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

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Authors’ contributions

This work was carried out in collaboration between all authors. Authors MFO and OAP designed the study and wrote the protocol. Authors MFO, TA and SGO performed the statistical analysis. Authors MFO, OPO, TA and SGO wrote the first draft of the manuscript, managed the analyses of the study and managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJBCRR/2018/38873

Editor(s):
(1) Richard A. Manderville, Professor, Departments of Chemistry and Toxicology, University of Guelph, Canada.

Reviewers:
(1) Ahmed Bakillah, SUNY Downstate Medical Center, USA.
(2) Ngoc-Anh Le, USA.
(3) Francesco Saverio Sorrentino, Ospedale Maggiore Bologna, Italy.

Complete Peer review History: [http://www.sciencedomain.org/review-history/23840](http://www.sciencedomain.org/review-history/23840)

Received 9th November 2017
Accepted 17th January 2018
Published 27th March 2018

**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 Apo B/Apo A ratio and decreased plasma Apo A in Diabetes mellitus patients with or without retinopathy which was found to be more intense in those patients with retinopathy.

**Keywords:** Retinopathy; lipid metabolic disorders; Diabetes mellitus.

1. **INTRODUCTION**

**Diabetes Mellitus** is a group of metabolic diseases as a result of inadequate secretion or absolute lack of insulin or resistance of a 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** is 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 a 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, signalling, 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 possible lipid and Apo-lipoprotein abnormalities in Diabetes mellitus Patients with or without Retinopathy in Saki- West Local Government area of Oyo state-Nigeria hence the justification for this work. This work was therefore designed to determine the 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-

2. **MATERIALS AND METHODS**

2.1 Materials

**2.1.1 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, 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.

2.1.2 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.

2.1.3 Ethical consideration

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

2.1.4 Consent disclaimer

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

2.1.5 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 than others, while others may preferentially increase high-density lipoprotein (HDL) known as "the good cholesterol".

2.1.6 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.

2.1.7 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].

2.2 Methods

2.2.1 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 fertile needle and syringe, a venous puncture sufficiently deep to allow free flow of blood was made and the plunger backwards 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.

2.2.2 Methods of biochemical analysis

2.2.2.1 Measurement of apolipoproteina1 using abcam’s kit by ELISA technique

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

2.2.2.2 Principle and procedure

An Apolipoprotein Al specific antibody has been precoated onto 96-well plates and blocked. Standards or test samples are added to the wells and subsequently, a 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 Al captured in plate.

2.2.2.3 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.

2.2.2.4 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 polyethene-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 polyethene-glycol ether (PEGME) and the reaction results in inaccessibility of LDL, VLDL and CM by cholesterol oxidase (CHOD) and cholesterol esterase (CHER). The enzymes selectivity reacts with HDL to produce H2O2 which is measured.

2.2.2.5 Plasma fasting glucose

Patients are instructed not to consume anything but water during the fasting period. Fasting time is between 8-12 hours or 14 hours [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.

2.2.2.6 Principle

Glucose oxidase catalyses 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 reacts with an oxygen acceptor such as ortho toluidine which itself converted to a coloured compound, the amount of which can be measured colourimetrically.

2.2.3 Total cholesterol

2.2.3.1 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 colour. Absorbance is measured at 500nm. The colour intensity is proportional to cholesterol concentration.

2.2.3.2 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 glycerol oxidase and H2O2, one of the reaction product is measured as same as cholesterol. Absorbance is measured at 500 nm.

2.3 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.

3. 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 Diabetes mellitus patients without Retinopathy than the control non-diabetic subjects without retinopathy with p<0.05 (Tables 1 and 2, Figs. 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 in patient with Diabetes mellitus and Retinopathy when compared to the control non-diabetic subjects without retinopathy with p<0.05 (Tables 1 and 2, Figs. 1, 2).

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

However there was no significant difference in the plasma value of HLDL-C when the values obtained in the subjects were compared (p>0.05; Tables 1 and 2, Figs. 1, 2).

Table 1. Mean and standard deviation of the values of plasma lipid profile including apoproteins obtained in the subjects

<table>
<thead>
<tr>
<th></th>
<th>Non-Diabetes mellitus subjects without vision defect (Control) (n=100)</th>
<th>Diabetes mellitus patients without retinopathy (n=100)</th>
<th>Diabetes mellitus patients with retinopathy (n=61)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>64.0±10.0</td>
<td>65.0±10.0</td>
<td>65.0±11.0</td>
</tr>
<tr>
<td>FPG (mg/dL)</td>
<td>90.0±12.0</td>
<td>171.0±11.0</td>
<td>250.0±12.0</td>
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<tr>
<td>TC (mg/dL)</td>
<td>131.0±5.0</td>
<td>191.0±10.0</td>
<td>301.0±15.0</td>
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<tr>
<td>LDL-C (mg/dL)</td>
<td>46.0±2.0</td>
<td>79.0±4.0</td>
<td>193.0±5.0</td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
<td>68.0±2.0</td>
<td>79.0±10.0</td>
<td>67.0±9.0</td>
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<tr>
<td>VLDL (mg/dL)</td>
<td>20±1.0</td>
<td>32.0±2.0</td>
<td>41.0±3.0</td>
</tr>
<tr>
<td>TG (mg/dL)</td>
<td>102.0±4.0</td>
<td>166.0±8.0</td>
<td>204.0±8.0</td>
</tr>
<tr>
<td>ApoA1 (mg/dL)</td>
<td>169.0±4.0</td>
<td>162.0±8.0</td>
<td>131.0±10.0</td>
</tr>
<tr>
<td>Apo B (mg/dL)</td>
<td>68±7.0</td>
<td>98.0±8.0</td>
<td>146.0±11.0</td>
</tr>
<tr>
<td>Apo B/Apo A1 (mg/dL)</td>
<td>0.4±0.02</td>
<td>0.61±0.01</td>
<td>1.0±0.1</td>
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</table>
Table 2. Comparative analysis of the plasma lipid profile including apoproteins obtained in the subjects

<table>
<thead>
<tr>
<th></th>
<th>Diabetes mellitus patients without retinopathy (n=100)</th>
<th>Diabetes mellitus patients with retinopathy (n=61) Vs control</th>
<th>Diabetes mellitus patients with retinopathy (n=61) Vs diabetes mellitus patients without retinopathy (n=100)</th>
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</thead>
<tbody>
<tr>
<td>FPG (mg/dL)</td>
<td>-4.96</td>
<td>-9.42</td>
<td>-4.85</td>
</tr>
<tr>
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<td></td>
<td></td>
</tr>
<tr>
<td>“p”</td>
<td>0.02*</td>
<td>0.005**</td>
<td>0.02*</td>
</tr>
<tr>
<td>TC (mg/dL)</td>
<td>-5.37</td>
<td>-11.12</td>
<td>-6.27</td>
</tr>
<tr>
<td>“t”</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>“p”</td>
<td>0.02*</td>
<td>0.004**</td>
<td>0.01*</td>
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<tr>
<td>LDL-C (mg/dL)</td>
<td>-7.38</td>
<td>-27.29</td>
<td>-17.80</td>
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<tr>
<td>“t”</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>“p”</td>
<td>0.009**</td>
<td>0.0007***</td>
<td>0.002**</td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
<td>-1.08</td>
<td>0.12</td>
<td>0.89</td>
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<tr>
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<td></td>
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<tr>
<td>“p”</td>
<td>0.20</td>
<td>0.46</td>
<td>0.23</td>
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<tr>
<td>VLDL (mg/dL)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>“p”</td>
<td>0.02*</td>
<td>0.01*</td>
<td>0.06</td>
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<td>TG (mg/dL)</td>
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<td>-11.40</td>
<td>-3.21</td>
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<tr>
<td>“t”</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>“p”</td>
<td>0.01*</td>
<td>0.004**</td>
<td>0.04*</td>
</tr>
<tr>
<td>ApoA1 (mg/dL)</td>
<td>0.78</td>
<td>3.52</td>
<td>2.42</td>
</tr>
<tr>
<td>“t”</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>“p”</td>
<td>0.26</td>
<td>0.04*</td>
<td>0.07</td>
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<tr>
<td>Apo (mg/dL)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>“p”</td>
<td>0.07</td>
<td>0.01*</td>
<td>0.06</td>
</tr>
<tr>
<td>B (mg/dL)</td>
<td>-9.46</td>
<td>-6.23</td>
<td>-4.02</td>
</tr>
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<tr>
<td>“p”</td>
<td>0.005**</td>
<td>0.01*</td>
<td>0.03*</td>
</tr>
</tbody>
</table>

Fig. 1. Comparative description of the lipid profiles including ApoA1 and ApoB obtained in the subjects

There was also no significant difference in the plasma value of VLDL, Apo B and Apo A1 in patients with Diabetes mellitus and Retinopathy when compared with patients with Diabetes mellitus without Retinopathy (p>0.05; Tables 1 and 2, Figs. 1, 2).

4. 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, hypertriglycerideremia, 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].

5. 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.

6. 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.

COMPETING INTERESTS
Authors have declared that no competing interests exist.
REFERENCES


