Research Article

International Journal of Pharmaceutical Biological and Chemical Sciences

LIPOPPROTEIN LIPASE AND RISK OF CARDIOVASCULAR DIESEASES IN TYPE 2 DIABETICS: A CASE CONTROL STUDY

A.A.Momin[1*], M. P. Bankar[2], G. M. Bhoite[3], P. D. Zende[1]

[1]Assistant Professor, Department of Biochemistry,
Institute of Medical Sciences & Research, Mayani, Satara, Maharashtra, India
[2]Professor, Department of Biochemistry, B. J. Government Medical College, Pune, Maharashtra, India
[3]Assistant Professor, BVDU Dental college, Pune, Maharashtra, India
*Corresponding Author Email: rahaman.momin@gmail.com

ABSTRACT:

Diabetes is a metabolic disorder characterized by hyperglycemia, due to insulin deficiency or insulin resistance. Dyslipidemia is very common in type 2 diabetes. Lipoproteins play an important role in the pathogenesis of cardiovascular diseases, which are released from triglyceride in triglyceride rich lipoproteins to form HDL particles by the action of enzyme lipoprotein lipase. The present case control study was designed to evaluate the role of lipoprotein lipase in type 2 diabetes. Total 50 type 2 diabetic patients and 50 age and sex matched control subjects were included in this study. We investigated the blood glucose, glycosylated haemoglobin, serum cholesterol, triglycerides, LDL, VLDL and HDL cholesterol in study and control group. We found significant increase in the levels of fasting blood glucose, serum total cholesterol, LDL & VLDL cholesterol and triglyceride levels in patients with type 2 diabetes than controls. The HDL cholesterol and lipoprotein lipase activity was significantly lower in type 2 diabetics than in controls. These findings suggest that in type 2 diabetes patients the deficiency of lipoprotein lipase enzyme play an important role in the formation of dyslipidemia, thereby increasing the risk of cardiovascular diseases.

KEYWORDS:

Lipoprotein lipase, Lipid profile, type 2 diabetes mellitus, Triglyceride, HDL cholesterol, cardiovascular disease.

INTRODUCTION

The prevalence of diabetes is rapidly rising all over the globe at an alarming rate. India leads the world with largest number of diabetic subjects earning the dubious distinction of being termed the "diabetes capital of the world".[1] Type 2 Diabetes Mellitus is a heterogeneous condition characterized by the presence of both impaired insulin secretion and insulin resistance.[2] Patients with type 2 diabetes have increased risk of cardiovascular disease associated with the presence of dyslipidaemia.[3] Positive relationship is observed between hyperglycemia and atherosclerosis in type 2 diabetics.[4] The prevalence of dyslipidemia in diabetes mellitus is noted to be 95%.[5] A major lipid transported with lipoproteins is triglycerides, which is carried in large chylomicrons and VLDL particles. Lipoprotein lipase (LPL) hydrolyses triglycerides present in chylomicrons and VLDL, into two free fatty acids and one monoacylglycerol molecule, and move the hydrolyzed product, readily within the tissue down a concentration gradient. The concentration gradient is created by their use or re-esterification within cells.[6] The dyslipidemia in type 2 diabetes mellitus, particularly hypertriglyceridemia, is exacerbated by poor glycemic control, lipid abnormalities cannot be explained merely by hyperglycemia.[3]

LPL plays a crucial role in the regulation of lipoprotein metabolism by hydrolysing the core triglycerides of circulating chylomicrons and VLDL.[7] The function of LPL could be associated with dyslipidemia and characterized by hypertriglyceridemia and low HDL cholesterol levels.[8] Type 2 diabetes may influence the activity of the enzyme LPL and plasma lipoprotein concentrations and that this effect is superimposed on the similar changes produced by obesity alone.[9]

The rationale of this study was to detect the activity of LPL, and its association with triglyceride level in type 2 diabetic patients and to evaluate the etiology of dyslipidemia in type 2 diabetics.

MATERIALS AND METHODS

Subjects

The present case control study was carried out in Department of Biochemistry, B. J. Government Medical College, Pune. Study consisted of total 100 subjects which included type 2 diabetics (n=50) and age & sex matched healthy control subjects (n=50). Informed consent was taken from all subjects and study was approved by institutional ethics committee. Type 2 diabetic patients were recruited from Department of medicine, B. J. Government Medical College, Pune. The patients were free from any

clinical evidence of retinopathy, nephropathy, neuropathy, coronary heart disease, or hypertension. The control subjects were with no history of diabetes and without any medication affecting lipid metabolism.

Fasting blood samples were collected from study and control groups, for laboratory investigations using standard protocols for estimation of fasting glucose, glycated haemoglobin, serum total cholesterol, serum triglycerides, HDL cholesterol, Serum lipoprotein lipase activity.

METHODS

Fasting blood glucose was determined in patients with type 2 Diabetes mellitus and healthy controls using modified Glucose Oxidase-Peroxidase method[10], serum lipase activity was measured using Methyl-Resorusin method[11], serum triglycerides by Glycerol Oxidase Peroxidase method[12], serum total cholesterol by Cholesterol Oxidase Peroxidase method[13] and HDL cholesterol by enzymatic Cholesterol Oxidase Peroxidase method[14] after precipitation of chylomicrons, LDL & VLDL cholesterol. Serum Glycated hemoglobin using Boronate Affinity assay.[15] And LDL & VLDL cholesterol levels were calculated using Friedwald's formula.[16]

Statistical Analysis

The results were expressed as mean ± standard deviation (SD). Independent samples 't' test was used to compare mean values. P<0.05 was considered statistically significant.

RESULTS

In the present study patients presenting type 2 Diabetes mellitus were evaluated with the following results. Among 50 patients of type 2 Diabetes 21 patients were males and 29 were females. The mean ± SD of duration of diabetes among patients was found to be 4.29 ± 1.37 years. The BMI values were between 2.75 to 5.69 kg/m^2 with 95% confidence interval with mean BMI of 26.68 ± 3.66 kg/m^2 in patients and 22.46 ± 3.73 kg/m^2 in controls with P<0.0001. The difference in WHR was found to be significant with p value P<0.0001 as shown in **Table 1**.

Table 1: Demographic values of patients with type 2 diabetes mellitus and controls

	Controls (n=50)	Patients (n=50)	'P' value
Gender (Male/Female)	26/24	21/29	-
Age (years)	56.22 ± 14.15	53.70 ± 11.80	P = 0.3360
BMI (kg/m2)	22.46 ± 3.73	26.68 ± 3.66	P < 0.0001
WHR	0.86 ± 0.06	0.79 ± 0.05	P < 0.0001
Duration of Diabetes (years)	-	4.29 ± 1.37	-

The mean ± SD of fasting blood glucose level of patients was found to be 220.06 ± 79.78 mg/dl and in controls it was 99.48 ± 13.02. The difference between blood glucose level was significantly high at p<0.0001 when compared patients and the controls. The patients had also significantly increased values of HbA_{1c} (P<0.0001), the range of HbA_{1C} in type 2 diabetic patients was 3.40-11.50% as shown in **Table 2**.

Table 2: Biochemical parameters of patients with type 2 diabetes mellitus and controls.

	Controls (n=50)	Patients (n=50)	'P' value
Glucose (mg/dL)	99.48 ± 13.02	220.06 ± 79.78	P<0.0001
HbA_{1c} (%)	4.63 ± 1.10	8.20 ± 2.04	P<0.0001
Serum cholesterol	184.56 ± 31.60	226.98 ± 33.00	P<0.0001
Triglyceride (mg/dL)	144.08 ± 37.80	171.54 ± 38.52	P=0.0005
HDL cholesterol (mg/dL)	42.48 ± 3.98	35.35 ± 3.48	P<0.0001
LDL cholesterol	113.25 ± 30.51	157.32 ± 33.68	P<0.0001
VLDL cholesterol	28.81 ± 7.56	34.30 ± 7.70	P=0.0005
Lipoprotein lipase (IU/L)	48.70 ± 20.34	28.48 ± 12.60	P<0.0001

The difference between mean levels of serum cholesterol was found to be significant with 95% confidence interval from 29.59 to 55.24 mg/dl. The t test value for serum total cholesterol was 6.56 with 98 degrees of freedom and associated P value p<0.0001. The mean ± SD levels of triglycerides of the diabetic patients and controls were 171.54 ± 38.52 mg/dl and 144.08 ± 37.80 mg/dl respectively, with 95% confidence interval 12.31 to 42.60 mg/dl and 't' value of 3.6 and the difference was found to be significant at P=0.0005. The levels of LDL cholesterol in patients and controls were 157.32 ± 33.68 mg/dl and 113.25 ±

30.51 mg/dl respectively, with the confidence interval of 31.31 to 56.81 mg/dl, t value of 6.85 and associated P value of P<0.0001. The difference in mean values of VLDL cholesterol in patients (34.30 ± 7.70 mg/dL) and controls (28.81 ± 7.56 mg/dL) was found to be significant with P=0.0005.

The mean ± SD values of HDL cholesterol were 35.35 ± 3.48 mg/dl and 42.48 ± 3.98 mg/dl in diabetics and controls respectively with 95% confidence interval 8.62 to 5.65 mg/dl and associated P value P<0.0001. The enzyme activity of lipase in the serum of patients and controls significantly differed (P<0.0001) with the mean values of 28.48 ± 12.60 IU/L & 48.70 ± 20.34 IU/L respectively, with 95% confidence interval of 13.50 to 26.93 IU/L and t value 5.98. The levels of triglyceride and LPL activity in type 2 diabetes mellitus patients were found to be negatively associated with the correlation coefficient 0.12.

DISCUSSION

Type 2 diabetes is associated with a clustering of interrelated plasma lipid and lipoprotein abnormalities, which include reduced HDL cholesterol, a predominance of small dense LDL particles, and elevated triglyceride levels. Each of these dyslipidemic features is associated with an increased risk of cardiovascular disease.[3]

Control of diabetes improves the dyslipidemia, and if it is uncontrolled, there is marked abnormality in lipid status. In type 2 diabetes the major disturbances in lipoprotein metabolism are reflected by an increase in plasma triglyceride & LDL cholesterol and low HDL Cholesterol.[2]

Lipoprotein lipase plays a central role in lipid metabolism and is widely distributed in various tissues. In addition to its effect on the lipid metabolism, LPL is also directly or indirectly implicated in some pathophysiological conditions such as insulin resistance and type 2 diabetes mellitus.[3,17,18,19]

LPL enzyme activity is insulin dependent, and therefore it varies in diabetes according to ambient insulin level and insulin sensitivity. In untreated Type 2 diabetic patients subnormal LPL activity may contribute to the elevation of serum triglycerides, LDL cholesterol and to the reduction of HDL level.[19]

Results from our study have shown that glycated hemoglobin and blood glucose level in the diabetic patients significantly increased than normal healthy controls as expected. Serum total cholesterol, serum triglyceride, LDL & VLDL cholesterol were significantly higher in diabetic subjects than healthy controls and HDL cholesterol and LPL activity was significantly lower in type 2 diabetic patients compared to control group, this suggests that type 2 diabetes patients are predisposed to the development of cardiovascular diseases. We found a negative correlation between serum triglyceride level and LPL activity.

Similar results were found by Mingrone G et al[20] suggesting an inverse association between LPL and triglycerides. Our results are in accordance with Taskinen MR[19] who shown LPL activity is associated with increased serum VLDL & triglyceride levels, and decreased HDL concentration in both Type 1 and 2 diabetes mellitus.

Reduction of LPL activity is observed in patients with type 2 diabetes and individuals with insulin resistance.[17] The Gly188Glu substitution has a relatively large effect, while the more common Asp9Asn and Asn291Ser substitutions have moderate effect on decreasing LPL enzyme activity, leading to increase in plasma triglycerides, and decrease in plasma HDL cholesterol.[20] Monsalve MV et al[7] also stated that missense mutation Gly188Glu within exon 5, is responsible for LPL deficiency. Another potential factor is advancing age where a decrease in LPL activity has been reported.[21]

A reduction in LPL activity would therefore result in an increase in plasma triglycerides, through lower triglycerides hydrolysis, and a decrease in HDL due to decline in the rate of surface component influx into the HDL pool.[3, 6, 21]

CONCLUSION

Our results show, the increased triglycerides, LDL cholesterol and reduced HDL cholesterol due to decreased activity of lipoprotein lipase has increased risk of cardiovascular events in type 2 diabetics. There can be improvement in the preventive measure of dyslipidemia in type 2 diabetes mellitus, if cause of decreased LPL activity and potential role of insulin resistance is evaluated.

REFERENCES

1. Mohan V, Sandeep S, Deepa R, Shah B, Varghese C: Epidemiology of type 2 diabetes: Indian scenario. Indian J Med Res 2007; 125:217-230
2. Ahmed N, Khan J, Siddiqui TS: Frequency of dyslipidaemia in type 2 diabetes mellitus in patients of hazara division. J Ayub Med Coll Abbottabad 2008;20(2):51-54
3. Roberto T, Dodesini AR, Lepore G: Lipid and Renal disease, J Am Soc Nephrol. 2006; 17:S145-S147
4. Yaomin H, Yan R, Robert ZL, Xiang M, Xiujun L, Xu C et al: Novel mutations of the lipoprotein lipase gene associated with hypertriglyceridemia in members of type 2 diabetic pedigrees. J. Lipid Res. 2007; 48:1681–1688
5. Chattanda SP, Mgonda YM: Diabetic dyslipidemia among diabetic patients attending specialized clinics in Dares Salaam. Tanzania Med. J. 2008; 23(1):8-11
6. Eckel RH, Yost TJ, Jensen DR: Alterations in lipoprotein lipase in insulin resistance. International journal of obesity and related metabolic disorders: journal of the International Association for the Study of Obesity 1995, 19 (Suppl 1):S16–S21.

7. Monsalve MV, Henderson H, Roederer G, JulIen P, Deeb S, Kastelein JJP et al: A missense mutatio1n at codon 188 of the human lipoprotein lipase gene is a frequent cause of lipoprotein lipase deficiency in persons of different ancestries. J. Clin. Invest. 1990; 86:728-734
8. Yaomin H, Wei L, Rong H, Xiaoying Z: A systematic review and meta-analysis of the relationship between lipoprotein lipase Asn291Ser variant and diseases. J. Lipid Res. 2006; 47:1908–1914
9. Taskinen MR, Nikkilä EA, Kuusi T, Harmo K: Lipoprotein lipase activity and serum lipoproteins in untreated type 2 (insulin-independent) diabetes associated with obesity. Diabetologia. 1982; 22(1):46-50
10. Trinder, P: Glucose assay: A colorimetric enzyme-kinetic method assay. Ann. Clin Biochem 1969; 6: 24.
11. McNeely M. Lipase. Kaplan A et al. ClinChem The C.V. Mosby CoSt Louis. Toronto. Princeton 1984; 1130-1134, 892.
12. Bucolo G, David H: Quantitative determination of serum triglycerides by the use of enzymes. Clin Chem, 1973; 19(5):476-482
13. Allain CC, Poon LS, Chan CS, Richmond W, Fu PC: Enzymatic determination of total serum cholesterol. Clin Chem, 1974: 20(4):470-475
14. Warnick GR, Nauck M, Rifai N: Evolution of method for measurement of HDL cholesterol: from ultracentrifugation to homogenous assays. Clin Chem, 2001, 47(9):1579-1596
15. Jeppsson JO, Kobold U, Barr J, Finke A, Hoelzel W, Hoshino T et al: Approved IFCC reference method for the measurement of HbA1c in human blood. Clin Chem Lab Med, 2002; 40(1):78-89
16. Friedewald WT, Levy RI, Fredrickson DS: Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972; 18:499-502.
17. Yifan H, Xiaoxia L, Maoqing W, Hua N, Lima A, Ying L et al: Lipoprotein lipase links vitamin D, insulin resistance, and type 2 diabetes: a cross-sectional epidemiological study. Cardiovascular Diabetology 2013, 12:17
18. Wang H, Eckel RH: Lipoprotein lipase: from gene to obesity. Am J Physiol Endocrinol Metab. 2009; 297: E271–E288
19. Taskinen MR: Lipoprotein lipase in diabetes. Diabetes Metab Rev. 1987; 3(2):551-70.
20. Mingrone G, Henriksen FL, Greco AV, Krogh LN, Capristo E, Gastaldelli A et al: Triglyceride induced diabetes associated with familial lipoprotein lipase deficiency. Diabetes 1999, 48(6):1258–1263.
21. Henderson HE, Kastelein JJP, Zwinderman AH, Gagné E, Jukema JW, Reymer PWA et al: Lipoprotein lipase activity is decreased in a large cohort of patients with coronary artery disease and is associated with changes in lipids and lipoproteins. Journal of Lipid Research. 1999; 40:735-743

***Corresponding author address:**
Abdulrahaman A. Momin
Assistant Professor,
Department of Biochemistry,
Institute of Medical Sciences & Research, Mayani,
Satara, Maharashtra.
Phone number- +919975966972
e_mail ID: rahaman.momin@gmail.com