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RESEARCH ARTICLE

Apolipoprotein A-I and Apolipoprotein B: Better Indicators of Dyslipidemia in Diabetic Retinopathy Patients?

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ABSTRACT

Diabetic retinopathy (DR) remains the leading cause of mortality and disability in adults with diabetes. Recently apolipoprotein A-I (Apo A-I) and apolipoprotein B (Apo B) have been found to be associated with biophysiological changes of DR than traditional lipids. The objective of the present study was to evaluate the lipid profile including Apo A-I, Apo B, and Apo B/Apo A-I levels in diabetes patients with or without retinopathy. The present study was conducted at Adichunchanagiri Institute of Medical Sciences and Hospital, India. The total numbers of subjects were 90, divided into three groups. Group I included 30 healthy controls, group II included 30 cases of diabetes mellitus (DM) without retinopathy, and group III had 30 cases of DR. Blood samples were drawn under aseptic precautions from study subjects. The investigations carried out were fasting plasma glucose (FPG), postprandial plasma glucose (PPPG), and lipid profile including Apo A-I and Apo B in all subjects. The FPG, PPPG, lipid profile, and apolipoproteins (Apo A-I and Apo B) were estimated using autoanalyzer EM 200.There was significant increase in FPG, PPPG, total cholesterol, triglycerides (TGs), low-density lipoproteins (LDLs) and no significant decrease in high-density lipoprotein (HDL) levels in group II and III subjects. There was significant decrease in Apo A-I and increase in Apo B levels and Apo B/Apo A-I ratio in group II and III subjects. There is a suggestive association of TGs, LDL, and Apo B/Apo A-I ratio in diabetic subjects with and without retinopathy. The Apo A-I, Apo B, and ratio of Apo B/Apo A-I are strong indicators of dyslipidemia in diabetic and DR patients. The ratio of Apo B/Apo A-I is better associated with DR and may contribute to development and progression of DR.

Keywords: Apolipoprotein A-I, Apolipoprotein B, Diabetic retinopathy.

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INTRODUCTION

Diabetic retinopathy is a well-characterized, sight-threatening chronic microvascular complication that eventually afflicts all patients with DM.[1] It remains the leading cause of mortality and disability in working adults with diabetes. However, this morbidity is largely preventable and treatable and if managed with timely intervention, the quality-of-life can be preserved.[2-4]

The DR has a significant impact on the world's health system, resulting in blindness of over 10,000 individuals with diabetes per year. If prompt action is not taken, the number of people with DR will grow from 126.6 million in 2010 to 191.0 million by 2030, and it is estimated that the number with vision-threatening DR (VTDR) will increase from 37.3 million to 56.3 million.[5]

The major predictor associated with the development of DR is the duration of the disease and chronic hyperglycemia. However, some patients do not develop DR even with poor glycemic control and long duration of diabetes, whereas others with tight glycemic control and short duration of diabetes have progressed to DR.[6]

Dyslipidemia is one of the major biochemical changes observed in DM. Studies to examine the relationship of DR with traditional lipids have produced conflicting results.[7] Elevated lipid levels lead to endothelial dysfunction via a local inflammatory response with release of cytokines and growth factors, activation of oxygen-sensitive biological changes in vessel walls, with consequent increase in LDL oxidation and quenching of nitric oxide.

Currently, there has been an interest in relationship of Apo A-I and Apo B with DR. Few studies have shown association of apolipoproteins and DR.[3,6]

Apolipoprotein A-I is the major antiatherogenic apolipoprotein present in HDL cholesterol, which plays an important role in HDL metabolism. Lecithin cholesterol acyltransferase enzyme present in HDL is activated by Apo A-I, which catalyzes the reaction forming cholesterol esters (CEs). This CE-rich HDL clears the cholesterol from peripheral tissue and transports it to liver (reverse cholesterol transport).[8]

Apolipoprotein B is a major apolipoprotein of very low-density lipoprotein (VLDL) and LDL, the atherogenic lipoproteins. It helps in solubilizing the cholesterol within the LDL complex, which, in turn, increases the transport capacity of LDL for subsequent deposition of cholesterol

in the arterial wall. Only one molecule of Apo B exists per lipoprotein particle. Thus, the quantity of Apo B is a direct measure of VLDL and LDL particles. Due to wide variations in the amount of cholesterol in these lipoproteins, measurement of Apo B has better relevance to the concentration of atherogenic lipoprotein particles than LDL cholesterol or non-HDL cholesterol levels. Although the measurements of apolipoproteins have not been used routinely, there seems to have distinct and significant associations with DR than traditional lipids.[9]

However, the extent to which these apolipoprotein measurements can be used to identify the risk of DR in individuals with DM remains unknown.

Hence, the present study aimed to evaluate the changes in traditional lipid profile and apolipoproteins in type II DR cases and compare them with type II DM patients without complications and healthy controls.

MATERIALS AND METHODS

Randomly, 90 subjects were selected from the Ophthalmology outpatient and inpatient departments, which included 30 cases of clinically diagnosed type II DM without retinopathy, 30 funduscopically diagnosed type II DR, and 30 numbers of age- (above 18 years) and sex-matched healthy controls; they were divided into three groups. Group I included healthy controls, group II included DM cases without retinopathy, and group III included DR cases. Each gave informed consent and the study was approved by the Ethical and Research Committee of the institution. Patients suffering from acute and chronic inflammatory conditions, other metabolic conditions like ketoacidosis, cerebrovascular accidents, preeclamptic patients, preexisting chronic kidney disease, chronic renal failure, chronic glomerulonephritis, nephrotic syndrome, smokers, alcoholics, patients with psychiatric disorders, primary hypertensives, pregnant women, and those with gestational DM (GDM) were excluded.

About 5 mL of fasting blood sample (3 mL in plain tube and 2 mL in fluoride tube) was drawn from all subjects under the aseptic precautions, and 2 mL of blood in fluoride tube was drawn in postprandial period. Fasting samples were analyzed for routine blood parameters, FPG, serum lipid profile, and serum apolipoproteins A-I and B. Postprandial sample was analyzed for PPPG. Plasma glucose was measured by glucose oxidase–peroxidase method (Trinder's method), serum TC by cholesterol oxidase/peroxidase aminophena zone method, HDL by modified polyvinyl sulfonic acid and polyethylene glycol methyl ether coupled classic precipitation method, and triacylglycerol (TG) by glycerol phosphate oxidase Trinder method using standard kits from ERBA diagnostics on EM-200 auto-analyzer. Very low-density lipoprotein was calculated by dividing TG with five (TG/5). The LDL levels were calculated using Friedwald's formula, LDL = TC – (HDL + TG/5). Serum apolipoproteins by turbidimetric immunoassay using QUANTIA Apo A-I and Apo B reagents were purchased from Tulip.[10]

STATISTICAL ANALYSIS

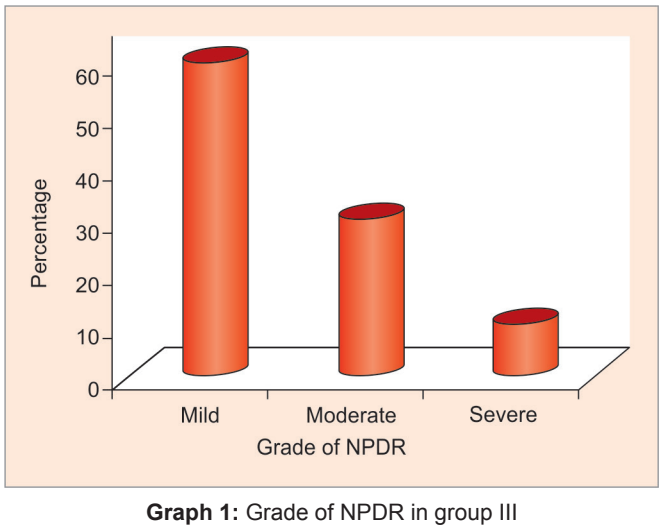
Descriptive and inferential statistical analyses were carried out in the present study. Results on continuous measurements are presented as mean + SD (Min–Max) and results on categorical measurements are presented as number (%). Significance was assessed at 5% level of significance.

Student's t-test (two-tailed, independent) was used to find the significance of study parameters on a continuous scale between two groups (intergroup analysis). Analysis of variance was used to find the significance of study parameters between three groups. Logistic regression analysis was done to study the association of lipid parameters in DR.

RESULTS

The age distribution pattern of controls (group I) and cases (groups II and III) under study ranged from 19 to 70 years with mean age of 50.43 ± 11.74, 52.97 ± 12.46, and 56.16 ± 7.93 for groups I, II, and III, respectively, and the difference is not statistically significant (p = 0.130). About 56.7, 60, and 53.3% were males in groups I, II, and III, respectively, and gender distribution among the groups was not statistically significant (p = 0.873). The mean duration of diabetes was 4.93 ± 3.68 and 8.37 ± 4.81 in diabetic patients without retinopathy and DR patients, respectively, which was significantly different (p = 0.003).

Graph 1 shows the grade of retinopathy in group III cases. Based on the funduscopic lesions, all the retinopathy cases were of nonproliferative DR (NPDR) type. Out of 30 cases, 60% had mild (n = 18), 30% had moderate (n = 9),



Graph 1: Grade of NPDR in group III

Table 1: Comparison of mean values of plasma glucose and lipid profile in three groups studied with *post hoc* test

					Significance p-value		
	Group I	*Group II*	*Group III*	*Overall p-value*	*Group I– Group II*	*Group I– Group III*	*Group II– Group III*
FPG (mg/dL)	91.77 ± 13.35	174.43 ± 67.96	219.70 ± 101.13	<0.001**	<0.001**	<0.001**	0.040*
PPPG (mg/dL)	122.43 ± 15.01	236.53 ± 86.45	341.73 ± 94.44	<0.001**	<0.001**	<0.001**	<0.001**
TC (mg/dL)	181.03 ± 32.66	193.33 ± 39.41	211.17 ± 46.42	0.016*	0.460	0.012*	0.199
TG (mg/dL)	157.33 ± 66.01	220.87 ± 77.56	213.20 ± 74.00	0.002**	0.003**	0.010**	0.912
HDL (mg/dL)	44.00 ± 8.82	39.47 ± 10.78	39.70 ± 10.94	0.160	0.204	0.239	0.996
VLDL (mg/dL)	31.10 ± 13.15	43.63 ± 15.37	42.27 ± 14.87	0.002**	0.003**	0.010**	0.929
LDL (mg/dL)	105.57 ± 32.64	115.03 ± 41.77	137.27 ± 38.15	0.005**	0.596	0.005**	0.063+

+Suggestive significance (0.05 < p < 0.10); *Moderately significant (0.01 < p < 0.05); **Strongly significant (p < 0.01)

Table 2: Comparison of mean values of Apo A-I, Apo B, and Apo B/Apo A-I in three groups studied with *post hoc* test

					Significance p-value		
	Group I	*Group II*	*Group III*	*Overall p-value*	*Group I– Group II*	*Group I– Group III*	*Group II– Group III*
Apo A-I (mg/dL)	145.97 ± 26.36	116.17 ± 32.96	114.07 ± 20.86	<0.001**	<0.001**	<0.001**	0.952
Apo B (mg/dL)	114.57 ± 20.08	150.50 ± 47.97	162.93 ± 47.30	<0.001**	0.003**	<0.001**	0.464
Apo B/Apo A-I	0.83 ± 0.32	1.4 ± 0.56	1.46 ± 0.45	<0.0001**	<0.001**	<0.0001**	0.65

**Strongly significant (p < 0.01)

Table 3: Association of lipid profile with diabetic retinopathy

	Odds ratio (95% CI)	*p-value*
TC	3.29 (1.08–9.95)	0.035*
TG	4.57 (1.45–14.39)	<0.0001**
HDL	2.04 (0.71–5.89)	0.187+
LDL	3.26 (1.09–9.95)	0.03*
VLDL	3.5 (1.11–11.01)	0.032*
Apo A-I	4.5 (1.09–18.5)	0.037*
Apo B	21.0 (0.05–87.38)	<0.0001**
Apo B/Apo A-I	29.57(6.85–127.64)	<0.0001**

+Suggestive significance (0.05 < p < 0.10); *Moderately significant (0.01 < p < 0.05); **Strongly significant (p < 0.01); TC: Total cholesterol; TG: Triglyceride; HDL: High density lipoprotein; VLDL: Very low density lipoprotein; LDL: Low density lipoprotein; Apo A-I: Apolipoprotein A-I; Apo B: Apolipoprotein B

Table 4: Association between serum lipids and diabetic patients with and without retinopathy

	Odds ratio (95% CI)	*p-value*
TC	1.73 (0.62–4.84)	0.299
TG	3.06 (0.97–9.66)	0.056+
HDL	1.0 (0.36–2.78)	1.00
LDL	2.75 (0.93–8.10)	0.067+
VLDL	0.88 (0.32–2.41)	0.796
Apo A-I	0.86 (0.30–2.45)	0.787
Apo B	2.05 (0.71–5.89	0.187
Apo B/Apo A-I	3.86 (0.93–16.05)	0.064+

+Suggestive significance (p-value: 0.05 < P < 0.10)

10% had severe NPDR (n = 3), and none of the patients had proliferative DR (PDR).

Table 1 shows the mean values of FPG and PPPG, which showed statistically significant difference (p < 0.001). Also, statistically significant difference was observed when the mean values of FPG and PPPG levels are compared between groups I *vs* II, I *vs* III, and II *vs* III.

Statistically significant alteration in the lipid profile was observed in diabetic patients showing increased levels of TC, TG, VLDL, and LDL in group II subjects and a further increase in group III when compared with group I subjects. The serum HDL levels were lowered in both groups II and III subjects, which was not statistically significant. Significant difference was seen in TG and VLDL when values were compared between groups I *vs* II and I *vs* III. The TC and LDL values also show significant difference when compared between groups I *vs* III. There was no statistically significant difference observed when lipid profile was compared between groups II *vs* III.

Table 2 shows statistically significant decrease and increase in Apo A-I and Apo B, respectively, were observed in group II and III subjects when compared with group I subjects. Significant difference was there in both Apo A-I and Apo B when mean values are compared between groups I *vs* II and I *vs* III. This was not statistically significant when compared between groups II *vs* III. There was a significant increase in the ratio of Apo B/Apo A-I in groups II and III when compared with group I and was statistically significant (p < 0.001). Significant difference was seen in the ratio of Apo B/Apo A-I when compared between groups I *vs* II (p < 0.001) and I *vs* III (p < 0.001). There was no statistically significant difference observed when compared between groups II *vs* III (p value = 0.65).

Table 3 shows logistic regression analysis with moderately significant association of TC, LDL, VLDL, and Apo A-I and strongly significant association of TGs, Apo B, and Apo B/Apo A-I ratio with DR.

Table 4 shows association of lipid parameters in DR subjects compared with DM subjects without retinopathy. There is suggestive significant association of TG, LDL,

and Apo B/Apo A-I ratio with and no association in all other lipid parameters.

DISCUSSION AND CONCLUSION

The DR is the most common long-term complication of DM. Nonproliferative DR, the early stage of DR, could progress to PDR, which causes severe visual impairment in diabetic patients. This is the leading cause of blindness and visual impairment in diabetic patients worldwide. Dyslipidemia is known to potentially contribute to microvascular disease. Thus, the control of blood lipid levels is one of the cornerstones in the treatment of DM.[11]

Recently, apolipoproteins Apo A-I, a HDL constituent, and Apo B, a constituent of LDL, VLDL, and lipoprotein (a), have been found to be more directly associated with biophysiological changes of DR than traditional lipids, and they are the better predictors of risk assessment of dyslipidemia.[3]

The present study was undertaken to measure the traditional lipid parameters and serum apolipoprotein Apo A-I, Apo B, and Apo B/Apo A-I ratios in DR patients.

In the present study as shown in Table 1, though there was increase in atherogenic traditional lipids (TC, TG, VLDL, and LDL), there was no significant decrease in antiatherogenic HDL levels. Also, *post hoc* test analysis showed no significant difference in serum lipids in diabetic patients with and without retinopathy.

The study also demonstrated statistically significant association of TC, TG, LDL, and VLDL with DR patients, but HDL did not show any association (Table 3). A suggestive association of TG and LDL was seen in DR against diabetic patients without retinopathy (Table 4). A South Indian–Chennai Urban Rural Epidemiology (CURE) study reported significant association of TG with DR and LDL with diabetic macular edema after adjusting for hemoglobin A1c (HbA1c).[12] Ajith et al[13] established significant association of TC, TG, LDL, and HDL with DR.

Serum Apo A-I and Apo B are constantly involved in the progression and severity of DR, independent of age, sex, and other known risk factors. Higher values of Apo A-I suggest the protective role within the retina against deposition of lipids and inflammation-induced toxicity. Thus, low level of this protective agent leads to DR. Increased levels of Apo B are associated with severity of DR and may reflect higher lipoprotein-related toxins, which are destructive to retinal vasculature.[3]

In the present study, even though there was no significant decrease in HDL, there was a strongly significant decrease in Apo A-I, a constituent of HDL and a strongly significant increase in Apo B, a constituent of LDL and VLDL denoting that, they are better assessment indicators of dyslipidemia in DM and DR patients. The present study also compared the ratio of Apo B/Apo A-I among the three groups, and the difference in the ratio was statistically highly significant ($p < 0.001$) (Table 2).

In addition, there was highly significant association of Apo B and Apo B/Apo A-I and significant association of Apo A-I with DR (Table 3). However, there was no difference in association of these apoproteins between DM with and without retinopathy patients, but there was suggestive association of Apo B/Apo A-I in DR patients [odds ratio (OR) 3.86; 95% confidence interval (CI), 0.93–16.05), $p = 0.064$] (Table 4). The lack of difference between these two groups could be due to most of the cases being NPDR of mild grade.

Ajith et al[13] showed a significant association of high Apo B/Apo A-I ratio with DR, even though they did not find any significant independent association of Apo A-I and Apo B with DR. They hypothesized that Apo A-I has anti-inflammatory and antioxidant action and, in addition, it specifically inhibits oxidation of LDL. Hence, increased oxidized LDL may contribute to the deterioration of antiplatelet and anti-inflammatory function of endothelium. Hence, Apo B/Apo A-I ratio may reflect a net effect produced from interactions of Apo A-I and Apo-B and the increased ratio may be crucial in the pathogenesis of microangiopathies leading to DR.

In a similar study, Sasongko et al[3] demonstrated inverse association of Apo A-I and positive association of Apo B and high Apo B/ApoA-I ratio in DR and also significant differences in association with different stages of retinopathy. Hu et al[6] reported association of low Apo A-I/Apo B ratio with severity of DR in type II diabetics.

In another study, Deguchi et al[14] concluded that Apo B/ApoA-I ratio may contribute to PDR progression in patients with DR. Thus, Apo A-I, Apo B, and ratio of Apo B/Apo A-I include both damaging and protective lipoprotein pathways and have better ability to discriminate the presence or absence of DR than traditional lipids.

In summary, the study reaffirms dyslipidemia in diabetic patients with and without retinopathy with Apo A-I, Apo B, and ratio of Apo B/Apo A-I as strong indicators of dyslipidemia. The study did not show any significant association of apoliproteins between DM patients with and without retinopathy; however, there was suggestive association of increased ratio of Apo B/Apo A-1 with DR.

To conclude, the ratio of Apo B/Apo A-I is better associated with DR and may contribute to the development and progression of DR.

The study had few limitations, such as a small sample size; all cases were of NPDR with majority being mild-grade cases. Also, HbA1c, which is better correlated with retinopathy, has not been measured.

A well-designed, large sample study is required to establish correlation between the apolipoproteins, pathogenesis, and progression of DR.

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