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ApoM/HDL-C and apoM/apoA-I ratios are indicators of diabetic nephropathy in healthy controls and type 2 diabetes mellitus

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ABSTRACT

Background: Apolipoprotein M (apoM) concentrations were decreased in type 2 diabetes mellitus (T2DM). ApoM was selectively expressed in renal tubular epithelial cells. We investigated the changes in plasma apoM concentrations in diabetic nephropathy (DN) patients and the potential of apoM as a biomarker of DN.
Methods: A total of 96 DN patients and 100 age- and sex-matched diabetic non-nephropathy (non-DN) patients and 110 healthy controls were included. All T2DM patients were divided into 3 groups according to urinary albumin excretion: normoalbuminuria (n = 100), microalbuminuria (n = 50) and macroalbuminuria (n = 46). Plasma apoM concentrations were measured by enzyme-linked immunosorbent assay.
Results: DN Patients had higher plasma apoM concentrations than those in non-DN patients (22.23 ± 11.69 vs. 18.96 ± 7.85 ng/μl, $P < 0.05$). In addition, microalbuminuria group showed higher plasma apoM concentrations than those in normoalbuminuria group (22.67 ± 11.40 vs. 18.96 ± 7.85 ng/μl, $P < 0.05$). The areas under curve (AUC) of apoM using a receiver-operating characteristic (ROC) curve analysis showed that plasma apoM concentrations were not indicators for identification of DN from healthy people (AUC = 0.478, P = 0.585) and from T2DM (AUC = 0.563, P = 0.125). DN patients had higher ratios of apoM/HDL-C and apoM/apoA1 than those in healthy controls and in non-DN patients. ApoM/HDL-C and apoM/apoA1 ratios could be used as indicators for identification of DN from healthy people (AUC = 0.597, P = 0.016; AUC = 0.665, P = 0.000, respectively) and from T2DM (AUC = 0.580, P = 0.050; AUC = 0.601, P = 0.015, respectively).
Conclusions: ApoM/HDL-C and apoM/apoA1 ratios could be used as indicators for identification of DN from healthy people and from T2DM patients.

1. Introduction

Apolipoprotein M (apoM), a member of the lipocalin protein family, plays a critical role in the formation of preβ-high density lipoprotein (preβ-HDL) particles with potential antioxidant activity by promoting intracellular cholesterol efflux [1,2]. Moreover, apoM acts as a biased agonist on the biologically active lipid mediator sphingosine 1-phosphate receptor 1, inhibits vascular inflammation and has a function in cardiovascular protection [3]. Recent research showed that ligand-free apoM protein has an open upper hydrophilic binding pocket, resulting in complete closure of the lower hydrophobic cavity by adjusting the gate for ligand access [4]. Plasma apoM (~23 mg/l) is mainly enriched in HDL and associated with HDL, low-density lipoprotein (LDL), total cholesterol (TC), apolipoprotein A-I (apoA-I) and apolipoprotein B (apoB) [5,6].

The occurrence of type 2 diabetes mellitus (T2DM), a chronic metabolic disorder caused by insulin resistance, is predicted to increase by 15% worldwide in 2025 [7]. Evidence of the strong association between apoM and T2DM has been demonstrated by in vivo and in vitro models [5,8,9]. Our previous research revealed that plasma apoM concentrations significantly decreased in Han Chinese with T2DM compared with healthy controls [10]. Low plasma apoM concentrations were also observed in Swedes and Caucasians and Chinese with T2DM [5,8,11]. The apoM promoter region single-nucleotide polymorphism (SNP) T-778C confers an increasing risk of T2DM among northern Han Chinese [12]. We also reported that SNP C-724del in the apoM promoter

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region is related to T2DM among eastern Han Chinese [10]. Many factors can affect the metabolism of apoM in T2DM, such as hyperglycaemia [13,14], hyperlipidemia [11], insulin [9,13], glucosamine [15] and intralipids [16], but the mechanisms are poorly understood.

Although apoM has been found recently in the human heart, brain, spleen and colon, it is predominantly expressed in the liver and renal tissues [17–19]. Moreover, apoM concentrations in the kidneys increase in streptozotocin-induced diabetic mice, indicating that hyperglycaemia can promote apoM expression in kidneys [9]. An ischaemia–reperfusion injury model of rat showed that plasma apoM concentrations increase during ischaemia–reperfusion injury, indicating that urinary apoM can be used as a biomarker of acute renal injury [20]. Diabetic nephropathy (DN) is one of the most common microvascular complications of T2DM, and it is associated with high morbidity and mortality in cardiovascular diseases [21]. DN patients not only have glomerular injury but also tubular degeneration and interstitial fibrosis, which are associated with DN progression [22,23]. However, the changes in plasma apoM concentration in DN patients remain unclear.

2. Methods and materials

2.1. Subjects

All subjects were recruited from The First and The Second Affiliated Hospital of Wannan Medical College and provided written informed consent. This research was divided into 3 groups: DN group (n = 96), non-DN group (n = 100) and healthy controls group (n = 110). The protocol in this study was approved by the Medical Ethics Committee of Wannan Medical College. T2DM was diagnosed using the 1999 World Health Organization criteria: fasting blood glucose (FBG) ≥7.0 mmol/l or 2-h blood glucose ≥11.1 mmol/l following an oral glucose tolerance test [24]. Subjects with non-type 2 diabetes (type 1 diabetes and drug-induced diabetes), hyperlipidemia, pregnancy, thyroid disease, severe liver disease, nephrolithiasis, azotaemia, renal failure and acute infectious diseases were excluded from the study. DN was diagnosed using the 2007 National Kidney Foundation criteria: T2DM patients were determined as DN if their two of 3 specimens of urinary albumin to creatinine ratio (ACR) > 30 mg/g in 3- to 6-month period or non-DN if their ACR was >30 mg/g [25]. The exclusion criteria were non-diabetic kidney disease, renal transplant or dialysis. The inclusion criteria of the healthy controls group were good general health, insignificant past medical history and documented normal FBG, glucose intolerance and renal function. All patients with T2DM were divided into 3 groups according to their ACR as follows: normoalbuminuria (<30 mg/g, n = 100), microalbuminuria (30–300 mg/g, n = 50) and macroalbuminuria (>300 mg/g, n = 46) [26]. Glomerular filtration rate (GFR ml/min/1.73m^2) = 186 × (CR mg/dl)$^{-1.154}$ × (Age)$^{-0.203}$ × (0.742 if female). Chronic kidney disease (CKD) stages were determined by GFR: GFR = 60 to 89 ml/min/1.73 m^2 was defined as stage 2; 30 to 59 ml/min/1.73 m^2 was defined as stage 3; 15 to 29 ml/min/1.73 m^2 was defined as stage 4; GFR < 15 ml/min/1.73 m^2 was defined as stage 5 [25].

2.2. General conditions of patients

Age, sex, systolic blood pressure (SBP), diastolic blood pressure (DBP), diabetic mellitus (DM) duration was collected from medical record. Plasma and urine samples were collected in the morning after the participants fasted for at least 12 h. ACR, creatinine (Cr), cystatin C (CYS-C), superoxide dismutase (SOD), fasting plasma glucose (FPG), TC, triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), LDL-C, apoA-I, apoB and lipoprotein (a) (Lp(a)) were measured by Hitachi 7600 biochemistry autoanalyzer (Hitachi, Tokyo, Japan). Hemoglobin A-Ic (HbA-Ic) was measured using a Bio-Rad Variant II analyzer (Bio-Rad Laboratories, Hercules, CA, USA), and presented as National Glycohemoglobin Standardization Program (NGSP) units (%) and with The International Federation of Clinical Chemistry and Laboratory

Table 1
The comparison of clinical characteristics and plasma parameters among healthy controls, DN and non-DN.

	Healthy controls (n = 111)	DN (n = 96)	non-DN (n = 100)	P value
N (M/F)	58/53	51/45	58/42	NS
Age (y)	58.87 ± 8.80	60.54 ± 10.55	58.92 ± 8.10	NS
SBP (mm Hg)	120.07 ± 13.56	146.59 ± 25.07***	133.85 ± 17.37$^{\#\#\#}$	0.000
DBP (mm Hg)	76.51 ± 8.39	85.11 ± 13.21***	82.16 ± 10.56	0.000
DM duration (y)	–	8.58 ± 5.87	7.28 ± 6.20	
ACR (mg/g)	–	524.75 ± 760.57	11.84 ± 10.89	
HbA1c (%(mmol/mol))	–	9.6 ± 2.4(81 ± 26)	9.0 ± 2.4(75 ± 27)	
Cr (umol/l)	63.23 ± 14.23	99.02 ± 61.50***	64.90 ± 18.50$^{\#\#\#}$	0.000
CYS-C (mg/l)	1.02 ± 0.26	1.52 ± 0.73***	1.01 ± 0.22$^{\#\#\#}$	0.000
SOD (U/ml)	107.16 ± 10.22	71.92 ± 20.50***	83.06 ± 16.74$^{\#\#\#}$	0.000
FPG (mmol/l)	5.37 ± 0.41	9.43 ± 3.42***	8.73 ± 2.79	0.000
TC (mmol/l)	4.40 ± 0.58	4.48 ± 1.08	4.36 ± 1.47	NS
TG (mmol/l)	1.27 ± 0.61	2.15 ± 1.56***	2.03 ± 1.39	0.000
HDL-C (mmol/l)	1.44 ± 0.25	1.22 ± 0.34***	1.25 ± 0.35	0.000
LDL-C (mmol/l)	2.38 ± 0.46	2.56 ± 0.83	2.42 ± 0.75	NS
apoA-I (g/l)	1.67 ± 0.29	1.28 ± 0.39***	1.34 ± 0.35	0.000
apoB (g/l)	0.79 ± 0.21	0.94 ± 0.33***	0.87 ± 0.31	0.001
LP(a) (mg/l)	120.74 ± 105.95	202.72 ± 263.11***	141.87 ± 155.92$^{\#}$	0.005
CKD stage				
Stage 2 (%)		16.7		
Stage 3 (%)		42.7		
Stage 4 (%)		20.8		
Stage 5 (%)		4.1		
Medications				
ACEI (%)		82.3	14$^{\#\#\#}$	
Insulin (%)		62.5	16$^{\#\#\#}$	
Biguanides (%)		5.2	56$^{\#\#\#}$	
Sulfonylurea (%)		54.2	42	

SBP, systolic blood pressure; DBP diastolic blood pressure; DM, diabetes mellitus; ACR: albumin-to-creatinine ratio; HbA1c, glycated hemoglobin A1c, is presented as National Glycohemoglobin Standardization Program (NGSP) units (%) and with The International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) units (mmol/mol) in parentheses; Cr, creatinine; CYS-C, cystatin C; FPG, fasting plasma glucose; TC, total cholesterol; TG, triglycerides; LP(a), lipoprotein (a); CKD, Chronic kidney disease; ACEI, angiotensin converting enzyme inhibitors. P value: *:vs. healthy controls group,*P < 0.05,**P < 0.01,***P < 0.001; $^{\#}$:vs. DN group, $^{\#}$P < 0.05, $^{\#\#}$P < 0.01, $^{\#\#\#}$P < 0.001.