Associations between apolipoprotein CIII concentrations and microalbuminuria in type 2 diabetes

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Abstract. Microalbuminuria (MAU) is a strong predictor of diabetic nephropathy (DN), which is the main cause of morbidity and mortality in patients with diabetes mellitus (DM). Dyslipidemia exists in the majority of patients with DM and contributes to micro- and macrovascular complications associated with DM. Apolipoprotein CIII (apoCIII) is an inhibitor of the activity of lipoprotein lipase, which metabolizes triglyceride (TG) in very low-density lipoprotein (VLDL) and facilitates its clearance from plasma. The aim of the present study was to investigate the associations between apoCIII and MAU and the effects of atorvastatin in type 2 diabetes. In total, 120 subjects were divided into type 2 diabetes and type 2 DN groups, while 60 healthy subjects were selected as controls. The patients with DN were administered 20 mg atorvastatin daily for 16 weeks. Blood pressure, body mass index (BMI) and levels of HbA1c, FBG, TG, VLDL-cholesterol (VLDL-C), apoCIII and MAU were markedly elevated in the type 2 diabetes and type 2 DN groups compared with those in the control group (P<0.01), while high-density lipoprotein-cholesterol (HDL-C) levels were decreased significantly (P<0.01). All patients with type 2 DN showed significantly elevated blood pressure, apoCIII levels, MAU, course of the disease and rate of stroke and retinopathy compared with the patients with type 2 diabetes (P<0.01). MAU was significantly positively correlated with the course of the disease, systolic blood pressure, diastolic blood pressure, BMI and HbA1c, FBG, TG, total cholesterol, low-density lipoprotein-cholesterol, VLDL-C and apoCIII levels (P<0.05), whereas negatively correlated with HDL-C levels (r=-0.194, P=0.020). Logistic regression analysis showed that apoCIII levels were independently associated with MAU (odds ratio, 1.100; 95% confidence interval, 1.037-1.153; P<0.001). Atorvastatin improved the lipid profile and MAU in patients with type 2 DN (P<0.01). Therefore, the present study demonstrated that an independent positive correlation exists between the levels of apoCIII and MAU in patients with type 2 diabetes. Furthermore, atorvastatin may be used to improve the lipid profile and MAU in type 2 DN.

Introduction

Diabetic nephropathy (DN) is a major cause of end-stage renal disease (ESRD). One of the early markers of DN and vascular disease in patients with diabetes is the presence of microalbuminuria (MAU). Without specific intervention, 20-40% of type 2 diabetic patients with MAU progress to overt nephropathy (1). Therefore, screening for MAU and timely therapeutic intervention has become the standard in care worldwide.

Plasma triglyceride (TG) levels are increased in patients with DN. The abnormal synthesis and clearance of TG and the lipoprotein particles associated with TG may be involved in the development of DN (2). There is evidence suggesting that TG-rich lipoprotein (TRL) particles predominantly containing apolipoproteins (apos) E, C and B may be major promoters of DN (2). In patients with DN, plasma TG levels may increase, partly due to the reduced activity of hepatic lipase (HL) and lipoprotein lipase (LPL), which hydrolyze TG (3).

ApoCIII is a protein composed of 79 amino acid residues that is predominantly synthesized in the liver, although synthesis also occurs in the intestine. ApoCIII is an exchangeable protein moiety between chylomicron remnants, very low-density lipoproteins (VLDLs) and high-density lipoproteins (HDLs) (4,5). In the blood circulation, ApoCIII is associated mainly with TRLs, HDLs and, to a lesser extent, low-density lipoproteins (LDLs) (6-8). Total plasma apoCIII levels have been found to be key determining factors of serum TG levels. Studies involving gene variation and circulating protein levels have implicated apoCIII as a risk factor for cardiovascular disease (9-12). ApoCIII is an inhibitor of the activity of LPL (13), which metabolizes TG in VLDL and facilitates its clearance from plasma. In addition, ApoCIII reduces the plasma clearance of VLDL and LDL by inhibiting their interaction with hepatic lipoprotein receptors (14,15).

There is considerable information regarding the effects of apoCIII on lipoprotein metabolism (10,16,17). Statins have been suggested to lower plasma apoCIII levels. In 27 patients with primary hypertriglyceridemia, administration of 20-40 mg atorvastatin for four weeks reduced plasma apoCIII levels by 18-30% (18). However, little information is available regarding
the association between microalbuminuria (MAU) and circulating apoCIII levels in DN. To enhance the understanding of this issue, the present study aimed to assess serum apoCIII levels and the association between apoCIII levels and MAU, and to evaluate the effectiveness of statins in lowering apoCIII levels and therefore decreasing MAU in type 2 diabetes.

Materials and methods

Patients. Subjects with type 2 diabetes (n=120) aged 48-66 years were recruited from Hebei General Hospital (Shijiazhuang, China). A group of 60 healthy subjects aged 47-65 years (30 male and 30 female) was selected as control subjects. The study was approved by the Institutional Ethic Committee of Hebei General Hospital and written informed consent was obtained from all subjects. Written informed consent was obtained from all subjects. The baseline characteristics of the type 2 diabetes and type 2 DN groups are shown in Table I. The patients with DN were administered atorvastatin (20 mg) daily. Patients were interviewed every four weeks during treatment to assess drug adherence up to a total of 16 weeks.

Samples. A blood sample was collected following an overnight fast of ≥8 h and prior to insulin administration from the 120 patients with type 2 diabetes and the 60 healthy subjects. Fasting blood samples were taken for the measurement of serum glucose, glycosylated hemoglobin (HbA1c) and the lipid profile. Timed urine samples (24 h) were collected from all patients for the determination of MAU. Fasting blood glucose (FBG) was determined on a Beckman CX9 automatic analyzer (Beckman Coulter, Miami, FL, USA) using a glucose oxidase method. HbA1c levels were determined by high-performance liquid chromatography, using the BioRad Variant Hemoglobin Analyzer (Bio-Rad, Hercules, CA, USA).

For the lipoprotein studies, blood was placed on ice in polypropylene tubes containing a solution of lipoprotein preservatives comprising 2.8 mmol/l EDTA, 62 µmol/l chloramphenicol, 50 µg/ml gentamycin sulfate, 10 mmol/l 2-aminocaproic acid and 100 mmol/l 5,5’-dithiobis-(2-nitrobenzoic acid) (final concentrations). Samples were immediately centrifuged at 1,008 x g for 25 min to sediment blood cells and were subsequently stored at -80°C until analysis. Total cholesterol (TC) and TG levels were determined using the enzymatic colorimetric method on the Beckman CX9 analyzer and HDL-cholesterol (HDL-C) was measured using a direct enzymatic HDL-C method based on polyethylene glycol-modified enzymes on the Beckman CX9 automatic analyzer. VLDL-cholesterol (VLDL-C) and LDL-cholesterol (LDL-C) levels were estimated using the Friedewald formula (19). ApoCIII concentration in the serum was determined using a competitive ELISA method (BioWorld Technology, Shanghai, China) (18). The standard curve was fitted to a four-parameter sigmoidal curve. The inter- and intra-assay variability for the assay was <7%.

Body mass index (BMI) and blood pressure (BP) were measured for all the subjects. Obesity was defined as BMI ≥25 kg/m². Hypertension was defined as BP >140/90 mmHg or the administration of anti-hypertensive drug treatment. Fasting blood samples were measured for biochemistry and metabolic profile analysis. Dyslipidemia was defined as TG ≥1.7 mmol/l and/or HDL-C < 1.1 mmol/l in males and <1.3 mmol/l in females and/or LDL-C > 2.6 mmol/l or treatment with lipid-lowering drugs. DN was defined as MAU ≥30 mg/24 h in two collections of timed urine samples (24 h) and serum creatinine (Cr) ≤132 µmol/l. Patients with urinary tract infection, hematuria shown by urine microscopy or obstructive uropathy shown by kidney ultrasound suggestive of non-diabetes-related causes were excluded. Type 2 diabetic patients without DN had serum Cr ≤132 µmol/l and MAU <30 mg/24 h in two collections of timed urine samples. Control subjects without diabetes had no known history of diabetes, exhibited normal glucose tolerance at the 75 g oral glucose tolerance test (1998 World Health Organization criteria) (20) and had normal blood biochemistry. The blood and urine samples of patients with type 2 DN were collected after 16 weeks of therapy.

Table I. Baseline characteristics of the two groups.

| Variable               | Type 2 diabetes | Type 2 DN | P-value |
|------------------------|-----------------|-----------|---------|
| Number (male/female)   | 60 (28/32)      | 60 (33/27) | 0.361   |
| Age, years*            | 55.3±9.8        | 56.0±9.5  | 0.692   |
| Course of disease, years* | 5.8±3.1       | 7.8±3.0   | 0.001   |
| Coronary heart disease, % | 8.3            | 13.3      | 0.378   |
| Stroke, %              | 5.0             | 16.7      | 0.040   |
| Retinopathy, %         | 28.3            | 55.0      | 0.003   |

*Values are presented as the mean ± standard deviation. DN, diabetic nephropathy.

Comparisons of basic clinical data. Compared with the type 2 diabetes group, the type 2 DN group showed a longer course of disease (P<0.01). The stroke and retinopathy rates in the patients with type 2 DN were significantly higher than those
in patients without DN (P<0.05). However the occurrence rate of coronary heart disease exhibited no statistical difference between the two groups (P>0.05) (Table I).

Comparisons of BP, BMI, HbA1c, FBG, lipid profile, apoCIII and MAU. Compared with the control group, systolic BP (SBP), diastolic BP (DBP), BMI and levels of HbA1c, FBG, TG, VLDL-C, apoCIII and MAU were markedly elevated in the type 2 diabetes and type 2 DN groups (P<0.01), while HDL-C levels were decreased significantly (P<0.01). All patients with type 2 DN showed significantly elevated TC and LDL-C levels (P<0.05) (Table II). Although the BMI and levels of HbA1c, FBG, TG, TC, LDL-C and VLDL-C were elevated in the type 2 DN group compared with those in the type 2 diabetes group, the differences were not significant (P>0.05). However, the levels of SBP, DBP, apoCIII and MAU in this group were more notably increased (P<0.01) (Table II).

Associations between MAU and age, gender, course of the disease, BP, BMI, HbA1c, FBG, lipid profile and apoCIII in type 2 diabetes and type 2 DN patients. MAU was significantly positively correlated with the course of the disease, SBP, DBP, BMI, HbA1c, FBG, TG, TC, LDL-C and VLDL-C and apoCIII, whereas negatively correlated with HDL-C. MAU exhibited no association with age or gender (Table III).

Multiple regression analysis for MAU in type 2 diabetes and type 2 DN patients. To further clarify the independent associations of age, gender, course of the disease, BP, BMI, HbA1c, FBG, lipid profile and apoCIII with MAU, logistic regression analysis was performed. This revealed that only apoCIII was independently associated with MAU (OR, 1.100; 95% CI, 1.037-1.153; P<0.001). The other variables were not independent predisposing factors for MAU in type 2 diabetes (Table IV).

Atorvastatin improves the lipid profile and MAU levels in patients with type 2 DN. After 16 weeks of atorvastatin administration in patients with type 2 DN, levels of TG, TC, LDL-C, VLDL-C and apoCIII were significantly decreased compared with those prior to treatment, while HDL-C levels were found to have increased markedly (P<0.05).
MAU levels were observed to have decreased significantly (P<0.01) (Table V).

Discussion

There is currently a global epidemic of type 2 diabetes mellitus (DM), which accounts for 40-50% of all new cases of ESRD. Among the clinical signs of nephropathy, the appearance of low but abnormal levels (>30 mg/day) of albumin in the urine, known as MAU, is first to occur. MAU is a leading cause of DM-related morbidity and mortality (21). In type 2 diabetes, lipotoxicity and gluotoxicity increase the risk of diabetic micro- and macrovascular complications (3,22).

In the majority of type 2 diabetic patients, insulin resistance is a key pathophysiological feature. Lipases are insulin-sensitive enzymes that hydrolyze TG in TRL particles. Diabetic dyslipidemia is typically characterized by high TG and low HDL-C levels. The high TG levels in turn can alter the composition of LDL-C and HDL-C, making these lipid particles more atherogenic (23). Nephritis can be exacerbated through increased TG levels and oxidized remnant lipoprotein particles, which induce mesangial cells to proliferate and secrete cytokines (24). Following the initiation of proteinuria, the loss of lipoprotein particles in the urine can enhance their synthesis in the liver. Furthermore, the lipoprotein particles can cause renal damage by binding to glomerular basement membranes and renal tubular cells. This can initiate a vicious cycle of proteinuria and dyslipidemia (22). Patients with renal disease, whether associated with diabetes or not, have increased TG and remnant lipoprotein levels and decreased LPL and...
Patients with type 2 DM may have been due to comprehensive treatment. In conclusion, in type 2 diabetes, circulating apoCIII levels were independently correlated with MAU. Atorvastatin improved the lipid profile in patients and may contribute to decreasing MAU levels in type 2 DN.

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