Scholars Journal of Applied Medical Sciences (SJAMS) **ISSN 2320-6691 (Online)**
Sch. J. App. Med. Sci., 2014; 2(3D):1119-1122 **ISSN 2347-954X (Print)**

(An International Publisher for Academic and Scientific Resources)
www.saspublishers.com DOI: 10.36347/sjams.2014.v02i03.051

Research Article

The Study of lipid Profile and Oxidised-LDL in Type 2 Diabetes Mellitus

P.V. Kiranmayi[1*], N. Vivekanand[2], Vijaya Laxmi Pandit[3]
[1]Associate Professor, Department of Biochemistry, Govt. E. N. T. Hospital, Koti, Hyderabad, India
[2]Associate Professor, Department of Pathology, Rajiv Gandhi Institute of Medical Sciences, Adilabad, India
[3]Rtd. Professor of Biochemistry, Gandhi medical college, Secunderabad.

***Corresponding author**
P.V. Kiranmayi
Email: kiranmayi.asp@gmail.com

Abstract: Aim of the study was to determine the usefulness of lipid profile& oxidized low density lipoprotein (LDL) in the identification of coronary artery disease (CAD) in Type 2 diabetes mellitus (T2DM) Lipid profile was estimated by using enzymatic methods and levels of oxidized LDL were measured by baseline diene conjugation method. Compared with control subjects, T2DM patients had higher levels of-1) Oxidized LDL (P<0.001), and the Lipid profile shows Increased levels of 2) cholesterol (p<0.001), 3) Triglycerides & VLDL (P<0.001),4) LDL-cholesterol(p<0.001) & decreased HDL cholesterol(p<0.05). The sensitivity for coronary artery disease was 81% for circulating oxidized LDL. Thus, circulating oxidized LDL is a sensitive marker of coronary artery disease. Consideration of oxidized LDL as a major risk factor and its addition to the list of established risk factors will facilitate and improve cardiovascular risk prediction to a very large extent.
Keywords: Low density lipoprotein, Coronary artery disease, Type 2 diabetes mellitus, Oxidized LDL (OXLDL), Atherosclerosis

INTRODUCTION

Type 2 diabetes exists in all populations but with varied prevalence [1]. In 2011 census, 5.3% of the Indian population was >65 years of age. Prevalence has risen as the population ages and becomes more obese. The prevalence rate of T2DM in elderly population is 30.42%. Almost equal numbers of both the sexes are affected, the ratio being (1:0.97). 64.04% have central obesity [2]. Aphenomenon called urbanization may contribute for the increasing incidence.

Seventy to eighty percent of morbidity and mortality in T2DM is due to premature atherosclerosis, i.e., diabetic macro-angiopathy which is the cause for Coronary artery disease, cerebrovascular disease and Peripheral vascular disease.

Though atherosclerosis is multifactorial, in T2DM patients, apart from hyperglycemia , dyslipidemiaseems to be an important risk factor along with hypertension and smoking. T2DM accounts for>90% of cases of metabolic defects is related to insulin resistance and relative insulin deficiency.

Insulin resistance is the characteristic feature of human obesity. Obesity is the major risk factor for diabetes mellitus and as many as 80% of patients with NIDDM have central obesity [3]. In one of the recent studies, out of all the female patients studied 84.84% had abdominal obesity, whereas only 15.15% were normal as per the WHR indicator. In case of male patients 40.70 % of the patients had abdominal obesity. These results clearly indicate that the overweight/abdominal obesity persists in T2DM patients which is used as a major indicator in assessing diabetes [4].

This combination of insulin resistance & failure of beta cell compensation is characteristic of NIDDM. Insulin resistance in obesity is seen in each of the major tissues involved in regulation of glucose homeostasis including liver, muscle and fat cell.

The Abdominal (subcutaneous and intra abdomen) fat is metabolically more active. Insulin resistance leads to increased lipolysis resulting in higher fluxes of Free fatty acid(FFA) reaching the liver and to the peripheral circulation [5]. High hepatic fluxes of FFA lowers the hepatic insulin extraction and thereby inducing hepatic insulin resistance and induces peripheral hyperinsulinemia which results in insulin resistance peripherally by down regulating the insulin receptors. Further high circulating FFAs competitively inhibit glucose uptake by the muscles thus inducing peripheral insulin resistance [6]. Apart from these elevated FFA levels, the metabolically active adipocytes are the

sources of pro-inflammatory cytokines namely IL-6 and TNF-α which may be directly responsible for induction of insulin resistance.TNF-α blocks the auto-phosphorylation of tyrosine kinase, the first step in insulin action [7].

The characteristic dyslipidemia in metabolic syndrome which favors atherogenesis is an elevated VLDL, lowered HDL and qualitatively altered LDL which becomes small and dense which is more readily oxidized and more atherogenic, being non recognized by LDL receptors but taken up by macrophages which intern become the foam cells.

The increased FFA fluxes to the liver resulting in increased synthesis of VLDL [8]. Because of hepatic insulin resistance even postprandial VLDL synthesis is not suppressed. Apart from increased hepatic VLDL synthesis, its clearance too is impaired because of impaired lipoprotein lipase activity due to insulin resistance. Normal activity of cholesterol ester transport protein results in transfer of cholesterol esters between VLDL, LDL and HDL. Thus VLDL becomes cholesterol rich and LDL and HDL become rich in triglycerides. Then they reach liver, lose the triglyceride merely by the action of hepatic lipase, whose activity is normal despite insulin resistance, thus converting into small dense LDL& small dense HDL which are poor cholesterols scavengers because of impaired reversed cholesterol transfer.

The new and emerging risk factor for coronary artery disease in these patients is oxidized low density lipoproteins. The increased serum concentration of this modified LDL is the cause for increased atherogenesis and hence coronary artery disease.

The major cy**t**otoxin of oxidized LDL is 7β-OOH cholesterol, which is shown to interfere with cell cholesterol metabolism, whose presence was demonstrated in human carotid atherosclerotic lesion. Cell death was postulated to occur due to lipid-peroxidation via a sequence involving lipid hydro peroxide induced formation of alkoxyl, lipid and peroxyl radicals. This cell injury by oxidized LDL & its toxic moiety, i.e. 7β-OOH cholesterol, indicate the specific intervention believed to be in the vascular lesion department [9].

70%-80% of morbidity and mortality in type 2 diabetes is because of premature accelerated, diffuse atherosclerosis, otherwise known as macro-angiopathy presenting as coronary artery disease, cerebrovascular disease and peripheral vascular disease.

Hyperglycemia, hypertension, smoking and dyslipidemia are the major risk factors for the diabetic macro-angiopathy apart from platelet – coagulation disorders, dysfibrinolysis and endothelial dysfunction.

Type 2DM is only a component of the metabolic syndrome(insulin resistance syndrome).Hence the lipid abnormalities noted in type2DM are because of insulin resistance, resulting in high VLDL, small dense LDL and small dense HDL. Oxidized LDL is not recognized by native LDL receptor, hence cleared by scavenger receptors particularly by macrophages, making them as foam cells the first lesion of atherosclerosis.

Further there is increased oxidative stress in diabetics because of auto-oxidation of glucose, increased polyolpathway and glycation of proteins. This leads to oxidation of LDL apart from oxidative damage to various components of cell and tissues.

Oxidized LDL: It has been suggested for 20 years that oxidative stress, and particularly LDL oxidation, could induce atherosclerosis and used to assess the development of atherosclerosis in patients. Circulating oxidized LDL is additive to the global risk score based on age, sex, total and HDL cholesterol, diabetes mellitus and hypertension as a useful marker for identifying persons at risk for CAD. Since oxidized LDL is the major component of atherosclerotic process and since atherosclerosis is the major complication in T2DM, the study is aimed to measure the lipid components, particularly the oxidized LDL levels in Type2 diabetics compared with non-diabetics [10].

MATERIALS AND METHODS

In this study patients suffering from T2DM i.e., NIDDM were taken as subjects for study from Dept. of Endocrinology (outpatient) at Gandhi Hospital along with healthy age matched controls. A total of 20 subjects with Type 2 diabetes mellitus&10 age-matched controls were studied. Samples were collected in plain bottles and serum extracted from centrifugation was used to perform—1) Lipid profile using enzymatic methods and 2) levels of oxidized LDL --- by Baseline diene conjugation method.

Oxidized LDL and Baseline diene conjugate were estimated specrophotometrically in the dept. of Biochemistry, National institute of Nutrition (NIN) of Hyderabad. Lipid profile was performed on the same day.

Method

By Baseline diene conjugation in LDL lipids (LDL-BDC)using buffered Heparin. Here selective ppt. of LDL was done using buffered Heparin by reducing plasma pH to 5.05thus HDL and VLDL remain in solution &LDL is isolated.

RESULTS

The Mean serum oxidized LDL levels in normal controls were 7.0+0.59 m.mols/dl and Standard deviation (S.D.) was 0.5908. The Mean serum oxidized LDL levels in cases of T2DM were significantly raised, the value being 12.69+ 2.18 mg% and the S.D. was

2.186. Lipid profile was estimated enzymatically in normal healthy controls& inT2DM cases. All the five parameters of lipid profile with the Mean serum values and S.D. are shown below (Table 1).

Comparison values of serum oxidized LDL levels of normal controls with the oxidized LDL levels of cases of T2DM—there is a rise of 81.28% change of serum oxidized LDL having a highly significant p-value of <0.001 (Table 2).

Values of lipid profile of normal persons with the lipid profile of cases of T2DM were compared. Total cholesterol: There is a rise of 51.99% change of total cholesterol, having a highly significant p-value of< 0.001. HDL cholesterol: There is rise of 13.17% change of HDL cholesterol, having a highly significant p-value of <0.05. Triglycerides: There is a rise of 58.05 % change of triglycerides, having a highly significant p-value of <0.001. VLDL cholesterol: There is a rise of 54.94 change of VLDL cholesterol, having a highly significant p-value of<0.001 LDL cholesterol: There is a rise of 60.34% change of LDL cholesterol, having a highly significant p-value of <0.001 (Table 3).

Table 1: Parameters of lipid profile with the mean serum values and SD

Factor	Mean values& S.D. of controls	Mean values& S.D. of cases
Total cholesterol	163.9±14.0115mg%	249.12± 27.30mg%
HDL cholesterol	35.45 ± 4.2440mg%	40.12±6.066mg%
Triglycerides	129.20±33.1354mg%.	204.2±38.67mg%
VLDL cholesterol	25.92±6.5985mg%	40.16 ± 7.046mg%
LDL cholesterol	103.42±11.0954mg%	165.82±23.81mg%

Table 2: Comparison of values of oxidised LDL levels of normal subjects with type 2 diabetes mellitus cases

Factor	Units mg/dl	Healthy cases	T2DM	% Change	p-value
Oxidized LDL	Mean ± S.D.	7.00 ± 0.59	12.69 ± 2.186	81.28	<0.001

Table 3: Comparison of values of lipid profile of normal subjects with type 2 diabetes mellitus cases

Sl. No.	Factor	Units mg/dl	Healthy controls	T2DM cases	% Change	p-value
1	Total cholesterol	Mean ± S.D.	163.9 ± 14.0115	249.12 ±27.30	51.99	<0.001
2	Triglycerides	Mean ± S.D.	129.203± 0.1354	204.2 ± 38.67	58.05	<0.001
3	HDL cholesterol	Mean ± S.D.	35.45 ± 4.2440	40.12 ± 6.066	13.17	<0.05
4	LDL cholesterol	Mean ± S.D.	103.421 ± 0.0954	165.822 ± 3.81	60.34	<0.001
5	VLDL cholesterol	Mean ± S.D.	25.92 ± 6.5985	40.16 ± 7.046	54,94	<0.001

DISCUSSION

Central obesity, Hypertension, Dyslipidemia in T2DM the important risk factors for CAD [11] the association explains 70%-80% morbidity and mortality in T2DM due to accelerated atherosclerosis.

There are new emerging risk factors such as hyper-homocystinemia, hyperfibrinogenemia, increased lipoprotein (a), insulin resistance, C-reactive protein and most importantly oxidized –LDL which are proved to be atherogenic in various stages of atherosclerotic process. The role of their measurement in clinical practice remains unclear. High-sensitivity C-reactive protein is considered as an essential atherosclerosis marker in patients with cardiovascular disease, but not as an insulin resistance marker in type 2 diabetes mellitus patients [12]. A new biological marker of the study, that is - The'' oxidized LDL'' inT2DM which is the indicator of both the states of insulin resistance and atherosclerosis is required to facilitate optimal health management of diabetic patients. According to the American Diabetes Association 2013 guidelines, Insulin resistance has been established as a precursor and acts as a strong premature and accelerated atherosclerotic factor linking T2DM with CAD & CVD [13, 14].

Central obesity and state of diabetes determine the degree and type of lipid abnormality. Significantly increased levels of the study showing--Hypercholesterolemia, hypertriglyceridemia and low HDL with HDL/TC ratio of above 4.5 have been shown as risk factors for CAD [15]. Insulin resistance and type 2 diabetes are associated with interrelated plasma lipid and lipoprotein abnormalities, which include reduced HDL cholesterol, and a predominance of elevated triglyceride levels is associated with an increased risk of cardiovascular disease [16]. National cholesterol education program, (NCEP) has identified LDL as the major atherogenic lipoprotein [17]. HDL cholesterol, the anti atherogenic lipoprotein influences the retardation of atherosclerotic process and its low levels contribute to CAD in Diabetes mellitus [8]. Thus the study including oxidized LDL and lipid profile levels in Type 2DM in comparison with normal subjects will help the clinician in preventing the complications i.e., atherosclerosis (CAD) to meet the goals of management of type 2 diabetes mellitus [18].

Results obtained in this study demonstrated a rise of oxidized LDL levels with a statistical significance of

$p<0.001$ when compared to control individuals. Role of abnormal lipid profile: Endothelial dysfunction triggered by persistent inflammation due to increased levels of triglycerides (TRYL), free fatty acids (FFA) and low-density lipoprotein (LDL), and decreased levels of high-density lipoprotein (HDL) that eventually leads to alteration of insulin signaling and glucose uptake in muscles and adipocytes. Inefficient glucose uptake by muscle and adipose tissue leads to insulin resistance, a precursor of accelerated atherosclerosis leading to CAD.

REFERENCES

1. King H, Rewers M; Global estimates for prevalence of diabetes mellitus and impaired glucose tolerance in adults. WHO Ad Hoc Diabetes Reporting Group. Diabetes care, 1993; 16(1):157-177.
2. Jain A, Paranjpe S; Prevalence of type 2 diabetes mellitus in elderly in a primary care facility. Indian J Endocrinol Metab., 2013; 17(Suppl 1): S318-S322.
3. Kisseba AH, Vydelingum N, Murray R; Relation of body fat distribution to the complications of obesity. J Clin Invest., 1982; 54(2): 254-260.
4. Sharma S, Jain S; Prevalence of obesity among type 2 diabetics. J Hum Ecol., 2009; 25(1): 31-35.
5. Boden G, Chen X; Effects of fat on glucose uptake and utilization in patients with NIDDM. J Clin Invest., 1995; 96(3): 1261-1268.
6. Boden G, Chen X, Ruiz J, White JV, Rossetti L *et al.*; Mechanisms of Fatty acid-induced inhibition of glucose uptake. 1994; 93(6): 2438–2446.
7. Hola Misligin G, Murry D, Choy LN, Spiegelman BM; Tumour necrosis factor inhibits signaling from the insulin receptor Proc Natl Acad Sci. USA, 1994; 91(11): 4854-4858.
8. Greenfield M, Kolterman O, Olefsky J, Rcaven GM; Mechanism of hypertriglyceridemia in diabetic patients with fasting hyperglycemia. Diabetologia, 1980; 18: 441-446.
9. Coffey MD, Cole RA, Colles SM, Chisolm GM; In vitro cell injury by oxidized low density lipoprotein involves lipid hydroperoxide-induced formation of alkoxyl, lipid, and peroxyl radicals. J Clin Invest., 1995; 96(4): 1866-1873.
10. Carmena R, Duriez P, Fruchart JC; Atherosclerosis: Evolving Vascular Biology and Clinical Implications, Atherogenic Lipoprotein Particles in Atherosclerosis. Circulation, 2004; 109: III-2-III-7.
11. Laakso M; Cardiovascular Disease in Type 2 Diabetes from Population to Man to Mechanisms. Diabetes care, 2010; 33(2): 442–449.
12. Theuma P, Fonseca VA; Ac Inflammation and emerging risk factors in diabetes mellitus and atherosclerosis Current Diabetes Reports, 2003; 3(3): 248-254.
13. Roglic G, Unwin N; Mortality attributable to diabetes: estimates for the year 2010. Diabetes Research and Clinical Practice, 2010; 87(1): 15–19.
14. Stumvoll M, Goldstein BJ, van Haeften TW; Type 2 diabetes: principles of pathogenesis and therapy. The Lancet, 2005; 365: 1333–1346.
15. Austin MA, Horanson JE, Edwards KL; Hypertrygliceridemia as a cardiovascular risk factor. Am J Cardiol., 1998; 81(4A): 7B-12B.
16. Krauss RM; Lipids and Lipoproteins in Patients with Type 2 Diabetes. Diabetes Care, 2004; 27(6): 1496-1504.
17. Grundy SM, Chairman; Expert panel on detection, evaluation and treatment of high blood cholesterol and NCEP report on detection, evaluation and treatment of high blood cholesterol in adults. Circulation, 1995; 89; 1329-1445.
18. Ikmal SIQS, Huri HZ, Vethakkan SR, Ahmad WAW; Potential biomarkers of insulin resistance and atherosclerosis in Type 2 diabetes mellitus patients with coronary artery disease. International Journal of Endocrinology, 2013; Article ID 698567.