnitine 1-g daily, plasma concentrations of trimethylamine-N-oxide continue to rise after 2 weeks.[68] Therefore, it does not seem that the regimen we designed this study to prescribe L-carnitine 1-g thrice daily for only 4 days induce significant accumulation of toxic metabolites. Since we did not access to intravenous formulations of L-carnitine in our country, we used oral formulation.

In this study, baseline plasma levels of carnitine were lower in patients with DGF compared with patients without DGF. This finding suggests that lower levels of carnitine may increase the risk of DGF in kidney transplant recipients and a connection maybe exists between L-carnitine supplementation and DGF. However, supplementation with L-carnitine could not decrease the risk of DGF in these patients. One suggested reason is that our study did not provide enough L-carnitine to show complete preventive effects against DGF. Some possible causes for low efficacy of antioxidants against I/R injury may be the short half-life of reactive oxygen species that necessitate the need for antioxidant with rapid reaction kinetic, multiple sites of reactive oxygen species generation and restricted cellular uptake of antioxidant agents.[69]

There were no significant differences in dietary intake of carnitine before transplantation between the L-carnitine and placebo groups or between patients with or without DGF. Although we reported the analysis of data about daily dietary intake of carnitine, the content of carnitine in foodstuff is based on an old and inadequate methodology.[20] In addition, the FFQ used in this study was not validated in CKD population. The validation for the FFQ of average daily intake of carnitine in CKD population seems necessary. Therefore, the shortcoming of well-documented data about dietary carnitine intake makes discussion on dietary carnitine and its relationship with DGF very difficult. In addition, differences in cooking methods can affect the amount of carnitine in foods and subsequently on dietary intake of carnitine.

As the majority of patients included in this study were on maintenance hemodialysis, high levels of plasma total carnitine before transplantation are acceptable because in hemodialysis patients, plasma total carnitine concentration is normal or elevated.[25] Although not statistically significant, plasma total carnitine concentrations decreased in both L-carnitine and placebo groups 96 hours after transplantation. Nevertheless, the amount of decrease in serum carnitine level was lower in the L-carnitine group. This decrease in serum carnitine level may be due to increased carnitine need and utilization as an antioxidant and energetic source after I/R insult of kidney transplantation. Animal studies have also shown that carnitine levels in kidney tissues decrease following I/R insult especially after reperfusion.[37,39] L-Carnitine supplementation in hemodialysis patients increases plasma total, free, and acylcarnitine levels and improves the acylcarnitine to free carnitine ratio. This finding suggests that carnitine continues to bind acyl residues that are present in excess in dialysis patients.[70] Also, in transplantation setting, measurement of acyl and free forms of carnitine in future studies will help to suggest better explanation about plasma levels of L-carnitine after oral administration.

The majority of our subjects were on maintenance dialysis for more than 1 year before transplantation. It has been demonstrated that carnitine stores decline in maintenance dialysis patients.[27] In fact, decreases in both free carnitine and plasma acylcarnitines have been reported after only 6 months of dialysis treatment.[71] Useful effects of L-carnitine in studies on dialysis patients (such as effects on anemia, hypotension, muscle weakness, improved lipid profiles, decreased oxidative stress, and reduced hospitalization) have been observed after several months of L-carnitine administration.[27,71,72] Therefore, it seems that the short period and acute administration of L-carnitine may be insufficient to see desirable antioxidant effects.

Keeping the cold ischemia time at the lowest possible time is unlikely to be sustainable in kidney transplant programs. Even though prolonged cold ischemia time is a well-known risk factor that may impair kidney function in the early posttransplantation period, but its impact on graft survival is controversial in studies.[73-75] Also, there is no consensus about what threshold values of cold ischemia time actually indicate an increased risk of graft failure.[73] There was a wide range of time for cold ischemia time in these studies.[74,75] One of these studies demonstrated that even a short lengthening in cold ischemia time may increase the risk of graft failure and also the risk of mortality. Each additional hour of cold ischemia time was associated with 1.3% increased risk of graft failure and 1.8% increased risk of death.[74] Unfortunately, we did not record the exact times of cold and warm ischemia for each transplantation to evaluate the effect of ischemia time on end points of this study.

Oral L-carnitine administration was well tolerated during this study. Nausea and vomiting in the first day after transplantation were reported by 1 patient that resulted in discontinuation of L-carnitine, although nausea and vomiting within first hours after surgery are multifactorial.

This study encountered several limitations including limited sample size and short-term frame for oral

L-carnitine administration. Larger clinical trials using intravenous administration of L-carnitine for a longer period and started earlier before transplantation or allograft storage in L-carnitine-containing solutions are recommended. Most candidates for kidney transplantation in this study were not carnitine deficient; however, lower serum carnitine levels were detected in patients who underwent DGF. This finding may necessitate evaluation of carnitine deficiency in a larger population of patients with the ESRD who are a candidate for kidney transplantation.

In this study, reported better 3-month outcome with carnitine could be due to changes in tissue carnitine levels. However, the proof of this hypothesis needs studies to measure changes in tissue carnitine levels. We suggest renal biopsy for histological examination of kidneys of L-carnitine-treated patients in future studies will help to measure carnitine level in renal tissue and to evaluate carnitine effects on kidney tissue early after kidney transplantation in human.

Practical Application

This study could not illustrate positive effects of L-carnitine on plasma levels of NGAL, a biomarker for DGF. However, the observation of less 3-month graft loss in L-carnitine group suggests that L-carnitine may be a promising agent for evaluation in larger clinical trials about DGF risk. This study also provided safety data for future research on the use of L-carnitine for DGF prevention and recovery.

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Supplementary Data

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