Original article:

Lipids and lipid peroxidation in diabetes mellitus with complications

Dr.G. Vijay Kumar[1*], Dr.Ch.Ratna Kumar[1], Dr.R.Suryanarayana Raju[1],Dr.G.Srilaxmi[1], Dr. A. Yohoshuva[1] , Dr.G.V. Benerji[1], Farid Babu. M.[2] , Rekha Kumari .D.[2]

[1]Faculty,Department of Biochemistry, RMC, Kakinada, AP

[1]Faculty,Department of Biochemistry, GEMS,Srikakulam,A.P

[1] Faculty,Department of Pharmacology, KIMS & RF,Amalapuram,A.P

[1]Faculty,Department of Pharmacology, RMC, Kakinada, AP,

[1]Faculty,Department of Biochemistry,SMC,Vijayawada,A.P

[1]Faculty,Department of Biochemistry, KIMS & RF, Amalapuram, AP

[2] Faculty,Department of Biochemistry, KIMS & RF, Amalapuram, AP

[2]Faculty,Department of Biochemistry, KIMS & RF, Amalapuram, AP

Corresponding author : Farid Babu

Date of submission: 07 April 2014 ; Date of Publication: 10 December 2014

Abstract:

Diabetes Mellitus comprises a group of heterogenous metabolic disorders that share a phenotype of hyperglycemia. Due to hyperglycemia, the metabolic disorders are progressed to lead into a endothelial dysfunction, atherosclerosis and alterations in the large blood vessels. Dyslipidemia is one of the important pathogenic factor for micro (nephropathy) and macro vascular diseases (Cardiac complications). Hyperglycemia is widely known cause of enhanced plasma free radical concentrations of oxidative stress. Malondialdehyde (MDA) is the important marker for the oxidative stress.

In the present study, an attempt was made to study the influence of dyslipidemia during the complications of Diabetis Mellitus and how the lipid peroxidation will induce the damage to various organs in diabetics. During the study, 120 Type-2 Diabetic patients were selected under test group and estimated the parameters like Fasting Plasma Glucose, Urea, Creatinine, Triglycerides, Total Cholesterol, HDL, LDL, VLDL fractions of Cholesterol by standard kit methods and Malondialdehyde by TBA reaction using spectrophotometer and compared the values with age, sex matched controls number 50. It was observed that a significant increase in the TG (P>0.001) and Total Cholesterol (P>0.001), VLDL (P> 0.001) during all the three complications due to Diabetes Mellitus and lower levels of HDL cholesterol was observed. LDL Cholesterol was elevated significantly during the complications of Dermatological, Ophthlmic and Nephrological diseases. Malondialdehyde elevation seen in above disease conditions indicating the increased free radical production. Enhanced MDA has been proposed to be one of the mechanism that influenced the rapid progression of diabetic vasculopathy. The peroxidative lipid damage can increase coagulability, alter phospholipid organisation and reduce the survival of cell, due to the formation of advanced glycation end products (AGEs), which might generate toxic reactive oxygen species.

Key words: Diabetes Mellitus, Malondialdehyde, HDL, VLDL and LDL

Introduction:

Diabetes Mellitus is defined by the American Diabetes Association (ADA) expert committee in their 1997 recommendations as "a group of metabolic diseases charecterised by hyperglycemia resulting from defects in insulin production or action or both . The chronic hyperglycemia is associated with long term damage , dysfunction and failure of various organs, especially the eyes, kidney, nerves, heart and blood vessels."[1].

Diabetes from the Greek word for a siphon, because the fluid does not remain in the body, but uses the man's body as a channel wherby to leave it. The Hindu physician, Charak and Sushrut, who wrote between 400 and 500 BC, were probably the first to recognise the sweetness of diabetic urine. Indeed, the diagnosis was made by testing the urine or noting that ants congregated round it [2]. The Edinburgh trained surgeon, John Rollo(1809) was the first to apply the adjective "Mellitus" (Rome Greek and Latin words meaning Honey [3] . Diabetes Mellitus is of three types Type I (IDDM) and Type II (NIDDM), and Type 3, Gestational Diabetes. By 2025 , 300 million people are expected to be diabetic [4]. This will be greatly increased by 19 to 57 million people. Due to the metabolic derangements several metabolisms are severely affected , like lipid metabolism , lipid peroxidation leading to free radical generation (ROS) triggers the complications like cardiac (atherosclerosis) , neurological (Diabetic Foot Ulcer), Nephrological(Nephropathy), Vascular (Micro and Macro) etc., Chronic Diabetes Mellitus leads to the impairment of microcirculation[5, 6]. And there is an alteration in structures of glycosaminoglycans , advanced glycation end products (AGEs), Glycoproteins , collagen, polyol pathways which ultimately brings the change in the blood vessels leads to the hardening of vessels . Alteration in the carbohydrate and lipid metabolism leads to the decreased antioxidants and increased generation of free radicals and oxidative stress. Finally, increased coagulability of the endothelial surface lead to micro thrombus formation and luminal occlusion with atherosclerotic plaques[7]. There are multiple causes for diabetic neuropathies, including metabolic, vascular, autoimmune, oxidative stress and neuro hormonal growth factor deficiency.

Free radicals are constantly being generated in the body, as a result of the normal metabolic process. Under physiological conditions, damage due to free radicals is countered by antioxidants. Sometimes excessive free radical formation occurs in the body, and the anti oxidant system in the body cannot cope with the situations, ie., the pro oxidants overwhelm the antioxidants. This situation is known as oxidative stress. MDA is the product formed from free radicals as a marker for oxidative stress. In Diabetics antioxidant systems like GSH, vitamin- C, Magnesium, Vitamin- E are lowered which aggrevates the lipid peroxidation[8].

Materials and methods:

In the present study 120 diabetic patients of both sexes aged 45yrs to 60 yrs were included under test group (DM no – 50, DM with skin problems no- 24 , DM with optthalmicical no -9, and DM with nephrological no- 11) , who are admitted to Rangaraya Medical college and Hospital , Kakinada, A.P India. All are Type 2 Diabetes mellitus with the duration of 10 ± 5 yrs. 5o Age, Sex matched healthy individuals were selected under control group. All the subjects are informed for consent. Other systemic illness, Alcoholics, tobacco chewers, Patients on insulin were excluded from this study. Experiments were done according to Helsinki declaration of 1975.

Biochemical Analysis: Fasting venous blood samples were collected from both the groups and separated the serum after appropriate time and Glucose (GOD-POD Method) [9,10, 11], Urea (Urease- GLDH Method)[12,13] , Creatinine (Jaffe's Method)[14,15] , Lipid profile- HDL-c(Phosphotungstic Acid Method)[16], LDL-c,VLDL-c(by Friedewald's Formula) [17], TG (GPO-Trinder Method)[18,19]and Total Cholesterol(CHOD-PAP Method)[20,21]were measured by KEMWELL fully automated analyser by standard kits. MDA (Malondialdehyde) is

www.ijbamr.com P ISSN: 2250-284X , E ISSN : 2250-2858

estimated Spectrophotometrically by TBA Reaction (THIOBARBITURIC ACID, MAHALOUZ et.al., 1978) [22].

Measurement of MDA: 1 ml of of serum is pipette into a test tube and 1 ml of 0.9% saline and 2 ml of 20% TCA are added to the serum. Mix well. And centrifuged at 3000 rpm for 30 mins. 2 ml of supernatant was collected into another new test tube, 0.5 ml of 0.67% TBA is added. Tubes are kept in boiling waterbath for 45 mins and cooled immediately. Absorbance is measured at 530 nm and values are calculated from the standard curve.

Data are expressed as mean ± SD, $p<0.05$ was considered as statistically significant. Statistical analysis was performed using SPSS software.

Results:

When Diabetes mellitus patients are compared with the healthy individuals (Table -1) we observed a significant elvation in the levels of Glucose, (p value ,0.001), Triglycerides (p>0.001), Total cholesterol (p>0.001), VLDL cholesterol (p>0.001), and MDA (p> ,0.001) in test group. There is no significance in the Urea, Creatinine , LDL and HDL cholesterol.

When Diabetes mellitus patients with Dermatological complications are compared with healthy individuals, we observed the significant elevation of glucose levels (p>0.001), urea (p>0.001), Triglycerides (p>0.001), Total cholesterol (p>0.001), HDL cholesterol (p>0.001), VLDL cholesterol (p>0.001) and MDA (p>0.001) in the test group. No significant difference is observed in creatinine and LDL cholesterol(Table-2).

When diabetes with ophthalmic complications are compared with healthy individuals, we observed significant elevation of plasma glucose (p>0.001), Triglycerides (p>0.001), Total cholesterol (p>0.001), HDL cholesterol (p>0.001), VLDL (p>0.001), MDA (p>0.001) in the test group. There is no significant difference in Urea, creatinine and LDL cholesterol in the test group(Table-3).

When diabetes with Nephrological complications are compared with healthy individuals, we observed significant elevation in the plasma glucose (p>0.001), urea (p>0.001), creatinine (p>0.001), Triglycerides (p>0.001), Total cholesterol (p>0.001), LDL (p>0.001), VLDL (p>0.001), MDA (p>0.001). No significant difference is found in HDL cholesterol level in the test group(Table-4).

Late complications of Diabetes Mellitus is ulceration of foot. Commonly occur in both Type-I and Type-II DM. Peripheral vascular disease and minor trauma leads to ulceration, infection and gangrene. Patients with retinopathy and nephropathy are at increased risk of foot ulceration.

Table-1

Parameters		Controls n = 50	Diabetes Mellitus n = 50	't'-value	'p'-value
Age	years	45 ± 10	49 ± 10	-2.37	<0.01
Glucose	mg%	82 ± 12	150 ± 57	-8.34	<0.001
Urea	mg%	25 ± 5.4	31 ± 12	-0.08	NS
Creatinine	mg%	0.9 ± 0.15	1.1 ± 0.7	-2.00	NS
Triglycerides	mg%	108 ± 21	181 ± 104	-4.899	<0.001
T.Cholesterol	mg%	175 ± 19	220 ± 56	-5.54	<0.001
HDL Cholesterol	mg%	37 ± 4.5	47 ± 15	-2.134	NS
LDL Cholesterol	mg%	117 ± 16	133 ± 55	-2.018	NS
VLDL Cholesterol	mg%	22 ± 4.2	38 ± 22	-5.095	<0.001
MDA	nmol/dl	222 ± 44	464 ± 238	-7.127	<0.001

Table -2

Parameters		Controls n= 50	Dermatological Complications due to DM. n=24	't'-value	'p'-value
Age	years	45±10	48±13	-1.094	NS
Glucose	mg%	82±12	141±41	-9.238	>0.001
Urea	mg%	25±5.4	29±7	-2.7	<0.005
Creatinine	mg%	0.9±0.15	0.9±0.2	0	NS
Triglycerides	mg%	108±21	169±94	-5.328	>0.001
T. cholesterol	mg%	175±19	199±49	-4.92	>0.001
HDL cholesterol	mg%	37±4.5	51±14	-6.45	>0.001
LDL cholesterol	mg%	117±16	114±40	-0.46	NS
VLDL cholesterol	mg%	22±4.2	34±19	-4.284	>0.001
MDA	nmol/dl	222±44	508±245	-8.045	>0.001

www.ijbamr.com P ISSN: 2250-284X , E ISSN : 2250-2858

Table-3

Parameters	Controls n=50	Ophthalmic complications due to DM n=9	't'-value	'p'-value
Age years	45±10	53±6.7	-2.299	<0.002
Glucose mg%	82±12	170±84	-7.281	>0.001
Urea mg%	25±5.4	25±6.4	0	NS
Creatinine mg%	0.9±0.15	0.8±0.3	-1.538	NS
Triglycerides mg%	108±21	173±81	-4.977	>0.001
T. cholesterol mg%	175±19	229±36	-6.722	>0.001
HDL cholesterol mg%	37±4.5	62±14	-10.301	>0.001
LDL cholesterol mg%	117±16	133±33	-2.288	NS
VLDL cholesterol mg%	22±4.2	35±16	-5.0	>0.001
MDA nmol/dl	222±44	604±305	-8.696	>0.001

Table-4

Parameters	Controls N=50	Nephrological complications due to DM n=11	't'-value	'p'-value
Age years	45±10	49±8	-1.238	NS
Glucose mg%	82±12	161±73	-7.417	>0.001
Urea mg%	25±5.4	51±20	-14.178	>0.001
Creatinine mg%	0.9±0.15	1.4±0.2	-9.404	>0.001
Triglycerides mg%	108±21	216±79	-8.592	>0.001
T. cholesterol mg%	175±19	241±55	-6.953	>0.001
HDL cholesterol mg%	37±4.5	39±9	-1.086	NS
LDL cholesterol mg%	117±16	158±58	-4.399	>0.001
VLDLcholesterol mg%	22±4.2	44±20	-14.27	>0.001
MDA nmol/dl	222±44	556±140	-14.284	>0.001

Discussion:

In the present study an attempt is made to study the influence of dyslipidemia in the complications of diabetes mellitus and how the lipid peroxidation will induce the damage to various organs in the Diabetic Patients. The cause of Diabetic complications is not clearly known and may be Multifactorial. The major emphasis has been placed on polyol pathway, where in glucose is reduced to sorbitol by enzyme aldolase reductase. Sorbitol, which appears to function as a toxin, has been implicated in the pathogenesisof retinopath[29,30,] neuropathic cataract, nephropathy and vascular diseases sorbitol accumulation is associated with a decrease in the myo inositol content, Abnormal phosphoinositide metabolism and a decrease in Na+, K+, ATP ase activity. Hyperglycemia Is a widely known cause of enhanced plasma Free

www.ijbamr.com P ISSN: 2250-284X , E ISSN : 2250-2858

Radical concentrations. There are many ways by which hyperglycemia may increase the generation of free radicals. Development of atherosclerosis based on metabolic disorders is accompanied by a smoothened response of morphologically unaltered blood vessels to endothelium dependant vasodilators such as acetylcholine and Bradykinin[23].If endothelial dysfunction promotes atherosclerosis, it may be a link between hyperglycemia and alterations of large blood vessels. In the present study HDL cholesterol levels were normal, no change was observed in nephrological cases[35]. The results are in agreement with those reported in some earlier studies by Giri et al.,(1999)[24]and Vanizor et al., (2001)[25,34]. These cases were already established as complicated cases and were under treatment. This might be the reason to have a normal HDL cholesterol in some cases. Increased lipid peroxidation in DM has been reported in various studies Giri. et. Al.,(1999), Turk et .al., (2002) [26,31], and Kumar et al.,(2003)[27,32], increased enhanced production of MDA has been proposed as one of the mechanism that fosters rapid progression of diabetic vasculopathy (jenero, 1990)[28,33]. Peroxidative lipid damage can increase coagulability alter , phospholipid organisation and reduce survival of cell, due to the formation of advanced glycation end products (AGEs) which generate toxic reactive oxygen species(ROS).

Conclusion:

From the above study we can conclude that poor glycemic control and dyslipidemia are the major causes for the complications of the Diabete Mellitus. Serum MDA levels are significantly elevated which shows the key role of oxidative stress in the pathogenesis of Diabetic complications.

References:

[1].Expert Committee 1997 on the diagnosis and classification of diabetes mellitus. Report of the expert committee on the diagnosis and classification of diabetes mellitus. Diabetes care; 20;1183-97.

[2].Aljada A, Thusu K, Armstrong D,et al 1995: increased carbonylation of proteins in diabetes mellitus(Abstract 420). Diabetes 44:113A

[3]. Chevreul ME., Note sur le Sucre du diabetes, Ann. Chim (Paris) 1815;95: 319-20

[4].King H ., Aubert ER, Herman WH (1998); Global burden of diabetes 1995-2025: prevalence, numerical estimates and projections. Diabetes care;21: 1414-1431.

[5].Nathan D.,1994 Relationship between metabolic control and long term complications of diabetes in: Kahn CR,Weir G,eds.Joslin's Diabetes, Philadelphia : Lea& Febiger,:620-30

[6]. Skyler J., 1996 Diabetic complications: the importance of glucose control. Endocrinol metab clin north Am ;25:243-54

[7].Paolisso G,D' Amore A ,Volpe C et al.1994 Evidence for a relationship between oxidative stress and insulin action in non insulin dependant diabetic patients.Metabolism:43:1426-1429

[8].Paolisso G,D'Amore A,Galzerano D et.al 1993 daily vit.E supplements Improve metabolic control but not insulin secretion in elderly type ll diabetic patient care:16:1433-1437

[9.]Penncock C.A et al ,1973Clin.chem.Acta.48(193)

[10].Pileggi, V.j. and Szuskiewiz, 1974 C .P clinical chemistry ,principles and Techniques(1288), Harper & Row, Hogerstoown MD

[11].Tietz, N.W 1976 Fundamentals of clinical chemistry (243), W>B Saunders &Co.philadelphia PA

[12]. Talke , H.and Schubert, G.E Klin,Wochschr, (1965)19,43:174

[13].Tietz. N (ed) , 1976 Fundamentals of clinical chemistry , W.B.Saunders Co., Philadelphia,PA

[14].Bartel ,H .(1972)Clin.Chem,Acta 37:193

[15].Bowers L.D 1980 clin chem. 26:551

[16].Burstein M, scholnic H.R., Morfin R.1970.j.lipid Res

[17].ICMR Bulletin,(2002): Mathematical models for the control of optimizing and predicting outcomes of intervention measures for the control of Lymphatic Filariasis Vol –3

[18].Fossati P.Ann (1969) Clin Biochem;:24-7

[19].McGowan MW et al(1983)clin chem.;29:538.

[20].Allain C.C., Poon L.S., chan C.S.G., Richmond W.and Fu P1974., clin chem.., 20(470)

[21].Roeschlau P., Bernt E., and Gruber W.A., 1974 clin biochem 12(226)

[22].Mahalouz et. Al., 1978

[23].Cohen RA,(1993):Dysfunction of vascular endothelium in diabetes mellitius. Circulation:87(suppl V): 67-76

[24].Giri R.Kesavulu MM,Kameswara Rao B ,Ramana V,Appa Rao Ch.1999 hyperlipidemia ,increased lipid peroxidation and changes in antioxidant enzymes, NA+ k+ATPase in erythrocytes of type 2 diabetic patients in Andhra Pradesh , india journal of clinical biochemistry;14(2) :168-175.

[25].Vanizor B, Orem A, Karahan SC, et.al(2001)Decreased Nitric oxide end products and its relationship with high density lipoprotein and oxidative stress in people with type 2 diabetes without complications. Diabetes research and clinical practice;54:33-39

[26].Turk HM,sevine A,camci C et.al.2002 plasmalipid peroxidation products and antioxidant enzyme activities in patient with type 2 Diabetes mellitus.Acta Diabetol:sep:39(3):117-22

[27].Kumar AP and Rajagopal G.2003 lippid peroxidationiin Erythrocytes of patients with type 2 Diabetes mellitus.india j of clinical biochemistry :18(1):71-74

[28].Jenero DR.1990 Malaondialdehyde and Thiobarbituric acidreactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury.free rad boil med :9:515-540

[29].Armstrong D, Al-Awadi F1991: lipid peraxidation and retinopathy in streptozotocin induced diabetes. Free Radic Biol Med; 11:433-436.

[30].Hammes HP, Martin S, Federlin K et. Al., 1991 Amino Guanidine treatment inhibits the development of experimental diabetic retinopathy. Proc Natl Acad Sci. USA; 88:11555-11559

[31].Brownlee M,(1995) Advanced protein glycation in diabetes and aging.Ann.Rev.Med, ;46:223-34.

[32].Lane PH., Steffes MW, et. Al1993: Renal expansion in insulin dependant diabetes mellitus Kidney lnt, 43:661-667

[33].Osterby R et.al 1993 : ZGLomerular structure and function in Proteinuric type 2 diabetic patients . Diabetologia ; 36:1064-1070

[34].Scitt AW, Li YM., Gariner TA et. Al., 1995 Advanced glycation and products (AGEs) co-localize with AGE infused rats . AM J. Pathjol;150:523-528

[35] Dobson M. 1776 Experiments and observations on the urine in diabetes. Md. Obs inq;5:298-316

www.ijbamr.com P ISSN: 2250-284X , E ISSN : 2250-2858