Correlation of ApoB/ApoA1 with diabetic nephropathy

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ABSTRACT

Introduction: Diabetic nephropathy is a microvascular complication and is the leading cause of diabetes related morbidity, mortality and important cause of end-stage kidney disease. Both microalbuminuria and macroalbuminuria are associated with increased risk of cardiovascular disease. Evidence has been accumulating from clinical trials that assessing the levels of apolipoprotein B (ApoB), a constituent of atherogenic lipoproteins: ApoA1, a component of anti-atherogenic high density lipoprotein (HDL) cholesterol; and the ApoB/ApoA1 ratio will provide better prediction of future cardiovascular events than measuring serum low-density lipoprotein (LDL)-cholesterol levels. There is paucity of published data linking ApoB/ApoA1 ratio to diabetic nephropathy especially from developing countries, hence this study was carried out. **Materials and Methods:** The present study was conducted in the Department of Medicine, CSM Medical University, Lucknow between August 2009 and July 2010. Patients with type 2 Diabetes Mellitus (DM) attending the Diabetic and Medical Out-Patient clinics or who were admitted to the medical wards of Gandhi Memorial and Association Hospital CSM University, Lucknow were included. One hundred patients were enrolled; 64 of those were cases (Micro– and Macroalbuminuria groups) and 36 without nephropathy (Normoalbuminuria) were controls. The cut-off value for higher ApoB/ApoA1 ratio for male was 0.97 and for female was 0.86. **Results:** Older age, durations and control of DM were significantly correlated with degree of albuminuria. Fifty-six patients (56%) had raised ApoB/ApoA1 ratio, 19.4% in the Normoalbuminuria group (n=7/36), 71.4% in the Microalbuminuria group (n=30/42), and 86.4% in the Macroalbuminuria group (n=19/22). There were no statistical differences in the mean total cholesterol, HDL, LDL, triglycerides among the groups. **Conclusion:** In our study higher ApoB/ApoA1 ratio was significantly correlated with diabetic nephropathy.

Keywords: Apolipoprotein A1, apolipoproten B, complication, diabetes mellitus, nephropathy

INTRODUCTION

Diabetic nephropathy is a microvascular complication of Diabetes Mellitus (DM) and is leading cause of DM related morbidity, mortality and also important cause of end stage renal disease. Both micro and macroalbuminuria are associated with increased risk of cardiovascular disease. [1] Several studies have shown that microalbuminuria is not only a predictor of proteinuria and DM nephropathy but also a prognostic indicator of early mor-

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tality from cardiovascular disease. [2, 3, 11] However, not all microalbuminuric patients will develop cardiovascular disease. So, there will be a definite role of other risk factors such as dyslipidemia, smoking, sedentary lifestyle which leads to manifestation of cardiovascular diseases. In the same way, not all microalbuminuric patients will develop nephropathy. [3]

DM nephropathy is divided into four phases: microalbuminuria, macroalbuminuria, the nephrotic syndrome, and chronic renal failure. Microalbuminuria (urine albumin 30 to 300 mg/d or <300 mg/g creatinine) is the first clinical sign of DM damage to the kidney. Macroalbuminuria (urine albumin >300 mg/d or >300 mg/g creatinine) usually denotes significant DM nephropathy and will be followed by a decline in glomerular filtration rate (GFR).

Atherogenic dyslipidemia, often called DM dyslipidemia in people with DM, is a condition associated with insulin resistance. This type of dyslipidemia is characterised by high levels of triglycerides (hypertriglyceridemia), high levels of small LDL particles and low levels of HDL. Even though most patients with diabetes don't have marked elevations of LDL cholesterol, their levels are high enough to support the development of atherosclerosis. [4]

For a number of years, evidence has been accumulating from clinical trials that assessing the levels of apolipoprotein B (ApoB), a constituent of atherogenic lipoproteins; ApoA1, a component of antiatherogenic high density lipoprotein (HDL) cholesterol; and the ApoB/ApoA1 ratio will provide better prediction of future cardiovascular events thanmeasuring serum low-density lipoprotein (LDL)-cholesterol levels. [5] There is paucity of published data linking ApoB/ApoA1 ratio to diabetic nephropathy especially from developing countries, hence this study was carried out.

The present study was conducted by the Department of Medicine, CSM University, Lucknow between August 2009 and July 2010. Patients with type-2-DM attending the Diabetic Out Patient Department clinics, Medical Out Patient Department clinics or who were admitted into the Medical wards of Gandhi Memorial and Association Hospital CSM University Hospital, Lucknow and fulfilling the inclusion criteria were included for the study. After informed consent, 100 patients were enrolled: 64 patients divided into two groups (Micro– and Macroalbuminuria) and 36 as controls (Normoalbuminuria).

MATERIALS AND METHODS

This was a cross sectional observational study conducted in various clinics and medical wards of several institutions.

Inclusion criteria

1) Patients with DM of five years or more.
2) Patients with estimated GFR >60ml/min by the Cockcroft Gault formula.
3) Patients able to give consent.

Exclusion criteria

1) Patients not meeting above mentioned criteria.
2) Patients having proteinuria due to other causes (i.e. urinary tract infection, congestive heart failure, pregnancy).
3) Patients on lipid lowering drugs.
4) Patients were selected as per inclusion criteria from the study population.

After taking detailed history and thorough physical examination, consent was taken from the patients or their relatives. Fasting

blood samples were taken from each patient for total cholesterol, HDL, LDL, triglycerides and non-fasting blood samples were taken for HbA1c, ApoB/ApoA1.

For the assessment of microalbuminuria/albuminuria, a 24 hours urine sample was collected in a five litre clean plastic container. All subjects were provided a labelled container containing 5ml toluene as preservative and a bag in which to carry the container. The patients were instructed to refrain from exercise for at least 24 hours before urine collection was started in the morning at 8:00 am after discarding the first urine passed, and then all urine produced for remainder of the day and overnight was added to specimen container till the next morning at 8:00am. Microalbuminuria was measured by Nephelometry method. On the basis of 24 hours urinary albumin patient were divided into three groups. Patients having albuminuria levels <30 mg/24 hrs were grouped as Normoalbuminuria, having albuminuria levels between 30-299 mg/24 hrs were grouped as Microalbuminuria and patients whose albuminuria levels were above 300 mg/24 hours were grouped as Macroalbuminuria. Normoalbuminuria patients were labelled as controls whereas microalbuminuria and macroalbuminuria patients were labelled as cases.

ApoB and ApoA1 were measured with the Nephelometry method from non-fasting blood samples of the patients. This method is based upon a comparison of the intensity of light scattered by the sample under defined condition with intensity of light scattered by a standard reference suspension. The higher the intensity of scattered light, the higher the turbidity. A standard suspension of formazin was used for calibration. The cut-off value for higher ApoB/ApoA1 ratio for male was 0.97 and for female was 0.86. [6]

RESULTS

The mean age of patients was 59.2 ± 10.4 years. Patients with macroalbuminuria were significantly older than those with normoalbuminuria and microalbuminuria and the mean duration DM was significantly longer among those with macroalbuminuria compared to the

Table 1: Comparisons between patients (microalbuminuria and macroalbuminuria) with controls (normoalbuminuria).

Characteristics	Normo (n=36) (28 M, 8 F)	Micro (n=42) (19 M, 23 F)	Macro (n=22) (11 M, 11 F)	*p* value for trend
Mean age (years)	58.1 ± 10.3	57.7 ± 10.9	63.5 ± 9.0	0.087
Mean duration of DM	6.3 ± 1.5	8.7 ± 5.8	11.9 ± 4.1	0.001
Mean GFR (ml/min)	83.5 ± 17.7	83.2 ± 19.7	84.5 ± 16.3	0.96
Median 24hrs urine albumin excretion (mg)	18 (6.5 to 27.0)	131 (31 to 280)	387 (310 to 4572)	
Hypertension	9 (25%)	13 (30.9%)	7 (31.8%)	
Mean HbA1c (%)	6.8 ± 0.6	8.3 ± 1.2	8.6 ± 1.4	0.0001
Mean LDL (mg/dl)	98 ± 10.5	99 ± 7.6	101 ± 10.6	0.50
Mean HDL (mg/dl)	42 ± 3.5	41 ± 2.7	41 ± 4.6	0.38
Total cholesterol (mg/dl)	167 ± 25	170 ± 27	171 ± 36	0.84
Triglycerides (mg/dl)	125 ± 22	132 ± 29	134 ± 21	0.32

Legends: M: Male, F: female, DM: diabetes mellitus, GFR: glomerular filtration rate, LDL: low density lipoprotein, HDL: high density lipoprotein

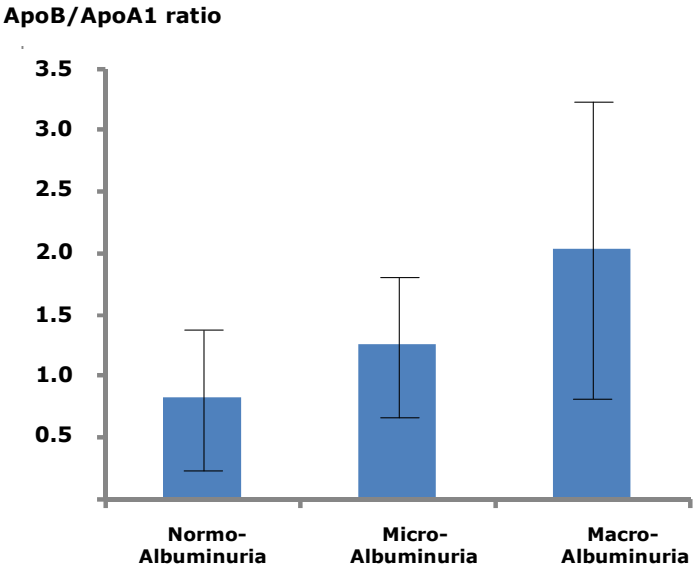

Fig. 1: The mean ApoB/ApoA1 for the three groups of patients ($p < 0.05$ for trend).

other groups.

The estimated GFR were comparable between the three groups and ranged between 60 ml/minute and 123.9 ml/min with a mean GFR of 83.6 ± 18.1 ml/min. The median 24 hour urinary albumin excretion and the mean HbA1c correlated with the degree of albuminuria. There was no correlation with the mean total cholesterol, HDL, LDL, triglycerides and hypertension among the groups.

Fifty-six patients had elevated Apo-B/ApoA1 ratio and there was a positive correlation with the degree of albuminuria (Figure 1). These differences were statistically significant (p <0.001).

DISCUSSION

Our study showed correlations between urinary albumin excretions or degree of nephropathy with several parameters in patients with type-2-DM seen in medical centres in Southern India. There were positive significant correlations and age, duration of DM, the mean HbA1c level and the ApoB/ApoA1 ratio but not the others parameters assessed. Mulec *et al.* also demonstrated that elevated serum cholesterol, triglycerides and ApoB correlated with decline in renal function among Swedish patients with type-1-DM. [7] Attman *et al*. also demonstrated higher ApoB levels in subjects with type-1-DM with nephropathy in comparison to subjects without nephropathy. [8] However, it is uncertain if there are any differences between type-1 and 2-DM.

Table 2: ApoB/ApoA1 ratio among the groups.

ApoB/ApoA1 ratio	Normoalbuminuria (n=36)	Microalbuminuria (n=42)	Macroalbuminuria (n=22)	Total (n=100)
Normal (Male <0.97 : Female <0.86)	29 (80.6%)	12 (28.6%)	3 (13.6%)	44
High	7 (19.4%)	30 (71.4%)	19 (86.4%)	56

X^2 = 31.8132 (df 2); p<.001

Microalbuminuria is regarded as a measure of generalised endothelial damage and it reflects trans-vascular albumin leak–age (endothelial permeability). Theoretically, such abnormality may lead to increased lipid insudation into the walls of blood vessels, thereby linking microalbuminuria to atherogenesis. [9] High level of ApoB and low level of ApoA1 lead to atherogenesis and may contribute to the development of nephropathy. Tamsma *et al*. showed that a higher interstitial ApoB/ApoA1 ratio in diabetic nephropathy patients compared to normoalbuminuria diabetic patients and control. [10] Our study also showed that the ApoB/ApoA1 ratio significantly correlated with the degree of nephropathy.

Apart from ApoB/Apo1 ratio, we also showed positive correlations with age, duration of DM and the sugar control with degree of albuminuria. Duration of DM indicates the length of time that patients have been exposed to the underlying pathology and age is also a reflection of duration of underlying DM. Chronic poor sugar control (serum HbA1c level) is an important marker of development of nephropathy.

Interestingly, despite finding a correlation between ApoB/ApoA1 ratio, we did not find any correlations between the lipid profiles with the degree of nephropathy. This may be due to the rapid turnover of the lipid parameters and they do not provide any indications of long control given that dyslipidemia can now be easily controlled with medications. Based on our findings, ApoB/ApoA1 may be helpful in selecting patients for treating dyslipidemia where traditional lipid profiles are within normal range.

There are several limitations to this study. The sample size is small and the groups were not well matched. There were several other confounding factors like significant differences in duration of DM and control as reflected by HbA1c among the groups. Further studies with control of confounding factors are required to establish the role ApoB/ApoA1 in diabetic nephropathy.

In conclusion, our study showed that higher ApoB/ApoA1 ratio was significantly associated with diabetic nephropathy. ApoB/ ApoA1 ratio is supposed to be predictor of coronary artery disease and it's relation with diabetic nephropathy supports the view that diabetic nephropathy is at least an expression of generalised atherogenesis. Further studies with larger sample sizes are needed to establish the role of ApoB/ApoA1 in diabetic nephropathy. It may also have therapeutic implications in the prevention of disease progression.

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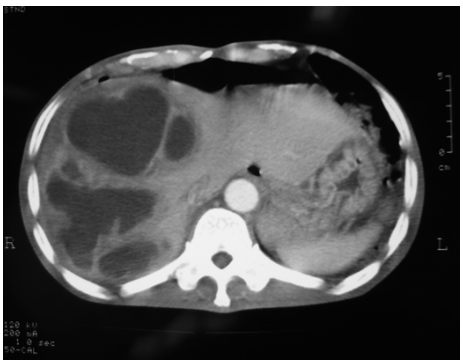
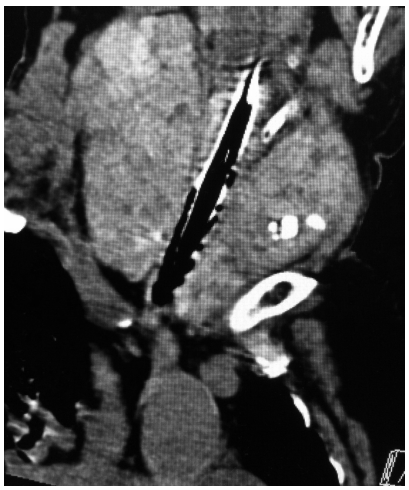
HUMERUS

ICB/VHC





First a Hearty Liver, then a Hearty Thyroid