

The Role of Small Dense Low-Density Lipoprotein in Diabetic Retinopathy

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Abstract:

Background: Diabetic retinopathy is the primary ocular outcome of diabetes mellitus, a serious disease known to have a significant impact on global health. Micro-vascular complications arise from damage to small blood vessels as retinopathy, nephropathy, cardiomyopathy, and neuropathy. Inconsistent findings exist in epidemiological research that specifically examines lipid levels and diabetic retinopathy. The small dense Low-Density Lipoprotein particles are a specific subset of Low-Density Lipoprotein that possessed several pro-atherogenic characteristics.

Objectives: to evaluate the usefulness of serum small dense low-density lipoprotein level as a biomarker for prediction of patients with type II diabetes mellitus who suffer from proliferative and non-proliferative diabetic retinopathy.

Methods: The study involved (160) individuals divided into four groups: (40) patients with non-proliferative diabetic retinopathy, (40) patients with proliferative diabetic retinopathy, (40) controlled diabetic patients without diabetic retinopathy, and (40) healthy controls.

Description of the statistical methods, such as t-tests or ANOVA, employed to compare the means or proportions between groups, depending on the nature of the data.

Results: The study revealed that small dense low-density lipoprotein levels in the proliferative diabetic retinopathy group were significantly higher than those in the non-proliferative diabetic retinopathy, diabetic without retinopathy, and healthy control groups. The mean and standard deviation in patients with proliferative diabetic retinopathy was $4.70\pm1.96 \ \mu mol/L$, in non-proliferative diabetic retinopathy was $3.00\pm0.90 \ \mu mol/L$, in diabetic patients without retinopathy was $2.51\pm0.53 \ \mu mol/L$ and in healthy controls was $2.45\pm0.48 \ \mu mol/L$.

Conclusion: Smalldense, low-density lipoprotein and triglyceride play roles in the progression of diabetic retinopathy in patients with type II diabetes mellitus. The small dense low-density lipoprotein levels and triglycerides levels were highest in the proliferative diabetic retinopathy and lowest in the healthy control. **Keywords:** Diabetes mellitus; Non-proliferative diabetic retinopathy; Proliferative diabetic retinopathy; Small dense low-density lipoprotein; Triglyceride.

Introduction:

One of the most common complications of diabetes is diabetic retinopathy (DR), which continues to be the world's most prevalent cause of vision loss and blindness (1). Diabetic retinopathy (DR) is characterised by a detrimental cycle of small vessel pathology and neuroretinal changes that result in significant mitochondrial and retinal cell apoptosis, chronic inflammation, neovascularisation, and impaired visual field, finally culminating in blindness (2). Nonproliferative diabetic retinopathy (NPDR) and proliferative diabetic retinopathy (PDR) are the two stages of the disease. An early phase of DR is called NPDR. Early morphological hallmarks of nonproliferative diabetic retinopathy (NPDR) include basal membrane thickening, tight junction deterioration, blood retina barrier (BRB) disintegration, microaneurysm formation, small hemorrhages, cotton wool patches, and capillary

* Corresponding author: nawar.mazen2209p@comed.uobaghdad.edu.iq. Retinal neovascularization brought on by ischemia and hypoxia is a hallmark of the proliferative stage of diabetic retinal disease. Due to their relative fragility, newly created blood vessels might rupture and cause retinal and vitreous hemorrhage, which can result in tractional retinal detachment and vision loss (3). Diabetes mellitus (DM) is a complex metabolic disorder characterised by elevated blood glucose levels due to diminished insulin secretion, action, or both, resulting in impaired metabolism of fats, proteins, and carbs (4). 80% to 90% of patients with diabetes are type II diabetes (5). Over 100 genetic variants have been associated with T2DM. Insulin resistance is a condition characterized by a change in the cellular response to insulin, leading to a reduced ability to reduce glucose levels in blood. This disorder inhibits utilization of glucose for energy and metabolism in somatic cells of tissues such as muscle, adipose tissues and liver (6). Alterations in some trace elements may disrupt insulin function and glucose metabolism or increase oxidative stress, potentially resulting in insulin resistance and the development of type II diabetes mellitus (7). Osteoporosis and bone

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fractures are results of insulin resistance and diabetes (8). Alongside abnormal blood-glucose metabolism, obesity, dyslipidaemia, inflammation, and insulin resistance are posited to facilitate the onset and progression of problems related to diabetes mellitus (9). Substantial data indicate a correlation between diabetic retinopathy (DR) and the risk of cardiovascular disease (CV). There exists a link between an increased risk of cardiovascular disease and the severity and progression of diabetic retinopathy. Secondly, diabetic retinopathy (DR) may exhibit a substantial correlation with peripheral arterial disease, vascular disease, calcification in the coronary arteries, and arterial stiffness, as indicated by several independent clinical trials. Third, in persons with type 1 and type 2 diabetes, the burden of atherosclerotic carotid plaques has been associated with advanced manifestations of diabetic retinopathy (DR) (10). Low density lipoprotein (LDL) is composed of small dense (sd) LDL and large-buoyant (lb) LDL particles. Small-dense low density lipoprotein (sdLDL) particles are more aggressive in promoting atherosclerosis compared to large particles (11). One of the main features of diabetic dyslipidemia is high serum level of sdLDL (12). The atherogenic damage of small dense low-density lipoprotein (sdLDL) particles is mostly due to their greater vulnerability to oxidation, glycation of apolipoprotein B, and enhanced absorption by the artery wall. Nevertheless, elucidation of other elements contributing to the elevated atherogenicity of sdLDL is still pending (13). The atherogenicity of sdLDL molecules is contingent upon several factors. Their diminutive size facilitates entry into the arterial wall, and their robust affinity for the proteoglycans within the arterial wall extends their residence in the subendothelial zone. However, the clearance of sdLDL from blood plasma is delayed because to its reduced affinity for LDL receptors compared to larger LDL-C particles (14).

The study aims to evaluate the usefulness of serum small dense low density lipoprotein level as a biomarker for predicting patients with type II diabetes mellitus who suffer from proliferative and nonproliferative diabetic retinopathy.

Subjects and Methods:

Subjects: The present study is a case-control study. The patients were diagnosed by an ophthalmologist at AL-Emamein AL-Kadhumein Medical City and the Endocrinology Center in AL-Kindy Hospital in Baghdad, Iraq, between January 2024 and June 2024. They were individuals aged between 30 and 70 years old with a body mass index categorized as either normal or overweight.

In this study, 160 persons were divided into four groups:

- 40 type II diabetic patients with nonproliferative diabetic retinopathy (NPDR).
- 40 type II diabetic patients with proliferative diabetic retinopathy (PDR).

- 40 controlled type II diabetic patients without diabetic retinopathy (NODR).
- 40 healthy control persons.

Exclusive criteria

- Cardiovascular diseases and hypertension.
- Drugs: Fibrate, statin 40 mg, and insulin.
- Eye diseases other than diabetic retinopathy and recent ocular interventions.
- Primary hyperlipidemia.
- Unstable medical conditions.
- Pregnant or breast feeding.
- Allergies or sensitivities.

Blood sample: Ten milliliters of venous blood were applied to a gel tube and left to clot for 10 minutes. It was then centrifuged with a force of 2000 times for 10 to 15 minutes. The resulting serum was separated and stored at -20 °C in Eppendorf tubes.

Measurement of biochemical parameters: Serum concentrations of small dense low-density lipoprotein in humans were quantified using an Enzyme-linked Immunosorbent assay (ELISA), although serum low-density lipoprotein, serum cholesterol, and serum triglyceride levels were manually assessed using a spectrophotometer.

Statistical analysis: Data analysis was performed with SPSS software version 16.0. Data were presented as Mean \pm standard deviation to compare serum levels based on descriptive statistics, ANOVA results, and post hoc comparisons for various biomarkers. Pearson correlation analysis was performed to see if there was a significant association between the parameters. The alpha level for statistical significance was established at a threshold of p < 0.05. Description of the statistical methods, such as t-tests or ANOVA, employed to compare the means or proportions between groups, depending on the nature of the data.

Results:

The study was focused on analyzing biochemical markers measured in both patient and control groups. Each marker was evaluated individually.

Descriptive statistic			ANOVA	and post l	hoc comparis	ons		
Variable	Group	Mean±SD	NPDR	PDR	NODR	Control	P value	Sig.
sdLDL µmol/L	NPDR	$3.00{\pm}~0.90$		В	А	А	< 0.001	Yes
	PDR	4.70 ± 1.96						
	NODR	$2.51{\pm}0.53$	A					
	Control	2.45 ± 0.48						
	NPDR	$208.83{\pm}57.77$		0	0 0	0	0.06	No
Chol. mg/dl	PDR	$197.93{\pm}47.54$	0					
	NODR	$182.18{\pm}34.96$	0					
	Control	$191.65{\pm}34.55$						
TG mg/d	NPDR	236.58 ± 104.99		В	AB A	А	< 0.001	Yes
	PDR	$274.08{\pm}\ 102.50$	D					
	NODR	221.10 ± 121.03	—В					
	Control	$176.08{\pm}42.40$						
	NPDR	$124.20{\pm}~58.10$		0	0	0	0.06	No
LDL mg/d	PDR	111.76 ± 40.68						
	NODR	$98.10{\pm}34.98$	0					
	Control	114.06± 32.93						

Table 1. Mean and standard deviation of biomarkers								
Descriptive statisti	c		А					
Variable	Group	Mean±SD	N					
		• • • • • • •						

ANOVA results comparing biomarker and lipid profile levels across the four groups, along with significance levels (Pr > F) and post hoc comparisons indicating significant differences (denoted by different letters).

indicated a highly significant difference in sdLDL levels across the groups (P < 0.001). Post hoc comparisons revealed that sdLDL levels in the PDR group were significantly higher than those in the NPDR, NODR, and Control groups as shown in Figure 1.

The sdLDL levels were highest in the PDR group and lowest in the Control group. The ANOVA test



Figure 1. Mean and confidence intervals of sdLDL by groups

The cholesterol levels varied among the groups, with NPDR exhibiting the highest mean and NODR the lowest. The ANOVA test yielded a p-value of 0.06, indicating that the differences in cholesterol levels across the groups were not statistically significant as shown in Figure 2.



Figure 2. Mean and confidence intervals of serum total cholesterol by groups

Triglyceride levels showed significant differences across the groups, as indicated by the ANOVA P-value of <0.001. The PDR group had the highest mean TG level, followed by NPDR and NODR. The Control group had the lowest mean TG level. Post hoc

comparisons revealed significant differences between the groups, with the Control group differing significantly from the NPDR, PDR, and NODR groups as shown in Figure 3.



Figure 3. Mean and confidence intervals of serum TG by groups

The LDL levels across the groups were not statistically significant among the groups. The

ANOVA test produced a *P*-value of 0.06 as shown in Figure 4.



Figure 4. Mean and confidence intervals of serum LDL cholesterol by groups

Our analysis revealed important findings about a sdLDL ability to distinguish between two stages of diabetic retinopathy - proliferative (PDR) and nonproliferative (NPDR). Using ROC curve analysis, sdLDL demonstrated nearly 80% accuracy in differentiating between PDR and NPDR cases, The area under the curve (AUC) was 0.797, which indicates a good discriminatory ability of the parameter in distinguishing between the two conditions. When we set the diagnostic threshold at 3.0969, the sensitivity was 72.50%, and the specificity was 77.50%, suggesting that the test correctly identified 77.5% of NPDR cases. This level of specificity indicates a relatively strong ability to rule out PDR in patients with NPDR, though some false positives may be present.

Discussion:

It was found that the serum level of small dense lowdensity lipoprotein in patients with proliferative diabetic retinopathy was significantly higher than those in non-proliferative diabetic retinopathy, diabetic patients without retinopathy, and the control group; these results similar to (15). This study revealed that sdLDL can be used as a very responsive indicator for the incidence of cardiovascular events and the necessity of laser therapy in individuals with hypercholesterolemia and diabetic retinopathy. In individuals who are at risk for cardiovascular events and diabetic retinopathy, it is important to assess the levels of sdLDL. These levels can serve as a reliable indicator of the worsening of diabetic retinopathy.

The reported differences in cholesterol levels among the groups did not reach statistical significance. It is similar to (16). Who did not find an effect of cholesterol on the risk of diabetic eye disease. Also, it is similar to (17). Although blood cholesterol levels were not shown to be linked to diabetic retinopathy (DR), their correlation with clinically severe macular edema was determined to be substantial. The erratic correlation between serum cholesterol parameters and diabetic retinopathy (DR) is attributed to the fact that HDL and LDL levels solely reflect the lipid composition of the lipoproteins. The cholesterol content of HDL and LDL particles may vary, but they both consist of a fixed quantity of apolipoprotein molecules. Therefore, the evaluation of conventional cholesterol properties is affected by the modification and distribution of lipid particles that constitute the cholesterol. Nevertheless, the quantification of apolipoprotein offers a more precise assessment of the true quantities of lipoprotein cholesterol. Furthermore, the quantification of serum cholesterol is affected by dietary intake, pre-analytic fasting guidelines, lipid-reducing drugs, and metabolic changes inherent in diabetes. It is not similar to (18). Who found that both statins and fenofibrate have been shown to have beneficial effects on inhibiting diabetic retinopathy. Statins can lower total cholesterol and LDL-C, while fenofibrate is likely to reduce triglyceride.

Triglyceride levels showed significant differences across the groups, as indicated by the ANOVA Pvalue of < 0.001. The PDR group had the highest mean TG level, followed by NPDR and NODR. The Control group had the lowest mean TG level. Post hoc comparisons revealed significant differences between the groups, with the Control group differing significantly from the NPDR, PDR, and NODR groups. It is not similar with (19). The study revealed that elevated levels of HDL, triglyceride, and remnant cholesterol variability were linked to a greater susceptibility to nephropathy and neuropathy. However, after accounting for possible confounding variables, no significant correlation was found between any lipid variability indicator and retinopathy. It is similar to (18). Who have demonstrated that both statins and fenofibrate have advantageous effects in suppressing diabetic retinopathy. Statins can decrease levels of total cholesterol and LDL-C, whereas fenofibrate is expected to lessen triglyceride levels.

The mean LDL levels across the groups were 124.20 mg/dl (NPDR), 111.76 mg/dl (PDR), 98.10 mg/dl (NODR), and 114.06 mg/dl (control). The ANOVA test produced a P-value of 0.06, suggesting that the differences in LDL levels were not statistically significant among the groups. It is not similar to (20). In which the overall pooled results showed significantly higher lipid levels, including total cholesterol, triglyceride, and LDL-C, in patients with later onset of DR than in patients without DR.

It is not similar with (21). The investigation confirms that the impact of lipids on the progression of diabetic retinopathy (DR) is still uncertain because of the limited statistical significance of the established results in standard clinical trial. Only the positive impact of LDL-cholesterol in the development of diabetic retinopathy (DR) was demonstrated. It agreed with (22). The prognostic value of circulating levels of various apolipoproteins for the advancement of diabetic retinopathy was shown to be higher than that of serum levels of total cholesterol or LDL-C isolated. Furthermore, the current research elucidates that despite the absence of a precise link between traditional lipid indicators and diabetic retinopathy, lipid-lowering therapies might be recognized as very promising therapeutic agents for diabetic retinopathy. Lipid-lowering drugs, such as statin and fenofibrate, exert their effects through different pathways that may be associated with the advancement of atherosclerosis and its associated coronary heart diseases. Furthermore, it suggests the presence of novel mechanisms via which these drugs inhibit diabetic retinopathy, such as activating systemic inflammatory reactions.

Limitation of Study

Further studies on a larger number of diabetic retinopathies with type II diabetes mellitus.

Studies on proliferative and non-proliferative diabetic retinopathy in patients with type I diabetes mellitus.

Conclusion:

The PDR group had the highest small dense low density lipoprotein and triglycerides levels, followed by NPDR, then NODR, while the healthy control group had the lowest levels so sdLDL and triglycerides are regarded as predictor for deterioration of retina in patients with type II diabetes mellitus.

Authors' declaration:

We confirm that all the Figures and Tables in the manuscript belong to the current study. Besides, the Figures and images, which do not belong to the current study, have been given permission for republication attached to the manuscript. Authors sign on ethical consideration's Approval-Ethical Clearance: The project was approved by the local ethical committee in (AL-Emamein AL-Kadhumein Medical City and the Endocrinology Center in AL-Kindy Hospital in Baghdad, Iraq) according to the code number (231) on (18/08/2023).

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Authors' Contribution:

Study conception & design: (Nawar M. Jawad, Halla G. Mahmood, Aber A. Mohamad). Literature search: (Nawar M. Jawad, Halla G. Mahmood, Aber A. Mohamad). Data acquisition: (Nawar M. Jawad, Halla Ghazi Mahmood, Aber A. Mohamad). Data analysis & interpretation: (Nawar M. Jawad, Halla G. Mahmood, Aber Abdul Amir Mohamad). Manuscript preparation: (Nawar M. Jawad, Halla Ghazi Mahmood, Aber A. Mohamad). Manuscript eview: (Nawar M. Jawad, Halla G. Mahmood, Aber A. Mohamad). Manuscript editing & review: (Nawar M. Jawad, Halla G. Mahmood, Aber A. Mohamad).

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دور البروتين الدهني قليل التكثف منخفض الكثافة في اعتلال الشبكية السكري

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الخلاصة

الخلفية:

يُعد اعتلال الشبكية السكري النتيجة العينية الرئيسية لمرض السكري، وهو مرض خطير يُعرف بتأثيره الكبير على الصحة العالمية. تنشأ المضاعفات الدقيقة للأو عية الدموية نتيجة تلف الأو عية الدموية الصغيرة، مثل اعتلال الشبكية، واعتلال الكلية، واعتلال عضلة القلب، واعتلال الأعصاب. وتوجد نتائج متضاربة في الأبحاث الوبائية التي تدرس تحديد مستويات الدهون واعتلال الشبكية السكري. وتُعتبر جزيئات البروتين الدهني منخفض الكثافة الصغيرة والكثيفة فئة فرعية محددة من البروتينات الدهنة منخفضة الكثافة، وتتميز بالعديد من الخصيص المؤدية لتشرايت الدراسة إلى تقييم مدى فائدة مستوى البروتينات الدهنية منخفضة الكثافة، وتتميز بالعديد من الخصائص المؤدية لتصلب الشرايين. وتهدف هذه الدراسة إلى تقييم مدى فائدة مستوى البروتين الدهني منخفض الكثافة الصغير والكثيف في مصل الدم كمؤشر حيوي للتنبؤ بمرضى داء السكري من النوع الثاني الذين يعانون من اعتلال الشبكية السكري التكاثري و غير التكثيف في مصل الدم كمؤشر حيوي للتنبؤ بمرضى داء السكري من

شملت الدراسة (160) فردًا مُقسَّمين إلى أربع مجمو عات: (40) مريضًا مصابًا باعتلال الشبكية السكري غير التكاثري، (40) مريضًا مصابًا باعتلال الشبكية السكري التكاثري، (40) مريضًا مصابًا بالسكري دون اعتلال الشبكية السكري، و(40) مجموعة سليمة. وصف للأساليب الإحصائية، مثل اختبار (t)أو تحليل التباين(ANOVA)، المُستخدمة لمقارنة المتوسطات أو النسب بين المجموعات، اعتمادًا على طبيعة البيانات.

النتائج :كشفت الدراسة أن مستويات البروتين الدهني منخفض الكثافة صغير الكثافة في مجموعة اعتلال الشبكية السكري التكاثري لدى مرضى السكري من النوع الثاني كانت أعلى بشكل ملحوظ من تلك الموجودة في اعتلال الشبكية السكري غير التكاثري ومرضى السكري دون اعتلال الشبكية ومجموعات التحكم الصحية. وكان متوسط مستوى البروتين الدهني منخفض الكثافة صغير الكثافة والانحراف المعياري لدى مرضى اعتلال الشبكية السكري التكاثري 4.70± 1.06 ميكرومول/لتر وفي اعتلال الشبكية السكري غير التكاثري كان 3.00± 0.00 ميكر ومول/لتر وفي مرضى السكري دون اعتلال الشبكية كان 2.51± 1.55 ميكرومول/لتر وفي مجموعة التحكم الصحية كان 2.45± 0.40 ميكرومول/لتر.

الأستنتاج :إن البروتين الدهني منخفُضُ الكَثْافةُ والصغيرُ والكَثيفُ يلعب دورًا في تطوّر اعتَّلال الشبكية السكري لدى مرضّى السكري من النوع الثاني. وكانت مستويات البروتين الدهني منخفض الكثافة والصغير والكثيف و كذلك مستوى الدهون الثلاثية أعلى في مجموعة اعتلال الشبكية السكري التكاثري وأدنى في مجموعة التحكم الصحية .

الكلماتُ المفتاحيةُ: داء السكري؛ اعْتلال الشبكية السكري غير التكاثري؛ اعتلال الشبكية السكري التكاثري؛ البروتين الدهني منخفض الكثافة صغير الكثافة؛ الدهون الثلاثية .