

Small dense low-density lipoprotein as a potential risk factor of nephropathy in type 2 diabetes mellitus

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ABSTRACT

Background: The risk for diabetic nephropathy in type 2 diabetes is about 30-40%, and it is considered the leading cause of end-stage renal disease. Small dense low-density lipoprotein (sdLDL) particles are believed to be atherogenic, and its predominance has been accepted as an emerging cardiovascular risk factor. This study aimed to assess small dense LDL as a potential risk factor and a possible predictor for diabetic nephropathy in type 2 diabetic patients. **Patients and Methods:** According to microalbuminuria test, 40 diabetic patients were categorized into two groups: Diabetic patients without nephropathy (microalbuminuria negative group) and diabetic patients with nephropathy (microalbuminuria positive group), each group consists of 20 patients and all were non-obese and normotensive. The patients were re-classified into three sub-groups depending on the glomerular filtration rate (GFR). **Results:** The mean of small dense LDL level in the microalbuminuria positive group was higher than that in the microalbuminuria negative group, but without statistical significance. It was significantly higher in patients with either mild or moderate decrease in estimated GFR than in patients with normal estimated GFR. There was statistically significant correlation between small dense LDL and albuminuria and significant inverse correlation between small dense LDL and estimated GFR in all patients in the study. Based on microalbuminuria, the sensitivity and specificity of small dense LDL in the diagnosis of diabetic nephropathy was 40% and 80%, respectively, with cutoff values of small dense LDL >55.14 mg/dl. On the other hand, based on GFR, the sensitivity and specificity were 88.24% and 73.91% respectively, with cutoff values of small dense LDL >41.89 mg/dl. **Conclusion:** Small dense LDL is correlated with the incidence and severity of diabetic nephropathy in type 2 diabetic patients. It should be considered as a potential risk factor and as a diagnostic biomarker to be used in conjunction with other biochemical markers for early diagnosis, assessment, and follow-up of diabetic nephropathy.

Key words: Albuminuria, diabetes mellitus, diabetic nephropathy, small dense LDL

INTRODUCTION

Diabetes mellitus (DM) is a common disease worldwide. Chronic complications are the major outcome of type 2 DM (T2DM) progress, which reduces the quality of life of patients, incurs heavy burdens to the healthcare system, and increases diabetic mortality.^[1-6] After adjusting for age, the

death rate of people with T2DM is about twice as high as their non-diabetic peers.^[7] About 50-80% of all individuals with diabetes die of cardiovascular and cerebrovascular diseases. Kidney failure is among the leading causes of death. Chronic renal insufficiency is reported twice as frequently in persons with diabetes.^[7-9]

The fall of glomerular filtration rate (GFR) in case of diabetic nephropathy is usually rapid and appears to be linear with time. Thus, factors other than hyperglycemia have been suggested to contribute to such progression. Hyperlipidemia has received attention as one of the factors incriminated in this process by participation in the progression of glomerular injury.^[10] More rapid decline of renal function has been observed in diabetic nephropathy patients with hyperlipidemia than in those without it.^[11]

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Prospective studies suggested that an adverse lipid profile might cause nephropathy in both type 1 and type 2 diabetic patients through possible mechanisms, including mesangial cell proliferation, recruitment of macrophages, altered cytokine responses, and increased matrix deposition.^[12-14]

Low density lipoprotein (LDL) consists of a heterogeneous spectrum of particles with highly variable atherogenic potential.^[15] Small dense LDL (sdLDL) particles are believed to be particularly atherogenic due to increased susceptibility to oxidation,^[16,17] high endothelia permeability,^[18] decreased LDL receptor affinity,^[19] and an increased interaction with matrix components.^[20] On the other hand, LDL size seems to be an important predictor of cardiovascular events and progression of coronary artery disease, and a predominance of sdLDL has been accepted as an emerging cardiovascular risk factor by the National Cholesterol Education Program Adult.^[21]

A number of studies have demonstrated a high prevalence of sdLDL particles in T2DM patients with nephropathy as compared with that in T2DM without nephropathy or in non-diabetic controls.^[22] The high prevalence of small-sized LDL may in part explain the high incidence of coronary heart disease in diabetes with nephropathy.^[23] In view of all these considerations, this study aims to assess sdLDL as a potential risk factor and a possible predictor for diabetic nephropathy in T2DM.

PATIENTS AND METHODS

The study was conducted on diabetic attendants of outpatient diabetes and general medicine clinics of Suez Canal University hospital from January 2011 to June 2012. After exclusion of those at risk of developing sdLDL in blood or urinary albumin excretion (e.g., with positive suggestive history or criteria of type 1 diabetes, hypertension, obesity, high-fat intake or on lipid-regulating agents, beta blockers or ACE inhibitors therapy, chronic liver disease, heart failure, and post-streptococcal glomerulonephritis), 20 consecutive adults with T2DM and persistent microalbuminuria were enrolled for the study (Group A). Another 20 adults with T2DM who were free of microalbuminuria and age and gender matched were included in Group B. Patients of both the groups underwent a detailed history taking, physical examination, and the following laboratory investigations: 24-h urine collection for microalbuminuria, glycated hemoglobin (HbA1c), fasting blood sugar (FBS), serum creatinine, blood urea, triglyceride, total cholesterol, HDL-C, and small dense LDL-C. The analyses were done by standard protocols at the Department of Clinical Pathology in Suez Canal University Hospitals. Samples were collected from each

individual at a single time point and kept at -70°C until analysis.

The cornerstone for the diagnosis of diabetic nephropathy was the measurement of microalbuminuria. Cutoff value of 20 mg/l is recommended by the European Diabetes Policy Group. All abnormal tests were confirmed in two out of three samples collected over a 3-6-month period. Tests were not performed in the presence of conditions that could increase microalbuminuria, such as hematuria, urinary tract infection, acute febrile illness, short-term obvious hyperglycemia, and vigorous exercise.^[24]

According to Modified Diet in Renal Disease (MDRD), which estimates GFR, diabetic patients were categorized into other three subgroups:

- Patients with normal GFR (GFR >90 mL/min/1.73 m²)
- Patients with mild decrease in GFR (GFR of 60-89 mL/min/1.73 m²)
- Patients with moderate decrease in GFR (GFR of 30-59 mL/min/1.73 m²). The recommended equation by the National Kidney Foundation is that of the MDRD (Modified Diet in Renal Disease):

$\text{GFR (mL} \cdot \text{min}^{-1} \cdot 1.73 \text{ m}^{-2}) = 186 \times [\text{serum creatinine (mg/dl)}]^{-1.154} \times \text{age (years)}^{-0.203} \times (0.742 \text{ if female}) \times (1.210 \text{ if African American})]$

Ethical consideration

Informed consent was obtained from the patients. The study was approved by the Ethics Committee of Faculty of Medicine, Suez Canal University.

Statistical analysis

The final study results were stated using the SPSS program version 14. Student's *t*-test, correlation coefficient, and Chi-square test were used to evaluate the results presented through tables and diagrams. The sensitivity, specificity, and positive and negative predictive values were calculated according to the standard formulae. The power of the study was 85%, with 95% confidence interval. Statistical significance was considered at $P < 0.05$ and highly significance at $P < 0.001$.

RESULTS

In this study, 40 T2DM patients were studied (22 males and 18 females, mean age: 46.7 years, and mean duration of diabetes: 7.5 years). No significant differences were observed between both the groups in terms of age, gender distribution, duration of diabetes, body mass index, and blood pressure. Except for waist circumference and HDL, all mentioned clinical parameters were nearly similar in both male and female patients.

Laboratory data analysis showed that sdLDL serum level was insignificantly higher in diabetic patients with microalbuminuria than in diabetic patients without microalbuminuria (the mean level of sdLDL was 50.08 in diabetic patients with the microalbuminuria group and 43.66 in diabetic patients without the microalbuminuria group, $P > 0.05$). However, statistically significant correlation between sdLDL and albuminuria in all patients in the study - if considered as a one group- was found ($P < 0.05$), [Figure 1].

Forty patients were divided into other three groups according to their estimated GFR by MDRD formula, and the sdLDL level was significantly higher in patients with either mild or moderate decrease in estimated GFR than in patients with normal estimated GFR ($P < 0.05$). (The mean level of sdLDL was 34.43 in patients with normal estimated GFR, 60.28 in patients with mild decrease in estimated GFR, and 74.85 in patients with moderate decrease in estimated GFR). There was statistically significant inverse correlation between sdLDL and estimated GFR in all patients in the study ($P < 0.05$) [Figure 2]. Significant inverse correlation between microalbuminuria and estimated GFR in all patients in the study ($P < 0.05$) was recorded.

It was found that sensitivity and specificity of sdLDL as a predictor of diabetic nephropathy in T2DM were 40% and 80% respectively, with cutoff values of sdLDL > 55.14 mg/dl regarding albuminuria. On the other hand, the sensitivity and specificity of sdLDL were 88.24% and 73.91% respectively, with cutoff values of sdLDL > 41.89 mg/dl based on the decreased GFR [Table 1].

DISCUSSION

In this study, 40 T2DM patients were studied. sdLDL

serum level was slightly higher in type 2 diabetic patients with microalbuminuria than in type 2 diabetic patients without microalbuminuria, with significant correlation with the microalbuminuria values. This is in accordance with findings observed by Hirano *et al.*, who found that LDL particle diameter was significantly smaller in type 2 diabetic patients with nephropathy as compared with in those without nephropathy.^[22] In addition, the current data are in agreement with those in previous studies that documented that all multiple lipoprotein abnormalities described in diabetic patients with nephropathy become more accentuated with increasing urinary albumin excretion.^[23-26]

In addition to highly significant inverse correlation between microalbuminuria and estimated GFR, statistically highly significant inverse correlation between sdLDL and estimated GFR were observed in this study in all patients. This is in agreement with Chowta *et al.*, who found that creatinine clearance negatively correlated with microalbuminuria.^[27] In contrary, another study suggested that the high prevalence of sdLDL in T2DM with nephropathy is not directly associated with kidney damage.^[28]

Serum creatinine and urea were significantly higher in the microalbuminuria positive group than in the microalbuminuria negative group. Although there was no statistically significant correlation between urea and sdLDL in each group, the correlation between serum

Table 1: Small dense LDL sensitivity and specificity as diagnostic marker for nephropathy

	Sensitivity (mg/dl)	Specificity (mg/dl)	Cutoff value (mg/dl)
Based on microalbuminuria	40	80	55.14
Based on GFR	88.24	37.91	41.89

LDL: Low-density lipoprotein, GFR: Glomerular filtration rate

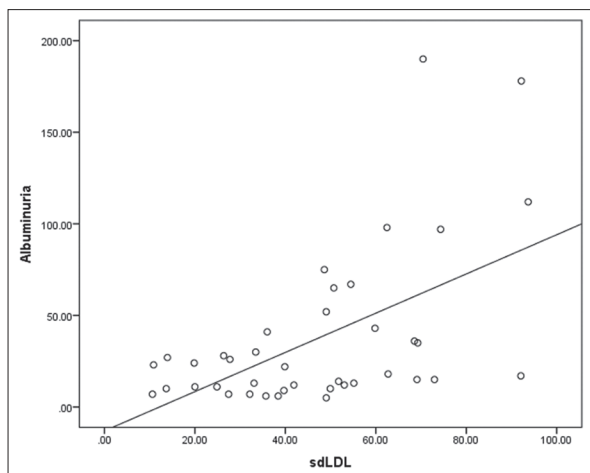


Figure 1: Correlation between sdLDL and albuminuria in all patients ($n = 40$)

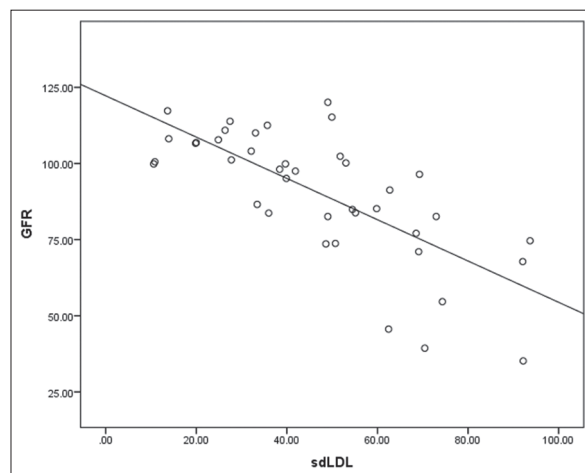


Figure 2: Correlation between sdLDL and GFR in all patients ($n = 40$)

creatinine and sdLDL was statistically significant. This is in accordance with other different studies that revealed that all multiple lipoprotein abnormalities described in diabetic patients with nephropathy become more accentuated with decreasing renal functions.^[24-26]

Statistically significant positive correlation between degree of diabetes control as presented by either FBG or HB A1c and sdLDL is shown in all patients. On the other hand, FBG and HBA1c were insignificantly higher in the microalbuminuria positive group than in the microalbuminuria negative group. Moreover, no statistically significant correlation was found between duration of diabetes and either sdLDL or microalbuminuria; this does not agree with the findings of Chowta *et al.*, who found that average FBS was significantly higher in microalbuminuria patients than in normo-albuminuric patients and suggested significant relationship between both severity and duration of diabetes and microalbuminuria.^[27] The conflict between both the studies may be explained by the variations of the sample size and duration of diabetes. Patients sample size and duration of diabetes was relatively low in the current study. In addition, those with short-term pronounced hyperglycemia at the time of sampling were excluded from the study.

The correlation between either age or gender and sdLDL was insignificant. This is in agreement with American Diabetes Association, which state that the gender difference of small dense LDL-C disappears after adjustment for TG, which is a significant determinant of small dense LDL-C.^[29] This was in contrast with the findings of Hirano *et al.*, who found that small dense LDL-C was higher in males than in females.^[22]

Cholesterol, triglycerides, and LDL-C are higher in the microalbuminuria positive group than in the microalbuminuria negative group, although high-density lipoprotein cholesterol (HDL) was lower; this was in accordance with other studies.^[22,30] Observations recorded by other studies state that this becomes more apparent when diabetic nephropathy is present.^[31,32] On the other hand, statistically highly significant correlation between sdLDL and cholesterol, triglycerides, and LDL had a highly significant inverse correlation between sdLDL and HDL. This is agree with American Diabetes Association, which report that sdLDL levels are positively correlated with serum triglyceride and LDL-C and that these levels were inversely correlated with HDL-C values.^[29]

The sensitivity of sdLDL in the diagnosis of diabetic nephropathy based on estimated GFR was higher than its specificity in nephropathy diagnosis based on

microalbuminuria. On the other hand, specificity of both the aspects were relatively similar. sdLDL cutoff values >55.14 mg% and >41.89% may be considered as a predictor values for nephropathy based on microalbuminuria or on GFR, respectively. sdLDL is suggested to be the diagnostic biomarker used in conjunction with other biochemical markers for early diagnosis, assessment, and follow-up of diabetic nephropathy among T2DM. To investigate this hypothesis, further studies should be undertaken to evaluate sdLDL levels in correlation to kidney histopathological profile in a relatively larger number of patients with diabetic nephropathy.

CONCLUSION

sdLDL levels in the microalbuminuria positive group was insignificantly higher than those in the microalbuminuria negative group, but it was significantly higher in patients with either mild or moderate decrease in estimated GFR than in patients with normal estimated GFR. There was a significant correlation between sdLDL and albuminuria, and a highly significant inverse correlation between sdLDL and estimated GFR. From all of the previously mentioned data, we can suggest that sdLDL can be considered as a potential risk factor for diabetic nephropathy and subsequent changes in the renal function. Given that sdLDL positively correlated with microalbuminuria and negatively with GFR, LDL can be considered as a diagnostic biomarker and predictor in conjunction with other parameters for early diagnosis and follow-up of diabetic nephropathy among type 2 diabetic patients.

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