Dyslipidemia and lipid peroxidation of Saudi type 2 diabetics with proliferative retinopathy

Yousef H. Aldebasi, BSc, PhD, Abdelmarouf H. Mohieldein, MSc, PhD, Yousef S. Almansour, MBBS, SSc (Ophth), Barakat L. Almutairi, MBBS, SB Med.

ABSTRACT

Objectives: To assess lipid profile and lipid peroxidation in type 2 diabetics with proliferative retinopathy (PDR), and investigate the association between these biochemical parameters and PDR.

Methods: This study was conducted between June 2011 and February 2012 in the Research Laboratory, College of Applied Medical Sciences, Qassim University, Qassim, Kingdom of Saudi Arabia. The study included 54 patients with type 2 diabetes (21 with PDR and 33 controls) and 30 healthy subjects. The biochemical parameters were measured using standard laboratory procedures.

Results: Patients with PDR characterized by significantly (p<0.05) increased levels of serum cholesterol, triglyceride, low density lipoprotein (LDL-C), plasma malondialdehyde; decreased levels of serum high density lipoprotein (HDL-C) and apolipoprotein A1 (Apo A1); positive correlation of malondialdehyde with triglyceride, but negative with HDL-C, Apo A1. In logistic regression, malondialdehyde, LDL-C, and Apo A1 were not associated with PDR. However, triglyceride (OR = 1.745; p=0.000), total cholesterol (OR = 0.079; p=0.000), and HDL-C (OR = 10.676; p=0.000) were independent risk factors for developing PDR.

Conclusion: Dyslipidemia and lipid peroxidation may play a role in pathogenesis of diabetic retinopathy. Patients with PDR displayed marked lipid abnormalities and increased lipid peroxidation. The control of lipid alterations through glycemic control and/or lipid lowering medication is required for type 2 diabetics at least to postpone or prevent loss of vision from retinopathy.


From the Department of Optometry (Aldebasi), the Department of Medical Laboratories (Mohieldein), College of Applied Medical Sciences, Qassim University, Qassim, the Ophthalmology Department (Almansour), and the Diabetes and Endocrinology Center (Almutairi), King Fahad Specialist Hospital, Buinadah, Kingdom of Saudi Arabia.

Received 18th February 2013. Accepted 12th May 2013.

Address correspondence and reprint request to: Dr. Yousef H. Aldebasi, Dean, College of Applied Medical Sciences, Qassim University, PO Box 6699, Buinadah 51452, Kingdom of Saudi Arabia. Tel. +966 503490004. Fax. +966 (6) 3801628. E-mail: dbasy@qu.edu.sa
Diabetes mellitus (DM) is a serious metabolic disease, with an increasing prevalence worldwide, and the number of affected people may reach 300 million by 2025. In Kingdom of Saudi Arabia, the prevalence of DM was estimated as 23.7% with more significant prevalence in males compared to females (26.2% and 21.5% respectively). Individuals with diagnosed diabetes are at an increased risk for vascular disease, including microvascular complications (namely, retinopathy, neuropathy, and nephropathy) and macrovascular complications (namely coronary heart disease and stroke), and lower extremity amputations. Patients with type 2 diabetes (T2DM) frequently have significant diabetic retinopathy (DR) at first diagnosis with prevalence rates vary from 5-35% due to a time lag between onset and clinical diagnosis. Diabetic retinopathy is a microangiopathy affecting the retinal capillary arterioles, capillaries and venules, namely, changes in the vascularization of the retina represent characteristic feature of DR. Based on severity of the disease, DR can be divided into background and proliferative retinopathy. Background retinopathy involves microaneurysms, intraretinal hemorrhages, clinically significant macular edema, venous beading, cotton wool spots, intraretinal microvascular abnormalities, and circinate retinal abnormalities. Proliferative diabetic neuropathy can include surface neovascularization, vitreous hemorrhaging, and tractional retinal detachments and eventually leads to complete blindness. The prevalence of proliferative retinopathy is somewhat higher among those with T2DM who require insulin to control their disease and is lower among those who do not. In diabetes, increased flux of glucose and free fatty acids is associated with mitochondrial reactive oxygen species (ROS) overproduction and, as a consequence, increased oxidative stress. Many studies have shown that a strong association exists between dyslipidemia and increased risk of macrovascular disease, including coronary heart disease, cerebrovascular disease, and peripheral vascular disease. However, the association of dyslipidemia with the pathogenesis of DR remains unclear, though it is thought to be a risk factor for its development.

Recently, it is reported that low serum apolipoprotein A1/B ratio was associated with proliferative diabetic retinopathy (PDR) in T2DM. Hardly, we had found a previous report in literature investigated the lipid level alterations and lipid peroxidation among Saudi diabetics with proliferative retinopathy. In the present study, we aimed to assess lipid profile and lipid peroxidation (measured by malondialdehyde [MDA]) in type 2 diabetics with proliferative retinopathy compared to those without retinopathy and to healthy subjects. Also, we investigated the correlations between lipid peroxidation and glucose lipid profile. Moreover, the association between the mentioned parameters and PDR was explored.

Methods. Literature search. We used PubMed and Google Scholar as scientific searching engines. The keywords, which we included in computer searches, were: type 2 diabetes, diabetic retinopathy, proliferative diabetic retinopathy, lipid profile, dyslipidemia, malondialdehyde, Apo A1, Saudi Arabia. According to the content of abstracts, the relevant full-text articles were downloaded. Only articles published in English language were considered and cited. For those articles, which need subscription, we utilized the e-journals databases of the academic digital library, Qassim University and Saudi Digital library (SDL).

Study design and subjects. This case-control study was conducted in the research laboratory, College of Applied Medical Sciences, Qassim University, Qassim, Saudi Arabia between June 2011 and February 2012. Twenty-one type 2 diabetics (age between 45 and 66 years) were examined and labeled as PDR by an ophthalmologist, Ophthalmology Department, King Fahad Specialist Hospital, Buraidah, Al-Qassim Province, Kingdom of Saudi Arabia. The inclusion criteria for patients with PDR include: Saudi citizen, male or female, history of T2DM, age more than 40 years, duration of diabetes more than 10 years. We excluded all secondary causes of retinal neovascularization, namely hypertensive retinopathy, patients with history of occlusive vascular diseases, history of uveitis.

Thirty-three type 2 diabetics were recruited as positive controls from the diabetes clinic of the same hospital. The inclusion criteria for type 2 diabetics were: Saudi citizen, male or female, having fasting blood glucose level equal to or more than 126 mg/dl based on the American Diabetes Association (ADA) criteria, have not been previously diagnosed with retinopathy, nephropathy or other diabetes complications. Those patients with type 1 diabetes were excluded from the study.

In addition, 30 healthy non-diabetic controls of both gender from general places were randomly selected as negative controls. Initially, they verbally informed that they had no history of diabetes or taking any hypoglycemic agents. Those participants with fasting blood glucose equal to or more than 126 mg/dl were excluded and not considered as negative controls. After informed consent were obtained, a questionnaire was used to obtain basic information.
from each participant, including age, educational level, current cigarette smoking status, and the occupation. The height and body weight were measured for each participant; then body mass index (BMI) was calculated as weight (kg) divided by height (m²).

**Diagnosis for retinopathy and labeling of proliferative retinopathy.** Type 2 diabetics underwent retinal examination by an ophthalmologist with indirect ophthalmoscope (condensing lenses like 78D, and 90D) after Pupil dilatation with cyclopentolate eye drops. Patients were labeled as suffering from PDR based on the following criteria: new vessels on the optic disc or within one disc diameter (NVD) and/or new vessels elsewhere on the retina along the vascular arcades (NVE).

**Blood sampling.** For each subject, fasting blood samples were collected in one EDTA vacutainer (4 ml) for preparation of plasma to determine malondialdehyde (MDA) and glucose; and one plain vacutainer (4 ml) for preparation serum to estimate total cholesterol (TC), triglycerides (TG), high-density lipoprotein-cholesterol (HDL-C), and apolipoprotein A1 (Apo A1). After centrifugation at 3000 RPM for 10 minutes, plasma and serum were separated and stored in Cryovials® at -80°C until analysis.

**Measurement of blood glucose and hemoglobin A1c.** Blood glucose was determined by the glucose oxidase-peroxidase (GOD-POD) method with commercial kits purchased from Human Diagnostics (Wiesbaden, Germany) using Hospitex Eos Bravo clinical chemistry analyzer. Glycated hemoglobin (HbA1c) was measured on whole blood samples by an immunoturbidimetric method using commercial kit (Vital Diagnostic, Italy).

**Measurement of lipid profile and apolipoprotein A1.** Serum TC, TG, and HDL-C were analyzed using enzymatic methods with commercial kits purchased from Human Diagnostics (Wiesbaden, Germany). Low-density lipoprotein-cholesterol (LDL-C) was calculated using Friedewald formula: [LDL-C = TC - HDL-C - TG/5 (mg/dl)]. Serum Apo A1 concentration was determined by an immunoturbidimetric method (Vital Diagnostic, Italy) with kits purchased from the same company. Dyslipidemia was defined as per the National Cholesterol Education Program (NCEP) Adult Treatment Panel (ATP) III guidelines: total cholesterol ≥200 mg/dL; LDL cholesterol ≥100 mg/dL; TG level ≥150 mg/dL; HDL cholesterol ≤40 mg/dL.¹⁶

**Measurement of malondialdehyde.** Plasma MDA was measured spectrophotometrically as the similar method applied in previous work¹⁷ using NWK-MDA kit (Northwest Company, Vancouver, USA). The method is based on the reaction of MDA with thiobarbituric acid (TBA); forming an MDA-TBA2 adduct that absorbs strongly at 532 nm.

**Ethical consideration.** The study was approved by Institutional Review Committee, College of Applied Medical Sciences, Qassim University, Qassim, KSA. The study was carried out according to principles of Helsinki Declaration. Participation was voluntary and verbal consent was acquired from each participant. Confidentiality of all participants was maintained as no names were requested.

**Statistical analysis.** All statistical analyses were performed using the statistical package for social sciences (SPSS) software (version 13). Continuous data were expressed as mean ± standard deviation (SD) while categorical data as number. Comparison of variables between 2 groups performed with an unpaired t-test for continuous data and chi-square for categorical data. The Pearson correlation was used to examine the relation between MDA and lipid profile parameters, blood sugar, and HbA1c. Univariate and multivariate analyses were performed; with PDR as the dependent variable, and parameters of lipid profile/peroxidation as independent variables. P-value less than 0.05 was considered significant.

**Results. Characteristics of the study participants.** The gender ratio was 1.0 male: 1.6 female for patients with PDR. While, in control groups the male to female ratio was 1:1.4 for diabetic controls and was 1:1 for healthy subjects. Regarding age of participants, diabetics with and without retinopathy were significantly older than healthy subjects (p=0.000 and p=0.021). Interestingly, diabetics seemed to have less education compared to healthy subjects (p=0.003) in that approximately 90% of patients with PDR and 81% of T2DM controls were having less than secondary education. Almost all participants in this study were overweight or obese with BMI ≥25 Kg/m². Moreover, all diabetics with or without retinopathy have poor glycemic control indicated by the values of glycated hemoglobin which were more than 7% as well as high blood glucose levels. Diabetics with retinopathy have longer duration of the disease than diabetics in the control group. Demographic and clinical characteristics of all participants showed in Table 1.

**Determination of lipid profile, apolipoprotein A1, and malondialdehyde.** As showed in Table 2, diabetics with or without retinopathy had significantly high levels of TG, TC, LDL-C compared to healthy subjects (p<0.05). In contrast to these findings, concentrations
of HDL-C and Apo A1 were significantly lower in diabetics with or without retinopathy when compared to healthy subjects. Moreover, the lipid peroxidation marker, MDA, was significantly higher (p=0.000) in diabetics with proliferative retinopathy than in subjects in control groups. When compared to diabetics without retinopathy; data showed that patients with PDR characterized by significantly low HDL-C (p=0.017) and high MDA (p=0.000).

**Correlation between malondialdehyde, lipid profile, blood glucose, and hemoglobin A1c.** In patients with PDR, Pearson correlation analysis revealed that the plasma concentration of MDA was positively correlated to the serum concentrations of TG (r=0.352, p=0.003) and HbA1c (r=0.295, p=0.010), and negatively correlated to the serum concentrations of HDL-C (r=-0.302, p=0.030) and Apo A1 (r=-0.413, p=0.045) (Table 3).

### Table 1 - Demographic and clinical characteristics of the study participants.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Proliferative diabetic retinopathy n=21</th>
<th>Diabetics without retinopathy n=33</th>
<th>Healthy controls n=30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (M/F)</td>
<td>08/13</td>
<td>14/19</td>
<td>15/15</td>
</tr>
<tr>
<td>Age (years)</td>
<td>60.38±5.4</td>
<td>51.82±9.9</td>
<td>44.57±10.9</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>77.76±26.1</td>
<td>81.39±14.8</td>
<td>69.63±14.0</td>
</tr>
<tr>
<td>Height (m)</td>
<td>152.67±19.0</td>
<td>160.76±11.5</td>
<td>155.50±15.5</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>29.71±5.9</td>
<td>31.35±6.4</td>
<td>29.77±7.7</td>
</tr>
<tr>
<td>Education **</td>
<td>&lt;Secondary</td>
<td>≥Secondary</td>
<td></td>
</tr>
<tr>
<td>Blood glucose level (mmol/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;7 mmol/l b</td>
<td>14.25±3.6</td>
<td>11.10±4.2</td>
<td>5.77±2.1</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>10.06±2.4</td>
<td>8.55±1.9</td>
<td>5.15±1.5</td>
</tr>
<tr>
<td>Duration of DM (year)</td>
<td>17.05±6.0</td>
<td>8.21±5.5</td>
<td>-</td>
</tr>
</tbody>
</table>

Data expressed as mean ± standard deviation (SD) for all variables except for gender (M/F) and education which expressed as number. P compared diabetics without retinopathy to healthy control, P compared diabetics with proliferative retinopathy to healthy control, P compared diabetics with proliferative retinopathy to diabetics without retinopathy. p<0.05, **Pearson Chi-Square = 0.003, *Normal range of weight based on WHO classification of body mass index.16

### Table 2 - Lipid profile and lipid peroxidation (MDA) in diabetics with proliferative retinopathy compared to diabetics without retinopathy and to healthy controls.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Proliferative diabetic retinopathy</th>
<th>Diabetics without retinopathy</th>
<th>Healthy controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglyceride (mg/dl) &lt;150a</td>
<td>170.12±5.3 (1.93mmol)</td>
<td>153.25±9.1 (1.74mmol)</td>
<td>133.76±5.6 (1.52mmol)</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl) &lt;200</td>
<td>207.81±4.5 (5.38mmol)</td>
<td>200.78±4.1 (5.18mmol)</td>
<td>181.47±4.0 (4.55mmol)</td>
</tr>
<tr>
<td>High density lipoprotein cholesterol (mg/dl)</td>
<td>36.12±1.8 (6.88mmol)</td>
<td>38.78±3.8 (1.01mmol)</td>
<td>48.17±1.1 (1.25mmol)</td>
</tr>
<tr>
<td>Low density lipoprotein cholesterol (mg/dl)</td>
<td>137.95±4.4 (3.59mmol)</td>
<td>132.10±5.5 (3.44mmol)</td>
<td>106.9±3.1 (2.78mmol)</td>
</tr>
<tr>
<td>Apolipoprotein A1 (mg/dl)</td>
<td>109.00±2.3</td>
<td>117.8±9.3</td>
<td>122.38±3.5</td>
</tr>
<tr>
<td>Malondialdehyde (µmol/l)</td>
<td>3.43±0.1</td>
<td>2.79±0.1</td>
<td>2.64±0.1</td>
</tr>
</tbody>
</table>

Data expressed as mean ± Standard error of mean (SEM), P compared diabetics without retinopathy to healthy control, P compared diabetics with proliferative retinopathy to healthy control, P compared diabetics with proliferative retinopathy to diabetics without retinopathy. p<0.05, *National Cholesterol Education Program-Adult Treatment Panel III desirable reference range.
Logistic regression analysis. Univariate analysis in patients with PDR showed significant association between PDR and parameters of lipid profile and lipid peroxidation (TG, TC, HDL, LDL, MDA) while ApoA1 was not significantly associated with PDR. Considering all variables together, the multivariate analysis revealed that only TG (OR=1.745, \( p < 0.001 \)), TC (OR=0.079, \( p = 0.000 \)), and HDL (OR=10.676, \( p = 0.000 \)) were associated with PDR (Table 4).

Discussion. New cases of blindness among 20-74 years old adults are attributed mostly to DR. Moreover, Over 60% of type 2 diabetics have retinopathy during the first 20 years of the disease.\(^{20,21}\) The Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR) showed that 1.6% of older-onset patients (type 2 diabetes) were legally blind in which one-third were due to diabetic retinopathy.\(^{22}\) Recently we have reported that hyperglycemia and poor glycemic control were obviously associated with DR.\(^{17}\) However, accelerated DR occurs in some individuals with modest hyperglycemia, while others never progress in spite of poor glycemic control over many years.\(^{23}\) This may imply the possibility of other factors that may play an important role in the pathogenesis of DR. Dyslipidemia as a metabolic abnormality is frequently associated with diabetes mellitus. Its prevalence is variable, depending on the type and severity of diabetes, glycaemic control, nutritional status, age and other factors.\(^{24}\) In agreement with recently published reports,\(^{14,25}\) our data demonstrated clear occurrence of lipid abnormalities in diabetics with proliferative retinopathy. In the Early Treatment of Diabetic Retinopathy Study (ETDRS); dyslipidemia, especially elevated triglycerides and LDL cholesterol, was found to be associated with increased risk of forming hard (lipid) exudates which are often found in the macular region.\(^{7}\) The origins of the dyslipidemia in diabetes are complex but derive from specific abnormalities in lipoprotein metabolism and abnormalities in insulin action. Insulin resistance as in abdominal obesity induces the release of free fatty acids (FFAs) into the circulation from adipocytes. These FFAs transported to the liver where they are assembled and secreted as very low density lipoprotein (VLDL), the secretion of which is not suppressed by meal-related insulin surges, resulting in hypertriglyceridemia.\(^{26}\) In addition to VLDL overproduction, reduced lipoprotein lipase activity and apolipoprotein (apo) C-III enrichment of VLDL may retard VLDL and remnant clearance. Moreover, cholesterol ester transfer protein stimulates exchange of cholesterol ester for triglyceride between both HDL and LDL and VLDL.\(^{27}\) Hydrolysis of triglyceride-enriched LDL and HDL by hepatic lipase, also considered to be upregulated in type 2 diabetes, results in the formation of small, cholesterol-poor HDL and LDL particle.\(^{25}\) In recent years, there has been an interest in the relationship of Apo A1 with DR.\(^{28}\) Apo A1 is the primary protein component...
of HDL particles. Apolipoprotein A1 interacts with HDL receptor and stimulates lecithin-cholesterol acyltransferase enzyme resulting in esterification of cholesterol, the essential step in the process of reverse cholesterol transport. Moreover, Apo A1 as well as HDL manifest anti-inflammatory and antioxidant effects. From this study, serum concentrations of Apo A1 and HDL were significantly low in patients with PDR compared to controls. Consistent with our findings, Hu et al recently reported significantly low apoA1 level \( (p=0.0304) \) in type 2 diabetics with proliferative retinopathy who have the disease over 15 years. These findings may indicate a role of Apo A1 in the pathogenesis of diabetic retinopathy although it is not fully elucidated. Interestingly, both apoA1 and Apo B were claimed by Sasongko et al to be more directly relevant to the biophysiological changes associated with diabetic retinopathy than the traditional lipids (namely, HDL and LDL cholesterol and triglycerides).

Furthermore, this study revealed increased lipid peroxidation in terms of MDA in diabetics with proliferative retinopathy when compared to controls. The plasma concentration of MDA was correlated positively with TG, HbA1c; and negatively with HDL-C, Apo A1. Since both HDL-C and ApoA1 have been reported to have anti-oxidant effects, the inverse correlation between MDA and HDL-C, ApoA1 may point to the role of oxidative stress in pathogenesis of diabetic retinopathy. There are a few biochemical mechanisms that explain the reason for such elevation of MDA. Hyperglycemia and dyslipidemia in diabetes induce increased lipid peroxidation and peroxyl radical formation, an important mechanism in genesis of microangiopathy. Again, the increase in the blood free fatty acid levels (depending on degree of lipolysis) along with a lack of insulin contribute to high plasma MDA levels. Moreover, retina, a tissue rich in polyunsaturated fatty acids with high glucose oxidation and oxygen uptake, experiences increased lipid peroxidation and oxidative stress result in biochemical changes contribute to both functional and structural changes in the retina microvasculature. Structural changes range from basement membrane thickening and microvascular cell loss to capillary closure and acellular capillary formation. In addition; in vitro studies have consistently shown that photochemical retinal injury is attributable to oxidative stress.

Data after univariate analysis revealed significant association of blood glucose, HbA1c, serum lipids and lipid peroxidation with PDR. While multivariate analysis revealed that only TG, TC, and HDL were associated with diabetic retinopathy and may be considered as independent risk factors for developing PDR. Consistent with our findings; Rema et al reported from regression analysis after adjusting age, gender, duration of diabetes that total cholesterol, non-HDL cholesterol, and triglycerides were significantly associated with diabetic retinopathy in type 2 diabetics.

The mechanisms by which dysglycemia and dyslipidemia use to cause diabetic vascular complications could be explained by that hyperglycemia can irreversibly induce glycation of both intracellular and extracellular proteins and lipids results in the formation of advanced glycation end-products (AGEs) which initiate oxidative reactions that promote the formation of oxidized low-density lipoprotein (ox-LDL) which is more atherogenic than naive LDL. It has been reported that LDL from diabetic patients displayed significantly elevated levels of both apoB- and lipid-linked AGEs, which correlated with the level of ox-LDL. Moreover; glycation of LDL is significantly increased in diabetic patients compared with the levels in normal subjects, even in those with good glycemic control. This may explain why accelerated diabetic retinopathy occurs in some individuals with modest hyperglycemia.

**The limitation of study.** One of the limitations of the current study is the small sample size of patients and controls. In addition, cases and controls were not matched. Therefore, we think that these findings could be considered as a preliminary report which may be replicated with a big sample size and well-matching between cases and controls.

**Implications of the result findings for future research.** We believe that there is a need for a future longitudinal prospective study to follow up the development, progress, and prognosis of retinopathy in type 2 diabetics (controlled and uncontrolled) who will receive lipid-lowering medications as well as anti-oxidants as supplementation to their treatment.

In conclusion, the pathogenesis of diabetic retinopathy is not completely understood at a cellular and molecular level; however, data from the present study pointing that dyslipidemia and lipid peroxidation may have a role in pathogenesis of DR. Type 2 diabetics with proliferative retinopathy displayed marked lipid abnormalities and increased lipid peroxidation. The control of lipid alterations through glycemic control and/or lipid lowering medication is required for type 2 diabetics at least to postpone or prevent loss of vision from retinopathy.
References


