

Possible biochemical abnormalities of plasma lipids and Apo-lipoprotein in *Diabetes mellitus* Patients with or without Retinopathy In Saki- West Local Government area of Oyo state- Nigeria

ABSTRACT

Study Background: Possible metabolic disorders of lipid, carbohydrate and protein are associated with *Diabetes mellitus* which may include complications such as retinopathy considering the pathophysiology of this metabolic disease. **Aims and Objectives:** This work was designed to determine apolipoproteins (ApoA, ApoB), Total cholesterol (TC), Total Triglyceride (TG), Low-density lipoprotein (LDL-C), Very Low-density Lipoprotein (VLDL), High-density Lipoprotein (HDL-C) in *Diabetes mellitus* patients with or without retinopathy. **Materials and Methods:** 261 subjects were recruited for this study from the Medical outpatient and ophthalmology clinic of Baptist Medical Center Saki-Nigeria. The subjects were classified into; Non- *Diabetes mellitus* without retinopathy Control subjects (n=100 : Female-50; Male-50), *Diabetes mellitus* patients without retinopathy (n=100: Female-50; Male-50) and *Diabetes mellitus* with retinopathy (n=61: Female-22; Male-39) aged 50 to 76 years. The Body mass index (BMI) of the subjects ranged between 18.2 – 19.6 Kg/m². Plasma Total cholesterol (TC), Total Triglyceride (TG), Low-density lipoprotein (LDL-C), Very Low density Lipoprotein (VLDL), High-density Lipoprotein (HDL-C) were determined biochemically using chemistry auto-analyser – COBAS C111 while ELISA technique determined apolipoproteins (ApoA, ApoB). **Results:** The result obtained showed a significantly higher mean plasma value of Fasting Plasma Glucose, Total cholesterol, LDL-C, VLDL, TG and Apo B/Apo A1 in a patient with *Diabetes mellitus* without Retinopathy than the control non-diabetic subjects without retinopathy with $p<0.05$. There was a significantly higher plasma value of Fasting Plasma Glucose, Total cholesterol, LDL-C, VLDL, TG Apo B and Apo B/Apo A1 and a significantly lower plasma value of Apo A1 in patients with *Diabetes mellitus* and Retinopathy when compared to the control non-diabetic subjects without retinopathy with $p<0.05$. There was also a significantly higher mean plasma value of Fasting Plasma Glucose, LDL-C, , TG, Total cholesterol, and Apo B/Apo A1 in *Diabetes mellitus* patients with Retinopathy than the *Diabetes mellitus* without Retinopathy with $p<0.05$. **Conclusion:** This work has been used to reveal a significant association between increased plasma Total cholesterol, Triglyceride, LDL-C, VLDL, ApoB and ApoB/ApoA ratio and decreased plasma ApoA in *Diabetes mellitus* patients with or without retinopathy which was found to be more intense in those patients with retinopathy

38 **Keywords:** Retinopathy, Lipid metabolic disorders, *Diabetes mellitus*

39 **Introduction**

40 *Diabetes Mellitus* is a group of metabolic diseases as a result of inadequate secretion or
41 absolute lack of insulin or resistance of a body to the action of insulin. This condition is
42 associated with hyperglycaemia and symptoms like polyuria, Polydipsia, and polyphagia [1]..
43 *Diabetes mellitus* may cause microvascular and macrovascular complications which manifest
44 in the eyes, kidneys, brain, extremities and other parts of the body [2].

45 Diabetic Retinopathy (DR) is caused by complications of diabetes mellitus which can
46 eventually lead to blindness especially in its advanced stage. Diabetic Retinopathy could lead
47 to visual impairment[1][3]. It is majorly a microangiopathy in which small blood vessels are
48 susceptible to damage by hyperglycemia. Excess glucose *Diabetes mellitus* is converted to
49 fat. Excessive production of fat from excess glucose could narrow the blood vessel
50 transporting blood to the eye which will as a consequence reduce the blood flow to the eye
51 including reduction of the supply of nutrients and other essential materials to the retina which
52 may cause retinopathy [4]. It could be mild, moderate and severe non-proliferative diabetic
53 retinopathy (NPDR) to proliferative diabetic retinopathy (PDR) [5]. Lipids are substances
54 soluble in a nonpolar solvent which include fats, waxes, sterols, fat-soluble vitamins (such as
55 vitamins A, D, E, and K), monoglycerides, diglycerides, triglycerides, phospholipids, and
56 others. Their major functions include: storing energy, signalling, and acting as structural
57 components of cell membranes [4-9]. Apolipoproteins are proteins transporting lipids through
58 lymphatic and circulatory systems, coenzymes (C-II for lipoprotein lipase and A-I for
59 lecithin-cholesterol acyltransferase) and ligands for interaction with lipoprotein receptors in
60 tissues(apoB100 and apoE for LDL-receptors, apoA-I for HDL receptors). Apolipoproteins
61 include A (apo A-I, apo A-II, apo A-IV, and apo A-V), B (apo B48 and apo B100), C (apo C-
62 I, apo C-II, apo C-III, and apo C-IV), D, E and H[10-12].

63 There is little information on the possible lipid and Apo-lipoprotein abnormalities in *Diabetes*
64 *mellitus* Patients with or without Retinopathy in Saki- West Local Government area of Oyo
65 state- Nigeria hence the justification for this work. This work was therefore designed to
66 determine the Possible biochemical abnormalities of plasma lipids and Apo-lipoprotein in
67 *Diabetes mellitus* Patients with or without Retinopathy In Saki- West Local Government area
68 of Oyo state- Nigeria

69 **Materials and methods**

70 **Materials**

71 **Area of Study**

72 Saki West is a Local Government Area in Oyo State, Nigeria. Its headquarters are in the town
73 of Saki. It has an area of 2,014 km² and a population of 278,002 at the 2006 census. Shaki,
74 Nigeria is located at the extreme end of Oyo state. It has a Resettlement center of 2nd

Mechanised Division of Nigerian Army, Baptist School of Nursing and Midwifery, School of Medical Laboratory Technology, Baptist Medical Centre, Saki, School of Basic Midwifery, Muslim Hospital, Saki, Baptist Medical Centre, Saki, State Hospital, Saki, Muslim Hospital, Saki, The Oke-Ogun Polytechnic, and a Technical college. Shaki, Nigeria is also one of the largest city in Oyo state. The postal code of the area is 203

Study Population

The study population include: Apparently healthy Non- *Diabetes mellitus* without retinopathy were recruited as Control subjects (n=100: Female-50; Male-50), *Diabetes mellitus* patients without retinopathy (n=100: Female-50; Male-50) and *Diabetes mellitus* with retinopathy (n=61: Female-22; Male-39) aged 50 to 76 years were recruited through the medical outpatient and ophthalmology department of Baptize Medical Centre Saki, Oyo State-Nigeria. The Body mass index (BMI) of the subjects ranged between 18.2 – 19.6 Kg/m²

Ethical Consideration

The proposal of this research work was reviewed, modified and approved by Research and Ethical Committee of Baptize Medical Center, Saki-Nigeria

Consent Disclaimer:

As per international standard or university standard written patient consent has been collected and preserved by the author(s).

Inclusion Criteria: *Diabetes mellitus* patients with and without DR. Patients who were not on any lipid-lowering medication were included as these medications have different effects on cholesterol as some may lower the "bad cholesterol" that is low density lipoprotein (LDL) more so than others, while others may preferentially increase high-density lipoprotein (HDL) known as "the good cholesterol".

Exclusion Criteria: *Diabetes mellitus* patients with the history of glaucoma, liver disease, previous vitreoretinal surgery and those with media opacity including those on any medication to treat hyperlipidemia were excluded from the study.

Recruitment of Patients with Diabetic Retinopathy

This was carried out utilising fasting blood glucose level, Medical History and Consultant ophthalmologist diagnostic reports on eye examination that includes:

- *Visual acuity test*: This test uses an eye chart to measure how well a person sees at various distances (*i.e.*, visual acuity).
- *Pupil dilation*: The eye care professional places drops into the eye to dilate the pupil. This allows him or her to see more of the retina and look for signs of diabetic retinopathy. After the examination, close-up vision may remain blurred for several hours [13]
- *Ophthalmoscopy or fundus photography*: Ophthalmoscopy is an examination of the retina in which the eye care professional: (1) looks through a slit lamp biomicroscope with a special magnifying lens that provides a narrow view of the retina, or (2) wearing a headset (indirect ophthalmoscope) with a bright light, looks through a special magnifying glass and gains a wide view of the retina. Hand-held ophthalmoscopy is insufficient to rule out significant and treatable diabetic retinopathy. Fundus photography captures considerably more significant areas of the fundus and has the advantage of photo documentation for future reference, as well as availing the image to be examined by a specialist at another location and time [14]
- *Fundus Fluorescein angiography (FFA)*: This is an imaging technique which relies on the circulation of Fluorescein dye to show staining, leakage, or non-perfusion of the retinal and choroidal vasculature.
- *Optical coherence tomography (OCT)*: This is a visual imaging modality based upon interference, and analogous to ultrasound. It produces cross-sectional images of the retina (B-scans) which can be used to measure the thickness of the retina and to resolve its significant layers, allowing the observation of swelling [15-19].

The eye care professional will look at the retina for early signs of the disease, such as:

1. leaking blood vessels,
2. retinal swelling, such as macular oedema,
3. pale, fatty deposits on the retina (exudates) – signs of leaking blood vessels,
4. damaged nerve tissue (neuropathy), and
5. any changes in the blood vessels[15-19].

Methods

134 **Sample collection**

135 Venous blood was collected via the median cubital vein. The area was tied with a tourniquet
136 to allow blood flow, cleansed with cotton wool moistened with methylated spirit and allowed
137 to dry, using a sterile needle and syringe, a venous puncture sufficiently deep to allow free
138 flow of blood was made and the plunger backwards to obtain the blood. The blood was
139 placed in fluoride oxalate and lithium heparin bottles. The plasma was separated from the
140 packed cell by centrifugation.

141 **Methods of Biochemical analysis**

142 **Measurement of ApolipoproteinA1 using Abcam's Kit by ELISA Technique**

143 Abcam's Apolipoprotein AI Human (APOA1) *in vitro* competitive ELISA (Enzyme-Linked
144 Immunosorbent Assay) kit is designed for the quantitative measurement of Apolipoprotein AI
145 levels in plasma and serum.

146 **Principle and procedure:** An Apolipoprotein AI specific antibody has been precoated onto
147 96-well plates and blocked. Standards or test samples are added to the wells and
148 subsequently, biotinylated Apolipoprotein AI is added and then followed by washing with
149 wash buffer. Streptavidin-Peroxidase Complex is added and unbound conjugates are washed
150 away with wash buffer. TMB is then used to visualize Streptavidin-Peroxidase enzymatic
151 reaction. TMB is catalyzed by Streptavidin-Peroxidase to produce a blue colour product that
152 changes into yellow after adding acidic stop solution. The density of yellow colouration is
153 inversely proportional to the amount of Apolipoprotein AI captured in plate.

154 **Measurement of ApolipoproteinB using Abcam's Kit by ELISA Technique**

Abcam's Apolipoprotein B (APOB) Human *in vitro* ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the quantitative measurement of Apolipoprotein B concentrations in Human plasma, serum, CSF and cell culture samples.

Principle and procedure: An Apolipoprotein B specific antibody has been precoated onto 96-well plates and blocked. Standards or test samples are added to the wells and subsequently, an Apolipoprotein B specific biotinylated detection antibody is added and then followed by washing with wash buffer. Streptavidin-Peroxidase Complex is added and unbound conjugates are washed away with wash buffer. TMB is then used to visualize Streptavidin-Peroxidase enzymatic reaction. TMB is catalyzed by Streptavidin-Peroxidase to produce a blue color product that changes into yellow after adding acidic stop solution. The density of yellow colouration is directly proportional to the amount of Apolipoprotein B captured in plate.

Plasma Fasting Glucose, Total Triglyceride, Total Cholesterol, Low-Density Lipoprotein (Cholesterol), Very low-Density Lipoprotein and High-Density Lipoprotein These were carried out using CobasC111 Chemistry auto-analyzer using the reagent kit of Roche.

LDL: LDL, VLDL, and chylomicron (CM) react with polyvinyl sulfonic acid (PVS) and polyethylene-glycol ether (PEGME) and the reaction results in inaccessibility of LDL, VLDL and CM by cholesterol oxidase (CHOD) and cholesterol esterase (CHER), whereas HDL reacts with the enzymes. Addition of specific detergent releases LDL from the PVS/PEGME complex. The released LDL reacts with the enzymes to produce H₂O₂ which is quantified.

HDL: LDL, VLDL and chylomicron (CM) react with polyvinyl sulfonic acid (PVS) and polyethylene-glycol ether (PEGME) and the reaction results in inaccessibility of LDL, VLDL and CM by cholesterol oxidase (CHOD) and cholesterol esterase (CHER). The enzymes selectively reacts with HDL to produce H₂O₂ which is measured.

180 **Plasma Fasting Glucose.**

181 Patients are instructed not to consume anything but water during the fasting period.

182 Fasting time is between 8-12 hours or 14hours [20].

183 Glucose oxidase is an enzyme highly specific for glucose and does not react with blood
184 saccharides. So it has been employed for the estimation of blood glucose.

185 **Principle**

186 Glucose oxidase catalyses the oxidation of Beta D- glucose present in the plasma to D
187 glucono - 1,5 – lactone with the formation of hydrogen peroxide; the lactone is slowly
188 hydrolysed to D- gluconic acid. The hydrogen peroxide produced is then broken down to
189 oxygen and water by a peroxidase enzyme. Oxygen then reacts with an oxygen acceptor such
190 as ortho toluidine which itself converted to a coloured compound, the amount of which can
191 be measured colourimetrically.

192 **Total Cholesterol**

193 **Principle**

194 Cholesterol is measured enzymatically in serum or plasma in series of coupled reactions that
195 hydrolyse cholesterol esters and oxidize the 3-OH group of cholesterol. One of the reaction
196 by-product, H₂O₂ is measured quantitatively in a peroxide catalyzed reaction that produces a
197 colour. Absorbance is measured at 500nm. The colour intensity is proportional to cholesterol
198 concentration.

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201 **Triglyceride**

202 It is measured enzymatically in serum or plasma in series of coupled reactions in which
203 triglycerides are hydrolysed to produce glycerol. Glycerol is then oxidized using

204 glyceroloxidase and H₂O₂, one of the reaction product is measured as same as cholesterol.
205 Absorbance is measured at 500nm.

206 **Statistical Analysis**

207 The Statistical Package for Social Science (SPSS) 19.0 version was used for the analysis of
208 the data appropriately. The level of significance will be taken at 95% confidence interval and
209 P value less than 0.05 will be considered significant.

210 **Results**

211 The result obtained showed a significantly higher mean plasma value of Fasting Plasma
212 Glucose, Total cholesterol, LDL-C, VLDL, TG and Apo B/Apo A1 in *Diabetes mellitus*
213 patients without Retinopathy than the control non-diabetic subjects without retinopathy with
214 $p < 0.05$ (table 1 and 2, figure 1, 2).

215 There was a significantly higher plasma value of Fasting Plasma Glucose, Total cholesterol,
216 LDL-C, VLDL, TG Apo B and Apo B/Apo A1 and a significantly lower plasma value of Apo
217 A1 in patient with *Diabetes mellitus* and Retinopathy when compared to the control non-
218 diabetic subjects without retinopathy with $p < 0.05$ (table 1 and 2, figure 1, 2).

219 There was also a significantly higher mean plasma value of Fasting Plasma Glucose, LDL-C,
220 TG, Total cholesterol and Apo B/Apo A1 in *Diabetes mellitus* patient with Retinopathy
221 than the *Diabetes mellitus* without Retinopathy with $p < 0.05$ (table 1 and 2, figure 1, 2).

222 However there was no significant difference in the plasma value of HDL-C when the values
223 obtained in the subjects were compared ($p > 0.05$; table 1 and 2, figure 1, 2).

224 There was also no significant difference in the plasma value of VLDL, Apo B and Apo A1 in
225 patients with *Diabetes mellitus* and Retinopathy when compared with patients with *Diabetes*
226 *mellitus* without Retinopathy ($p > 0.05$: (table 1 and 2, figure 1, 2).

227 **Table 1: Mean and standard deviation of the values of plasma Lipid Profile including**
 228 **Apoproteins obtained in the subjects**

	<i>Non-Diabetes mellitus</i> subjects without vision defect (Control) (n=100)	<i>Diabetes mellitus</i> patients without Retinopathy (n=100)	<i>Diabetes mellitus</i> patients with Retinopathy (n=61)
Age	64.0±10.0	65.0 ±10.0	65.0±11.0
FPG (mg/dL)	90.0±12.0	171.0±11.0	250.0±12.0
TC (mg/dL)	131.0±5.0	191.0±10.0	301.0±15.0
LDL-C (mg/dL)	46.0±2.0	79.0±4.0	193.0±5.0
HDL-C (mg/dL)	68.0±2.0	79.0±10.0	67.0±9.0
VLDL (mg/dL)	20±1.0	32.0±2.0	41.0±3.0
TG (mg/dL)	102.0±4.0	166.0±8.0	204.0±8.0
ApoA1 (mg/dL)	169.0±4.0	162.0±8.0	131.0±10.0
Apo B(mg/dL)	68±7.0	98.0±8.0	146.0±11.0
Apo B/Apo A1 (mg/dL)	0.4±0.02	0.61±0.01	1.0±0.1

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237 **Table 2: Comparative Analysis of the plasma Lipid Profile including Apoproteins obtained**
 238 **in the subjects**

	<i>Diabetes mellitus</i>	<i>Diabetes mellitus</i>	<i>Diabetes mellitus</i> patients
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		patients without Retinopathy (n=100) Vs Control	patients with Retinopathy (n=61) Vs Control	without Retinopathy (n=100) Vs <i>Diabetes mellitus</i> patients with Retinopathy (n=61)
FPG (mg/dL)	“t”	-4.96	-9.42	-4.85
	“p”	0.02*	0.005**	0.02*
TC (mg/dL)	“t”	-5.37	-11.12	-6.27
	“p”	0.02*	0.004**	0.01*
LDL-C (mg/dL)	“t”	-7.38	-27.29	-17.80
	“p”	0.009**	0.0007***	0.002**
HDL-C (mg/dL)	“t”	-1.08	0.12	0.89
	“p”	0.20	0.46	0.23
VLDL (mg/dL)	“t”	-5.37	-6.64	-2.50
	“p”	0.02*	0.01*	0.06
TG (mg/dL)	“t”	-6.60	-11.40	-3.21
	“p”	0.01*	0.004**	0.04*
ApoA1 (mg/dL)	“t”	0.78	3.52	2.42
	“p”	0.26	0.04*	0.07
Apo B (mg/dL)	“t”	-2.37	-5.98	-2.52
	“p”	0.07	0.01*	0.06
Apo B/Apo A1 (mg/dL)	“t”	-9.46	-6.23	-4.02
	“p”	0.005**	0.01*	0.03*

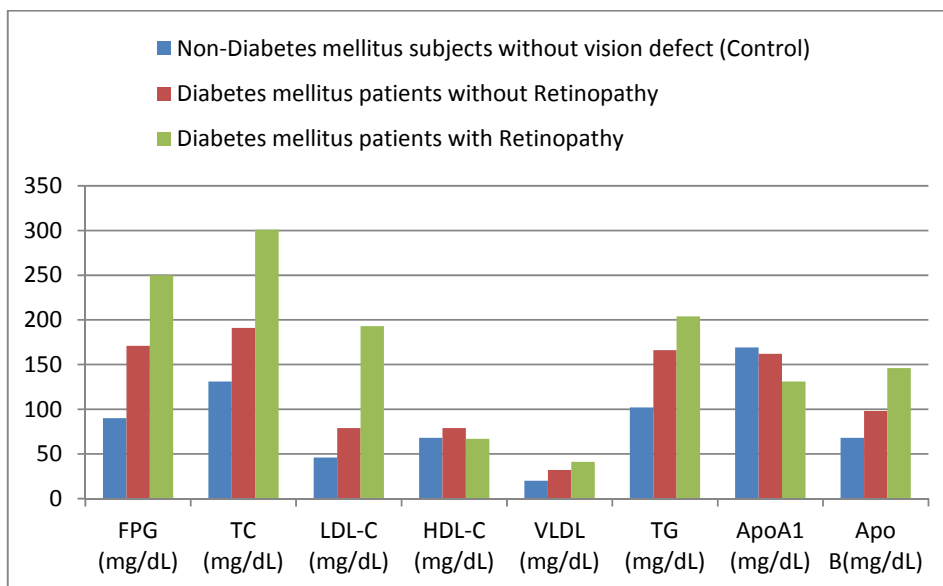


Figure 1; Comparative Description of the Lipid Profiles including ApoA1 and ApoB obtained in the subjects

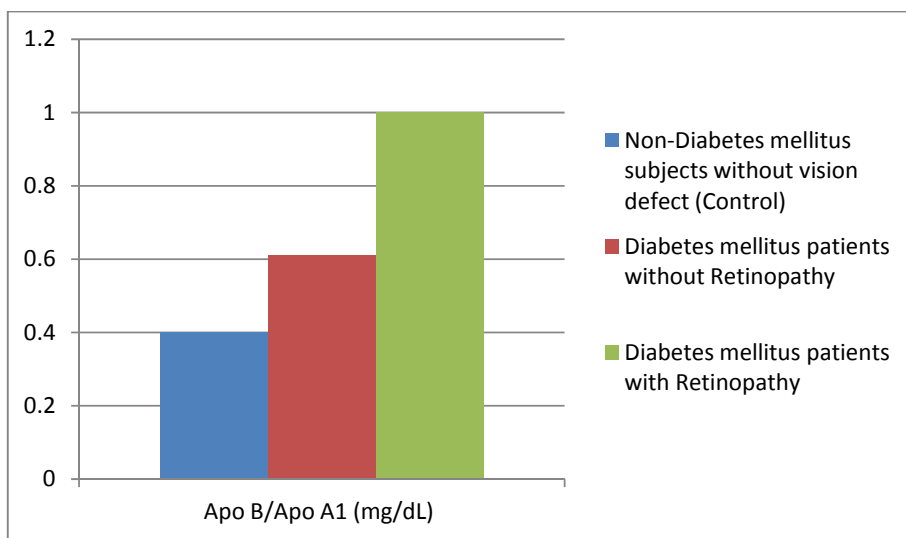


Figure 2; Comparative Description of ApoA1/ApoB obtained in the subjects

Discussion

The results obtained showed a significantly higher mean plasma value of Fasting Plasma Glucose, Total cholesterol, LDL-C, VLDL, TG and Apo B/Apo A1 in *Diabetes mellitus* patients without Retinopathy than the control non-diabetic subjects without retinopathy. There was a significantly higher plasma value of Fasting Plasma Glucose, Total cholesterol, LDL-C, VLDL, TG Apo B and Apo B/Apo A1 and a significantly lower plasma value of Apo A1 in *Diabetes mellitus* patient with Retinopathy when compared with the values obtained in the control non-diabetic subjects without retinopathy. These findings are consistent with the report of Maria and Ronald^[21] who reported elevated lipid in patients with *Diabetes mellitus*. In addition, Diabetic could be characterized by elevated triglyceride levels, decreased high-density lipoprotein cholesterol levels, and elevated low-density lipoprotein cholesterol (LDL-C) levels [22] which could be associated with this work. These findings could also be associated with the fact that Diabetes is characterized by chronic hyperglycemia and disturbances of carbohydrate, lipid and protein metabolism [23]. Aclan^[23] showed widespread lipid abnormalities in the course of diabetes triggered dyslipidemia as hypercholesterolemia, hypertriglyceridemia, elevated LDL and decreased HDL which also agrees with the findings of this study.

There was also a significantly higher mean plasma value of Fasting Plasma Glucose, LDL-C, TG, Total cholesterol, and Apo B/Apo A1 in *Diabetes mellitus* patients with Retinopathy than the *Diabetes mellitus* patients without Retinopathy. This result agrees with the report of Jyothi *et al.*^[24] who reported increase in plasma total cholesterol and LDL levels and that elevated serum lipids showed a significant association with retinal hard exudate formation.

Association of Diabetic retinopathy and elevated lipids including increase in ApoB100, ApoB100/ApoA ratio and decrease in ApoA could be associated with the fact that Diabetic retinopathy (DR) is a major cause of blindness worldwide. Previous studies have shown that

intensive control of risk factors such as high blood sugar and blood pressure can be helpful in reducing the onset and progression of DR [25]. High serum lipid levels have also been proposed as a risk factor for DR. High lipid levels are known to cause endothelial dysfunction due to a reduced bioavailability of nitric oxide and this endothelial dysfunction was suggested to play a role in retinal exudate formation in DR [26]. However large clinical studies showed a discrepancy about the association of serum lipids with the severity of DR or diabetic macular edema (DME). In ETDRS report, high total cholesterol and LDL levels were associated with retinal hard exudates; in the Chennai Urban Rural Epidemiology Study, serum lipids were higher in patients with DR than those without DR [27][28]. On the other hand, those findings were not confirmed by other large studies such as Multi-Ethnic Study of Atherosclerosis and the Australian Diabetes, Obesity, and Lifestyle Study [29][30]..

Conclusion

This work has been used to reveal a significant association between increased plasma Total cholesterol, Triglyceride, LDL-C, VLDL, ApoB and ApoB/ApoA ratio and decreased plasma ApoA in *Diabetes mellitus* patients with or without retinopathy which was found to be more intense in those patients with retinopathy.

Recommendation

Investigation of lipid profile and apoproteins in *Diabetes mellitus* is recommended to provide useful information for the management of *Diabetes mellitus* with or without Retinopathy

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