

Possible biochemical lipid and Apo-lipoprotein abnormalities in Patients with Retinopathy in *Diabetes mellitus*

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 patients with *Diabetes mellitus* with and 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), *Diabetes mellitus* patients without retinopathy (n=100) and *Diabetes mellitus* with retinopathy (n=61) aged 50 to 76 years. 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-analyzer – COBAS C111 while apolipoproteins (ApoA, ApoB) was determined by ELISA technique. **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 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 patient 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 patient with *Diabetes mellitus* with Retinopathy than the *Diabetes mellitus* without Retinopathy with $p<0.05$. **Conclusion:** This work has been used to revile a significant association of increase in Total cholesterol, Triglyceride, LDL-C, VLDL, ApoB and ApoB/ApoA ratio and decrease in plasma ApoA in patients with *Diabetes mellitus* with or without retinopathy and it was found to be more intense in those patients with retinopathy

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

Introduction

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

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

There is little information on the association of 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 patients with Diabetes mellitus with and without retinopathy hence the justification for this work. 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 patients with *Diabetes mellitus* with and without retinopathy

Materials and methods

Materials

Area of Study

Saki West is a Local Government Area in Oyo State, Nigeria. Its headquarters are in the town of Saki. It has an area of 2,014 km² and a population of 278,002 at the 2006 census. Shaki, 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,

76 Saki, The Oke-Ogun Polytechnic, and a Technical college. Shaki,Nigeria is also one of the
77 largest city in Oyo state. The postal code of the area is 203

78 **Study Population**

79 One Hundred *Diabetes mellitus* patients without retinopathy, 61 *Diabetes mellitus* patients
80 with retinopathy and 100 apparently healthy non- *Diabetes mellitus* subjects aged 50 to 76
81 years were recruited through the medical outpatient and ophthalmology department of
82 Baptize Medical Centre Saki, Oyo State-Nigeria.

83 **Ethical Consideration**

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

86 **Inclusion Criteria:** *Diabetes mellitus* patients with and without DR.

87 **Exclusion Criteria:** *Diabetes mellitus* patients with history of glaucoma, liver disease,
88 previous vitreoretinal surgery and those with media opacity were excluded from the study

89 **Recruitment of Patients with Diabetic Retinopathy**

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

- 92 • *Visual acuity test:* This test uses an eye chart to measure how well a person sees at
93 various distances (*i.e.*, visual acuity).
- 94 • *Pupil dilation:* The eye care professional places drops into the eye to dilate the pupil. This
95 allows him or her to see more of the retina and look for signs of diabetic retinopathy.
96 After the examination, close-up vision may remain blurred for several hours [13]
- 97 • *Ophthalmoscopy or fundus photography:* Ophthalmoscopy is an examination of the retina
98 in which the eye care professional: (1) looks through a slit lamp biomicroscope with a
99 special magnifying lens that provides a narrow view of the retina, or (2) wearing a
100 headset (indirect ophthalmoscope) with a bright light, looks through a special magnifying
101 glass and gains a wide view of the retina. Hand-held ophthalmoscopy is insufficient to
102 rule out significant and treatable diabetic retinopathy. Fundus photography generally
103 captures considerably larger areas of the fundus, and has the advantage of photo
104 documentation for future reference, as well as availing the image to be examined by a
105 specialist at another location and/or 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 an optical 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 major 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 edema,
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

Sample collection

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

Methods of Biochemical analysis

Measurement of ApolipoproteinA1 using Abcam's Kit by ELISA Technique

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

Principle and procedure: An Apolipoprotein AI specific antibody has been precoated onto 96-well plates and blocked. Standards or test samples are added to the wells and subsequently biotinylated Apolipoprotein AI 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 coloration is inversely proportional to the amount of Apolipoprotein AI captured in plate.

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 coloration 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

Plasma Fasting Glucose.

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

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

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

Principle

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

Total Cholesterol

Principle

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

Triglyceride

It is measured enzymatically in serum or plasma in series of coupled reactions in which triglycerides are hydrolysed to produce glycerol. Glycerol is then oxidized using glyceroloxidase and H₂O₂, one of the reaction product is measured as same as cholesterol. Absorbance is measured at 500nm.

Statistical Analysis

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

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 patient with *Diabetes mellitus* without Retinopathy than the control non-diabetic subjects without retinopathy with $p < 0.05$ (table 1 and 2, figure 1, 2).

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 patient with *Diabetes mellitus* and Retinopathy when compared to the control non-diabetic subjects without retinopathy with $p < 0.05$ (table 1 and 2, figure 1, 2).

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

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

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

205 **Table 1:** Mean and standard deviation of the Lipid Profiles including Apoproteins obtained
 206 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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215 **Table 2:** Comparative Analysis of the Lipid Profiles including Apoproteins obtained in the
216 subjects

		<i>Diabetes mellitus</i> patients without Retinopathy (n=100) Vs Control	<i>Diabetes mellitus</i> patients with Retinopathy (n=61) Vs Control	<i>Diabetes mellitus</i> patients 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*

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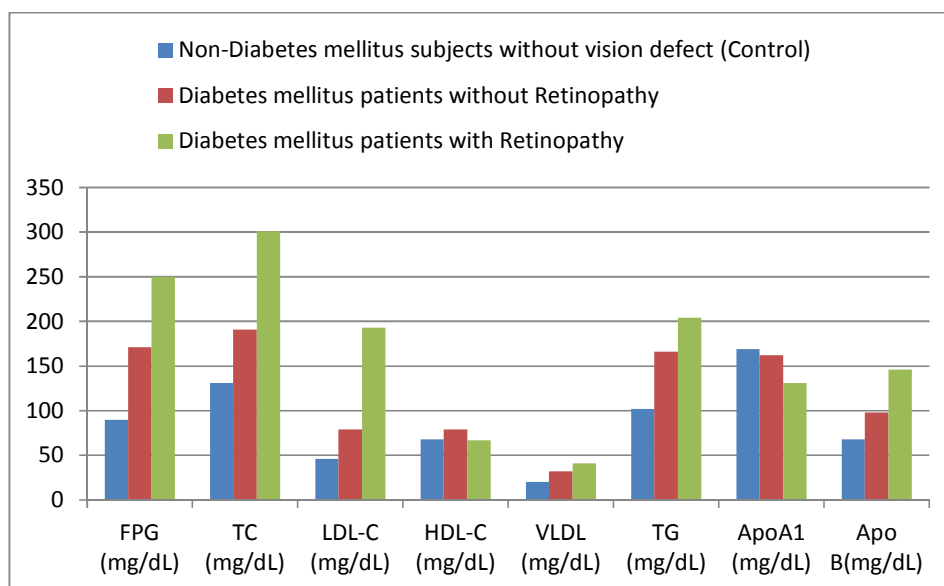


Figure 1; Comparative Description of the Lipid Profiles including ApoA1 and ApoB obtained in the subjects

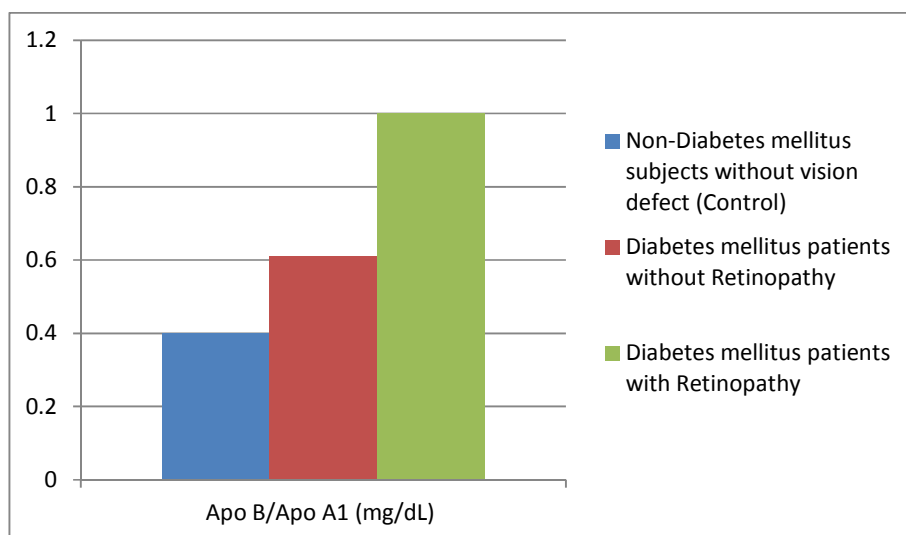


Figure 2; Comparative Description of ApoA1/ApoB obtained in the subjects

Discussion

The 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 patient with *Diabetes mellitus* 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 patient with *Diabetes mellitus* and Retinopathy when compared to 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. This study proposes the predominance of hyperlipidemia over increased prevalence of diabetic dyslipidemia. This report is 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 patient with *Diabetes mellitus* with Retinopathy than the *Diabetes mellitus* without Retinopathy. This result is consistent 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 ApoB1, ApoB1/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 revile a significant association of increase in Total cholesterol, Triglyceride, LDL-C, VLDL, ApoB and ApoB/ApoA ratio and decrease in plasma ApoA in patients with *Diabetes mellitus* with or without retinopathy and it 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 in the management of *Diabetes mellitus* with or without Retinopathy

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