SHORT COMMUNICATION

Effect of Oxidative Stress on Lecithin Cholesterol Acyl Transferase Activity in Newly Detected Type 2 Diabetes Mellitus

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Abstract:

Background: Oxidative Stress and Lecithin Cholesterol Acyl Transferase (LCAT) activity is widely accepted participant in the development and progression of diabetes and its complications. *Aim and Objectives:* The present study has been undertaken to evaluate the effect of oxidative stress on LCAT activity in newly detected type 2 Diabetes Mellitus (DM), and its role in development and progression of diabetes and its complications. *Materials and Methods:* 200 participants were enrolled in the study, 100 with newly detected type 2 DM were taken as study group and 100 age and sex matched healthy participants were taken as control group. Biochemical parameters like serum Malondialdehyde (MDA), Superoxide Dismutase (SOD), LCAT activity, Total cholesterol, Triacylglycerol, HDL, LDL, VLDL and erythrocyte reduced glutathione levels were analyzed in all the participants. *Results:* The patients with type 2 DM showed increased oxidative stress ($p<0.05$) and decreased LCAT activity ($p<0.001$) and HDL levels ($p<0.001$). *Conclusion:* Study concludes that increased oxidative stress might be responsible for the reduced LCAT activity. This may be involved in pathogenesis of atherosclerosis and could be an alarming finding for the risk of atherosclerosis in newly detected type 2 DM.

Keywords: Oxidative stress, LCAT activity, HDL, Diabetes Mellitus.

Introduction:

Diabetes Mellitus is a multisystem metabolic disorder showing the common underlying feature of hyperglycemia [1]. Oxidative stress is a condition in which there is either increased rate of free radical production or impaired antioxidant mechanisms [2]. Increased oxidative stress is one of the etiologies for the development and progression of diabetes and its complications [3]. Atherosclerosis is one of the major complications associated with type 2 diabetes. 50% of diabetic patients' deaths occur due to cardiovascular disease [4]. Low HDL is a strong risk factor for the development of atherosclerosis. The cardioprotective role of HDL is related to its role in Reverse Cholesterol Transport (RCT). LCAT plays a key role in RCT by mediating the production of most of the cholesteryl esters and efflux of free cholesterol from peripheral cells to HDL particles and its conversion to cholesteryl esters. The cholesteryl esters are internalized within the lipoprotein core for ultimate transport to liver for clearance or recycling [5]. The present study was undertaken to evaluate the effect of oxidative stress on LCAT activity in type 2 DM.

Material and Methods:

The study group comprised of 100 newly detected type 2 diabetic patients in the age group of 30-60 years, visiting Outpatient of Department of Medicine, Belgaum Institute of Medical Sciences (BIMS) Hospital, Belgaum. The diagnosis of diabetes mellitus was done by senior physicians. The diagnosis of type 2 DM was made by measuring Fasting Blood Glucose (>6.93 mmol/L) and 2 hour Oral Glucose Tolerance Test (>11.1mmol/L) values on two occasions as per American Diabetic Association's Revised Criteria [6]. As a control group 100 age and sex matched healthy participants were taken. Patients on hypolipidemic drugs, antioxidant supplements, steroids and oral contraceptives were excluded. Known cases of Hypothyroidism, Hyperthyroidism, Cushing's syndrome, Kidney diseases, hepatic diseases, alcoholics, smokers, tobacco chewers and patients with Type 1 Diabetes Mellitus were included.

The study was conducted from December 2011 to May 2013 in the Department of Biochemistry, BIMS, Belgaum. The research protocol has been examined and approved by institutional ethics committee. After obtaining informed written consent, 10 ml of 12 hours fasting blood sample was collected from diabetic patients and the control group under all aseptic conditions. The blood samples were used for measuring various parameters. LCAT activity was assessed by measuring the difference between esterified and free cholesterol [7]. Determination of free and esterefied cholesterol was done by using digitonin precipitation method [8]. HDL cholesterol level [7] and total cholesterol was measured by Cholesterol Oxidase Peroxidase method [8]. Triacylglycerol estimation was done by Glycerol 3-Phosphate Oxidase – Peroxidase method [8]. VLDL and LDL cholesterol were calculated by formula given in Tietz textbook [8]. Fasting blood Glucose was measured by Glucose Oxidase Peroxidase method [9]. Serum MDA levels were measured by method of Wilber KM by measuring Thiobarbituric acid reactive substances [10]. Serum SOD was measured by Marklund and Marklund method by autoxidation of pyrogallol [11]. Erythrocyte reduced glutathione (GSH) measurement was done by method of Beutler [12].

Statistical analysis:

The results were expressed as mean ± SD. The results were further subjected to students't' test, differences between means were considered significant at $p< 0.05$.

Results:

We found that serum MDA, LDL, VLDL, total cholesterol, triglycerides were significantly increased in type 2 DM when compared to control group. Erythrocyte reduced GSH, serum LCAT activity, SOD and HDL were significantly decreased in type 2 DM when compared to control group.

Table 1: Biochemical Parameters in Newly Detected Type 2 DM and Control Participants

Sr. No	Parameters	Controls (N=100)	Newly detected type 2 DM (N=100)	p value
1	**Serum MDA(nmol/ml)**	5.78±0.83	9.02±1.36	<0.05
2	**Serum SOD(units/ml)**	3.77±0.79	2.75±0.98	<0.05
3	**Erythrocyte reduced GSH (micro mol/g of Hb)**	6.03±0.95	4.54±0.32	<0.05
4	**LCAT Activity(IU/L)**	91.74±6.50	59.00±9.86	<0.001
5	**HDL(mmol/L)**	1.26±0.436	0.864±0.11	<0.001
6	**LDL(mmol/L)**	2.48±1.01	3.38±0.93	<0.01
7	**VLDL(mmol/L)**	0.77±0.51	1.04±0.44	<0.01
8	**Total Cholesterol(mmol/L)**	4.53±1.032	5.32±0.92	<0.05
9	**Triacylglycerol (mmol/L)**	1.75±1.21	2.56±1.19	>0.05

N = number of participants

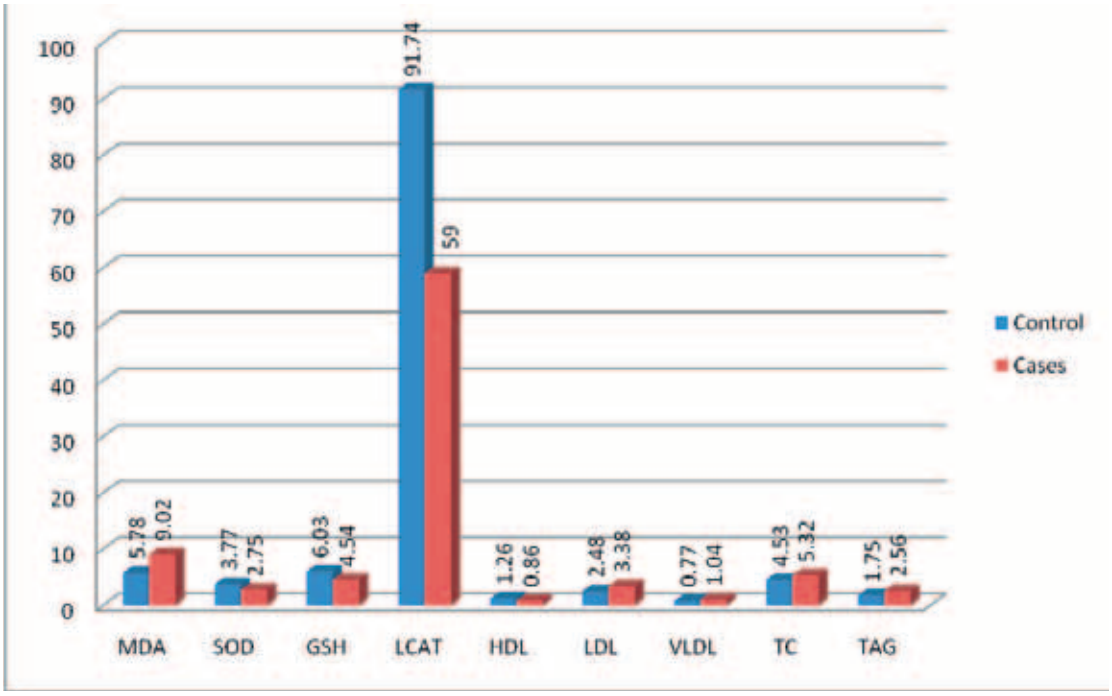

Fig. 1 Percentage Change Graph between Newly Detected Type 2 DM & Control

Discussion:

Oxidative stress is a widely accepted participant in the development and progression of diabetes and its complications. Diabetes Mellitus is associated with hyperglycemia which may lead to cellular damage; increased extravascular matrix production and vascular dysfunction which have been implicated in the pathogenesis of vascular disease in type 2 DM [13].

The study shows increased oxidative stress in newly detected type 2 DM in comparison with the control group. At present we found increased MDA levels in DM when compared to the control group.

Free radicals and oxidative stress may act as a common pathway to diabetes itself, as well as to its complications [14].

MDA levels are significantly ($p<0.05$) increased in type 2 DM when compared to the control group (Table 1). These findings are in agreement with the findings of Mahreen [15] and Ozdem [16]. Higher blood glucose level is associated with free radical mediated lipid peroxidation [17]. The peroxidative breakdown of phospholipids might lead to accumulation of MDA in type 2 diabetics. In healthy individuals, oxidative damage to tissue is prevented by a system of defense called antioxidant enzymes. Mechanisms involved in the increased oxidative stress in diabetes include not only oxygen free radical generation due to nonenzymatic glycation, autooxidation of glycation products, but also changes in the tissue content and activity of antioxidant defense systems [18].

In the present study serum SOD activity and concentration of erythrocyte GSH are significantly ($p<0.05$) decreased in type 2 DM as compared to the control group (Table 1). Reduced glutathione functions as a direct free radical scavenger as a co-substrate for glutathione peroxide (GPx) which explained decreased GSH concentration with increased oxidative stress. In diabetic patients, the autoxidation of glucose results in the formation of hydrogen peroxide which inactivates SOD [19] and this accumulated hydrogen peroxide may be one of the reactive oxygen species responsible for decreased activity of SOD in type 2 diabetic patients. Decreased activity of SOD and GPx may increase the oxidative stress in newly detected type 2 DM.

Present study found that in newly detected type 2 diabetes patients the activity of LCAT is significantly reduced ($p<0.01$) when compared to control group (Table 1). Durucan and coworkers found significantly lowered LCAT activity in diabetics [4]. Ghanei concluded that LCAT activity is considerably lower in diabetics compared with non-diabetics [20]. There is decrease in LCAT activity in diabetics, concluded by Nikam and Suman [21].

In present study the levels of HDL cholesterol were significantly decreased ($p<0.01$) in newly detected type 2 DM in comparison with the control group (Table 1). Smith *et. al.* [22] concluded that HDL cholesterol was significantly lower in diabetic subjects as compared to controls. In type 2 DM there is overproduction of active oxygen and

this is associated with auto-oxidation of glucose which results in glycation of LCAT, due to this structural change occurs in HDL [23, 24]. Thus the present study hypothesized that increased oxidative stress glycates LCAT and reduces the functional capacity of HDL in type 2 DM. This lowered HDL levels leads to decreased esterified cholesterol and might be responsible for higher free cholesterol levels in newly detected type 2 DM patients. This might result in increased risk of atherosclerosis in type 2 DM.

Limitations:

The duration of diabetes before the formal diagnosis was unknown. Because of limited resources the direct methods available for measuring LCAT activity could not be used. The LCAT activity was indirectly measured as the difference between esterified cholesterol and free cholesterol.

Conclusion:

Study concluded that increased oxidative stress might be responsible for the reduced LCAT activity. This may be involved in pathogenesis of atherosclerosis and could be an alarming finding for the risk of atherosclerosis in newly detected type 2 DM.

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References

1. Maitra A, Abbas, Robbins, editors. Pathologic basis of disease, 7th Ed, Philedelphia: Elsevier; 2004:1191–1192.
2. Irshad M, Chaudhari PS. Oxidant and antioxidant system: role and significance in human body. *Indian J Exp Biol* 2002; 40:1233–1239.
3. Ramakrishna V, Jailkhani R. Evaluation of oxidative stress in Insulin Dependent Diabetes Mellitus (IDDM) patients. *Diagn Pathol* 2007; 22:10-18.
4. Durucan M, Serdar Z, Sarand LE, Surmen Gur E. LCAT activity and cholesterol ester transfer rate in patients with Diabetes Mellitus. *Turk J Med Sci* 2003; 33:95-101.
5. Ganda OP, editors. Joslin's Diabetes Mellitus, Philadelphia: Lea & Febiger; 1985:219-230.
6. Nader RG, Warnick RG, editors. Teitz textbook of clinical and Molecular Diagnostics. 4th Ed. Philadelpia:W.B. Saunders; 2006: 820.
7. Gowland E, editors. Varley's Practical Clinical Biochemistry. 4th ed. New Dehli: CBS; 2005:311-313.
8. Nader RG, Warnick RG, editors. Teitz textbook of clinical and Molecular Diagnostics. 4th ed. Philadelpia:W.B. Saunders; 2006: 940-949.
9. Nader RG, Warnick RG, editors. Teitz textbook of clinical and Molecular Diagnostics. 5th Ed. Philadelpia:W.B. Saunders; 2012: 870.
10. Wilbur KM, Bernheim F, Shapiro OW. The thiobarbituric acid method for malondialdehyde estimation. *Arch Biochem Biophys* 1943; 250:305–213.

11. Marklund S, Marklund G. A simple assay for superoxide dismutase using autooxidation of pyrogallol. *Eur J Biochem* 1974; 47:469–472.
12. Beutler E, Duron O, Kelly BM. Improved method for the determination of blood glutathione. *J Lab Clin Med* 1963; 61:882–888.
13. Dalle-Donne. Biomarkers of oxidative damage in human disease. *Clin Chem* 2006; 52(4):601–623.
14. Wolff SP. Diabetes mellitus and free radicals. *Br Med Bull* 1993; 49(3):432–642.
15. Mahreen R, Mohsin M, Nasreen Z, Siraj M, Ishaq M. Significantly increased levels of serum malondialdehyde in type 2 diabetics with myocardial infarction. *Int J Diabetes Dev Ctries* 2010, 30:49−51.
16. Ozdemir G, Ozden M, Maral H, Kuskay S, Cetinalp P, Tarkun I. Malondialdehyde, glutathione, glutathione peroxidase and homocysteine levels in type 2 diabetic patients with and without microalbuminuria. *Ann Clin Biochem* 2005, 42:99−104.
17. Patchanans N, Peerapatdil T, Sriratanasathavorn C. Lipid peroxidation and antioxidant enzyme activity in erythrocyte of type 2 diabetic patients. *J Med Assoc Thai* 2010; 93:682-693.
18. Ceriello A. A hyperglycemia: the bridge between non enzymatic glycation and oxidative stress in the pathogenesis of diabetic complications. *Diabetes Nutr Metab* 1999; 12(1):42–46.
19. Nobar R, Pour R. Total antioxidant capacity, superoxide dismutase and glutathione peroxidase in diabetic patients. *Med J Islam Acad Sci* 1999; 12(4):109–114.
20. Ghanei A, Esfananian F, Esteghamati A, Behjati J, Hamadi S, Nakhjavani M. LCAT activity is decreased in type 2 Diabetes Mellitus. *Acta Medica Iranica* 2007; 45(6):481-486.
21. Shashikant Nikam, Suman Doddamani, Padmaja Nikam, Archana Dambal. Role of LCAT activity and HDL cholesterol levels in newly detected type 2 diabetes mellitus. *International Journal of Biochemistry* 2013; 108:140-143.
22. Sapna Smith, Alok M Lall. A study on lipid profile levels of diabetics and non-diabetics among Naini Region of Allahabad, India. *Turkish Journal of Biochemistry* 2008; 33(4):138-141.
23. Brites FD, Cavallero E, de Geitere C, Nicolaiew N, Jacotot B, Rosseneu M, Fruchart J C, Wikinski RL, Castro GR. Abnormal capacity to induce cholesterol efflux and a new LpA-I pre beta particle in type 2 Diabetic patients. *Clin Chim Acta* 1999; 279(1-2):1-14.
24. Atsunori Kashiwagi. Complications of Diabetes Mellitus and Oxidative Stress. JMAJ 2010; 44(12): 521–528.

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