THE ASSOCIATION OF OXIDIZED HIGH DENSITY LIPOPROTEIN 
AND OXIDIZED NON-HIGH DENSITY LIPOPROTEIN WITH THE 
DEVELOPMENT OF MICROALBUMINURIA IN DIABETIC 
NEPHROPATHY

1 Mohammed A. Al-Bayati, 2 Dina A. Jamil, 3 Hayder A. Al-Aubaidy*

1 Department of Chemistry & Clinical Biochemistry, College of Medicine, Al-Nahra
University, Iraq
2 School of Community Health, Charles Sturt University, NSW, Australia
3 School of Medicine, University of Tasmania, Hobart, Australia.

ABSTRACT
This study was undertaken to investigate the association of total lipid peroxides and oxidized HDL in the progression of microalbuminuria in diabetic nephropathy. Fifty-five patients with type 2 diabetes mellitus were recruited in this study and were divided into 2 main groups based on the presence of microalbuminuria, the first group (microalbuminuric group n=31) had a microalbuminuria between 30-299 ug/mg. The second group (normoalbuminuric group, n=29) had an albumin level less than 30 ug/mg. The two diabetic groups were compared to the control group (n=37). The results of our study showed significant elevation in the levels of serum lipid profiles and lipid peroxidation markers in the microalbuminuric group compared to the normoalbuminuric group at P<0.001. This was associated with significant changes in these markers between the normoalbuminuric group and the control group. In addition, there was also significant changes in the level of these markers in the microalbuminuric group compared to the normoalbuminuric group at P<0.001. The current study illustrates that the presence of microalbuminuria in type 2 DM can be regarded as an index of increased cardiovascular vulnerability and a signal for vigorous efforts at correction of the known risk factors to avoid the progression of diabetic nephropathy.

Keywords: Serum lipids, lipid peroxidation, microalbuminuria, type 2 diabetes mellitus.
INTRODUCTION
Due to the changes in the dietary habits and the increase in the incidence of obesity and overweight in both developed and developing countries, the prevalence of type 2 diabetes mellitus (T2DM) is growing at an exponential rate [1-3]. Previous studies showed that atherosclerosis is common in patients with diabetes mellitus (DM), and it causes an increase in incidences of microangiopathy and macroangiopathy [4, 5]. Macroangiopathy can in turn cause an accelerated form of atherosclerosis that affects important vessel sites, including the coronary, carotid, aortic, iliac, femoral popliteal tibial and peroneal vessels [4, 5].

Diabetic nephropathy (DN) is clinically defined by the presence of albuminuria, edema and hypertension. DN is the most common cause of renal failure in the western world [6, 7]. Dialysis and renal transplantation can have a devastating effect on quality and length of life of the patients [6, 7], besides, these are expensive which increase the financial burdens of the patients [8]. DN progresses from subclinical disease, through the earliest clinically detectable stage, characterized by microalbuminuria (urinary albumin 30 to 300 mg/day), to overt nephropathy with macroalbuminuria (urinary albumin >300 mg/day) [8].

Renal dysfunction is typically identified in the macroalbuminuria stage, and can progress over time to end stage renal disease [8]. Detection of patients with microalbuminuria can certainly help to prevent progression to later stages renal disease [6, 8] associated with cardiovascular complications and potential death [6].

Several risk factors have been contributed to the development of DN such as hyperglycemia, arterial hypertension and dyslipidemia [4]. Studies showed that microvascular changes begin when the diabetic state becomes overt (i.e. fasting blood glucose is at or higher than 126 mg/dL), and macrovascular changes begin many years earlier [9]. Microalbuminuria is associated with an increased risk for renal and cardiovascular morbidity and mortality in diabetic patients, patients with hypertension and in elderly subjects [9]. The presence of microalbuminuria is a strong predictor of DN and cardiovascular disease (CVD) in both type 1 and type 2 DM [10]. It has become increasingly clear, however, that microalbuminuria is not specific for diabetes or early nephropathy alone but is considered to reflect generalized vascular damage [11].

A number of studies have been conducted to determine the relative reliability of performing a protein- or albumin-to-creatinine ratio on a random urine sample as an alternative to using a
24-h urine sample. The protein- (or albumin-) to-creatinine ratio correlates to the 24-h total urine protein and significantly improves the reliability of the test results from random urine [11].

A study comparing the albumin-excretion rate to the albumin-to-creatinine ratio on a random sample found that the two results were essentially equal without the time and trouble involved in collecting an accurate 24-h sample [11]. In renal transplant patients, it was shown that the protein-to-creatinine ratio on a random urine sample was a useful and a convenient screening and longitudinal test for proteinuria (as compared to the 24-h total urine protein) [12, 13]. Albumin is normally present in urine at concentrations of less than 30 mg albumin/g creatinine (< 3.4 mg/mmol).

The current study aims to illustrate the association of oxidized high density lipoprotein and oxidized non-high density lipoprotein with the development of microalbuminuria in diabetic nephropathy

MATERIALS & METHODS

Study protocol and Participants

The study protocol was reviewed and approved by the Scientific and Ethics Committee of the College of Medicine, Al-Nahrain University. Informed consent was obtained from each subject. Study group comprised 55 patients (24 males and 31 females) with T2DM (mean age 51.6 ± 8.1 years). Patients were diagnosed according to the WHO definition [14] the patients were divided into two groups: microalbuminuric (group 1, n= 31 and normoalbuminuric (group 2), n = 24. All patients were recruited from the outpatient diabetes clinic of the Al-Kadhymia Teaching Hospital. The exclusion criteria included: Patients with any recent illness, impaired thyroid or renal function, diagnosis of renal disease, treatment with estrogen, glucocorticoids, or other drugs except oral hypoglycemic and/or beta blocker antihypertensive drugs. All patients included in the study were non-smokers; none were taking antioxidant supplements or drugs with known antioxidant activity. The mean duration of diabetes was (7.96 ± 3.45 years).

The control group consisted of 37 healthy, sex- and age-matched subjects (48.92 ± 8.9 years). They were recruited from the staff of the medical College of AL-Nahrain University and the Al-Kadhymia Teaching Hospital. The control group consisted of participants with no known medical history and with no family history of diabetes or nephropathy.
Blood Samples
Ten milliliters of venous blood samples were collected from each subject in the study after a 12 hour fasting. 2 milliliters were collected into EDTA containing tubes for glycated hemoglobin (HbA1c) assay. The remaining 8 milliliters were centrifuged at 3000 rpm for 10 minutes after about 30 minutes from the time of blood collection. Sera were separated for measurement of serum creatinine, serum lipids, serum malondialdehyde (MDA) level & oxidized HDL-C. Serum MDA level and oxidized HDL-C were assayed at the same day of blood collection, the sera were stored at -80 C°. All assays were obtained by running duplicates for the test, control and the standard.

Urine samples
Random morning urine Specimens were obtained from each subject in the study, to quantify albuminuria, creatinine and albumin to creatinine ratio. No urine preservatives were used; the samples were stored in appropriate containers and were kept at the refrigerator until the time of measurements.

Parameters of the study
A micro method was employed for the determination of urinary protein based upon the co precipitation of protein and ponceau S dye by trichloracetic acid (TCA), dissolution of the precipitate in dilute alkali and spectrophotometric determination of the dye in alkaline solution [15]. Serum and urine creatinine was estimated by the BioMerieux assay kit based on the method of Bartels et al [16].

Serum lipids were measure using BioMerieux assay kits for total cholesterol (TC), triglycerides (TG), high density lipoprotein cholesterol (HDL-C), and low density lipoprotein cholesterol (LDL-C).

Malonyldialdehyde (MDA), an end product of fatty acid peroxidation, can react with thiobarbituric acid reacting substance (TBA) to form a colored complex that has maximum absorbance at 532 nm. It serves as a convenient index of lipid peroxidation to measure total lipid peroxide and oxidized HDL-C.

Statistical analysis
Data are expressed as mean ± standard deviation of mean. Statistical significance was determined by ANOVA test followed by unpaired Student's t-test and Pearson’s correlation (r)
to test correlation of regression. P values equal or lower than 0.05 were considered statistically significant.

**RESULTS**

Diabetic patients (n = 55) were divided according to the urine protein (albumin) excretion measured in ug per mg creatinine (Table 1) into:

1. Patients with albumin-creatinine ratio that is equal to 30-299 ug/mg were considered to have microalbuminuric (n = 31)
2. Patients with albumin excretion less than 30 ug per mg creatinine were considered normoalbuminuric (n = 24)

All groups were closely age-matched, and the diabetic groups were well matched for duration of disease (Table 3-2).

**Table (1) Demographic and clinical data of the participants included in the study. Values were expressed as mean ± standard deviation. Significant difference was considered when P value equal or less than 0.05.**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>(Group 1) Diabetic Microalbuminuric</th>
<th>(Group 2) Diabetic Normoalbuminuric</th>
<th>(Group 3) Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>31</td>
<td>24</td>
<td>37</td>
</tr>
<tr>
<td>Male/female</td>
<td>11/20</td>
<td>13/11</td>
<td>15/22</td>
</tr>
<tr>
<td>Urine Albumin/creatinine ratio (ug/mg)</td>
<td>85.4±30.6 *</td>
<td>15.3±4.8*</td>
<td>11.4±2.5</td>
</tr>
<tr>
<td>Age/ years</td>
<td>49.5±7.6</td>
<td>52.2±8.2</td>
<td>48.9±8.9</td>
</tr>
<tr>
<td>Duration/ Years</td>
<td>8.0 (3.512) NS</td>
<td>7.83 (3.535) NS</td>
<td></td>
</tr>
<tr>
<td>HbA1c %</td>
<td>8.1±1.1*</td>
<td>7.68±0.9*</td>
<td>4.87±1</td>
</tr>
<tr>
<td>FBG/ mmol/L</td>
<td>8.9±2.4*</td>
<td>6.3±1.1</td>
<td>5.1±0.3</td>
</tr>
</tbody>
</table>

* Significant difference when P value equal or less than 0.05.

Serum lipids include TC, TG, HDL-C, LDL-C, atherogenic index (expressed as LDL-C/HDL-C) and LDL size index (expressed as TG/HDL-C) were measured in all the groups, Table (2). Group 1 showed a statistical significant elevation of TC compared to the control group (P<0.001). Such a relation was absent when compared Group 2 with the control group. However, there was a significant difference when comparing Group 1 and 2 (P=0.4).

There were significant elevation in serum TG and LDL-C in the diabetic groups (Group 1 & 2) compared to the control group at P<0.001. This was associated with a significant reduction
in the level of HDL-C in group 1&2 when compared to the control group (P<0.001) Table (2).

**Table (2): Serum Lipid Profile (mean ± SD) in Diabetic and Control Groups**

<table>
<thead>
<tr>
<th>Serum Lipids</th>
<th>(Group 1) Diabetic Microalbuminuric</th>
<th>(Group 2) Diabetic Normoalbuminuric</th>
<th>F Test</th>
<th>(Group 3) Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>T.C mmol/L</td>
<td>5.6±1*</td>
<td>5.29±0.78</td>
<td>0.04</td>
<td>4.4±0.58</td>
</tr>
<tr>
<td>TG mmol/L</td>
<td>1.9±0.2*</td>
<td>1.6±0.48*</td>
<td>&lt;0.001</td>
<td>1.28±0.4</td>
</tr>
<tr>
<td>HDLc mmol/L</td>
<td>1.09±0.2*</td>
<td>1.1±0.1*</td>
<td>0.8</td>
<td>1.4±0.2</td>
</tr>
<tr>
<td>LDLc mmol/L</td>
<td>3.5±1.1*</td>
<td>3.46±0.8*</td>
<td>0.03</td>
<td>2.3±0.5</td>
</tr>
</tbody>
</table>

* Significant difference when P equal or less than 0.05.

Both diabetic groups have increased atherogenic index (AI) and LDL size as compared with control group Figure (1). Furthermore, group 1 (microalbuminuric) had a significantly higher AI compared to the group 2 (normoalbuminuric) (P <0.05), Figure (1).

**Figure (1) Atherogenic Index (LDL-C/HDL-C) and LDL-C size Index (TG/HDL-C) in the studied groups.**

Lipid peroxides expressed as total malondialdehyde (MDA) and oxidized HDL was measured, then the value of oxidized non-HDL was obtained by subtracting the level of oxidized HDL from the total MDA. Serum MDA was significantly elevated in group 1 (microalbuminuric) compared to group 2 (normoalbuminuric) (P = 0.004) and also compared to the control group (P <0.001), Table (3).
Table (3): Lipid peroxidation and its fractions percentages in the two diabetic (group 1: microalbuminuric, group 2: normoalbuminuric) and the control group. Data were expressed as mean ± standard deviation.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Serum Malondialdehyde (umol/L)</th>
<th>Oxidized HDL-C %</th>
<th>Oxidized non-HDL-C %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetic Group 1</td>
<td>0.96±0.1*+</td>
<td>53.9±15.3*+</td>
<td>46.1±15.3*+</td>
</tr>
<tr>
<td>Diabetic Group 2</td>
<td>0.84±0.18*</td>
<td>72±11.6*</td>
<td>28±11.6*</td>
</tr>
<tr>
<td>Control Group 3</td>
<td>0.58±0.1</td>
<td>75.5±16</td>
<td>24.5±16</td>
</tr>
<tr>
<td>ANOVA Test</td>
<td>0.03</td>
<td>0.017</td>
<td>0.01</td>
</tr>
</tbody>
</table>

*Significant difference between the diabetic groups and the control group at P<0.05.
+Significant difference between the diabetic group 1 and 2 at P<0.05.

This was associated with a significantly reduction in the oxidized HDL% in group 1 (59.3±15.3%) compared to the control group (75.5 ± 16%), (P<0.001). The same correlation was seen when comparing group 2 (72±11.5%) with the control group at P<0.001, (Table 3, Figure 2).

Figure (2): The percentages of oxidized (HDL and non-HDL) cholesterol in two diabetic groups and the control group.

DISCUSSION

Our study proved that the degree of oxidative stress as represented by the MDA was significantly elevated in group 1 (microalbuminuric) compared to the group 2 (normoalbuminuric) and the control group. MDA was also increased significantly in group 2 compared to the control group. The present data confirms earlier reports [17, 18]. Jennings et
al, reported an increased serum conjugated diene levels in 26 diabetic patients with microangiopathy compared to 36 diabetic patients without microangiopathy[19].

Previous studies showed that plasma TBARS levels correlated with albumin excretion in type 1 and type 2 DM patients [20]. Plasma lipid peroxides were reported in human subjects with diabetes [21] and were found to be particularly elevated in patients with poorly controlled diabetes and with angiopathy [20].

The studies outlined above are consistent with increased oxidized lipids and lipoproteins in the plasma and tissues of certain categories of diabetic subjects. The studies discussed above together with the present results suggested that these increased levels may occur because lipids are more readily oxidized in the presence of increased glucose concentrations. This hypothesis is supported by the continued observations, in both animal models and humans, that in subjects with well-controlled diabetes (for example, in animals that receive insulin), lower levels of circulating lipid peroxides are found.

Mechanisms underlying impaired endothelial function in various disease states such as hypertension, diabetes mellitus, hypercholesterolemia, and atherosclerosis are multifactorial [4]. There is a growing evidence that oxidative stress contributes to the mechanisms of vascular dysfunction [22, 23]. These observations fit well with the recognition that increased oxidative stress may be central to the atherogenic process [23] and several different studies suggest that the development of microalbuminuria, type 2 diabetes, insulin resistance, or CVD may each precede the development of the others, providing support for a common etiology [4]. The accelerated atherosclerosis (atheroscleropathy) associated with the type 2 DM has been previously reviewed and is definitely a serious problem associated with the current epidemic of type 2 DM [24]. The mechanism of the link between microalbuminuria and cardiovascular mortality is unknown. However, increased urinary albumin loss has been postulated to be a marker of a generalized increase in the vascular permeability, which might predispose to greater penetration into the arterial wall of atherogenic lipoprotein particles [25]. Deckert’s hypothesis to understand the pathogenesis of albuminuria in diabetes is still well recognized [25]. This hypothesis has been usually cited as the steno hypothesis which implies that albuminuria reflects a wide spread of vascular damage due to a generalized vascular dysfunction in an overall vascular bed. However, microalbuminuria is thought to represent early generalized vascular (endothelial) damage [26]. In patients with microalbuminuria, the vascular endothelium fails to restrict the passage of macromolecules.
and loses its anticoagulant and profibrinolytic properties [26]. Microalbuminuria is an established marker of early renal damage in patients with type 1 DM. However, in type 2 diabetes mellitus, there is a clear association between albuminuria and mortality, with subclinical rises in albumin excretion being predictive of cardiovascular mortality [13, 24].

In conclusion, the presence of microalbuminuria in type 2 DM can be regarded as an index of increased cardiovascular vulnerability and a signal for vigorous efforts at correction of the known risk factors to avoid the progression of diabetic nephropathy.

REFERENCES
11. Rodriguez-Thompson, D. and E.S. Lieberman, Use of a random urinary protein-to-


