Correlation of Plasma Apolipoprotein and lipid profiles with different stages of type 2 diabetic nephropathy- A Hospital based study in North Indian Population

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Abstract

Background- The aim of this study was to evaluate the lipid abnormalities associated with different stages of albuminuria and also examine role of ApoB lipoprotein as a predictor of early diabetic nephropathy in type 2 diabetic patients.

Methods and Results- A total 259 diabetic patients (110 men and 149 women) with mean age group 62.5±11.2 years were studied. Normoalbuminurea (n=110), microalbuminurea (n=93) and macroalbuminurea (n=56) were defined as albumin to creatinine ratio of <30, 30-299 and >300µg/micro respectively. Lipid parameters included, total cholesterol, triglyceride (TG), high and low density lipoprotein cholesterol, apolipoproteins A1, and Apo B, Lipoprotein (a). Result showed that Apo B differed significantly (P<0.05) between normoalbuminurea and micro and macroalbuminurea. Lp(a) differed between normoalbuminurea/ microalbuminurea and macroalbuminurea. Triglyceride increases progressively with increasing albuminurea. In multivariate logistic regression analysis, only Apo B showed significant odd ratio (95% confidence interval) for microalbuminurea: 1.012 (1.003-1.023): and both TG and Lpa where as significant for macroalbuminurea [(respective odd ratio 1.996) (1.010-3.937) and 1.707 (1.119-2.431)].

Conclusion- Apo B and Lp(a) increases in the stages of microalbuminurea and macroalbuminurea respectively. However, Triglyceride increases significantly throughout the 3 stages of albuminurea.

Index Terms- Dyslipidemia, Nephropathy, Apo A1, Apo B, Triglyceride, Type 2 diabetes

I. BACKGROUND AND OBJECTIVES

The prevalence of diabetes mellitus has been increasing worldwide with an expected doubling of diabetic population from 171 million to 366 million between 2000-2030. The greatest relative increase will occur in Middle Eastern Crescent, Sub Saharan Africa and India.[1] This diabetic population is predisposed to an increase risk of both micro and macrovascular complications and some 50% of people with diabetes die of cardiovascular disease.[2] Atherosclerosis which may began early in presence of diabetes, lipid and lipoprotein abnormalities can be a cause of increased cardiovascular complications in such patients[3].

Diabetic patients are also known to be at increased risk of dyslipidemia which can contribute to the higher morbidity and mortality.[4] Dyslipidemia in diabetes is characterized by elevated triglycerides, low high density lipoprotein (HDL) cholesterol levels, and increased low density lipoprotein (LDL).[5] There are four major groups of lipoproteins that are known, namely chylomicrons, very low density lipoprotein (VLDL), low density lipoprotein (LDL) and high density lipoprotein (HDL).The protein moiety of a lipoprotein is known as apolipoprotein which can be of different types with different functions . Apo B is the major protein component of VLDL and LDL in serum, forms an important part of their structure, while Apo A1 is a major protein component of HDL .[6]

Microalbuminuria, an early marker of diabetic nephropathy, is an independent risk factor for cardiovascular disease. Microalbuminuria is excretion of albumin 30 to 300 mg/day in a 24 hour urine collection or 30 to 300 µgram/mg creatinine in a spot collection. Diabetic nephropathy is characterised by progressive increase of urinary albumin excretion rate (UAER). The increased levels of urinary albumin excretion may represent a more generalised vascular injury alone. During the past decade, the incidence of end stage renal disease has risen dramatically, primarily due to an increase in the incidence of diabetes.

The presence of microalbumin in the urine of persons with type 2 diabetes is perhaps the most important early signal heralding the onset of systemic vasculopathy and associated with target organ damage (Brain, heart and kidney) . Microalbuminuria also identifies patients who need more rigorous cardiovascular risk management, especially more intensive blood pressure control, strict attention to glycemic control and lipid level. Interestingly it has been suggested that hyperglycemia, hypertension, and dyslipidemia cause disorders of albumin excretion rate by damaging the podocyte and slit diaphragm protein scaffold with overproduction of and extracellular release of oxygen radical species at the glomerular level.[7]

A study conducted on 1253 patients of type 2 diabetes over seven years by Bruno et al[8] and showed the progression of 3.7% patients to overt nephropathy every year and microalbuminuria provided a risk increased by 42% as compared to normoalbuminuria. This raises the question as to which of the above statistics shows the importance of early diagnosis, treatment and prevention of microalbuminuria in type 2 diabetic patients. Significant changes in the metabolism of lipoproteins

occur with the progression of diabetic nephropathy, the lipid abnormalities associated with different stages of diabetic nephropathy could be different and should better be evaluated in the respective stages of UAER with appropriate control of confounders. The aim of this study was to evaluate the lipid abnormalities associated with different stages of albuminuria and also examine role of ApoB lipoprotein as a predictor of early diabetic nephropathy in type 2 diabetic patients.

II. MATERIAL AND METHOD

The present study was a cross sectional study done over a period of two year in the Department of Medicine, CSM Medical University, Lucknow India from August 2009 to July 2011. Patient having type 2 diabetes mellitus, attending diabetic clinic and admitted in indoor medical wards of Gandhi Memorial & Associated Hospital, CSM Medical University, and fulfilling the inclusion criteria were enrolled in the study. After written informed consent 259 patients were studied. The study was approved by the ethical & research committee of CSM Medical University Lucknow to use human subject in the research study.
Patient with duration of diabetes for 5 years or more with GFR more than 60ml/min were included in the study. Subject not fulfilling the above mentioned criteria, proteinuria due to other causes like urinary tract infection, congestive heart failure, pregnancy and patients on Angiotensin Converting Enzyme Inhibitor (ACEI) / Angiotensin Receptor Blocker (ARB) , lipid lowering drugs were excluded from the study. After detailed history and thorough physical examination, relevant investigations were done.

Measurement of Albuminurea: A 24 hour urine sample was collected in a five litre clean plastic container. All the subjects were provided with a labelled container containing 5 ml toluene as preservative and a bag in which to carry the container. The patients were instructed to refrain from exercise at least 24 hours before urine collection which was started in the morning at 8:00 Am. After discarding the first voided urine sample, then all the urine of day and overnight was added to the specimen container till the next morning at 8:00 Am was subjected for measurement of microalbuminuria. Microalbuminuria was measured by Nephelometry method. On the basis of 24 hours urinary albumin, patients were divided into three groups. Normoalbuminuria, albumin excretion less than 30 mg/24 hour, microalbuminuria having albumin levels between 30-300 mg/24 hour, and macroalbuminuria were above 300 mg/24 hour. Creatinine clearance rate (Ccr, ml/min) was calculated from the Cockcroft – Gault formula as: [(140-age in years)×body weight in kg/ 72 × serum creatinine in mg/dl)]. For women, the values were multiplied by 0.85.

Lipid parameters: About 5 ml of venous blood were drawn under aseptic precautions, in a sterile bulb from selected subject after a period of overnight fasting; serum was separated by centrifugation and used for analysis. Serum lipid profile which includes triglycerides (TG), total cholesterol (TC), high density cholesterol (HDL-C) were measured by enzymatic method and serum low density cholesterol (LDL-C) and very low density cholesterol (VLDL-C) were calculated by using Friedwald formula (LDL-C=TC (HDL-C + TG/2.2).[9] Lipid profile was analyzed by using ERBA kits in microlab semi analyzer of MERK Company, all the reagents used in the estimation were of analytical grade. Serum Lp (a) was measured by turbidimetric immunoassay method. Serum apolipoprotein A1 (ApoA1) and apolipoprotein B (ApoB) were measured by Nephelometry method from random blood samples of the patients. This method is based upon a comparison of the intensity of light scattered by the sample under certain defined conditions with the intensity of light scattered by a standard reference suspension. The higher the intensity of scattered light, higher the turbidity. A standard suspension of Formazin is used for calibration.

Confounders: Age, sex, body mass index (BMI), duration of diabetes, history of hypertension, smoking, systolic blood pressure (SBP), diastolic blood pressure (DBP), blood urea , serum creatinine , fasting plasma glucose (FPG), glycosylated haemoglobin (HbA_{1c}), were treated as potential confounder.
Blood pressure was measured in the right arm after 20 minute rest on a sitting position with a standard mercury sphygmomanometer. Body height in centimetres and body weight in kilograms (kg) were measured with light clothes and bare feet , and BMI in kg/m^2 was calculated. Blood urea, serum creatinine and plasma glucose levels were measured on an automatic biochemistry analyzer (Transasia GM360 India). All the reagents used in the estimation were of analytical grade. HbA_{1c} was measured by means of boronate affinity chromatography.

III. STATISTICAL ANALYSIS

Distribution of microalbumin, TG, and Lp(a) were highly skewed, the natural logarithms of these variables were used for statistical analysis. Continuous variables were expressed as the mean (standard deviation±SD) and categorical variables, as percentage. P <0.05 was considered as statically significant. Differences in the continuous variables among the three subgroup of albuminuria were tested by one way analysis of variance (ANOVA) followed by multiple comparison test using least significant difference (LSD) when ever the p values for one way (ANOVA) were <0.05. The χ^2 test was used when the variables were categorical. Correlation coefficients between microalbuminurea and the lipid parameters were generated in all patients, in patients without macroalbuminurea and in patients with abnormal albuminuria (macroalbuminurea + microalbuminurea).

IV. RESULTS

A total of 259 type 2 diabetic patients (110 men and 149 women) with mean age 62.5±11.2 years were studied. Table 1 shows base line characteristics. Comparisons of the characteristics among the three subgroups of albuminuria are shown in Table 2. In addition to age and sex, BMI, diabetic duration, SBP, GFR were selected as potential confounder for adjustment in the logistic regression analysis. Blood urea and serum creatinine were not selected because they were highly associated with creatinine clearance, and history of hypertension was not selected because of its association with systolic blood pressure. ApoB was elevated in the early microalbuminuric stage and remained high throughout the later stage macroalbuminurea. On the other hand Lp (a) levels were relatively similar for normoalbuminurea and microalbuminurea, but increased

significantly at the macroalbuminuric stage. TG differed significantly among the three subgroup of albuminuria.

The correlation coefficients between urinary albumin excretion (UAE) and lipid parameters are shown in Table 3. When analysed in all patients, UAE was correlated significantly with TC, TG, ApoB and Lp (a) in a positive pattern and HDL-C in a negative pattern. While excluding patients with macroalbuminuria, UAE was significantly correlated with TC, TG and ApoB. When analysed in patients with abnormal albuminuria, UAE was correlated with TG significantly and with Lp (a) with borderline significance.

Table 4 shows the adjusted odd ratios for microalbuminuria and macroalbuminuria. After adjustment for the selected potential confounders and the other lipid parameters, only ApoB showed significant association with microalbuminurea, and both TG, Lp(a) were associated with macroalbuminuria.

V. DISCUSSION

It has been predicted that world wide the prevalence of diabetes in adults would increase to 5.4% by the year 2025 from the prevalence rate of 4.0% in 1995. Consequently the number of adults with diabetes in the world would rise from 135 million in 1995 to 300 million in the year 2025[10]. It is expected that much of this increase in prevalence rate will occur in developing countries. While a 42% increase is expected in developed countries, a 170% increase is expected in the developing countries. In the latter, most of the diabetic patients are in the age range of 45–64 years, while in developed countries most of them are ≥65 years. Therefore diabetic patients in developing countries are even more vulnerable to develop the micro- vascular complications of diabetes including diabetic nephropathy.

Lp(a) is an independent risk factor for cardiovascular disease, cause vascular injury through activation of intercellular adhesion molecule-1 expression, vascular smooth muscle cell proliferation, interaction with macrophages, prothrombosis and inducing apoptosis with oxidative stress.[11] The factors leading to elevated Lp(a) in more advanced renal disease are unknown, although increased hepatic synthesis and decreased renal excretion might be responsible.[12] It is true that the increased Lp(a) was not explained by a decreased GFR in the present study, because the effect of Ccr had been adjusted (Table 4). Whether a decreased catabolic rate of Lp(a) can be responsible but it required further investigations.

This study indicated a lack of association between Lp(a) and the early stage of microalbuminuria, but it is a major lipid parameter associated with macroalbuminuria (Tables 2-4). On the contrary, a Spanish study observed that type 2 diabetic patients with microalbuminuria had higher Lp(a) than those with normoalbuminuria (15.7mg/dL vs. 4.5 mg/dL, $p<0.01$); and a significant correlation coefficient of 0.34 ($0<0.01$) existed between Lp(a) and AER.[13] Another study indicated that Lp(a) was definitely increased in diabetic patients with macroalbuminuria, but increase of Lp(a) was only observed in microalbuminuric patients with significant decrease of Ccr or increase of serum creatinine.[14]

TG differed significantly among the three subgroups of albuminuria (Table2) and was correlated with AER for all ranges of albuminuria (Table 3). Furthermore, TG was independently associated with microalbuminuria after adjustment for selected confounders (Tables 4). But with further mutual adjustment of the lipid parameters the association with microalbuminuria became insignificant, and became statistically significant for macroalbuminuria (Table 4). The association between TG and albuminuria could possibly reflect an association between the atherogenic small dense LDL particles and albuminuria, because TG level was inversely correlated with LDL size (γ=-0.66, $p<0.001$)(24) and a TG level >1.5 mM (or 134 mg/dL) is significantly associated with small dense LDL .[15] However, further investigations are required to confirm this speculation.

The strength of this study included the recruitment of a large number of cases in each subgroup of albuminuria and analysis of a variety of lipid parameters. Most of the commonly encountered confounders were also adjusted for in statistical analyses. However, some limitations existed. Since it was a cross-sectional design, the cause and effect relationship between lipid parameters and albuminuria was not certain. Referral bias could not be excluded because of the hospital based analyses. However, we were not able to provide such information due to the lack of its collection at the recruitment of patients. Because glycemic control appeared to be suboptimal in the study subjects with FPG of 151.7 (51.3) mg/dL and HbA_{1c} of 7.6 (1.6)% (Table1) and FPG seemed to increase (through not statistically significant) from normoalbuminuria to microalbuminuria and to macroalbuminuria (Table2), residual confounding by glycemic control could not be completely excluded. However, the adjusted odds ratios derived from the logistic regression models as shown in Table 4 did not change significantly even when FPG or HbA_{1c} was added into the models in secondary analyses. Therefore, the conclusions would not be different even after excluding the possible residual confounding by glycemic control.

In summary, during the development of diabetic nephropathy, different atherogenic lipoproteins may play significant role in different stages. Apo B was associated with the early development of microalbuminuria and remained elevated throughout the course of abnormal albuminuria. On the other hand, Lp(a) is not increased in the early microalbuminuric stage, it is highly associated with the later stage of macoralbuminuria. TG may increase throughout the three stages of albuminuria.

Table -1: Base line characteristics of study subjects (N=259)

Age (years)	62.5±11.2
Gender	
Male %	45
Female %	55
Body mass index (kg/m^2)	24.8±3.6

Diabetics duration (years)	11.5±7.6
Smokers %	28.4
Fasting plasma glucose (mg/dl)	152.5±51.5
HbA1C(%)	7.8±1.4
Hypertension (%)	39.5
Systolic blood pressure (mmHg)	135.5±15.4
Diastolic blood pressure (mmHg)	80.5±9.6
Estimated glomerular filteratinrate (ml/min)	64.5±26.5
Blood urea nitrogen (mg/dl)	21.3±9.6
Serum creatinine (mg/dl)	1.4±0.8
Total cholesterol (mg/dl)	206.9±38.5
Triglyceride (mg/dl)	212± 25.6
High density lipoprotein cholesterol (mg/dl)	49.5±14.3
Low-density lipoprotein cholesterol (mg/dl)	114.6±28.6
Apolipoprotein A1 (mg/dl)	140.5±32.8
Apolipoprotein B1(mg/dl)	114.8±31.5
Lipoprotein (mg/dl)	55±10.5
Urinary albumin to creatinine ratio (µg/mg)	156±12.5

Date are expressed as mean ±SD or %

Table-2: Comparison among the three subgroups of albuminurea

Characteristics	**Normoalbuminurea**	**Microalbuminurea**	**Macroalbuminurea**	**p-value**
N	110	93	56	
Age (years)	63.5±10.5	65.4±10.7	62.6	0.065[a]
Gender				
Male (%)	45	47	46	
Female (%)	55	53	54	
Diabetic duration (years)	8.5±2.4	12.5±7.5	13.6±6.8	0.025[b]
Smokers	25.2	28.5	35.6	>0.1
Fasting plasma glucose (mg/dl)	148.4±45.6	155.2±56.5	156.4±53.5	>0.1
HbA1C (%)	7.4±1.5	7.6±1.68	7.8±1.5	>0.1
Hypertension (%)	35	42	47	0.056
Systolic blood pressure (mmHg)	130.4±16.5	136.5±16.5	137.5±18.5	<0.001[a,b,c]
Diastolic blood pressure (mmHg)	77.5±8.5	79.5±9.8	79.6±9.2	>0.1
Estimated glomerular filteration rate (ml/min)	67.5±23.5	65.5±25.3	62.6±24.8	<0.001[a,b,c]
Blood urea nitrogen (mg/dl)	16±6.2	22.5±10.8	25.6±11.6	0.001[a,b,c]
Serum creatinine (mg/dL)	1.02±0.96	1.05±0.94	1.42±0.92	0.015[b]
Total cholesterol (mg/dl)	196.2±36.8	205.6±40.2	206.3±36.3	0.05
Triglceride (mg/dl)	225.6±25.8	340.2±35.7	422.7±38.6	<0.001[a,b,c]
High density lipoprotein cholesterol (mg/dl)	48.8±14.6	47.5±13.5	45.6±12.8	0.005
Low density lipoprotein cholesterol (mg/dl)	112.6±31.6	115.2±28.7	114.5±28.9	>0.1
Apolipoprotein A1 (mg/dl)	137.2±32.5	140.2±31.5	138.5±29.5	>0.1
Apolipoprotein B (mg/dl)	106.8±27.5	118.4±34.5	119.4±28.5	<0.001[a,b]
Lipoprotein(a) (mg/dl)	35.2±5.2	42.5±8.6	55.6±10.5	<0.012[b,c]

Data are expressed as mean ±SD or %, p* value for one way ANOVA p<0.005
a, b and c indicate p<o.o5 for normoalbuminurea vs microalbuminurea,
normoalbuminurea vs macroalbuminurea and microalbuminurea vs macroalbuminurea

Table-3: Correlation coefficient between lipid parameter and microalbuminurea

Lipid parameters	All patients	Excluding macroalbuminurea	Excluding normoalbuminurea
N	259		
Total cholesterol	0.108*	0.096*	0.065*
Triglyceride	0.170**	0.094*	0.155*
High density cholesterol	-0.085*	-0.052	-0.036
Low density cholesterol	0.035	0.031	0.30
Apolipoprotein A1	0.027	0.051	-0.024
Apolipoprotein B	0.158**	0.166**	0.030
Lipoprotein (a)	0.086*	0.011	0.107+

+:0.05<p<o.1; * : p<0.05; ** : p<0.01

Table-4: Adjusted odds ratio for microalbuminurea (excluding macroalbuminurea) and macroalbuminurea (excluding normal albuminurea) estimated by every one unit increment of lipid parameters in type-2 diabetics patients.

Models	Adjusted odds ratio (95% confidence under)	
	Microalbuminurea	Macroalbuminurea
Lipid parameters entered separately		
Total cholesterol	1.004(1.000-1.010)+	1.000(0.992-1.008)
Triglyceride	1.403(1.015-1.942)*	1.500(0.882-2.552)
HDL-cholesterol	0.985(0.971-0.997)*	0.987(0.965-1.014)
LDL-cholesterol	1.001(0.996-1.007)	1.003(0.994-1.016)
Apolipoprotein A1	1.004(0.998-1.009)	0.997(0.986-1.008)
Apolipoprotein B	1.014(1.008-1.018)**	1.000(0.991-1.008)
Lipoprotein-a	0.987(0.841-1.161)	1.576(1.112-2.198**)
Lipid parameters entered simultaneously		
Total cholesterol	1.004(0.994-1.014)	0.993(0.975-1.006)
Triglyceride	0.956(0.637-1.431)	1.996(1.010-3.937)*
HDL-cholesterol	0.985(0.986-1.005)	0.994(0.962-1.026)
LDL-cholesterol	0.992(0.981-1.003)	1.008(0.992-1.026)
Apolipoprotein A1	1.002(0.996-1.007)	1.001(0.995-1.008)
Apolipoprotein B	1.012(1.003-1.023)**	0.997(0.985-1.012)
Lipoprotein-a	1.008(0.845-1.208)	1.707(1.119-2.431)**

Adjusted variables age, sex, body mass index, diabetes duration, systolic blood pressure, and creatinine clearance rate, +:-0.05<p<0.1, * :p<0.05; ** :p<0.01.

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