Serum small-dense LDL abnormalities in chronic renal disease patients

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ABSTRACT

Cardiovascular disease (CVD) is the principal cause of mortality in chronic kidney disease (CKD) patients. Dyslipoproteinaemia is a common metabolic derangement in CKD and a traditional risk factor for CVD. This study investigates serum lipoprotein, especially small-dense low-density lipoprotein (sd-LDL), abnormalities in CKD patients. A total of 131 CKD patients (age: 59±12 years, male=64) diagnosed according to Kidney Disease: Improving Global Outcomes, 2004 (KDIGO) and 121 age- and gender-matched control subjects (age: 58±6 years, male=62) were recruited from Hong Kong and Macau. Serum total cholesterol (TC), triglyceride (TG), high-density lipoprotein-cholesterol (HDL-C) and direct LDL-C were assayed enzymatically. In addition, sd-LDL, together with very low density and intermediate-density lipoproteins (VLDL and IDL) were measured by US Food and Drug Administration (FDA)-approved polyacrylamide gradient gel electrophoresis. Compared to controls, CKD patients showed significantly decreased TC, LDL-C, normal-size LDL and HDL-C with increased TG, VLDL, IDL and sd-LDL (all $P<0.01$). The increased sd-LDL and decreased normal-size LDL fractions resulted in a significantly elevated sd-LDL:LDL ratio in CKD ($P<0.005$). In contrast to the low TC and LDL-C, sd-LDL and sd-LDL:LDL ratio were significantly elevated in CKD. Thus, sd-LDL will be used increasingly for CVD risk assessment in CKD and other diseases that show lipoprotein derangement.

KEY WORDS: Kidney diseases. Lipoproteins.

Introduction

The World Kidney Day was proposed by the International Society of Nephrology (ISN) and International Federation of Kidney Foundations (IFKF) in order to remind the public, government, medical and healthcare professionals that kidney disease is common, harmful and treatable, as well as being costly and preventable.[1] Chronic kidney disease (CKD) is a global public health problem causing great suffering and serious financial burden to patients and/or society.[2–8]

Currently in the USA, 13% (26 million) of non-institutionalised adults are estimated to have CKD. About a million patients are being treated for end-stage renal disease (ESRD), with half a million surviving on renal replacement therapy (RRT), while a worryingly higher proportion (15 million) are in the earlier stages of CKD that may escape timely diagnosis and intervention.[2–4] Prevalence rates are similar in Europe,[5] Australia[6] and Asia,[7] including China, where it has risen from 8% in 2005 to 11% in 2009.

The most serious adverse outcome of CKD is represented by debilitating metabolic complications of decreased glomerular filtration rate (GFR) progressing to renal failure. Moreover, it will lead to about 100-fold higher risk of cardiovascular disease (CVD) than that in the general population, which is a principal cause (approximately 58%) of mortality in ESRD patients.[9,10]

Dyslipoproteinaemia is a well-known common metabolic derangement of CKD and a traditional risk factor for CVD. The elevated triglyceride (TG) concentration and decreased high-density lipoprotein (HDL) concentration are two main dyslipidaemic derangements which may cause CVD.[11–13] They are seen from the early stages of CKD, as shown by the Mild and Moderate Kidney Disease (MMKD) study that followed 227 stage 2–3 CKD patients for seven years.[14] In these patients, there is decreased lipolysis caused by suppressed lipoprotein lipase (LPL) activity due to increased hepatic synthesis of apolipoprotein (apo) CIII, an inhibitor of LPL.[15] Delayed catabolism of triglyceride-rich lipoproteins including chylomicrons (CM), very low density lipoproteins (VLDL) and immediate-density lipoproteins (IDL) and their remnants causes hypertriglyceridaemia.[16] Subnormal HDL in CKD has been attributed to reduced hepatic synthesis of apo AI, the structural protein of HDL, and low activity of lecithin-cholesterol acyl transferase (LCAT), an enzyme that esterifies free cholesterol in nascent HDL, resulting in its maturation to spherical HDL3 and finally biologically active HDL2.[17,18]

Low-density lipoprotein cholesterol (LDL-C) is the main treatment target identified by the Adult Treatment Panel III (ATP III) of the National Cholesterol Education Programme (NCEP).[19] However, in CKD, total cholesterol (TC) and LDL-C, the routinely measured lipid parameters, are usually within their normal ranges or even low.[20] These 'atypical' manifestations have resulted from cytokine-driven chronic inflammation and malnutrition prevalent in CKD patients,

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causing increased catabolism, poor nutritional intake and accelerated atherosclerosis (MIA syndrome).[21,22] Consequently, CVD mortality and TC exhibit a U-shaped relationship with high mortality at both hypocholesterolaemic and hypercholesterolaemic concentrations.[23]

As circulating LDL particles show heterogeneity with respect to size, density and chemical composition, small-dense LDL (sd-LDL) has been identified as a more atherogenic subfraction due to its higher penetration into the arterial wall, lower binding affinity for LDL receptors, prolonged plasma half-life, and increased susceptibility to oxidative stress compared to that of normal-size LDL.[24–26] These post-ribosomal changes favour the development of LDL-enriched foam cells in atherosclerotic plaques.

Several publications have confirmed the presence of elevated sd-LDL in CVD and diabetes patients,[27,28] but not in CKD that is increasingly prevalent and associated with dyslipoproteinemia, and a high risk for CVD. Therefore, this study investigates serum lipoprotein, especially sd-LDL abnormalities, in CKD patients.

Materials and methods

Patients

This study was approved by the Institutional Research Board of the University of Hong Kong and Hong Kong Hospital Authority, Hong Kong West Cluster (No. UW-08-354). Adult CKD patients were recruited with informed consent from Hong Kong and Macau. A diagnosis of CKD was made according to the Kidney Disease: Improving Global Outcomes (KDIGO, 2004), based on GFR >60 mL/min/1.73 m^2.[29] The Modification of Diet in Renal Disease (MDRD) formula was used to calculate GFR using serum creatinine concentration, taking into account age, gender and ethnic parameters (if Chinese).[30] The MDRD formula was GFR (mL/min/1.73 m^2)=$186 \times Pcr^{-1.154} \times Age^{-0.203} \times 0.742$ (if female) $\times$ 1.227 (if Chinese)

The stages of CKD were defined in accordance with KDIGO as follows:

- Stage 3: moderate decrease in GFR (30–59 mL/min per 1.73 m^2)
- Stage 4: severe decrease in GFR (15–29 mL/min per 1.73 m^2)
- Stage 5: kidney failure (GFR <15 mL/min per 1.73 m^2).

Age- and gender-matched controls were recruited among healthy hospital laboratory and nursing personnel and their relatives who had no documented clinical history of CVD, CKD, diabetes and other endocrine or metabolic diseases, and other physical and mental illness.

Blood samples

A 12 mL clotted blood sample was collected by antecubital venipuncture from each patient and control subject after an overnight fast (at least 12 h). Serum was obtained by centrifugation (2000 x*g* for 15 min) and preserved at –80°C until analysis.

Measurement of serum lipids and lipoproteins

Serum creatinine, TC, TG, HDL-C and direct LDL-C concentrations were measured by appropriate enzymatic assays (*ci*8200 analyser, Abbott Laboratories, Illinois, USA).

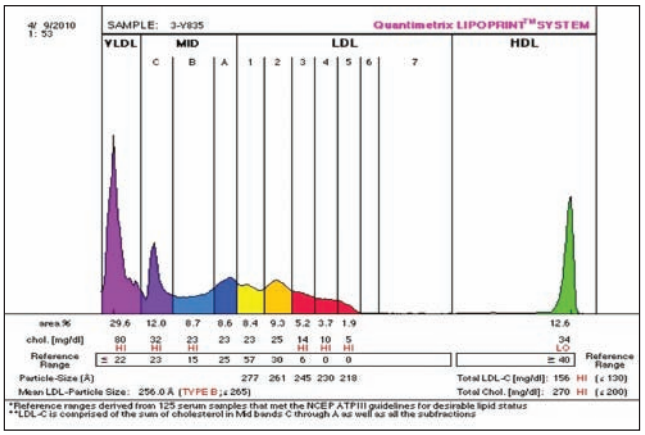

Fig. 1. Serum lipoprotein electrophoresis using the Lipoprint system.

All inter-assay coefficients of variation (CV) of the measurements were lower than 3%, fulfilling analytical reproducibility required by the NCEP ATPIII for lipid and lipoprotein measurements.[19]

Measurement of sd-LDL was by gradient gel electrophoresis (Lipoprint System, Quantimetrix, Redondo Beach, California, USA). Briefly, 25-µL serum and 200-µL liquid loading gel with lipophilic dye were loaded successively onto the high-resolution 3% polyacrylamide gel tube. After 30-min photopolymerisation, samples were electrophoresed at 3 mA/tube (500 V) for 1 h.

The amount of cholesterol in each of the VLDL, IDL, LDL and HDL bands was calculated by multiplying the relative area of each band by the sample TC. A scoring system was used to assess the seven LDL subfractions, with LDL bands 1–2 being considered normal-size LDL and bands 3–7 as sd-LDL (Fig. 1). The quality control samples provided by the manufacturer were analysed with each batch of test samples in order to establish analytical acceptability.

Statistical analysis

Statistical data analysis was performed using SPSS version 11.5 (SPSS, Chicago, Illinois, USA). Lipid and lipoprotein concentrations and areas of electrophoretic fractionation were compared using Student's *t*-test. All probability values were two-tailed, and values >0.05 were considered significant.

Results

In total, 131 CKD patients (mean age: 59±12 years, range: 22–77, male=64) and 121 control subjects (mean±SD age: 58±6 years, range: 47–75, male=62) were studied. The CKD patients included 61 with stage 4 and 70 with stage 5 disease. Forty-nine stage 5 patients were being treated by peritoneal dialysis, while the remaining 82 patients, who did not require dialysis, were generally treated with antihypertensive medication such as drugs that block the renin-angiotensin system (angiotensin-converting enzyme inhibitor or angiotensin receptor blocker) and retard the progression of CKD.

The average body mass index for all patients was 26±5 kg/m^2. Fourteen percent were current smokers, 16% were ex-smokers and 70% were non-smokers. The

background causes of CKD included diabetic nephropathy (n=44, 33.6%), chronic glomerulonephritis (n=32, 24.4%), tubulointerstitial disease (n=11, 8.4%), hypertensive nephrosclerosis (n=10, 7.6%), acute vasculitis or systemic lupus erythematosus (n=6, 4.6%), polycystic kidney disease (n=3, 2.3%) and obstructive uropathy (n=1, 0.8%). The cause was unknown in 24 cases (18.3%). Mean systolic and diastolic blood pressures in patients and controls were 135±18 and 78±13 mmHg, respectively.

Compared to control subjects, CKD patients showed significantly decreased serum TC, LDL-C, normal-size LDL and HDL-C, with increased TG, VLDL, IDL and sd-LDL (all P<0.01 [Table 1]). The increased sd-LDL and decreased normal-size LDL fractions quantified by electrophoresis resulted in a significantly elevated sd-LDL:LDL ratio (P<0.005).

Discussion

Chronic kidney disease is an increasing global health problem resulting in about 100-fold higher risk for CVD, causing over 50% mortality in ESRD.[4,31] Dyslipoproteinaemia is a common metabolic derangement of CKD and a traditional risk factor for CVD, causing progressive atherosclerosis of the coronary arteries.[32] Early studies, including that by Nobel Laureates Brown and Goldstein, established that elevated plasma TC and LDL-C concentrations accelerate atheroma formation.[33]

Targeting this pathological mechanism, statin therapy with HMG CoA reductase inhibitor (e.g., simvastatin, atorvastatin or rosuvastatin) has been highly effective in lowering plasma/serum TC, LDL-C and non-HDL-C by inhibiting intracellular cholesterol synthesis and up-regulating LDL receptor activity for accelerated clearance of apolipoprotein B-containing lipoproteins.[34] A meta-analysis of 14 statin clinical trials involving 90,056 patients showed that for every 1.0 mmol/L LDL-C decrease, statin significantly decreased all-cause mortality by 12%, CVD mortality by 19% and myocardial infarction by 23%.[35] The beneficial effects of statin therapy may even promote regression of coronary atheroma.[36]

However, therapeutic targeting is frequently not clear-cut. Currently, some plasma lipids and lipoproteins are measured and classified as causative of atherosclerosis (e.g., TC and LDL-C) or protective of CVD (HDL-C). However, there has been debate about whether or not these 'bad' and 'good' cholesterols are merely markers of some more fundamental variables.[37] As observed in the present study of CKD patients, and a previous study of diabetic patients, TC and LDL-C were low in these patients, potentially resulting in a false risk for CVD.

Substantial efforts have been made to fractionate lipoprotein particles to recognise those subfractions that are fundamentally more atherogenic. For example, sd-LDL is a newly identified LDL subfraction that has been epidemiologically proven from a 13-year follow-up study to result in enhanced risk for CVD.[38] It is highly atherogenic due to its high penetration into the arterial wall, low binding affinity for the LDL receptor, prolonged plasma half-life, and increased susceptibility to oxidative stress.

Recently, it has been reported that rosuvastatin at a dose of 40 mg/day is effective in lowering sd-LDL-C by more than 50%, along with LDL-C suppression by a similar percentage.[39] Measurement of sd-LDL by density gradient ultracentrifugation, non-denaturing gradient gel electrophoresis and nuclear magnetic resonance has been reported, but these methods require special equipment and expertise and are available only in specialist laboratories.[40]

Several years ago a fractional precipitation method was developed to measure sd-LDL-C directly.[41] It can be automated for use in a non-specialist laboratory and shows excellent reproducibility in analysing plasma/serum samples that have been stored at –80°C for up to two years. Therefore, sd-LDL has established itself as a new risk parameter for CVD which can be modified by drug therapy and can now be measured conveniently for assessment and monitoring.

The findings of the present study support the strategy that sd-LDL should be used increasingly for fundamental CVD risk assessment in CKD, as well as for other diseases that exhibit lipoprotein derangement. □

Table 1. Serum lipid and lipoprotein profiles of CKD patients and control subjects.

	CKD (n=131)	Control (n=121)	P value*
Lipid parameter			
TC (mmol/L)	5.2±1.0	5.6±0.8	<0.01
TG (mmol/L)	2.3±1.3	1.3±0.8	<0.001
HDL-C (mmol/L)	1.1±0.2	1.6±0.4	<0.001
LDL-C (mmol/L)	3.0±0.8	3.3±0.8	<0.001
Electrophoretic area			
VLDL (%)	22.7±5.2	16.6±4.0	<0.001
IDL (%)	30.9±4.5	27.0±3.8	<0.001
Normal size LDL (%)	24.4±5.6	31.9±4.3	<0.001
Small-dense LDL (%)	0.9±1.8	0.4±0.9	<0.01
HDL (%)	18.3±3.9	21.4±5.0	<0.001
sd-LDL:LDL	$(1.6\pm3.3)\times10^{-2}$	$(0.7\pm1.4)\times10^{-2}$	<0.005

Data expressed as mean±SD.
*Compared by Student's t-test.

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