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Original Article

Effects of pre-transplant L-carnitine supplementation on primary graft dysfunction in liver transplant recipients: a pilot, randomized, placebo-controlled clinical trial

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Abstract

Primary graft dysfunction (PGD) and non-function (PNF) happen in 8.7-24.7% and 0.9-7.2% of liver transplant recipients, respectively. These phenomena increase treatment cost and patients' death. This study assessed the effect of L-carnitine supplementation on the incidences of PNF/PGD in liver transplant recipients. This randomized, placebo-controlled, clinical trial was performed on adult liver transplant recipients. Patients took L-carnitine syrup 500 mg three times daily or placebo from the time of including in transplant waiting list until the day of transplant surgery (median 14 days, 1-192 days). Thirty-three patients in L-carnitine and 39 patients in placebo group completed the study. Although not statistically significant, PNF and PGD happened less frequently among recipients in L-carnitine compared with placebo group (3% *vs.* 12.8% for PNF; 15.2% *vs.* 30.8% for PGD). Alanine aminotransferase (ALT) and aspartate aminotransferase were lower in L-carnitine group at day 3 after transplantation. ALT declined more significantly within 48 h after transplantation in L-carnitine arm (median 120.50 *vs.* 79 IU/L; $P = 0.03$). One-month patients' survival was significantly higher in L-carnitine versus placebo group (97% *vs.* 74.4%; $P = 0.008$). The rates of PNF and PGD in L-carnitine group were approximately one-fourth and one-half of placebo group respectively. One-month patients' survival was higher in L-carnitine group.

Keywords: Ischemia reperfusion injury; L-carnitine; Liver transplantation; Primary graft.

INTRODUCTION

Liver transplantation is an effective treatment for patients with cirrhosis complications. Primary graft dysfunction (PGD) is a main problem after liver transplantation that leads to increased length of intensive care unit (ICU) and hospital stays, graft loss, recipients' death, and treatment cost (1). There is no standard pathologic diagnosis for PGD and no consensus on its definition (2). PGD has different stages based on severity including primary graft non-function (PNF) and initial poor function, the latter is also known as early allograft dysfunction (EAD). Although there are several different definitions for PNF, it is commonly accepted that without immediate re-transplantation, PNF leads to graft loss and recipient's death (1,2). In these patients during first few days after liver transplantation serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and bilirubin concentrations increase. Decreased allograft function in PNF-experienced patients leads to hepatic hemodynamic instability, hypoglycemia, coagulopathies, metabolic acidosis, absence of bile flow, and also renal and respiratory failure. There are also different definitions for EAD.

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The most commonly used definition is the presence of at least one of the following findings in the liver transplant recipient: serum bilirubin concentration of 10 mg/dL or higher at the end of first week after liver transplantation, international normalize ratio (INR) of 1.6 or higher at day 7 after transplantation or presence of serum aminotransferases of higher than 2000 IU/L within first week after transplantation (1).

Considering different definitions, the incidences of PGD and PNF vary among studies from 8.7% to 24.7% and 0.9% to 7.2%, respectively (3). Several donors' characteristics (older age, macrosteatosis of the liver, non-heart-beat donor, hypoxia), organ procurement, and transplant surgery factors (long cold and warm ischemia time, increased anhepatic phase) and recipients' characteristics (age, renal insufficiency before liver transplant, re-transplant, model for end-stage liver diseases (MELD) score of 20 or more) have been associated with PGD (1,3).

Ischemia/reperfusion (IR) injury is considered as the main cause of PGD (1). IR injury happens following reperfusion of flow-deprived organs. Molecular mechanism of IR includes oxidative stress due to mass production of reactive oxygen species by mitochondria and some enzymes such as xanthine oxidase, NADPH oxidase, and nitric oxide synthase, and increased inflammatory mediators (4). Therefore, interventions to reduce IR injury may be helpful for protecting the liver and reducing PNF and PGD occurrence. Several antioxidant and anti-inflammatory agents such as prostaglandin (PG) E1, PGI2, and N-acetyl cysteine (NAC) have been applied to reduce IR injury to hepatic allograft with different results (5-8).

L-carnitine was discovered in 1905 and its main function is transferring long-chain fatty acids from cytosol to mitochondria to generate adenosine triphosphate (ATP). In human, the main source of L-carnitine is exogenously achieved from animal diet and the lesser part is endogenously biosynthesized using methionine and lysine in different organs mainly in the liver, kidney, and brain (9). L-carnitine and its derivatives have substantial antioxidant, anti-inflammatory, and anti-apoptotic properties through several mechanisms (10). First, L-carnitine derivatives act as direct scavenger of free radicals. Second, L-carnitine chelates some metals such as cupper and iron that act as promoters of reactive oxygen species generation. Third, L-carnitine derivatives prevent reactive oxygen species formation by inhibition of some enzymes such as xanthine oxidase and NADPH-oxidase. Fourth, L-carnitine derivatives maintain mitochondrial integrity. Finally, L-carnitine derivatives affect redox-signaling via activation of nuclear factor erythroid 2-related factor 2 and peroxisome proliferator-activated receptor alpha, inhibition of nuclear factor kappa-light-chain-enhancer of activated B cells, and additional synthesis of antioxidant enzymes (10).

L-carnitine and its derivatives have been studied as antioxidant agents with potential hepatoprotective effects against hepatotoxicity of different drugs (11-13). L-carnitine derivatives have also shown protective effects against IR injury in different tissues and organs such as neuromuscular system, gastrointestinal tract, liver, and kidney (14-16). Addition of L-carnitine derivatives to cold storage solutions that are used to preserve donated organs during cold ischemia phase mitigated IR injury to hepatic tissue (17,18). In these animal studies, hepatic tissues that were preserved in L-carnitine-containing storage solutions released lower ALT and showed less hepatic mitochondrial injury after reperfusion (17,18). In addition, administration of oral L-carnitine to kidney transplant recipients during first three days after transplantation decreased the incidence of delayed graft function and increased 3-month graft survival in these patients (16). Based on above mentioned data it seems that assessing systemic administration of L-carnitine to reduce hepatic IR injury and PGD incidence in liver transplant patients is worth testing. In this study, for the first time, we evaluated

the effects of prophylactic oral supplementation with L-carnitine against PGD in liver transplant recipients.

MATERIALS AND METHODS

Study type and setting

This prospective, single-blinded, randomized, placebo-controlled, single-center, pilot clinical study was performed in liver transplant ward of Imam-Khomeini Hospital Complex affiliated to Tehran University of Medical Sciences, Tehran, I.R. Iran from March 2018 to March 2019.

Patients and interventions

Adult patients older than 14 years old with history of liver cirrhosis for any reason who were candidate to receive orthotopic liver transplantation from deceased donor were eligible to participate in this study since including in transplant waiting list of this center.

Patients who were candidate for liver re-transplantation, transplantation due to acute hepatic failure, simultaneous multi-organ transplantation or receiving split graft from living donor, pediatric patients, pregnant and nursing women, patients with history of seizure or hypersensitivity to L-carnitine were not eligible to participate in the study. In addition, patients with acute coronary syndrome or gastrointestinal bleeding within first few days after liver transplantation were excluded.

Eligible patients were randomized (1:1) to either L-carnitine or placebo group using computer generated sequences in block sizes of 4. Patients were blinded to the allocation arm. Patients in L-carnitine group took L-carnitine syrup 500 mg / 5 mL (Alborz Daru, Tehran, I.R. Iran) with dose of 5 mL three times daily from the time of including in transplant waiting list until the day of liver transplant surgery.

Patients in the placebo group received simple syrup with the same prescribed schedule as L-carnitine group. Same packaging of L-carnitine and placebo was done in the Faculty of Pharmacy, Tehran university of Medical Sciences, Tehran, I.R. Iran using L-carnitine syrups that were bought by researchers from a community pharmacy and simple syrup. All patients in both groups received their routine medical managements for their underlying liver disease and cirrhosis complications (such as lactulose syrup, antibiotic therapy for spontaneous bacterial peritonitis, diuretic therapy for ascites). At the time of transplantation and onwards, immunosuppressive regimen and infections chemoprophylaxis was prescribed according to the center protocol for all patients of the two groups.

In this center immunosuppressive regimen included a 1 g dose of intraoperative methylprednisolone intravenously followed by intravenous methylprednisolone 50, 40, 30, 20 mg QID, and 20 mg BID at first to fifth days after transplantation and oral prednisolone thereafter with rapid taper down/off based on underlying liver disease leaded to cirrhosis. Maintenance immunosuppressive regimen included a calcineurin inhibitor (cyclosporine or tacrolimus) with defined blood levels for different times after transplantation plus mycophenolate mofetil / sodium.

Infections prophylaxis consisted of oral trimethoprim-sulfamethoxazole for six months after transplantation to prevent *Pneumocystis jirovecci* pneumonia, 10-day oral fluconazole or voriconazole (based on patient's risk stratification) for fungal prophylaxis and pre-emptive cytomegalovirus surveillance for six months after transplantation or based on clinical indication.

The surgery was done using full size livers. Biliary reconstruction was carried out as a side-to-side anastomosis or choledochojejunostomy.

Measurements and definitions

The primary aim of this study was comparing the incidences of PGD within first week after transplant procedure between the two groups of study. Patients were considered as suffering PGD if they fulfilled the criteria of PNF or EAD occurrence. In this study Olthoff's definition (19) was used to detect the occurrence of EAD. This definition includes the presence of at least one of

the following findings in liver transplant recipient: bilirubin concentration of 10 mg/dL or more on day 7 post-transplant, INR of 1.6 or more on post-operative day 7, ALT or AST of more than 2000 IU/L within first week after transplant (19). PNF was defined as the need for re-transplantation within few days after liver transplant that was not due to technical complications (such as portal thrombosis, hepatic artery/vein/vena cava thrombosis or stricture and massive transfusions), biliary complications or hyperacute rejection. In addition, description of United Network for Organ Sharing (UNOS) was applied for assessing of PNF occurrence. UNOS describes PNF as the presence of AST ≥ 3000 IU/L in addition to at least one of the following findings: acidosis with arterial pH ≤ 7.3 or venous pH ≤ 7.25, serum lactate > 4 mmol/L, INR ≥ 2.5. In fact, the latter patients also need re-transplantation or would be dying (1,20). Expanded donor criteria was defined as the presence of following criteria in donors and donated organ: cold ischemia time of more than 10 h, warm ischemia time of more than 40 min, donor serum sodium concentration of higher than 155 mEq/L, donor age of more than 60 years, donor obesity (body mass index above 30 kg/m^2), and donor ICU stay of more than 5 days. The presence of each criterion was calculated as one score (6). All liver function tests (ALT, AST, INR, bilirubin, and alkaline phosphatase) were gathered daily within first week after liver transplantation from patients' medical records.

Patients' adherence to treatment was confirmed by tele-communicating with the patients at least once-weekly and by counting consumed syrup bottles during the time in transplant waiting list. Patients were considered to be compliant if at least 80% of predicted syrup bottles were taken.

Ethics consideration

This study followed the tents of the Declaration of Helsinki. The study protocol was approved by local ethics committee of Tehran University of Medical Sciences (IR.TUMS.TIPS.REC.1397.008) and was registered in Iranian Registry of Clinical Trials (IRCT ID: IRCT20100111003043N12). All patients signed written consent forms before participation.

Data analysis

Statistical analysis was performed using SPSS (SPSS Inc., Chicago, IL, USA) version 22. The Kolmogorov-Smirnov test was used to assess the normal distribution of variables. Comparisons of quantitative variable between the two groups of the study were performed using the unpaired Student's t-test and Mann-Whitney U-test for variables with normal and skewed distribution, respectively. Spearman test was used to assess correlations between quantitative variables. Chi-square and Fisher's exact tests were employed for analyses of nominal variables. Due to violence of most quantitative variables from normal distribution, repeated measure analysis was not performed. Logistic regression analysis was done for dependent variables PNF and PGD using independent variable that their difference between L-carnitine and placebo groups had a *P* value of 0.2 or less. Kaplan-Meier analysis was used for comparing 1-month patients' and grafts' survivals between the two groups of the study. *P* values of less than 0.05 were considered statistically significant.

RESULTS

Of 135 liver transplant cases in this center during the study period, 84 patients complied with the inclusion criteria. Thirty-three patients in L-carnitine group and 39 patients in the placebo group completed the study and were included in data analysis (Fig. 1). The two groups were comparable in terms of recipients', donors', organs' and surgery procedures' characteristics except for the history of spontaneous bacterial peritonitis that was more common among patients in the placebo arm of the study (Tables 1 and 2). The median dose of ingested L-carnitine was 24 g (1-128 g) for median duration of 14 days (1-192 days).

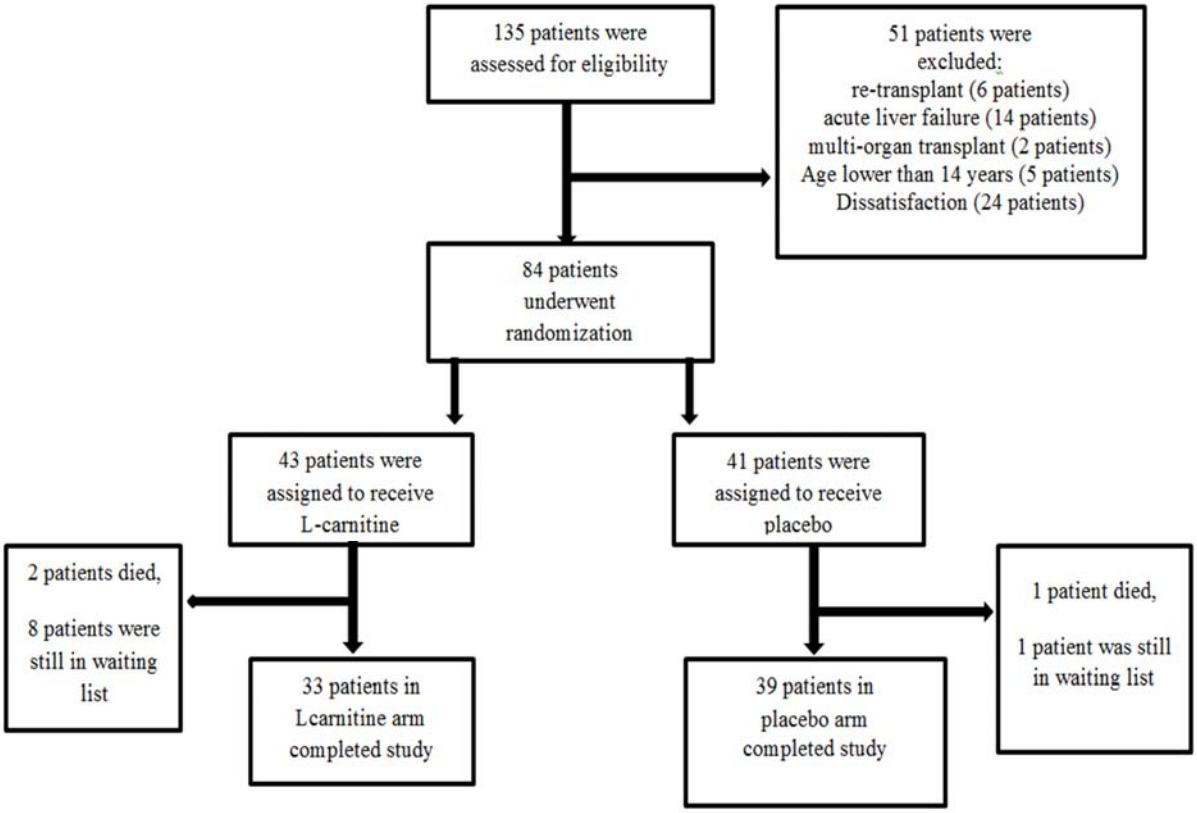

Fig. 1. Patients' inclusion and allocation.

Although not statistically significant, PNF and PGD happened less frequently among recipients in the L-carnitine group compared with patients in the placebo group. One out of 33 patients (3%) in the L-carnitine group *vs.* 5 out of 39 patients in the placebo arm (12.8%) suffered PNF ($P = 0.134$). Five of 33 patients (15.2%) in L-carnitine group compared with 12 out of 39 patients (30.8%) in the placebo group experienced PGD ($P = 0.120$).

Regarding liver enzymes, ALT and AST concentrations were significantly lower in L-carnitine group compared with placebo group at day 3 after transplantation (Table 3). Mean differences also showed that ALT but not AST concentration declined more significantly within 48 h after liver transplantation in L-carnitine arm compared with placebo group [for ALT: median -120.50; range (-626)-(+394) IU/L in L-carnitine group *vs.* median -79; range (-501)-(+567) IU/L in the placebo group; $P = 0.03$; for AST [median -380.50; range (-1644)-(+305) IU/L in L-carnitine group *vs.* median -366; range (-1956)-(+1720) IU/L in the placebo group; $P = 0.563$].

As seen in Table 1, less number of patients in L-carnitine group needed blood product administration during surgery. Significantly less albumin dose was needed for patients in the L-carnitine group compared with dose for patients in the placebo group. Furthermore, duration of mechanical ventilation was significantly lower for patients in the L-carnitine group (Table 1).

One-month patients' survival was significantly higher in L-carnitine group compared with placebo group (97% *vs.* 74.4%; $P = 0.008$; Fig. 2). During one month after transplantation only one patient died in the L-carnitine group following PNF; while 10 patients died in the placebo group, 4 due to PNF, 3 due to sepsis, and 3 due to cardiovascular events.

Table 1. Recipients' and transplant procedures' characteristics**.** Data are presented as mean ± SD and median (min-max) for normally distributed and skewed quantitative variables respectively; and number of patients (%) for nominal variables.

Characteristics	L-carnitine group (N = 33)	Placebo group (N = 39)	*P* Values
Age (y)	48.21 ± 12.61	48.54 ± 13.24	0.821
Sex (male)	19 (57.6)	25 (64.1)	0.571
Body mass index (kg/m^2)	24.97 ± 4.72	24.55 ± 3.91	0.683
Model for end-stage liver disease score	16.00 (10-35)	17.00 (6-39)	0.583
Model for end-stage liver disease-Na score	22.00 (15-38)	23.00 (11-39)	0.843
Serum albumin (g/dL)	3.16 ± 0.61	3.06 ± 0.62	0.501
Comorbidity			0.874
No comorbidity	18 (54.5)	24 (61.5)	
Diabetes mellitus	8 (24.2)	7 (18.1)	
Cardiovascular disease	2 (6)	2 (5.2)	
Kidney diseases	7 (21.2)	10 (25.7)	
Underlying disease leaded to cirrhosis			0.246
Autoimmune hepatitis	2 (6.1)	9 (23.1)	
Viral hepatitis	7 (21.2)	9 (23.1)	
Wilson's disease	3 (9.1)	1 (2.6)	
Primary sclerosing cholangitis	4 (12.1)	2 (5.1)	
Non-alcoholic steatohepatitis	13 (39.4)	11 (28.2)	
Others	4 (12.1)	7 (17.9)	
Cirrhosis complications			1.000
History of variceal bleeding	18 (54.5)	20 (51.3)	0.782
History of spontaneous bacterial peritonitis	2 (6.1)	17 (43.6)	< 0.001
History of encephalopathy	28 (84.8)	30 (76.9)	0.397
History of hepatorenal syndrome	5 (6.9)	10 (13.9)	0.275
History of paracentesis	20 (60.6)	24 (61.5)	0.936
Hemodynamic parameters during transplant surgery			
Central venous pressure (mmHg)	9.60 ± 2.24	9.30 ± 2.57	0.530
Mean arterial pressure (mmHg)	79.79 ± 8.87	77.70 ± 6.42	0.25
Systemic vascular resistance index (dyn.sec/cm^5/m^2)	1374 (683-3518)	1538 (693-2395)	0.619
Cardiac index (L/min/m^2)	4.53 (1.90-11.40)	4.39 (2.01-8.77)	0.651
Use of vasoactive agents during surgery	23 (69.7)	30 (76.9)	0.488
Surgery duration (min)	291.06 ± 50.4	289.2 ± 47.4	0.872
Patients needed blood products during surgery	28 (84.8)	38 (97.4)	0.087
Fibrinogen dose during surgery (g)	3.38 ± 2.06	3.71 ± 1.87	0.746
Packed cell during surgery (units)	2.00 (1-4)	2.00 (1-9)	0.617
Administered albumin during surgery (g)	27.50 (0.00-30.00)	30 (0.00-40)	0.033
Crystalloid solutions during surgery (L)	3.50 (2.50-5.00)	4 (1.50-6)	0.208
Length of ICU stay after transplant (days)	3.00 (1.00-7.00)	3 (1-17)	0.324
Length of hospital stay after transplant(days)	10 (1-28)	8 (2-32)	0.121
Mechanical ventilation duration after transplant (h)	10.20 (5.00-24.00)	12.00 (8.50-192.00)	0.006

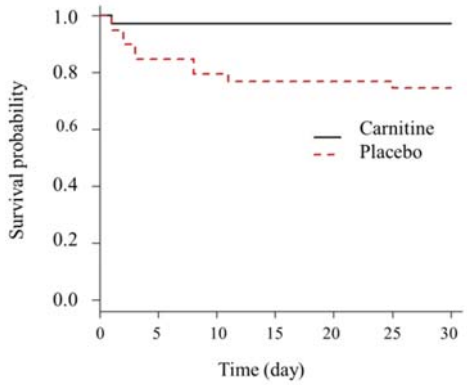

Fig. 2. Kaplan-Meier analysis for comparing 1-month patients' survivals between L-carnitin and placebo groups of the study. One-month patients' survival was significantly higher in L-carnitine group compared with placebo group (97% *vs.* 74.4%; $P = 0.008$).

Table 2. Donors' and organs' characteristics. Data are presented as mean ± SD and median (min-max) for normally distributed and skewed quantitative variables respectively; and n (%) for nominal variables.

Characteristics	L-Carnitine group (N = 33)	Placebo group (N = 39)	*P* Values
Age (y)	40.06 ± 15.79	42.36 ± 11.66	0.680
Sex (male)	27 (81.8)	26 (66.7)	0.146
Body mass index (kg/m^2)	24.95 ± 3.34	25.58 ± 1.97	0.344
Creatinine (mg/dL)	1.40 (0.60-7.10)	1.60 (0.70-9.90)	0.469
Aspartate aminotransferase (IU/L)	44 (11-415)	55 (7-293)	0.973
Alanine aminotransferase (IU/L)	35 (9-157)	38 (10-340)	0.982
Who were administered vasoactive agent	30 (90.9)	36 (92.3)	1.000
Type of vasoactive agent			0.756
Norepinephrine	9 (30)	8 (22.2)	
Dopamine	20 (66.7)	27 (75)	
Norepinephrine + dopamine	1 (3.3)	1 (2.8)	
Intensive care unit stay (days)	4 (1-18)	4 (1-21)	0.641
Cardiopulmonary resuscitation	11 (33.3)	7 (17.9)	0.133
Cause of brain death			0.892
Trauma	8 (24.2)	7 (17.9)	
Cerebrovascular accident	15 (45.4)	20 (51.3)	
Toxicity	5 (15.2)	7 (17.9)	
Others	5 (15.2)	5 (12.8)	
Cold ischemic time (min)	311.36 ± 57.54	313 ± 72.5	0.991
Warm ischemic time (min)	40.91 ± 12.58	40.95 ± 13.67	0.865
An hepatic phase time (min)	64.52 ± 22.47	64.95 ± 21.80	0.934
Expanded donor criteria	1 (0-3)	1 (0-2)	0.76

Table 3. Liver function tests. Data are presented as median (min-max).

Laboratory data	L-Carnitine group (N = 33)	Placebo group (N = 39)	*P* Values
Aspartate aminotransferase (IU/L)			
Day 1	500.00 (10.00-2073.00)	554.00 (20.00-3036.00)	0.159
Day 2	464.00 (10.00-1950.00)	470.50 (96.00-5321.00)	0.189
Day 3	168.00 (56.00-607.00)	286.50 (34.00-5650.00)	0.044
Day 4	94.00 (29.00-237.00)	81.00 (21.00-1452.00)	0.706
Day 5	64.00 (25.00-471.00)	62.00 (13.00-1230.00)	0.983
Day 6	49.00 (16.00-321.00)	35.50 (10.00-239.00)	0.234
Day 7	57.00 (17.00-153.00)	38.50 (17.00-413.00)	0.139
Alanine aminotransferase (IU/L)			
Day 1	337.00 (11.00-1293.00)	413.00 (20.00-2248.00)	0.127
Day 2	323.00 (24.00-1300.00)	487.00 (18.00-4200.00)	0.011
Day 3	220.00 (37.00-708.00)	352.50 (75.00-5239.00)	0.004
Day 4	205.00 (42.00-465.00)	215.00 (50.00-1310.00)	0.407
Day 5	151.00 (12.00-448.00)	182.00 (36.00-1193.00)	0.223
Day 6	124.50 (29.00-589.00)	140.50 (36.00 -716.00)	0.751
Day 7	150.00 (22.00-512.00)	108.50 (35.00-559.00)	0.606
Total bilirubin (mg/dL)			
Day 1	4.20 (1.20-37.00)	3.95 (1.00-23.60)	0.665
Day 2	2.80 (0.90-18.30)	3.30 (0.70-17.80)	0.968
Day 3	1.90 (0.60-10.50)	2.15 (0.50-13.50)	0.611
Day 4	1.70 (0.50-10.00)	1.60 (0.50-11.00)	0.604
Day 5	1.50 (0.50-9.10)	1.30 (0.60-12.30)	0.740
Day 6	1.60 (0.50-7.00)	1.20 (0.60-11.70)	0.116
Day 7	1.5 (0.5-7.5)	1.1 (0.5-35)	0.109
International normalized ratio			
Day 1	3.75 (1.38-7.77)	3.75 (1.05-7.80)	0.630
Day 2	2.36 (1.39-5.53)	2.50 (1.23-7.60)	0.337
Day 3	1.60 (1.20-2.40)	1.63 (1.20-8.00)	0.414
Day 4	1.38 (1.14-2.02)	1.42 (1.02-2.64)	0.988
Day 5	1.32 (1.05-2.16)	1.33 (1.00-2.63)	0.826
Day 6	1.22 (1.05-1.80)	1.27 (1.00-2.9)	0.799
Day 7	1.29 (1.00-1.98)	1.28 (1.00-2.20)	0.651

Table 4. Logistic regression analysis for comparing primary graft dysfunction and primary graft non-function incidences between L-carnitine and placebo groups adjusting for some covariates.

Covariates	Primary graft dysfunction		Primary graft non-function	
	Regression coefficient	*P* value	Regression coefficient	*P* value
Donor's gender	0.183	0.788	18.909	0.998
History of cardio-pulmonary resuscitation in donor	0.701	0.299	-0.275	0.826
Amount of administered albumin to recipient (g)	-0.054	0.222	-0.036	0.595
Recipients' needed blood product during surgery	0.602	0.611	19.109	0.999
Administered amount of crystalloids solutions in recipients (L)	0.264	0.502	1.329	0.065
History of spontaneous bacterial peritonitis in recipient	0.763	0.232	0.498	0.634
Duration of L-carnitine/placebo administration (day)	0.995	0.627	1.007	0.840
Group (L-carnitine/placebo)	0.475	0.285	0.187	0.181

Logistic regression analysis of dependent variables PNF and PGD using independent variable that their difference between L-carnitine and placebo groups had a *P* value of 0.2 or less (donor's gender, history of cardiopulmonary resuscitation in donor, history of spontaneous bacterial peritonitis in recipient, need for blood products in recipient and amount of infused albumin and crystalloid solutions in recipients during transplant surgery (Tables 1 and 2) revealed a non-significant higher risk of PNF (OR = 2.103; 95% CI: 0.538-8.224; *P* = 0.285) and PGD (OR = 5.358; 95% CI: 0.457-62.811; *P* = 0.181) in the placebo group. None of the covariates showed significant impact on the occurrence of PNF or PGD (Table 4). Including duration of L-carnitine or placebo administration in logistic regression analysis also showed no influence on PGD/PNF occurrence (Table 4). Due to wide variation in time on transplant waiting list and using L-carnitine/placebo, we assessed correlation between L-carnitine use duration and liver enzymes. No significant correlation was seen (data has not been shown).

Oral L-carnitine administration was well tolerated during the study. Only one patient reported nausea that was eliminated by consuming L-carnitine after meal with a glass of water.

DISCUSSION

This is the first clinical study that assessed the protective effect of L-carnitine administration during liver transplant waiting list on reducing the incidence of PNF and PGD in liver transplant recipients. The overall incidences of PGD and PNF in our center were 23.3% and 8.3% respectively that were comparable with those reported worldwide (1,3). Although differences in the incidences of PNF and PGD between L-carnitine and placebo groups did not reach statistical significance, the rate of PNF occurrence in L-carnitine group was one-fourth of that in the placebo group and the incidence of PGD in L-carnitine group was approximately one-half of that in the placebo group. These lower incidences of PGD and PNF in L-carnitine group may be of clinical importance. In addition, less blood products transfusion was needed for patients in the L-carnitine groups during transplant surgery that may be explained by cardioprotective effects of L-carnitine, balancing cardiac metabolism, regulating calcium influx, endothelial integrity, improving left ventricular function, and decreasing erythropoiesis resistance using L-carnitine (21,22). L-carnitine treated patients needed less mechanical ventilation duration that may be attributed to more ATP storage in respiratory muscles following L-carnitine supplementation. Based on Kaplan-Meier analysis, 1-month patients' survival rate was significantly higher in L-carnitine *vs.* placebo group. Death due to PNF was higher in the placebo group that can be explained by higher rate of PNF in this group. Sepsis and sepsis related death was seen in the placebo but not in the L-carnitine arm of the study. This difference may partly be related to the need for longer mechanical ventilation support for patients in the placebo group.

IR injury is considered as the main cause of PGD (1); therefore, some antioxidant agents with potential to ameliorate IR injury have been clinically studied for prevention of PNF in liver transplant recipients. With this hypothesis, Takaya *et al*. administered intravenous PGE1 perioperatively to 174 liver transplant recipients and compared the results with those of a cohort of previously liver transplant recipients in their center. They reported the incidence rate of only 1.1% for PNF among patients who took PGE1, while 5.9% of cohort patients who were not administered PGE1 showed PNF (OR: 0.188; $P < 0.05$) (5). During another clinical trial on efficacy of PGI2 during first week after liver transplant Bärthel *el al.* showed lower incidence of primary graft dysfunction in PGI2 users compared with controls (5% *vs.* 20%; $P = 0.087$). In their study 10% of patients in the control group underwent re-transplantation for PNF, while there was no case of PNF in PGI2 group (6). However, data on another antioxidant agent, NAC, are controversial (7,8). An Egyptian, randomized, placebo-controlled clinical trial revealed that preoperative administration of 150 mg/kg loading dose of intravenous NAC followed by 12.5 mg/kg/h NAC infusion for 4 h during surgery and continued by 6.25 mg/kg/h for 3 days after transplant surgery significantly reduced the incidence of PNF (1% in NAC group *vs.* 14% in the placebo group; $P = 0.03$) (7). In contrast, another randomized, double-blinded, placebo-controlled clinical trial in United States showed that intravenous NAC with a loading dose of 140 mg/kg over 1 h followed by 12 maintenance doses of 70 mg/kg every 4 h did not affect graft function in liver transplant recipients (8).

L-carnitine has been used as an antioxidant and anti-inflammatory agent to prevent IR injury to different organs in animal and human studies (14,15). Addition of L-carnitine derivatives to organ cold storage solution reduced IR injury to liver tissue (17,18). Oral L-carnitine has been used to prevent IR injury to kidney allograft in kidney transplant recipients. Although, delayed graft function between L-carnitine and placebo group did not differ significantly in that study, 3-month graft survival was significantly higher in patients who received L-carnitine within first few days after kidney transplantation (16).

Above studies showed the efficacy of prophylactic administration of L-carnitine before ischemia insult on IR injury (16-18), hence, we decided to assess the effect of prophylactic administration of L-carnitine on hepatic IR injury and subsequent PGD/PNF in liver transplant recipients. Since intravenous L-carnitine was not available in our region at the time of this study to be used exactly preoperatively in liver transplant recipients, oral route of L-carnitine administration was selected. Considering dose-dependent and saturable absorption of L-carnitine after oral bolus doses from the gastrointestinal tract (23,24), chronic oral L-carnitine dose of 1.5 g/day rather than a single, high oral dose exactly before transplant surgery was selected in this study. Although historic review of recipients' data of the center showed that most patients had been in transplant waiting list for 1 to 2 months, when we started the study we encountered much wider variation in time in waiting list that resulted in large variation in duration of L-carnitine or placebo administration. We tried to adjust the results by including this confounding variable in logistic regression analysis and in correlation test. To overcome this discrepancy, for future studies we suggest using high dose of intravenous L-carnitine exactly before transplant surgery instead of pretransplant oral supplementation.

In the present study liver transaminases decreased more rapidly in the L-carnitine group compared to the placebo group from the first liver enzymes assessment after transplantation surgery, but this difference disappeared from day 3 to 7 after transplantation. It has been noted that reperfusion injury may continue several hours to days after ischemia insult (25); therefore, more prolonged administration of antioxidants after liver transplantation might result in better hepatic protection and is worth assessing in future studies.

The main limitation of this study is small sample size that resulted in not enough study power (achieved power of 34%). As the pilot

study we only considered prophylactic administration of L-carnitine before transplantation. Based on our results that showed significant difference in liver transaminases during first three days after liver transplantation and disappearance of this difference after that, future studies with larger sample size and continuing L-carnitine supplementation to the end of first week after liver transplantation is recommended. Furthermore, considering unpredictable length of patients stay in transplant waiting list and need for oral L-carnitine, developing study on the effect of large intravenous doses of L-carnitine at the beginning of liver transplant surgery or during an hepatic phase and before allograft reperfusion instead of long-term pretransplant oral supplementation is recommended.

CONCLUSION

Although statistically non-significant, however, the rates of PNF and PGD in L-carnitine group were approximately one-fourth and one-half of placebo group, respectively. One-month patients' survival was significantly higher in the L-carnitine versus placebo group. Death due to PNF was higher in placebo group that can be explained by higher rate of PNF in this group. Sepsis and sepsis related death was seen in the placebo but not in the L-carnitine arm of the study. This difference may partly be related to the need for longer mechanical ventilation support for patients in the placebo group.

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