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Research Article

Progression of Increased Oxidative Stress and Inflammation in Chronic Kidney Disease Patients with Type 2 Diabetes Mellitus

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ABSTRACT
Type 2 diabetes mellitus (T2DM) patients have increased oxidative stress, inflammation and reduced antioxidant defenses mechanisms in the circulatory system. This study demonstrates the progression and correlation of oxidative stress and inflammation with chronic kidney disease (CKD), pre-hemodialysis and hemodialysis in T2DM patients. A total of 292 T2DM patients participated in the present study. These T2DM patients' diagnoses ranged from stage 1 to 5 according to their estimated glomerular filtration rate (eGFR). Serum levels of high-sensitive C-reactive protein (hs-CRP) and malondialdehyde (MDA) were significantly increased, but total antioxidant capacity (TAC) in these T2DM patients was decreased. The eGFR was inversely correlated with MDA, hs-CRP, NAG, TG/HDL-C and positively correlated with HDL-C and TAC. Hs-CRP and TG/HDL-C were positively correlated with MDA, and negatively correlated with TAC and HDL-C in the present study. Multiple forward stepwise linear regression analyses demonstrated independent predictors of eGFR were hs-CRP (β = -0.572, r^2 =0.425, p<0.001), Glu (β = -0.162, r^2 =0.458, p<0.001), MDA (β = -0.111, r^2 =0.479, p= 0.02) and TG/HDL-C ratio (β = -0.099, r^2 =0.486, p= 0.04). The CKD progression, as demonstrated by increase in BUN, CT and decreased eGFR, was concomitant with increased MDA, hs-CRP levels and TG/HDL-C ratio in each group, but slightly declined in the patients receiving hemodialysis. eGFR reduction was parallel with the increased of oxidative stress and inflammation when CKD developed and progressed. Conclusions: oxidative stress and inflammation may underlie the progression and decline of renal function and structural damage to kidneys in T2DM patients.

Keywords: Type 2 diabetes mellitus, chronic kidney disease, oxidative stress, inflammation, estimated glomerular filtration rate.

INTRODUCTION

Numerous studies have been made to identify the major causative manners in the pathogenesis of diabetic nephropathy, and these studies suggest that among the numerous systems activated during the course of the disease are the following: glucose autoxidation, hexosamine pathways, advanced glycation end-products (AGEs) formation, polyol pathway, and protein kinase-Cβ1/2 (PKCβ1/2)[1], renin–angiotensin system, vasoconstrictor systems, profibrotic and inflammatory cytokines and the most importantly, oxidative stress mediators, such as nicotinamide adenine dinucleotide phosphate oxidase (NADPH oxidase), all of these pathways can also increase oxidative stress in T2DM patients. Many research studies demonstrated that type 2 diabetes mellitus (T2DM) patients have over-production of ROS and also have reduced antioxidant defenses mechanisms, which can cause ROS-induced oxidative damage in the circulation and organs[2-5]. However, clinical strategies based on these systems for the management of diabetic nephropathy complication remain unsatisfactory, the number of patients with diabetic nephropathy is increased in each year[6]. Many studies demonstrated that diabetic nephropathy risk is higher in patients with poor metabolic control[7,8]. Diabetic nephropathy is the most common cause of end stage renal disease (ESRD) accounting for >50% of new cases of renal failure[9]. About 25–35% of T2DM patients will develop the diabetic nephropathy complication, associated with cardiovascular disease risk[10,11]. All constituents of the kidney including glomerular, mesangial cells, podocytes, endothelia and tubular epithelia are damaged or injured from oxidative stress in the chronic hyperglycemic condition. Glomerular hypertrophy, glomerular basement membrane thickness, mesangial cell expansion, renal hypertrophy, glomerulosclerosis, podocytes loss and tubulointerstitial fibrosis are the major pathological changes of these renal components during the chronic hyperglycemia which causes diabetic nephropathy. Diabetic nephropathy is characterized by reduction in glomerular filtration rate, progressive albuminuria, elevation of arterial blood pressure and fluid retention[12]. Chronic kidney disease or renal insufficiency (RI) and renal failure (RF) associated with increased oxidative stress and inflammation levels and associated with higher cardiovascular disease risk, and may promote the progression of renal disease in these

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Table 1a: Comparison of the clinical characteristics of the all groups and intergroup of the different stages of chronic kidney diseases in type 2 diabetes mellitus patients by using Kruskal-Wallis test and Mann-Whitney *U*-test.

Variable	Gr-1 eGFR≥60 ml/min/1.73 m^2 (n=87)	Gr-2 eGFR 30-59 ml/min/1.73 m^2 (n=22)	Gr-3 eGFR15-29 ml/min/1.73 m^2 (n=46)	Gr-4 eGFR<15 ml/min/1.73 m^2, non-dialysis (n=57)	Gr-5 eGFR<15 ml/min/1.73 m^2, dialysis (n=80)	*p*-value
Age (Years)	41.0(36.0-49.0)*	53.5(49.8-56.0)*	54.5 (45.8-59.0)*	53.0 (45.0-56.0)*	55.0 (48.5-57.5)*	<0.001
BMI (kg/m^2)	21.0(18.8-24.2)	25.5(22.1-28.2)	23.1(19.9-26.3)	22.3(19.5-26.4)	20.9(18.1-24.9)	0.006
WC (cm)	75.0(70.0-80.0)	86.3(79.4-100.0)	83.8(76.9-90.6)	87.5(80.0-100.0)	85.0(75.0-95.0)	<0.001
Syst BP (mmHg)	120.0(110.0-120.6)	135.5(114.8-150.8)	136.0(120.0-147.5)	140.0(120.0-156.5)	136.0(120.0-150.0)	<0.001
Diast BP (mmHg)	80.0(70.0-80.6)	70.0(63.0-81.3)	78.0(64.8-87.3)	80.0(67.0-89.5)	79.0(70.0-85.5)	0.310
Glu (mmol/l)	4.57(4.18-4.90)	5.64(4.95-6.93)	5.61(4.83-6.62)	5.89(4.81-8.39)	5.61(4.76-7.07)	<0.001
BUN (mmol/l)	3.92(3.21-4.64)	11.77(8.13-15.44)	15.16(10.98-21.40)	35.32(20.51-34.95)	25.32(16.94-29.24)	<0.001
CT (µmol/l)	60.99(52.16-76.91)	187.41(177.68-206.86)	289.95(239.56-358.02)	918.48(733.72-1155.39)	537.47(403.10-634.71)	<0.001
UA (mmol/l)	303.45(249.9-237.0)	487.9(428.4-571.2)	493.85(478.4-595.0)	493.85(410.55-624.75)	440.30(392.70-559.30)	<0.001
TC (mmol/l)	4.54(4.10-4.90)	4.21(3.48-5.40)	3.74(3.32-4.93)	4.23(3.55-4.80)	3.56(3.02-4.70)	<0.001
TG (mmol/l)	1.02(0.81-1.37)	1.44(1.28-2.02)	1.51(1.16-3.08)	1.77(1.14-2.48)	1.38(1.00-2.38)	<0.001
HDL-C (mmol/l)	1.35(1.17-1.58)	1.22(0.98-1.42)	0.97(0.85-1.24)	1.01(0.87-1.22)	0.83(0.67-1.05)	<0.001
LDL-C (mmol/l)	2.74(2.30-3.12)	2.47(1.67-3.01)	2.05(1.47-2.59)	2.22(1.73-2.69)	1.83(1.37-2.61)	<0.001
TG/HDL-C ratio	1.61(1.29-2.49)	2.62(2.27-5.01)	3.19(2.54-7.14)	4.15(2.12-6.15)	3.83(2.46-6.59)	<0.001
MDA (µmol/l)	6.45(4.88-7.88)	7.88(6.10-10.13)	8.25(6.19-11.53)	10.88(7.90-16.50)	10.31(7.88-12.19)	<0.001
TAC(µmol/l Trolox equiv/l)	430.0(320.0-530.0)	355.0(230.0-465.0)	350.0(227.5-432.5)	350.0(270.0-440.0)	290.0(210.0-390.0)	<0.001
HbA1c (%)	5.30(5.10-5.70)	5.85(5.20-6.93)	5.90(5.35-6.83)	5.70(5.30-6.80)	5.60(5.05-7.30)	<0.001
NAG (U/g CT)	10.65(6.97-14.44)	32.46(20.41-45.96)	38.37(24.45-56.48)	51.73(29.95-65.95)	34.66(24.99-48.31)	<0.001
hs-CRP (g/l)	1.30(0.99-1.92)	6.34(4.18-9.13)	7.86 (5.87-8.93)	8.87(6.41-8.99)	6.45(5.65-8.05)	<0.001

* median (interquatile)

T2DM patients[13-15]. Previous studies[16,17] has been discussed the effect of oxidative stress in different stages of CKD, and concluded that the antioxidant enzymes declined at the severe stage of CKD and in hemodialysis patients. Experiments have shown that oxidative stress and inflammation coexist together[18]. This study aims to evaluate the progression and correlation of oxidative stress and inflammation in T2DM patients with different stages of chronic kidney disease (CKD), predialysis and receiving hemodialysis stage.

MATERIALS AND METHODS

Subjects

This present study is a cross sectional study, with 292 T2DM patients. All T2DM patients (overt diabetes more than 5 years) were randomized from the Diabetes Care Clinic of Uttaradit Hospital during January 2009 to December 2009. All T2DM patients were receiving regular treatment with glycemic lowering, lipid lowering, and anti-hypertensive medication. The exclusion criteria were sustained heart failure, peripheral vascular disease, recent myocardial infarction, unstable angina, stroke, acute or

Table 1b: *p*-values

Variable	*p*-value of Gr- 1:2	*p*-value of Gr- 1:3	*p*-value of Gr- 1:4	*p*-value of Gr-1:5	*p*-value of Gr-2:3	*p*-value of Gr-2:4	*p*-value of Gr-2:5	*p*-value of Gr-3:4	*p*-value of Gr-3:5	*p*-value of Gr-4:5
Age (Years)	<0.001	<0.001	<0.001	<0.001	0.581	0.449	0.678	0.797	0.159	0.167
BMI (kg/m^2)	0.001	0.033	0.678	0.094	0.078	0.002	0.037	0.041	0.568	0.092
WC (cm)	<0.001	<0.001	<0.001	<0.001	0.141	0.386	0.738	0.577	0.030	0.084
Syst BP (mmHg)	<0.001	<0.001	<0.001	<0.001	0.922	0.978	0.429	0.723	0.219	0.364
Diast BP (mmHg)	0.755	0.342	0.071	0.215	0.458	0.219	0.278	0.295	0.631	0.498
Glu (mg/dl)	<0.001	<0.001	<0.001	<0.001	0.753	0.996	0.454	0.772	0.226	0.394
BUN (mg/dl)	<0.001	<0.001	<0.001	<0.001	0.019	<0.001	<0.001	<0.001	<0.001	0.251
CT (mg/dl)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
UA (mg/dl)	<0.001	<0.001	<0.001	<0.001	0.916	0.607	0.245	0.637	0.128	0.048
TC (mg/dl)	0.459	0.006	<0.001	<0.001	0.342	0.751	0.067	0.314	0.276	0.023
TG (mg/dl)	<0.001	<0.001	<0.001	<0.001	0.860	0.634	0.481	0.803	0.249	0.191
HDL-C (mg/dl)	0.044	<0.001	<0.001	<0.001	0.046	0.023	<0.001	0.744	0.001	0.001
LDL-C (mg/dl)	0.094	<0.001	<0.001	<0.001	0.173	0.454	0.045	0.307	0.341	0.022
TG/HDL-C ratio	<0.001	<0.001	<0.001	<0.001	0.206	0.296	0.131	0.858	0.716	0.795
MDA (µmol/l)	0.026	<0.001	<0.001	<0.001	0.018	0.494	0.001	0.045	0.156	<0.001
TAC (µmol/l Trolox equiv/l)	0.066	0.004	0.004	<0.001	0.829	0.961	0.184	0.946	0.080	0.048
HbA1c (%)	0.004	<0.001	<0.001	<0.001	0.870	0.591	0.559	0.559	0.603	0.800
NAG (U/g CT)	<0.001	<0.001	<0.001	<0.001	0.326	0.563	0.164	0.632	<0.001	0.006
hs-CRP (g/l)	<0.001	<0.001	<0.001	<0.001	0.042	0.718	0.189	0.006	0.196	0.092

chronic infection, cancer, hepatic disease, acute illness, blood glucose (Glu) and hemoglobin A1c (HbA1c) levels over 9.9 mmol/l (180 mg/dl) and 8.0% in the year of recruitment. All participants gave written informed consent. Our study protocol was approved by the Ethic committees of Naresuan University.

Physical and Biochemical examination

Physical examination was performed with anthropometry and blood pressure measurement. Height, weight, and blood pressure (BP) were measured and body mass index (BMI) was calculated. Waist circumference (WC) was measured at the midpoint between the rib cage and the top of lateral border of iliac crest at minimum respiration. BP was measured after the participants had been seated and rested for 5 minutes, as the mean value of at least two measurements for these participants on the same day with calibrated desktop sphygmomanometers. Venous blood samples were collected from all participants without stasis after 8–12 hour fast in a seated position. Blood specimens were processed and assayed in the clinical laboratory of Uttaradit Hospital, Uttaradit, Thailand. Plasma glucose (Glu), blood urea nitrogen (BUN), total cholesterol (TC), triglyceride (TG), high density lipoprotein-cholesterol (HDL-C) were measured by enzymatic method. Serum creatinine (CT) and urine creatinine (UCT) concentrations were determined based on the Jaffe reaction. Low density lipoprotein-cholesterol (LDL-C) concentrations were calculated with Friedewald's formula in specimens with TG levels <400 mg/dl. TG/HDL-C ratio was calculated by TG divided by HDL-C concentrations (expressed as mg/dl). Urine samples were collected in polyethylene bottles after physical examination and blood taken. A 30-ml of urine sample was collected and divided into two aliquots (5-10 ml each), one for microscopic investigation

Table 2: Bivariate correlation of the markers in type 2 diabetes patients with chronic kidney disease.

Correlation parameters	between	Correlation coefficient		Correlation parameters	between	Correlation coefficient	
		r	*p*- value			r	*p*- value
eGFR	Age	-0.498	<0.001	hs-CRP	Age	0.488	<0.001
	WC	-0.347	<0.001		BMI	0.161	0.045
	SystBP	-0.222	0.006		WC	0.344	<0.001
	Glu	-0.496	<0.001		SystBP	0.377	<0.001
	BUN	-0.816	<0.001		Glu	0.531	<0.001
	CT	-0.918	<0.001		BUN	0.751	<0.001
	UA*	-0.689	<0.001		CT	0.808	<0.001
	TG	-0.457	<0.001		UA	0.676	<0.001
	HDL-C	0.407	<0.001		TC	-0.205	0.011
	LDL-C	0.271	0.001		TG	0.498	<0.001
	TG/HDL-C*	-0.526	<0.001		HDL-C	-0.437	<0.001
	MDA*	-0.455	<0.001		LDL-C	-0.329	<0.001
	TAC*	0.206	<0.001		TG/HDL-C*	0.562	<0.001
	HbA1c	-0.267	0.001		HbA1c	0.274	0.001
	NAG	-0.585	<0.001		NAG	0.573	<0.001
	hs-CRP*	-0.851	<0.001		TAC	-0.185	0.021
MDA	Age	0.279	<0.001	NAG	Age	0.317	<0.001
	WC	0.218	0.006		BMI	0.180	0.025
	SystBP	0.190	0.018		WC	0.308	<0.001
	Glu	0.292	<0.001		SystBP	0.398	<0.001
	BUN	0.464	<0.001		Glu	0.424	<0.001
	CT	0.443	<0.001		BUN	0.543	<0.001
	UA	0.380	<0.001		CT	0.579	<0.001
	TG	0.341	<0.001		UA	0.429	<0.001
	HDL-C	-0.268	<0.001		TG	0.263	0.001
	TG/HDL-C*	0.380	<0.001		HDL-C	-0.174	0.031
	TAC	-0.195	0.015		LDL-C	-0.258	0.001
	HbA1c	0.158	0.049		TG/HDL-C	0.283	<0.001
	NAG	0.226	0.005		HbA1c	0.261	0.001
	hs-CRP*	0.431	<0.001	TG/HDL-C	Age	0.254	0.001
TAC	Age	-0.207	0.010		BMI	0.206	0.010
	Glu	-0.159	0.048		WC	0.235	0.003
	BUN	-0.188	0.019		SystBP	0.199	0.013
	CT	-0.179	0.026		Glu	0.338	<0.001
	TG	-0.172	0.032		BUN	0.439	<0.001
	HDL-C	0.272	0.001		CT	0.546	<0.001
	TG/HDL-C	-0.257	0.001		UA	0.506	<0.001
	HbA1c	-0.160	0.046		TG	0.918	<0.001
					HDL-C	-0.715	<0.001
					HbA1c	0.239	0.003

Table 3: Multiple forward stepwise linear regression analyses of the significant variables demonstrated that, hs-CRP, Glu, MDA and TG/HDL-C ratio were independent predictors of eGFR in these T2DM patients.

Variables	β	R^2	Adjusted R^2	*p*-value
hs-CRP	-0.572	0.425	0.423	<0.001
Glu	-0.162	0.458	0.454	<0.001
MDA	-0.111	0.479	0.473	0.020
TG/HDL-C	-0.099	0.486	0.479	0.040

and another aliquot for N-acetyl-β-D-glucosaminidase (NAG) and UCT determination.

Malondialdehyde (MDA) measurement

MDA level was determined by using the thiobarbituric acid substances (TBARS) assay, a spectroscopic technique as described in our previous study[5]. The method is based on the reaction of one molecule of MDA with 2 molecules of TBA to yield a pink chromophore with the maximum absorption at 532 nm.

Total antioxidants capacity (TAC) measurement

The assay method was based on the study of Miller et al.[19], by using the metmyoglobin reaction with hydrogen peroxide to form a free radical species of ferryl myoglobin. A radical cation which has a relatively stable blue-green color can be measured at 600 nm, after adding a

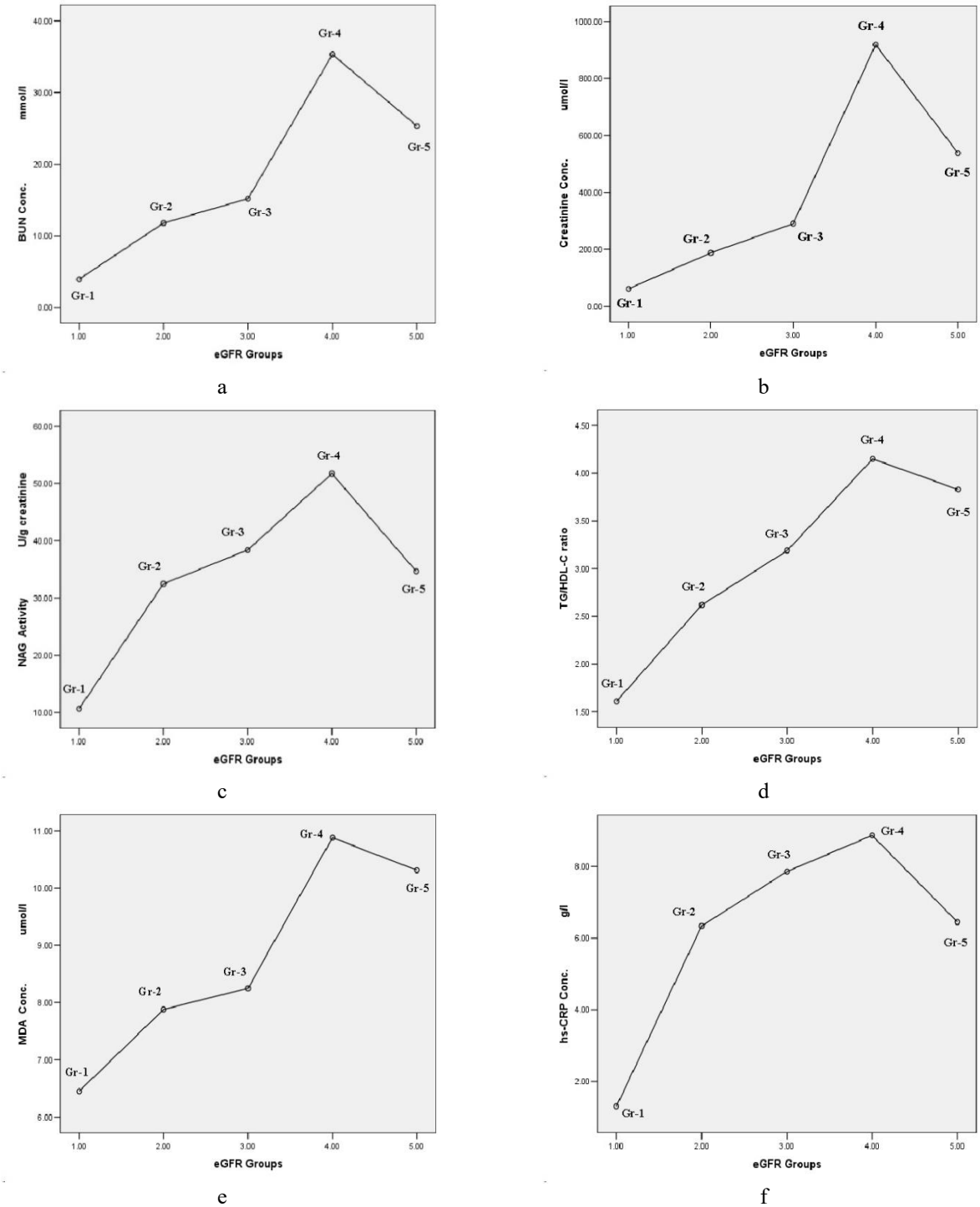

Figure 1: Demonstrated the progression of increased in (a) BUN, (b) creatinine, (c) NAG, (d) TG/HDL-C ratio, (e) MDA and (f) hs-CRP concentrations according to their eGFR stages and declined after hemodialysis stage. (*All marker levels in each group demonstrated as median levels of each marker).

chromogen of 2, 2'-amino-di-[3-ethylbenzthiazole sulphonate] to react with ferryl myoglobin. Any antioxidant capacity in the added serum sample can suppress this blue-green color production to a degree proportional to the antioxidant capacity as mmol/l trolox equivalent. The within–run coefficient of variation for the TAC assay in control material was 4.8% (n=10).

High sensitivity-C-reactive protein (hs-CRP) measurement

The hs-CRP levels were assayed by using latex-enhanced immunoneplelometric method on the Hitachi 912 auto-analyzer (Roche Diagnostic, Switzerland) that has been standardized against the World Health Organization reference.

N-acetyl-β-D-glucosaminidase (NAG) measurement

The assay is based on NAG in urine reacting with the substrate of p-nitrophenyl-N-acetyl- β -D-glucosaminide

in sodium citrate buffer (pH 4.4) at 37 ºC to liberate p-nitrophenylate ion, then adding 2-amino-2-methyl-1-propanol (AMP) buffer (pH 10.25) to the reaction and measuring the color reaction with spectrophotometer at 405 nm[20]. The within–run and between-run coefficient of variation in control material were 3.14% and 4.11% (n=10).

Estimated glomerular filtration rate (eGFR)

Estimated glomerular filtration rate was calculated by the Modification of Diet in Renal Disease (MDRD) equation[21]. The formula is: eGFR = 186 * [plasma creatinine $^{-1.154}$] * (age) $^{-0.203}$ * (0.742 if female) * (1.210 if African-American). Five eGFR stages were used: Stage I was normal eGFR (≥90ml/min/1.73 m^2); Stage II was mildly eGFR (60-89 mL/min/1.73 m^2); Stage III was moderately eGFR (30-59 ml/min/1.73 m^2); Stage IV was severely eGFR (<30 ml/min/ 1.73 m^2), and Stage V was end-stage renal disease: eGFR (<15 ml/min/1.73 m^2). An eGFR lower than 60 ml/min/1.73 m^2 (moderately eGFR) was defined as chronic kidney disease (CKD)[22].

Statistical analysis

All data are presented as median and interquartile range for non-normally distributed data and tested by using Shapiro-Wilk test. These T2DM patients were stratified to 5 stages according to the stage of eGFR by using the KDOQI criteria. We compared all clinical characteristics of these 5 groups of T2DM patients by using Kruskal–Wallis test. We also compared the differences of all clinical characteristics for the intergroup by using Mann-Whitney *U*-test. Bivariate correlation between eGFR, NAG, MDA, TAC, hs-CRP and TG/HDL-C with the other variables was assessed by using Spearman rank correlation test. Clinical variables that correlated with eGFR in these patients were tested as independent variables by using multivariate forward stepwise linear regression analysis. All tests were two tailed, and *p*-values less than 0.05 were regarded as statistically significant. All analysis was performed by SPSS version 13.0 (SPSS, Chicago, IL, USA).

RESULTS

Median age of 152 (52.1%) women was 51.0 (41.0–56.0) years and the 140 (47.9%) men had median age of 52.0 (43.0–56.0) years. We stratified the 292 T2DM patients into five groups according to their eGFR levels as follow: Group (Gr)-1, 87 (29.8%) T2DM patients had eGFR ≥ 60 ml/min/1.73 m^2; Gr-2, 22 (7.5%) T2DM patients had eGFR 30–59 ml/min/1.73 m^2; Gr-3, 46 (15.8%) T2DM patients had eGFR 15-29 ml/min/1.73 m^2; Gr-4, 57 (19.5%) T2DM patients had eGFR <15 ml/min/1.73 m^2, with no hemodialysis; Gr-5, 80 (27.4%) T2DM patients had eGFR <15 ml/min/1.73 m^2 with hemodialysis. Patients in Gr-1 were the youngest while those in Gr-5 were the oldest. This may be an indication of shorter duration since onset of T2DM in Gr-1 and longer duration since onset of T2DM in Gr-5. All T2DM patients had good glycemic and lipid control, demonstrated by low glucose, HbA1c and TC and TG levels. These T2DM patients also demonstrated higher levels of NAG, MDA, hs-CRP levels, TG/HDL-C ratio and lower HDL-C and TAC levels in eGFR stages 2 to 5. The comparison of all clinical characteristics of those 5 eGFR stages was significantly differenced in all clinical characteristics except diastolic blood pressure by using the Kruskal–Wallis test (p<0.001), as shown in Table 1. We also compared the difference of all clinical characteristics between groups. In Gr-1, non-CKD: Age, BMI, WC, Syst BP, Glu, HbA1c, UA, TC, TG, HDL-C, TG/HDL-C, NAG, MDA, TAC and hs-CRP are significantly different from the other groups. Results of the comparison between groups demonstrated that MDA and hs-CRP levels were significantly higher in Gr-2 than Gr-1, in Gr-3 higher than Gr-2, in Gr-4 higher than Gr-3, but lower in Gr-5 than Gr-4, which may be due to hemodialysis. These results demonstrated the progression increased in (a) BUN, (b) creatinine, (c) NAG, (d) TG/HDL-C ratio, (e) MDA and (f) hs-CRP concentrations according to their eGFR stages and those levels were declined after hemodialysis stage as shown in Fig. 1. The bivariate correlation between eGFR, NAG, MDA, hs-CRP, TAC, TG/HDL-C ratio with other variables in these T2DM patients was demonstrated in Table 2. These results demonstrate that hs-CRP, Glu, MDA and TG/HDL-C ratio and showed association with eGFR, which remained highly significant after adjusting for any clinical or laboratory confounding variables [hs-CRP (β = -0.572, r^2 =0.425, p< 0.001; Glu (β = -0.162, r^2 =0.458, p < 0.001; MDA (β = -0.111, r^2 =0.479, p = 0.02; TG/HDL-C ratio (β = -0.099, r^2 = 0.486, p=0.04] in these T2DM patients, as shown in Table 3.

DISCUSSION

The present study demonstrated a progression of increasing oxidative stress, inflammation and decreasing TAC in these T2DM patients with CKD, which decreased slightly after they received hemodialysis. Our results were in agreement with previous studies which associated the increased MDA levels in patients with CKD[16,17]. Many studies and experimental evidences indicate that diabetes is a state of oxidative stress, and it has been suggested that these oxidants are the causative link all of the major mediator pathways that are implicated in micro and macro-vascular complications, especially in glomerulosclerosis of diabetes[1]. Diabetic nephropathy is a major microvascular complication in the long duration of diabetes mellitus. Numerous pathways including NADPH oxidase, protein kinase C, renin-angiotensin aldosterone system, transforming growth factor-β, tumor necrosis factor-α, JAK/STAT pathway, adenosine, cannabinoid receptors and others are activated during the long-term of diabetes mellitus. These pathways individually or collectively play the major role in the induction and progression of glomerulosclerosis in diabetic nephropathy. This may the reason why the number of diabetic-nephropathy patients is increasing every year[6]. Endothelial nicotinamide adenine dinucleotide phosphate (NADPH) oxidase is a major source of the generation of endothelial or vascular ROS. It has an important pathogenic mechanism underlying endothelial or vascular dysfunction and complications in diabetes mellitus. The activation of endothelial NAD(P)H oxidase initiates and worsens the progression of diabetic nephropathy, especially in the

albuminuria development. These endothelial NAD(P)H oxidase activations and increased ROS generation in glomeruli cause endothelium glomerular dysfunctions[23]. Inflammation is another major cause of increased oxidative stress in renal disease patients[24] producing the inflammatory mediators (monocyte chemoattractant protein-1and interleukins (IL)) to induce oxidative stress[24]. IL-6 is one pro-inflammatory chemokine responsible for recruitment and activation of neutrophils, T cells, monocytes and macrophages. These conditions of IL-6 and other cytokines releasing can trigger CRP and fibrinogen synthesis from the liver as an acute phase response[25]. T2DM patients in Gr- 2 to Gr-5 of the present study also demonstrated lower HDL-C and higher TG/HDL-C ratio, indicating that these T2DM patients may be in the insulin resistance condition[26]. Many studies demonstrated that antioxidants have the prevention effect for renal hypertrophy and glomerular cells, albuminuria, glomerular expression of TGF-β1, extracellular matrix, and PKC pathway activation in the experimental diabetes[27]. Our previous study[28] demonstrated the effect of cinnamon (an antioxidant) supplementation to increase eGFR and reduce blood pressure (BP) in T2DM patients. This effect demonstrated that cinnamon regulates blood pressure via peripheral vasodilatation[29], which may be beneficial for renal function and BP. Thus, the suitable and effective antioxidants should be supplemented for T2DM patients to prevent the adverse effects to glomerular renal function and limit the activation of all vicious pathways[28,30]. A limitation of the present cross sectional study is that it did not measure the other cytokines and other anti-oxidants markers in circulation in these T2DM patients. Also all of our T2DM patients are only in one hospital and of similar ethic background.

CONCLUSION

The evidence shows increased oxidative stress and inflammation in progressive kidney disease and enhanced production of ROS even in in these T2DM patients with good glycemic control. Oxidative stress and inflammation may underlie the progression and decline of renal function and structure change.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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