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RESEARCH ARTICLE

SERUM APOLIPOPROTEIN B-100 IN TYPE 2 DIABETES MELLITUS

[1]Oinam Prabita Devi, [*,1]Davina Hijam, [1]Abhishek Dubey, [1]NingthoujamOmita Devi, [1]Sungdirenla Jamir, [1]Sumpi Percy, [1]Chubalemla Longkumer, [2]Taruni Ng and [1]Amuba Singh, M.

[1]Department of Biochemistry, Regional Institute of Medical Sciences, Imphal, Manipur-795004, India
[2]Department of Medicine, Regional Institute of Medical Sciences, Imphal, Manipur-795004, India

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ABSTRACT

OBJECTIVES: To evaluate the levels of serum apolipoprotein B-100 in type 2 diabetes mellitus with and without complications.

MATERIALS AND METHODS: The study was conducted on 96 Type 2 Diabetes Mellitus patients (thirty three with cardiovascular complications and another sixty three without cardiovascular complications) attending Diabetic Clinic and Medicine Ward RIMS, Imphal. Serum Apo-B was estimated by ELISA and enzymatic methods were used for estimation of serum lipid parameters (Triglycerides, Total Cholesterol and HDL cholesterol were measured). Fasting and postprandial blood sugar were measured by glucose oxidase method.

RESULTS: Serum apolipoprotein-B levels for Type 2 Diabetes Mellitus patients with and without complications were found to be 2 (0.1 – 6.5) g/l and 1.1 (0.2 – 7.4)g/l respectively. Both of these were significantly higher ($P < 0.05$) than normal value (0.85 g/l – 1.0 g/l) and patients with cardiovascular complications had higher serum apo-B levels than those without complications ($p < 0.05$). Serum apo-B was positively correlated with total cholesterol ($r = 0.339$, $P < 0.05$), Triglycerides ($r = 0.373$, $P < 0.05$), VLDL ($r = 0.367$, $P < 0.05$) and LDL-cholesterol level ($r = 0.356$, $P < 0.05$) and was negatively correlated with HDL-cholesterol level ($r = - 0.393$, $P < 0.05$).

CONCLUSION: Raised serum apolipoprotein B-100 level may have some relationship with vascular complications in Type 2 Diabetes Mellitus especially coronary heart disease. Serum apolipoprotein B-100 may be a preferred marker of coronary heart disease in Type 2 Diabetes Mellitus patients.

INTRODUCTION

Diabetes Mellitus is a metabolic disorder of multiple etiologies characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from decreased insulin secretion, decreased insulin action or both (Report of a WHO Consultation, 2012). Apolipoproteins are the protein components of lipoproteins. Each class of lipoproteins have several apolipoproteins in differing proportions (Sahay, 2008). It is now established that the apolipoproteins of various lipoproteins regulate lipoprotein metabolism and determine the unique roles of these lipoproteins in lipid metabolism (Shaw *et al.*, 2010). Apolipoprotein B (APO-B) levels show a positive correlation with total cholesterol and with LDL cholesterol. Disorders producing marked changes in serum concentrations of apo–B include familial hypercholesterolaemia, in which levels may sometimes exceed 3.0g/l. It was found to be very low in both homozygous hyperbetalipoproteinaemia and familial abetalipoproteinaemia (Alberti, 2001).

Corresponding author: Dr. Davina Hijam,
Department of Biochemistry, Regional Institute of Medical Sciences, Imphal, Manipur-795004, India.

The lipoprotein abnormalities commonly present in Type 2 Diabetes Mellitus (T2DM) include hypertriglyceridemia and reduced plasma HDL cholesterol. A number of studies using tracer kinetics in humans have demonstrated that liver production of apolipoprotein B, the major protein component of Very Low Density Lipoprotein (VLDL) and Low Density Protein (LDL) is increased in T2DM (Report of a WHO study group, 1985). An increase in apo-B may be a perfect marker of coronary heart disease (CHD), since each atherogenic lipoprotein particle contains one molecule of apo-B; relying on total LDL would bias the results towards lower risk. There are abundant evidences that the risk of atherosclerotic vascular disease is directly related to plasma cholesterol levels. On the contrary, risk appear to be more directly related to the number of circulating atherogenic particles that contact and enter the arterial wall than to the measured concentration of cholesterol in these lipoprotein fractions. Each of the atherogenic lipoprotein particles contains a single molecule of apo-B and therefore the concentration of apo-B provides a direct measure of the number of circulating atherogenic lipoproteins. The present study is therefore taken up to evaluate the serum apo-B levels in T2DM patients with CHD in this North-Eastern part of the country. This study has been carried out with aim to

estimate serum apolipoprotein B-100 level in patients with type 2 DM and to access its role as a biomarker of cardiovascular complication in patients with type 2 DM. And, also to determine correlation between apolipoprotein B-100 and lipid profile in patients with T2DM with and without cardiovascular complications.

MATERIALS AND METHODS

A cross-sectional study was conducted in the Department of Biochemistry in collaboration with department of Medicine, Regional Institute of Medical Sciences, Imphal, Manipur from October 2011 to September 2013. Study was approved by the institutional review board and all participants gave written informed consent. The study group comprised of ninety six cases which were selected randomly from the diabetic patients who attended Diabetic Clinic in Regional Institute of Medical Sciences and/or admitted in Medicine Ward/ICCU irrespective of sex, religion and socio-economic status. These were divided into 2 groups based on presence of cardiovascular complications. Group 1 (n=63) T2DM without cardiovascular complication and group 2 (n=33) T2DM with cardiovascular complications. All cases were aged 18 years and above. Each individual enrolled in study underwent a detailed history, clinical examination and laboratory examination designed for the study. Diagnosis of T2DM was based on WHO criteria i.e. 12 hour fasting glucose of ≥ 126 mg/dl(7.0 mmol/l) with symptoms of diabetes and 2hour postprandial glucose level of ≥ 200 mg/dl (11.1 mmol/l) after 75 gm oral glucose. Participants suffering from diabetes were included in this study irrespective of their glycemic status. Diagnosis of coronary artery disease (myocardial infarction, angina) and ischemic heart disease was based on history, clinical findings and findings from serial tracing of 12 lead ECG.

Patients with hormonal disorder, cancer, congenital heart disease, signs of congestive heart failure and pericardial disease, signs of chronic obstructive pulmonary disease and with evidence of severe renal and/or hepatic impairment and on lipid lowering drugs were excluded from this study. Five ml of venous blood was collected from antecubital vein of each individual after an overnight fasting of twelve hours. 4.0 ml was collected in sterilized plain vial for the estimation of serum apolipoprotein B-100 and lipid parameters. One ml was collected in fluoride vial for estimation of fasting blood sugar. For estimation of post prandial blood sugar, about 1ml blood was collected 2 hours after meal. Blood in plain vial was centrifuged immediately for the estimation of serum apolipoprotein B-100 and lipid parameters. All the tests were carried out on the same day or within the next 15 days by keeping the serum in the refrigerator at 4°C to 8°C. Serum apolipoprotein B estimation was done by Enzyme Linked Immunosorbent Assay (ELISA) method using ELISA kit for Human apolipoprotein B from Mabtech AB, Sweden as per protocol described by (Flevet *et al.,* 1984). Fasting and postprandial blood sugar was estimated quantitatively by glucose oxidase technique. Serum lipid parameters i.e. Total Cholesterol (TC), Triglycerides (TG) and HDL cholesterol were estimated enzymatically using reagents by HUMAN, Germany. Serum LDL cholesterol was calculated by the formula of Friedwald *et al.* (Powers, 2006).

Statistical analysis

Statistical analysis was performed using SPSS version 16. Data were expressed in Mean ± SD. Statistical tests like χ2-test, independent t-test,ANOVA (F-test) and correlation coefficient 'r' were applied whenever found suitable and necessary. The P-value less than 0.05 was considered significant.

RESULTS

Table 1 shows the distribution of the number of study subjects along with percentage over the two groups considered according to their socio-demographic and behavioral factors. It shows 34.4% of the study population had cardiovascular complications while remaining 65.6% were free from any cardiovascular complication. Males (69.7%) were more common than that of females (30.3%) in diabetic patients with CVS complications which is in contrast to patients of T2DM without CVS complications where the number of females

Table 1. Socio-demographic characteristics of the study subjects

PARAMETERS		Diabetic without CVS complications N_1=63 (65.6%) N(%)	Diabetic with CVS complications N_2=33 (34.4%) N(%)	χ^2 value	P-value
Sex	male	29 (46.0)	23 (69.7)	4.885	0.032
	female	34 (54.0)	10 (30.3)		
Occupation	employed	15 (23.8)	11 (33.3)	2.816	0.245
	Self-employed	11 (17.5)	2 (6.1)		
	unemployed	37 (58.7)	20 (60.6)		
Marital status	married	62 (98.4)	33 (100)	-	-
	unmarried	1 (1.6)	0 (0.0)		
Alcoholism	alcoholic	18 (28.6)	9 (27.3)	0.018	0.893
	Non-alcoholic	45 (71.4)	24 (72.7)		
Smoking	smoker	20 (31.7)	15 (45.5)	1.757	0.264
	Non-smoker	43 (68.3)	18 (54.5)		
Education	illiterate	13 (20.6)	11 (33.3)	2.487	0.478
	<10th std	20 (31.7)	11 (33.3)		
	10-12 std	18 (28.6)	7 (21.2)		
	>12 std	12 (19.1)	4 (12.2)		
Treatment	*OHA	60 (95.2)	30 (90.9)	0.693	0.411
	Insulin	3 (4.8)	3 (9.1)		

*OHA- Oral Hypoglycemic agents

(54%) is more than that of males (46%). These patterns of sex distribution among the groups were significant as evident by P < 0.05.

apoliporotien B-100 levels had a significant (P < 0.05) positive correlation with Total cholesterol (TC), Triglycerides (TG), VLDL and LDL in both the groups. Serum apolipoprotein B-

Table 2. Group wise comparison of studied parameters

Parameters	Diabetic without CVS complications N_1=63	Diabetic with CVS complications N_1=33	P-value
Age (yr)	57.33 ± 9.639	64.39 ± 11.538	0.002
Duration of DM*(yrs)	7 (1 – 20)	9 (3 – 20)	0.078
Systolic BP* (mmHg)	130 (70 -120)	130 (100-190)	0.061
Diastolic BP* (mmHg)	80 (70 - 120)	80 (60 – 120)	0.584
Fasting BS* (mg/dl)	112 (69 – 194)	110 (69 – 194)	0.757
Postprandial BS* (mg/dl)	191 (130 – 328)	190 (88 – 280)	0.835
BMI	24.59 ± 2.30	23.89 ± 2.58	0.183
TC (mg/dl)	208.67 ± 43.02	213.06 ± 42.76	0.635
TG (mg/dl)	142.22 ± 44.45	134.91 ± 38.48	0.425
VLDL (mg/dl)	28.40 ± 8.94	27.00 ± 7.71	0.446
HDL* (mg/dl)	40 (18 – 86)	40 (24 – 77)	0.526
LDL (mg/dl)	136.94 ± 42.74	144.50 ± 43.28	0.414
S. Apo B-100* (g/l)	1.1(0.2-7.4)	2 (0.1 – 6.5)	0.006

Data expressed as mean ± SD; t-test applied *data expressed as median (range); Mann Whitney U test applied
Significant at P < 0.05

Table 3. Group wise correlation between serum apolipoprotein B-100 and other parameters

Parameters	Diabetes with CVS complications		Diabetes without CVS complications	
	Correlation coefficient(r)	P - value	Correlation coefficient(r)	P - value
Age (years)	0.026	0.837	0.165	0.359
Duration of DM (years)	0.188	0.139	0.095	0.600
Systolic BP (mmHg)	0.088	0.494	0.006	0.975
Diastolic BP (mmHg)	0.026	0.842	0.098	0.586
Fasting BS (mg/dl)	0.035	0.784	- 0.258	0.147
Postprandial BS (mg/dl)	0.036	0.777	- 0.146	0.417
BMI (kg/m^2)	0.225	0.076	- 0.157	0.383
TC (mg/dl)	0.339	0.007	0.568	0.001
TG (mg/dl)	0.373	0.003	0.686	0.000
VLDL (mg/dl)	0.367	0.003	0.682	0.000
HDL (mg/dl)	- 0.393	0.001	- 0.363	0.038
LDL (mg/dl)	0.356	0.004	0.532	0.001

Correlation is significant at P < 0.05

It is evident from Table 2 that the mean age of T2DM with cardiovascular complication was significantly (P < 0.05) older (64 years) than that of T2DM without complications (57 years). Median duration of diabetes in the group with cardiovascular complications was 9 years as compared to 7 years in T2DM without cardiovascular complications (P < 0.05). Median systolic and diastolic blood pressure levels in both the groups showed similar trends. Fasting and Postprandial blood sugar levels were higher in T2DM without cardiovascular complications. Mean BMI was slightly lower (23.89 ± 2.58) among T2DM with complications as compared to T2DM without complications (23.59 ± 2.30). There was not much difference in the serum levels of lipid parameters viz., total cholesterol, triglycerides, VLDL, HDL and LDL in the two groups. However serum apolipoprotein B-100 level has a median value of 2.0g/l in diabetes with cardiovascular complications which was higher than that in diabetes without cardiovascular complications (1.1 g/l). As evident from table – 3, no significant correlation was found between serum apolipoprotein B - 100 and age, duration of DM, systolic BP, diastolic BP, fasting blood sugar, post prandial blood sugar and body mass index (BMI) in both the groups. However serum 100 levels were negatively correlated with HDL in the two groups.

DISCUSSION

In the present study, 54.2% of T2DM patients were males and 45.8% were females. Thus, highest prevalence of T2DM was seen in males. This was consistent with the findings of (Nayak *et al.,* 2011) who reported occurrence of diabetes mellitus more in males (16.9%) than in females (11.1%). In this study, it was found that there was slight difference in concentrations of serum lipid parameters i.e. total cholesterol, triglycerides, VLDL, HDL and LDL between T2DM with and without cardiovascular complications and it was not significant. This has shown that despite normal serum lipid parameters, there is risk for cardiovascular complications in patients with T2DM. (Walldius *et al.,* 2004) stated that individuals with seemingly low or normal LDL – cholesterol can still be at risk of cardiovascular events. Our findings were in agreement with the above statement.

In this study, there were increased serum apolipoprotein B – 100 concentrations in both the study groups. In a study conducted on a population of African American males (Sumner *et al.,* 1999) found a significant elevated apolipoprotein B – 100 level in insulin resistant individuals. They have concluded that insulin resistance is correlated with a decreased fractional clearance rate of apolipoprotein B – 100 in VLDL and IDL and elevated plasma levels of apolipoprotein B. Insulin resistance, which is a prominent feature of T2DM might be one of the factor responsible for elevated apolipoprotein B – 100 levels in our study subjects. In the present study, it was seen that the median serum apolipoprotein B – 100 level was significantly higher in diabetes with cardiovascular complications than without complications (Table 2). Similar finding was observed in the study conducted by (Jiang *et al.,* 2004), where apo B concentration was significantly higher in diabetic men with cardiovascular complications than in diabetic men without complications. Glycated apolipoproteins are associated with increased free radical generation and increased oxidative damage to the lipid core. With extravasation of these modified lipoproteins, covalent binding to vascular structure proteins occurs, which may cause direct damage to the vascular wall (Akanji, 2002). Apolipoprotein B is known to be a glycoprotein, with about 4 – 9 % of its mass as carbohydrate linked to asparagines (Vauhkonen *et al.,* 1986). There are 19 potential N – linked glycosylation sites on apo B, three of these are glycosylated (Yang *et al.,* 1989).

LDL particles, not simply LDL – C, play a central role in atherogenesis. The initiating process is the sub – endothelial retention of intact apo B containing particles (Tabas *et al.,* 2007). LDL particles move into arterial intima through a gradient driven process, and the rate of passive diffusion is increased when the concentration of circulating LDL particles is increased (Nordestgaard *et al.,* 1995). Once inside the intima, the LDL particles bind to proteoglycans and initiate a process whereby the LDL particles become oxidized or otherwise modified and are taken up by monocytes or macrophages to form foam cells (Rudd *et al*., 2002). The cholesterol molecules contained in the LDL are "passengers", but the intact particles drive the atherosclerotic process. Low density lipoprotein cholesterol (LDL - C) has been cornerstone measurement for assessing cardiovascular risk for nearly 20 years. Recent data demonstrate that apolipoprotein B is better measure of circulating LDL particle number (LDL - P) concentration and is a more reliable indicator of risk than LDL – C, and there is growing support for the idea that addition of apoB measurement to the routine lipid panel for assessing and monitoring patients at risk for cardiovascular disease would enhance patient management (Contois *et al*., 2009). The present study showed that increased apolipoprotein B – 100 level was the most consistent and significant finding in T2DM patients with and without cardiovascular complications despite normal levels of traditional lipid parameters.

Conclusion

The study showed that in the absence of diabetic dyslipidemia, the only biomarker which was elevated in the serum of Type 2 Diabetes mellitus patients was apolipoprotein B – 100. Thus, it can be concluded that apolipoprotein B – 100 can be used as an independent biomarker for identifying patients at risk for developing cardiovascular disease in T2DM.

However, further studies would be required to determine whether genetic or disease related to apolipoprotein B – 100 metabolism account for elevated serum apolipoprotein B – 100 levels observed in T2DM patients with and without cardiovascular complications.

REFERENCES

Akanji, A.O. 2002. Diabetic dyslipidemia in Kuwait. *Med Principles Pract.,* 11 (2): 47-55.

Alberti, G. 2001. Noncommunicable diseases: tomorrow's pandemics. *Bull world Health Organ*., 79 (10): 907

Contois, J.H., McConell, J.P., Sethi, A.A., Csako, G., Devaraj, S., Hoefner, D.M. *et al.* 2009. Apolipoprotein B and cardiovascular disease risk: position statement from the AACC lipoprotrins and vascular diseases division working group on best practices. *ClinChem*, 55 (3):407-19.

Flevet, C., Koffigan, M., Ouvry, D., Marcovina, S., Moschetto, Y. and fruchart, J.C. 1984. Noncompetitive enzyme-linked immunoassay for apolipoprotein B in serum. *Clin Chem,* 30(1): 98-100.

Jiang, R., Stampfer, M.J., Schulze, M.B., Rimm, E.B., Li, T., Hu, F.B. *et al*. 2004. Non-HDL cholesterol and apolipoprotein B predict cardiovascular disease events among men with type 2 diabetes. *Diabetes Care*, 27 (8): 1991-97.

Nayak, H.K., Vyas, S., Solanki, A. and Tiwari, H. 2001. Prevalence of type 2 diabetes in urban population of Ahmedabad, Gujarat. *Indian Journal of Medical Specialities*, 2 (2): 101-5.

Nordestgaard, B.G., Wootton, R. and Lewis, B. 1995. Selective retention of VLDL, IDL and LDL in the arterial intima of genetically hyperlipidemic rabbits in vivo: molecular size as a determinant of fractional loss from the intima-inner media. *ArteriosclerThrombBiol*, 15: 534-42.

Powers, A.C. 2008. Diabetes mellitus. ln: Fauci AS, Braunwald E, Kasper DL, Hauser SL, Longo DL, Jameson JL et al, editors. Harrison's principle of internal medicine. 17th ed. New York: *Mc Graw Hill,* (2). P. 2275-304.

Powers, A.C. 2006. Diabetes mellitus. ln: Jameson JL, editors. Harrison's endocrinology. USA: *Mc Graw Hill*, P. 283-331.

Report of a WHO Consultation. 2012. Definition, diagnosis and classification of Diabetes Mellitus and its complications. Available at http://whqlibdoc.who.imt/hq/1999/WHO_NCD_NCS_99.2 .pdf. Accessed September 12, 2012.

Report of a WHO study group. 1985. Diabetes mellitus. WHO Technical Report Series 727. World Health Organization, Geneva.

Rudd, J.H., Davies, J.R. and Weissberg, P.L. 2002. Atherosclerotic biology and epidemiology of disease. ln: Topol RJ, editor. Textbook of cardiovascular medicine. 2nd ed. Philadelphia: Lippincot, Williams and Wilkins. P. 2-12.

Sahay, B.K. 2008. Diabetes mellitus – basic consideration. ln: Shah SN, editor. API textbook of medicine. 8th ed. Mumbai: *The Association of Physics of India*., (2). P. 1042-44.

Shaw, J.E., Sicree, R.A. and Zimmet, P.Z. 2010. Global estimates of the prevalence of diabetes for 2010 and 2030. *Diabetes Res Clin Pract,* 87: 4-14.

Sumner, A.E., Falkner, B., Diffenderfer, M.R., Barrett Ph. and Marsh, J.B. 1999. A study of the metabolism of apolipoprotein B-100 in relation to insulin resistance in African American males. *ProcSocExpBiol Med*., 221 (4): 352-60.

Tabas, I., Williams, K.J. and Boren, J. 2007. Subendothelial lipoprotein retention as the initiating process in atherosclerosis: update and therapeutic implications. Circulation., 116: 1832-44.

Vauhkonen, M. 1986. Complex-type carbohydrates of apolipoprotein B of human plasma low-density lipoproteins. *Glycoconj J.*, 3: 35-43.

Walldius, G. and Jungner, I. 2004. Apolipoprotein B and apolipoprotein A-1: risk indicators of coronary heart disease and targets for lipid-modifying therapy. *J Intern Med,* 255: 188-205.

Yang, C.Y., Gu, Z.W., Weng, S.A., Kim, T.W., Chen, S.H., Pownall, H.J. *et al.* 1989. Structure of apolipoprotein B-100 of human low density lipoproteins. *Arteriosclerosis.,* 9: 96-108.

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