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The Effects of Coenzyme Q10 on Contrast-Induced Acute Kidney Injury in Type 2 Diabetes: A Randomized Clinical Trial

Ashkan Karbasi[1], Ali Abbasi[2], Abbas Mohagheghi[2], Jalal Poorolajal[3], Farzad Emami[4], Shirin Moradkhani[5], Iraj Khodadadi[1], Mahmoud Gholyaf[6], and Heidar Tavilani[1,7,*]

[1]Department of Clinical Biochemistry, School of Medicine, Hamadan University of Medical Sciences, Hamadan, [2]Department of Cardiology, Shariati Hospital, Tehran University of Medical Sciences, Tehran, [3]Department of Epidemiology, School of Public Health, Hamadan University of Medical Sciences, [4]Department of Cardiology, School of Medicine, Hamadan University of Medical Sciences, [5]Department of Pharmacognosy, School of Pharmacy, Medicinal Plants and Natural Products, Hamadan University of Medical Sciences, [6]Department of Internal Medicine, School of Medicine, Hamadan University of Medical Sciences, [7]Infectious disease Research Center, Hamadan University of Medical Sciences, Hamadan, Iran

Contrast-induced acute kidney injury (CI-AKI) is a frequent challenge following the injection of contrast media and its subsequent oxidative stress. The aim of the present study was to evaluate the preventive effects of coenzyme Q10 (Q10), as a mitochondrial-targeted antioxidant in CI-AKI in diabetic patients, who account for a large proportion of angiographic cases. A total of 118 diabetic patients were randomly assigned to receive 120 mg of oral coenzyme Q10 (Q10 group) or placebo (Placebo group) for four days, starting 24 hours before contrast media injection. Blood urea nitrogen (BUN), serum and urinary creatinine, estimated glomerular filtration rate (eGFR), urinary malondialdehyde (UMDA), urinary total antioxidant capacity (UTAC), and urinary mitochondrial to nuclearDNA ratios (mtDNA/nDNA ratio) were evaluated before and after the treatment period. Urine sediments were also evaluated to report the urine microscopy score (UMS).The levels of BUN, serum and urine creatinine, and UMS were similar in the Q10 and placebo groups. EGFR was lower in the Q10 group before the treatment ($p=0.013$) but not after. The urinary mtDNA/nDNA ratio was 3.05±1.68 and 3.69±2.58 in placebo and Q10 groups, but UTAC was found to be lower in Q10 both before ($p=0.006$) and after the treatment ($p < 0.001$). The incidence of CI-AKI was 14.40% and the mtDNA/nNDA ratio was similar between CI-AKI and non-CI-AKI patients. In conclusion, Q10 treatment shows no favorable effect on prevention of CI-AKI or a urinary mtDNA/nDNA ratio among diabetic patients.

Key Words: *Coenzyme Q10; Acute Kidney Injury; Type 2 Diabetes Mellitus; Mitochondrial DNA*

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Corresponding Author:
Heidar Tavilani
Department of Clinical Biochemistry, School of Medicine, Hamadan University of Medical Sciences, Hamadan 6516844541, Iran
Tel: +98-8138380572
Fax: +98-8138380208
E-mail: tayebinia@umsha.ac.ir

INTRODUCTION

Contrast-induced acute kidney injury (CI-AKI) is considered as the third frequent cause of AKI cases who are in need of hospital care. CI-AKI occurs nearly in 30% of cases being injected by iodinated contrast media, which is also linked to a high rate of mortality.[1] It is essential to clarify the unsettled pathogenesis of CI-AKI. The main known mechanism points to alterations in renal tubular and vascular endothelial cells, followed by oxidative stress in kidneys and eventually vasoconstriction.[2,3] The literature has demonstrated that routine treatments in the clinical settings offer minor benefits to CI-AKI patients and that the risk rate for kidney complications still remains substantially high.[4] Furthermore, there are no efficient therapeutics or preventive plans or recovery strategies for AKI,[5,6] meaning early diagnosis and effective treatment of CI-AKI is of the utmost clinical value.

Mitochondria, the "power plant" organelle, are the ulti-

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mate site in cells playing substantial roles to support proper cell functioning.[7] Since kidneys are rich in mitochondrial content[8] and this organelle normally deals with considerable levels of both oxygen and energy, damage-free mitochondria are crucial for renal health.[9] Impairment of this organelle provokes renal damages, namely AKI caused by nephrotoxic chemicals.[10-12] These circumstances are tightly bound to the pathological alterations of mitochondria in kidney tubules through the early stages until the recovery phases of AKI.[13,14] Subsequently, mitochondrial dysfunction leads to oxidative stress, inflammation, mitophagy, and eventually a sudden decline in renal function.[15,16] Also, damaged mitochondria can cause a buildup of a considerable amount of reactive oxygen species (ROS) which are harmful to the kidneys.[17] Coenzyme Q10 (Q10) a well-known potent antioxidant and a crucial mediator in mitochondrial function, could possibly modulate oxidative-induced injuries. It has been reported that Q10 in combination with other medication reduces the incidence of CI-AKI in cases of coronary artery disease displaying renal comorbidity.[18]

Both qualitative and quantitative defects regarding the performance of mitochondria have been reported in the diabetes mellitus outset, as reflected by the quantity of the mitochondrial DNA (mtDNA) content in different tissues.[19,20] Recently, an increase in urinary mtDNA content has been evaluated as a potential accessible and non-invasive biomarker to spot kidney injuries.[7,21,22] MtDNA alterations follow a unique mechanism independent form classical biomarkers of kidney function;[21] therefore, urinary mtDNA content could be referred as a biomarker of kidney damage in certain kidney disorders.[7,21,23] Beyond just mtDNA, the analysis of the urine sediment for cells as a simple and economical test is widely considered as a useful complementary tool to evaluate acute and chronic kidney injuries, especially as a quantitative approach.[24]

Since diabetic patients are potential candidates for the exposure to contrast media, and mitochondrial damage is a central challenge in CI-AKI, we hypothesized that Q10 might possibly reduce the CI-AKI risk among diabetic patients. Therefore, here we evaluated, for the first time, the preventive effects of Q10 against contrast-induced acute kidney injury in type 2 diabetic patients by measuring urinary mitochondrial to nuclear DNA ratio (mtDNA/nDNA ratio), determining the levels of UMDA and UTAC, and assessing urine microscopy score.

MATERIALS AND METHODS

1. Study design, registration, and participants

This study was a randomized, double-blind, clinical trial which was registered on January 29, 2020 in Iranian Registry of Clinical Trials portal (IRCT20120215009014N337). All participants were given informed written consent before the commencement of the study, which was approved by the ethics committee of Hamadan University of Medical Sciences (IR.UMSHA.REC.1398.875).

A total of 136 consecutive patients were enrolled in this study among those who admitted to Farshchian Cardiovascular (Hamadan-Iran) and Shariati (Tehran-Iran) hospitals to undergo elective CT angiography for diagnostic purposes through February 2020 to January 2022. All participants were adults suffering from type 2 diabetes mellitus for at least one year (fasting blood glucose $\geq$ 126 and HbA1c $\geq$ 6.5%). Patients had stable renal function, presenting estimated glomerular filtration rates (eGFR) greater than 60 mL/min/1.73 m^2 (based on the equation provided by Chronic Kidney Disease Epidemiology Collaboration, CKD-EPI) with no changes in their anti-diabetic medications during the last 60 days. Subjects in two groups were statistically evaluated based on the gender, current and ex-smoking status, opium addiction, and the medications they were taking. Exclusion criteria were refusal of consent, taking coenzyme Q10 or any other antioxidant supplements and any possible nephrotoxic medications within the past week, suffering from any other comorbidities, the requirement of any emergency procedure, renal failure leading to dialysis, exposure to contrast media within the past month, pregnancy, malignancies, and participation in any other concurrent trial (Fig. 1). The baseline characteristics of the participants are presented in Table 1.

The patients were randomly assigned to the Q10 and placebo groups by using the balance block randomization method. To achieve this goal, four cards were chosen, "Q10" and "Placebo" were written on two of each. The cards were pooled, placed in a container, and randomly picked for each participant with no replacement till all four cards were picked. Later, all the cards were placed back and the drawn again till the sample size was covered for statistical analysis. The allocations were kept masked throughout the trial. The patients and the outcome assessors were blind to the trial grouping. Patients orally received either Q10 capsules (120 mg/per day; Organika, Canada) or a placebo for 4 days; starting 24 hours before exposure to contrast media (iodixanol, 290 mOsm/kg H_2O, 320 mgI/mL; Cork-Ireland). Supplementation adherence was monitored by a daily phone call. Before and at the end of the treatment a random urine sample as well as fasting blood sample was collected. Height and weight were also measured.

2. Determination of renal function parameters

The measurement of blood urea nitrogen (BUN), serum creatinine (SCr) and urinary creatinine (UCr) was performed by using Chemistry Analyer BT-3000 (Biotecnica Instruments S.p.A., Italy) with commercial kits (Pars Azmoon, Iran). The occurrence of CI-AKI was considered as primary outcome and the remaining measured parameters were defined as secondary outcomes. The incidence of CI-AKI was defined in accordance with Kidney Disease: Improving Global Outcomes (KDIGO) guideline (Stage 1) where elevations of 25% or more in SCr within 48 hours indicates kidney injury.[2]

3. Urinary lipid peroxidation assay (UMDA)

Malondialdehyde (MDA) is one of the end products of lip-

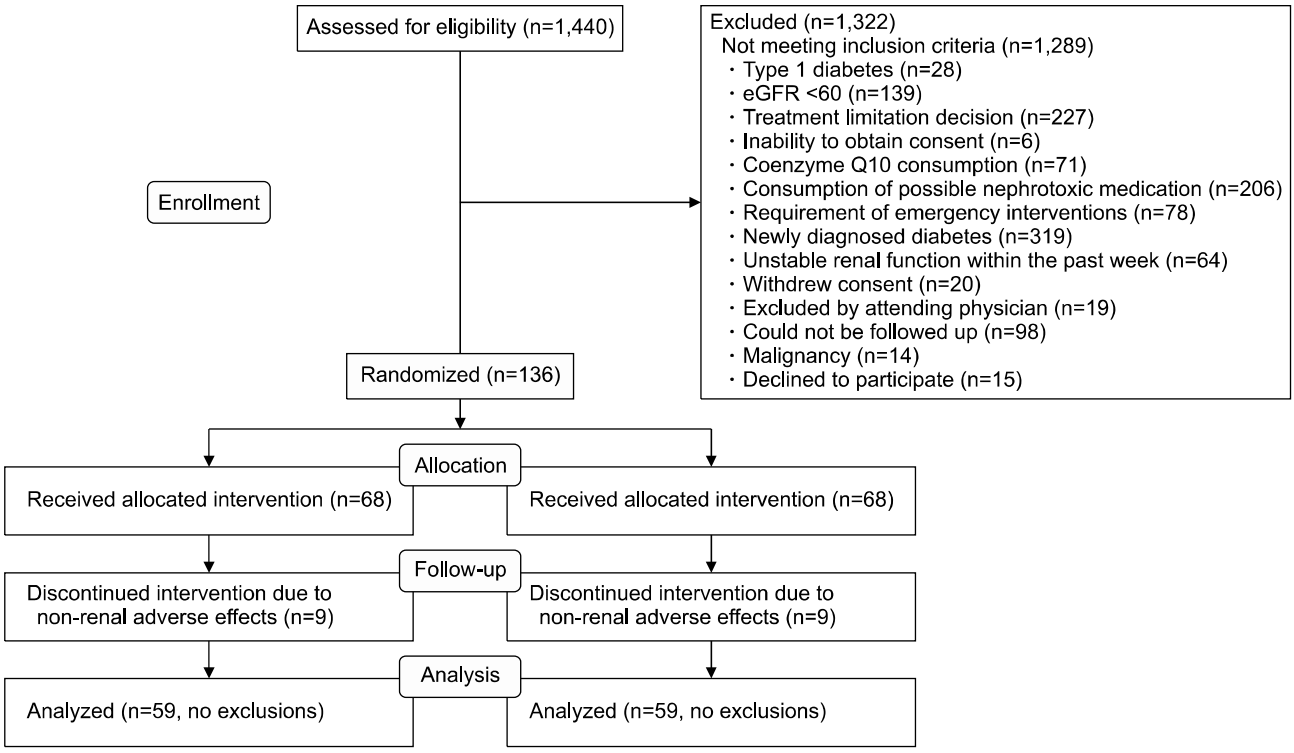

FIG. 1. Flow of enrollment of the patients through the study.

TABLE 1. Baseline characteristics of the intervention and control groups

Categorical variables	Placebo group	Q10 group	p-value
	Number (percent)	Number (percent)	
Gender			1.000
Female	20 (33.90)	20 (33.90)	
Male	39 (66.10)	39 (66.10)	
Ex-smoker			0.754
No	53 (89.83)	54 (91.53)	
Yes	6 (10.17)	5 (8.47)	
Current smoker			1.000
No	41 (69.49)	41 (69.49)	
Yes	18 (30.51)	18 (30.51)	
Opium user			1.000
No	50 (84.75)	50 (84.75)	
Yes	9 (15.25)	9 (15.25)	
Medications			
Biguanides			-
No	0 (0.00)	0 (0.00)	
Yes	59 (100.00)	59 (100.00)	
Sulfonylureas			0.091
No	40 (67.80)	31 (52.54)	
Yes	19 (32.20)	28 (47.46)	
Thiazolidinediones			0.057
No	32 (54.24)	42 (71.19)	
Yes	27 (45.76)	17 (28.81)	
Dipeptidyl peptidase IV inhibitors			0.398
No	46 (77.97)	42 (71.19)	
Yes	13 (22.03)	17 (28.81)	

TABLE 1. Continued

Categorical variables	Placebo group	Q10 group	p-value
	Number (percent)	Number (percent)	
SGLT2 inhibitors			0.545
No	40 (67.80)	43 (72.88)	
Yes	19 (32.20)	16 (27.12)	
Statins			0.424
No	20 (33.90)	16 (27.12)	
Yes	39 (66.10)	43 (72.88)	
Omega 3 supplements			0.769
No	7 (11.86)	6 (10.17)	
Yes	52 (88.14)	53 (89.83)	
Aspirin			1.000
No	25 (42.37)	25 (42.37)	
Yes	34 (57.63)	34 (57.63)	
ACE inhibitors			0.456
No	27 (45.76)	23 (38.98)	
Yes	32 (54.24)	36 (61.02)	
Continuous variables	Mean±SD	Mean±SD	p-value
Age (y)	67.94±4.45	67.55±4.70	0.645
Body mass index (kg/m^2)	26.08±2.74	25.36±2.61	0.213
Potassium (mEq/L)	4.35±0.28	4.28±0.33	0.228
Sodium (mEq/L)	141.01±2.62	140.30±2.58	0.139
HbA1c (%)	8.49±1.25	8.58±1.26	0.677
FBS (mg/dL)	200.47±43.43	199.08±53.02	0.876
TG (mg/dL)	407.86±38.99	416.08±43.14	0.279
Cholesterol (mg/dL)	178.59±27.57	208.28±56.53	0.006
LDL-cholesterol (mg/dL)	114.62±11.03	115.67±13.44	0.505
HDL-cholesterol (mg/dL)	26.59±5.16	24.67±6.19	0.070
ALT (IU/L)	26.62±9.22	28.28±8.19	0.303
AST (IU/L)	23.28±8.57	21.54±7.89	0.182
ALP (IU/L)	116.05±24.20	107.03±26.16	0.054
LDL-cholesterol/HDL-cholesterol ratio	4.49±1.09	5.12±2.18	0.064
AST/ALT ratio	1.09±1.00	0.79±0.33	0.103
TG/HDL-cholesterol ratio	16.02±4.08	18.61±9.07	0.035
WBC (cells per μL)	6,103.05±1,290.36	5,902.37±980.17	0.343
Neut (cells per μL)	3,175.71±732.03	3,093.37±576.05	0.498
Lym (cells per μL)	2,282.23±490.56	2,199.67±383.85	0.310
RBC (million cells per μL)	4.88±0.72	4.87±0.71	0.933
Hb (g/dL)	13.98±1.91	14.12±1.88	0.699
Plt (cells per μL)	221,593.20±60,151.28	226,186.40±54,324.53	0.546
Plt/Lym ratio	101.09±33.04	105.79±31.35	0.350
Neut/Lym ratio	1.39±0.18	1.41±0.19	0.630

id peroxidation (LPO) and is an indicator of free radical production and consequently, oxidative stress. For measuring the amount of LPO in urine samples, thiobarbituric acid (TBA) was used, which reacts with lipid peroxide molecules. Urine was mixed with trichloroacetic acid and the precipitate was dispersed in H_2SO_4. TBA was then added and incubated in 95℃. Thiobarbituric acid reacting materials were extracted by n-butanol, and the optical density was measured at 532 nm.[25] The results were expressed as nmol/mL.

4. Urinary total antioxidant capacity (UTAC)

UTAC was measured using the ferric reducing ability of plasma method for urine samples, regarding the ability of urine to transform Fe^{3+} to Fe^{2+} in the presence of tripyridyl-s-triazin.[26] The results were expressed as nmol/mL.

5. Determination of mitochondrial to nuclear DNA ratio (mtDNA/nDNA ratio) in urine by quantitative real-time PCR

After discarding the intact cells and cellular debris, supernatants were harvested and stored at −80℃. Total DNA was isolated and purified from urine samples (3.5 mL)

using column-based DNA Extraction commercial Kit (SinaPure, Sinaclon, Iran). The extraction was conducted according to the manufacturer's protocol. Each DNA concentration was measured by NanoDrop spectrophotometer. To identify the mtDNA/nDNA ratio as mitochondria-specific cellular damage, qPCR was performed (LightCycler® 96 System, Roche, Switzerland) to amplify conserved single-copy genes encoded in the mitochondrial genome and nuclear genome, with SYBR® Green as a fluorescent dye (Pishgam, Iran). The expression of mtDNA was normalized by that of nuclear DNA using the relative cycle threshold (△Ct) method. The qPCR reaction (10 μL) contained 0.5 μmol/L of each primer and 10 ng of DNA. The mitochondrially encoded NADH dehydrogenase 1 (NC_012920) was amplified as for the mtDNA, using forward 5'CCACTTT CCACACAGACATCA3' and reverse 5'GGTTAGGCTGGT GTTAGGG3' primers (product size=127 bp) and human beta-2-microglobulin (M17987) as for the nDNA (forward 5'TGCTGTCTCCATGTTTGATGTATCT'3 and reverse 5' TCTCTGCTCCCCACCTCTAAG'3, product size=86 bp). The following reaction conditions were used: 15 min at 9 5℃; and 40 cycles of 95℃ for 30 s, 60℃ for 30 s and 72℃ for 30 s. The assays were performed in duplicate for each DNA sample and negative controls without a template were run for each gene. The means of the cycle threshold values for mtDNA and nDNA were used to calculate delta Ct (△Ct) for each sample and $2^{-\triangle CT}$ was used for statistical analysis.[27,28] The efficiency of the assay for amplifying both nDNA and mtDNA was measured with standard curves generated by a dilution series of 10.00, 1.00, 0.10 and 0.01 ng of total genomic DNA. Negative controls without templates were run for each gene.

6. Assessment of urine microscopy score (UMS)

To evaluate UMS, fresh midstream urines were obtained in sterile bottles from the patients and were examined within one hour after voiding. Following centrifugation at 2,000 rpm for five minutes, the supernatants were retained for other evaluations and the pellet was resuspended in 0.5 mL of urine by gentle manual agitation, wet mounted on a glass slide, covered with a cover slip, and examined by an experienced clinical laboratory technician in low (×10) and high (×40) magnifications using bright field microscopy. Fifteen fields per each sample were analyzed to quantify granular casts and/or renal tubule epithelial (RTE) cells. The UMS system[29] was used for statistical analysis and points were assigned from zero to three.

7. Sample size and statistical analysis

The sample size was calculated as 52 patients for each group based on the eGFR results by Chen et al.[18] applying a 95% of confidence interval and a 80% for statistical power. To prevent possible loss of sample, we included 68 patients in each group but due to the exclusion of some participants during the study, the experiments were finally ended with 59 cases.

Chi-square and Fisher's exact tests were applied to analyze categorical parameters. Independent t-test or Mann-Whitney U tests were used to analyze continuous parameters as appropriate. To perform adjusted analyses, four-way ANOVA model was used. Statistical significance was defined as p-values of less than 0.05 and all statistical tests were two-sided. All these tasks were performed using Stata software version 16 (Stata Corp., TX, USA).

RESULTS

1. Baseline characteristics

A total of 59 patients (20 females and 39 males) were present in each group (Fig. 1). No statistical difference was found between two groups regarding age, body mass index, and hematological indices. However, significant differences were found in the cholesterol levels and TG/HDL-cholesterol ratios (Table 1).

2. Laboratory parameters

No significant difference was observed in BUN, SCr, UCr, eGFR and UMDA levels between Q10 and placebo groups, both before and after the treatment. The mean level of UTAC was significantly lower in treated group compared with controls both before and after the treatment ($p=0.006$ and $p<0.001$, respectively). No statistical significant difference was also found between groups after adjustments for UTAC, TG/HDL-cholesterol ratio, and cholesterol (Table 2).

3. Urinary mtDNA/nDNA ratio

The efficiency of the mtDNA and nDNA primers were 96% and 98%, respectively. Before the treatment, urinary mtDNA/nDNA ratios were 3.05±1.68 and 3.69±2.58 in placebo and Q10 groups, respectively ($p=0.407$). Following the treatment, the ratios became 3.55±1.96 and 4.31±5.39 in placebo and Q10 groups, respectively ($p=0.861$, Table 2).

4. UMS findings

Before the intervention, the UMS for the majority of the patients were normal (57 in placebo and 58 in Q10 group). The score shifted from normal to mild and moderate in a small number of patients following the treatments, however, the UMS were similar in both groups. No patient presented with severe score through the study (Table 3).

5. CI-AKI incidence and comparison within subgroups

The incidence of CI-AKI was defined as 25% increase in SCr. The parameters were compared between the patients with at least a 25% elevation in SCr (CI-AKI$^+$, 14.40%) and those with SCr elevation of less than 25% (CI-AKI$^-$, 85.59%). The comparison of the CI-AKI$^+$ and CI-AKI$^-$ groups without considering the placebo and Q10 treatment revealed that the levels of BUN, SCr, eGFR, UCr ($p<0.001$), and UMS ($p=0.003$) showed a significant difference. There were no significant differences between the CI-AKI$^+$ and CI-AKI$^-$ groups regarding UMDA, UTAC, or mtDNA/nDNA ratio.

TABLE 2. Comparison of the laboratory results between the intervention and control groups

Variables	Before			After			
	Placebo group	Q10 group	p-value	Placebo group	Q10 group	p-value[a]	p-value[b]
	Mean±SD	Mean±SD		Mean±SD	Mean±SD		
Blood urea nitrogen (mg/dL)	23.50±4.69	24.42±4.36	0.272	27.75±5.01	26.80±4.40	0.280	0.695
Serum creatinine (mg/dL)	0.89±0.17	0.90±0.18	0.786	1.05±0.21	1.04±0.24	0.678	0.524
Estimated glomerular filtration rate (mL/min/1.73 m^2)	86.25±9.30	83.61±9.21	0.090	72.54±11.68	73.18±13.20	0.779	0.813
Urinary creatinine (mg/dL)	93.58±12.77	94.25±13.66	0.786	105.83±15.56	104.53±18.40	0.678	0.524
Urinary malondialdehyde (nmol/mL)	6.14±2.28	6.19±2.37	0.889	6.05±2.35	5.85±2.20	0.618	0.239
Urinary total antioxidant capacity (nmol/mL)	62.22±4.17	60.11±4.04	0.006	61.48±4.50	58.07±4.46	<0.001	0.045
Urinary mtDNA/nDNA ratio ($2^{-\triangle CT}$)	3.05±1.68	3.69±2.58	0.407	3.55±1.96	4.31±5.39	0.861	0.014

[a]Unadjusted. [b]Adjusted for cholesterol, UTAC, and TG/HDL-cholesterol ratio.

TABLE 3. Comparison of the urine microscopy score (UMS) between the intervention and control groups

UMS	Before			After		
	Placebo	Q10	p-value[a]	Placebo	Q10	p-value[a]
Normal (0)	57	58	1.000	54	54	1.000
Mild (1)	2	1		4	4	
Moderate (2)	0	0		1	1	
Severe (3≤)	0	0		0	0	

[a]Fisher's exact test.

TABLE 4. Comparison of the laboratory results between groups based on presenting contrast-induced acute kidney injury (CI-AKI)

Variables	$CI\text{-}AKI^-$, n=101			$CI\text{-}AKI^+$, n=17		
	Placebo group	Q10 group	p-value	Placebo group	Q10 group	p-value
	Mean±SD	Mean±SD		Mean±SD	Mean±SD	
Blood urea nitrogen (mg/dL)	26.54±4.00	25.77±3.77	0.319	35.42±4.00	32.55±3.08	0.116
Serum creatinine (mg/dL)	1.00±0.16	0.97±0.21	0.427	1.38±0.17	1.39±0.10	0.894
Estimated glomerular filtration rate (mL/min/1.73 m^2)	74.58±10.97	75.80±12.39	0.603	59.50±6.76	58.66±6.65	0.801
Urinary creatinine (mg/dL)	102.3±12.25	99.81±15.56	0.427	130.07±12.55	130.75±7.75	0.894
Urinary malondialdehyde (nmol/mL)	5.95±2.39	5.86±2.23	0.847	6.71±2.06	5.74±2.21	0.368
Urinary total antioxidant capacity (nmol/mL)	61.52±4.60	58.02±4.61	<0.001	61.17±4.03	58.32±3.81	0.153
Urinary mtDNA/nDNA ratio ($2^{-\triangle CT}$)	3.65±1.99	4.14±5.53	0.561	2.95±1.75	5.23±4.74	0.360

Further analysis within each group was conducted (Table 4). The laboratory parameters revealed non-significant differences between placebo and Q10 groups among both $CI\text{-}AKI^+$ and $CI\text{-}AKI^-$ cases. Additionally, the UTAC level among $CI\text{-}AKI^-$ patients was higher in placebo group than Q10 group.

DISCUSSION

Many studies reinforce the significance of mitochondrial quality and quantity in the various forms of nephrotoxicity.[10-12,30-32] Here, we have provided findings demonstrating that Q10 as a mitochondrial-related antioxidant supplement, has minor beneficial effects on contrast-induced acute kidney injury in type 2 diabetic patients

Due to the high prevalence of diabetes mellitus and its macro-vascular complications, a considerable number of diabetic patients undergo contrast-mediated CT angiography in their lifetime. Unfortunately, this procedure has been known as a causative factor for the occurrence of CI-AKI with an incidence range of 5.7 to 29.4 percent.[33] Interestingly, we showed that the incidence of CI-AKI in the present study is 14.4%, which is in the line with previous reports. Although diabetes has usually been considered as an independent non-modifiable risk factor for development of CI-AKI, sufficient evidence is not available

to support this idea. Furthermore, the incidence of CI-CKI in this study falls to 0% by taking the absolute ≥ 0.5 mg/dL of SCr increase as the CI-AKI diagnosis criteria, rather than relative $\geq 25\%$ SCr increase. On this basis, diabetes does not seem to be essentially a risk factor for CI-AKI unless accompanied with other factors (i.e., diabetic/chronic kidney disease or eGFR < 30 mL/min/1.73 m^2).[33-35] Although the sensitivity to CI-AKI in healthy individuals and diabetic patients with preserved renal function are relatively equal,[36,37] our findings favor the notion that Q10 cannot prevent CI-AKI in the latter. A limited number of studies have evaluated the benefits of Q10 in CI-AKI in clinical settings. It is noteworthy that Q10 along with other medications has shown success in CI-AKI prevention among renal insufficiency (eGFR ≤ 60 mL/min/1.73 m^2) patients even in lower doses than other settings, i.e., percutaneous coronary intervention.[18] Such inconsistencies leave the Q10 effects with the need for further research. Q10 has been reported as a promising antioxidant in other forms of AKI.[38] Two mechanisms are more suitable to explain the pathology of CI-AKI. The first one points to the direct contact of contrast media to renal medulla and present its cytotoxicity to tubular cells via ROS generation and consequent vasoconstriction. The second mechanism is generally based on the permeability of the contrast media which is followed by slow blood flow of the renal medulla and eventually, ischemia. Both phenomena leave the renal tubular epithelial cells with damage and apoptosis.[39-41] No substantial direct damage to the kidneys occur in the early stages of diabetes, however, oxidative stress hypoxia-inducible factors are key to the changes in the renal blood flow during diabetes.[42,43]

Since the kidneys are rich in mitochondria and Q10 plays a crucial role in the integrity and function of mitochondria, we tried to find out whether Q10 improved the renal function against the toxicity of contrast media. Further, it was hypothesized that such beneficial effects would be reflected in the urinary mtDNA/nDNA ratio since the role of mtDNA in CI-AKI is poorly understood. The release of mtDNA in urine did not increase under contrast media exposure. Q10 is an antioxidant compound having a highly-hydrophobic ploy-isoprenoid chain. Due to the relatively high molecular weight and poor solubility, Q10 presents a poor bioavailability (5% percent or even less), which possibly makes its preventive effects a challenge in short periods of treatment,[44] as in the present study. Additionally, the transport system by which cells uptake Q10 from bloodstream and into the mitochondrial membrane is still a challenge that is unresolved. This issue has left Q10 to be ineffective in some clinical trials in chronic periods as well.[45] Besides the role in the electron transport chain in the mitochondria, the antioxidant action of Q10 in lipoproteins and intracellular environment against lipid damages is clearly understood[46] and the benefits of Q10 supplementation among statin takers is critical for cardiovascular health since statins inhibit the endogen synthesis of Q10.[47] A possible Q10 deficiency remains as an important question since a considerable percent of the participants in this study were taking statins.

This study focused on an important question about the diagnostic value of urinary mtDNA/nDNA ratio in patients injected by iodixanol, an iso-osmolar contrast media. From our observations, this novel biomarker was not a suitable choice in diabetic patients, at least not until the chronic renal complications are seen.[48,49] Furthermore, our results elucidated that the effect of Q10 is not reflected in UMS among diabetic patients with normal renal function, therefore, the value of UMS in CI-AKI deserves more consideration. MtDNA has been introduced as a valuable marker of mitochondrial damage in different renal diseases.[23,50-52] Though we value these studies, we favor the notion that urinary mtDNA/nDNA ratio cannot be considered as a biomarker in the diagnosis of CI-AKI, especially not in case with diabetes. Additionally, there is evidence suggesting that an atypical mtDNA/nDNA ratio increase occurs when diabetic complications are present, i.e., in diabetic nephropathy.[20] Contrary to the classical biomarkers of kidney function, no alterations were found in the urinary mtDNA/nDNA ratio in CI-AKI$^+$ group. This finding puts more emphasis on the notion that mtDNA content is possibly controlled by a separate mechanism to traditional biomarkers.[21] While it seems that the urinary mtDNA mainly originates from the kidneys, the literature offers different opinions, proposing the systemic mitochondrial damage and filtration of plasma mtDNA into the urine.[21,53] In addition, it is yet to be established whether the plasma or urinary mtDNA/nDNA ratios give more insight into the AKI.[54] While the urinary levels of mtDNA/nDNA ratio in other forms of kidney damage including glomerular has led to promising findings leading it to be considered as a valuable biomarker, the variation seems considerably high[54] which was a result that was similar to our analysis. This phenomenon indicates the need for more consideration in future studies to evaluate urinary mtDNA/nDNA ratios in kidney diseases.

In the preclinical settings, the exposure to contrast media leads to mitochondrial ROS production and subsequently inflammation, and oxidative DNA damage in kidneys, reflected as apoptosis of renal tubular epithelial cells (RTECs).[55] Unfortunately, our findings could not emphasize any benefits of UMS assays, however, their benefits are reflected in the literature. Accordingly, it had been mentioned that the more useful application of the UMS among hospitalized patients is in presenting the renal damage, i.e., in differentiating septic and non-septic forms of AKI.[24] Further, this scoring system is more suitable to detect severe AKI with high specificity following surgeries.[56] Apart from sepsis and surgery, AKI secondary to urinary tract infection could also be reflected by UMS,[57] indicating the possible effect of the etiology of AKI on the usefulness of this biomarker.

While the preventive effects of Q10 in CI-AKI were investigated in this study, several limitations are worth mentioning. Our work could benefit from a larger sample size, especially with regard to the measurement of kidney

laboratory parameters. Also, it would be advantageous to measure novel kidney injury/function biomarkers (i.e., cystatin-c, interleukin-18, lipocalin-2, fatty acid binding proteins, and kidney injury molecule-1) in plasma and urine to offer more information about the effects of Q10 in CI-AKI. Moreover, the measurement of urine albumin/creatinine and kinetic measurement of the biochemical parameters would be beneficial as well to understand the course of the kidney injury. Furthermore, not using plasmids to perform absolute quantification of mtDNA is an important limitation since it makes the copy number determination of the genes possible. In addition, the amplification of the mitochondrial genome could also be useful to evaluate mtDNA deletions. Future studies might focus on the comparison of mtDNA/nDNA ratio in plasma and urine to bring more insight into this matter in CI-AKI. Lastly, measuring other markers of oxidative stress as total thiol groups (TTG), ROS, and F2-isoprostanes in urine would also add value to the findings of this work. The authors tried but failed to measure TTG in urine by using Ellman's reagent according to the Hu method.[58]

Collectively, the results imply that Q10 supplementation is ineffective in preventing CI-AKI among diabetes mellitus patients with normal eGFR, reflected in both traditional (SCr, eGFR, BUN, and UMS) and novel biomarkers of kidney injury (mtDNA/nDNA ratio, UMDA, and UTAC) in serum and urine, respectively. Further studies are suggested to evaluate the prophylactic effects of Q10 in various groups and other forms of AKI.

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CONFLICT OF INTEREST STATEMENT

None declared.

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