ORIGINAL ARTICLE

"Study of Lipid Profile, Oxidative Stress, And Antioxidant Status, In Type-2 Diabetes Mellitus."

P. M. Kamble [1], S.C. Choudhari [2], A. S. Yadav [3]

M.Sc. Medical Student, Professor & Head, Biochemistry[1], Professor of Biochemistry.

Government Medical College & Hospital, Miraj [1,2] & BKL Walawlkar Rural Medical College, Dervan [3]

Abstract:

Background: Diabetes mellitus is regarded as a metabolic syndrome, a collection of disorders that have hyperglycemia as the hallmark. Type-2 Diabetes mellitus (Type-2 DM) is now epidemic in many countries undergoing modernization and industrialization. Diabetes is becoming the third killer of mankind, after cancer and cardiovascular diseases, because of its high prevalence, morbidity and mortality.

Aims and Objectives: The aim of this study was to assess the biochemical parameters in Type-2 Diabetes mellitus patients and normal healthy individuals (Control Group).

Material and Methods: A total 100 patients with Type-2DM from last 1-2 years and 100 normal healthy individuals were chosen as control group.

Results

The mean values of BSL (F), BSL (PP), TC, TG, and LDL were significantly increased ($P<0.001$) in patients as compared to control. Whereas the mean value of HDL was significantly decreased in patients as compared to control. The Mean value of Malondialdehyde (MDA) was significantly increased($P<0.001$) in patients as compared to control The Mean values of SOD, Vitamin C, E were significantly decreased in patients as compared to control.

Key Words: Type-2 Diabetes mellitus, hyperglycemia, Insulin resistance, lipid profile, superoxide dismutase , oxidative stress, malondialdehyde , reactive oxygen species.

Introduction:

Diabetes mellitus is regarded as a metabolic syndrome, a collection of

disorders that have hyperglycemia as the hallmark. Type-2 Diabetes mellitus (Type-2DM) is now epidemic in many countries undergoing modernization and industrialization. Diabetes is becoming the third killer of mankind, after cancer and cardiovascular diseases, because of its high prevalence, morbidity and mortality.[1] Diabetes mellitus is a common endocrine disorder characterized by hyperglycemia, metabolic abnormalities and long term complications afflicting the eyes, kidneys, nerves, and blood vessels. India, a developing country with fast industrialization and a modern lifestyle is facing a grave problem in having the largest number of people with Diabetes which is estimated to reach 80 million by the year 2030.[2,3] Type-2 Diabetes mellitus is the most rapidly growing chronic disease in the world. It is characterized by absolute or relative deficiencies in insulin secretion and/or insulin action associated with chronic hyperglycemia, disturbances in carbohydrate, lipid, and protein metabolism.[4] It is the major health problem throughout the world. Diabetes is classified into two types, type-1 DM (Insulin dependent Diabetes mellitus) and type-2 DM (Non insulin dependent Diabetes mellitus). In the early stages, the β-cells respond to the insulin resistance by secreting increased quantities of insulin and maintain blood sugar at normal levels. But gradually the beta cells begin to fail and the insulin levels decrease, resulting in rising levels of blood sugar. So it is a combination of insulin resistance and beta cell failure that causes Type-2 DM.[5] India is considered the diabetic capital of the world by 2020AD. In India the prevalence is 2-4% in rural and 4.0-11.6% in urban areas. Worldwide estimates project that in 2030 the gretest number of individuals with diabetes will be aged 45-64 years.[6] Type-2 DM leads to reduced quality of life and life expectancy, with a greater risk of heary disease, stroke, peipheral neuropathy, renal diseases, cataracts formation, amputation, ketoacidosis.[7] The development of Type-2 DM is caused by a combination of genetic factors related to insulin resistance, impaired insulin secretion and environmental factors such as obesity, lifestyle, mental stress and ageing.[8] Type-2 DM is associated with plasma lipid and lipoprotein abnormalities, including reduced High Density Lipoprotein(HDL) cholesterol, a predominance of small dense Low Density Lipoprotein (LDL) particles, and elevated Triglycerides (TGS).[9] During the study, serum MDA levels are measured, which is supposed to be an index of lipid

peroxidation. Malondialdehyde is the organic compound with the formula $CH_2(CHO)_2$. The structure of this species is more complex than this formula suggests. This reactive species occurs naturally and is a marker for oxidative stress. Malondialdehyde mainly exists in the enol form.[10] Superoxide dismutase (SOD) is dimeric antioxidant enzyme responsible for the quenching of superoxide radicals which are released during the chemical reactions of the various metabolic pathways. Vitamin c (Ascorbic acid) is a six carbon lactone that is synthesized from glucose in the liver of mammalian species but not by humans, human primates and guinea pigs. It acts as chain breaking antioxidant.[11]

Vitamin E is a lipid soluble antioxidant present in all cellular membranes protecting against lipid peroxidation . It acts as a chain breaking antioxidant.[12] With this background the present study was examine alterations in biochemical parameters such as blood glucose, lipid profiles, MDA, SOD, vitamin C, vitamin E in Type -2 diabetic patients.

Materials and Methods:

Present study was conducted as per the guidelines of Institutional Ethics Committee.The study includes total 200 subjects. This includes patients and control consists of 100 normal healthy individuals with age and sex matched with patients was selected from staff members and 100 patients with Type-2 DM from last 1-2 years attending OPD at Government Medical College and Hospital, Miraj, Maharashtra. The diagnosis of the patient was done on the basis of the patient's condition, clinical history, personal history, physical examination, laboratory investigations etc. Blood samples were collected twice for fasting & postprandial BSL.

Inclusion Criteria:-

The patients were having Type-2 Diabetes mellitus from last 1-2 years.

Exclusion Criteria: - The patients were having Type-2 DM more than 2 years, and having complications, smokers, and alcoholics are excluded.

Blood Collection:

Informed consent was obtained from all participants. To avoid contamination blood samples were withdrawn by using 20 gauge stainless steel disposable needles attached to 5 ml polythene disposable syringes, from anticubital vein with aseptic precautions. Needle was removed and, 3.5 ml blood was collected in plain bulb and serum was separated and used for the

estimation of Lipid peroxidation product, Lipid profile and Vitamin – E. The remaining 1.5 ml blood was collected in heparin bulb. The blood sample from heparinized bulb was centrifuged at 3000 rpm for 5 minutes. Clear plasma was separated and used for estimation of Blood sugar and ascorbic acid (Vitamin C) and RBCs were used for the preparation of hemolysate which was used in estimation of superoxide dismutase activity. Blood sugar, lipid profile (TC, TG, LDL, and HDL) were assayed using commercial kits from Erba on fully automated chemistry analyzer from Transasia. Malondialdehyde was estimation done by thiobarbituric acid method. Vitamin C assayed by (AYEKYAW 1978) method & Vitamin E assayed by (BAKER AND FRANK 1968) method by using colorimeter. SOD estimation was done by Marklund and Marklund (1974) method on spectrophotometer.

Statistical Analysis:

Data was compiled and analyzed using SPSSv10 software package. It was expressed

as mean +/- S.D. (standard deviation).

Results:

The subjects were distributed according to age groups as 41-60 & 61-80 years. Our study findings are as following.

Table No.1: Distribution of Patients and Control

Groups	**Number of cases**
Patients	100
Controls	100

Table No. 2 Distribution of Patients and Controls according to Age and Sex.

Age Group (in years)	Number of Patients		No. of Controls	
	Male	Female	Male	Female
41-60	38	23	38	23
61-80	22	17	22	17

Table No. 3:Biochemical Parameters in Patients and controls

Parametes	Patients(n=100) Mean± SD	Controls (n=100) Mean± SD	Statistical Significance
BSL (F) mg/dl	161.26±58.53	77.03±13.49	Highly significant
BSL(PP)mg/dl	260.47±79.36	126.26±13.67	Highly significant
TC mg/dl	210.78±25.49	160.87±5.3	Highly significant
TG mg/dl	187.98±42.28	119.81±11.54	Highly significant
HDL mg/dl	35.16±3.85	44.39±2.91	Highly significant
LDL mg/dl	160.66±17.53	134.92±6.01	Highly significant

P<0.001 Highly significant

Table No.4:Oxidant and antioxidants in Patients and Control

Parameters	Patients n=(100) Mean±SD	Control n=(100) Mean±SD	Statistical Significance
Serum MDA nM/ml	5.91±0.75	2.91±0.58	Highly significant
SOD U/gm of Hb	8.93±1.06	13.05±1.24	Highly significant
Vitamin C mg/dl	0.90±0.14	1.27±0.16	Highly significant
Vitamin E mg/dl	0.91±0.20	1.36±0.25	Highly significant

P<0.001 Highly significant

DISCUSSION

Type-2 DM is a heterogenous chronic disorder characterized by hyperglycemia and its complications. Type-2 DM is the most prevalent variant due to combination of insulin resistance and relative insulin deficiency, due to pancreatic beta cell failure.[13] Impaired insulin secretion is generally progressive, and its progression involves glucose toxicity and lipotoxicity. When untreated, these are known to cause a decrease in pancreatic cell mass. The progression of the impairment of pancreatic cell function greatly affects the long term control of blood glucose. While patients in early stages after disease onset chiefly show an increase in postprandial blood glucose as result of increased insulin resistance and decreased early phase secretion, the progression of the determination of pancreatic cell function subsequently causes permanent

elevation of blood glucose. Hyperglycemia leads to disturbances in carbohydrate, lipid and protein metabolism. Every cell in human body with diabetes is exposed to abnormally high glucose levels, but hyperglycemia selectively damage specific cell type, due to the failure in regulation of these cells for glucose uptake.[14] In view of this, we have investigated the biochemical parameters like BSL (F), BSL(PP), lipid profile(TC, TG, HDL, LDL), lipid peroxidation product as MDA, enzymatic antioxidant like SOD, and non enzymatic antioxidants such as vitamin C and E. We found significantly increased ($p<0.001$) BSL level in patients as compared to control, this may be due to impairment of insulin secretion or insulin resistance. Prolonged hyperglycemia in these patients may cause damage to the biomolecules and the biomembranes, thus leading to various diabetes associated complications. Other workers showed a highly significant increment in the level of BSL in Type-2 DM with and without coronary Arteriosclerosis[13]. We observed significantly increased ($p<0.001$) TC, TG, LDL levels and decreased HDL levels in patients as compared to control. Our study correlates with previous studies [16].Insulin resistance may play a vital role in the development of diabetic dyslipidemia, by influencing several factors like genetic, obesity etc. Insulin resistance increases efflux of free fatty acid from adipose tissue and impaired insulin causes decrease uptake of free fatty acid by skeletal muscles, causes fatty acid flux to the liver, kidney etc. In the presence of Insulin resistance, free fatty acid in the form of TG are deposited in muscle, liver, heart, and pancrease. Insulin resistance also increases the hepatic lipase activity, which is responsible for hydrolysis of TG in LDL and HDL particles and leads to smaller and denser LDL particles.[1, 17] In the present study MDA level was increased significantly in diabetic patients comparing to healthy subjects. Our results are similar to earlier workers [18] who found increased MDA level in serum of Type -2 DM patients. The rise in the MDA indicated increased oxidative stress, caused by free radical mediated lipid peroxidation in cell membrane. Therefore MDA is a good indicator for oxidative stress. All diabetics are showed hyperglycemia which can cause ROS generation. Glucose can undergo autooxidation and generate hydroxyl (OH*) radicals. Glucose reacts with proteins in a nonenzymatic manner leading to the formation of advanced glycation products (AGEs). ROS is generated at multiple steps during this process. In hyperglycemia there is increased metabolism of glucose through the polyol (sorbitol) pathway, which also results

in increase production of superoxide radicals. We observed relatively decreased activity of SOD enzyme in patients compared to control.

We evaluate antioxidant status by measuring the activity of the antioxidant enzyme like SOD in patients with Type-2 DM and in a healthy control group. A result of our study indicates the action of antioxidant enzyme SOD in diabetic patients which is a sign of oxidative stress. The mean erythrocyte SOD activity in Type-2 DM patients was significantly low as compared to normal control. Similar findings of decreased activity of SOD have been reported by other authors [5, 18].They suggest that there is an imbalance between plasma oxidant and antioxidant system in patients with Type-2 DM. The autooxidation of glucose results in the formation of hydrogen peroxide(H_2O_2) which decrease the activity of SOD. The activity of SOD decrease in erythrocytes of diabetic subjects duet to glycation of SOD enzyme.

In the present study decreased activity of SOD in the erythrocytes appears due to the change of protein synthesizing machinery in the erythrocyte. Diminished activity of SOD results into increased oxidative stress.

In our study the lower levels of vitamin C and E are seen in type-2 DM patients. Our results are similar to other researchers [15]. Vitamin C and E are diet derived and detoxify free radicals by chain breaking reaction. They also interact in recycling processes to generate reduced forms of the vitamins which may destroy free radicals.

CONCLUSION

In our study there is a disturbed lipid profile in Type-2 DM patients. Serum total cholesterol, Triglycerides, LDL, level found increased while HDL level decreased. Hence, high levels of TC, TG, LDL and low levels of HDL may be due to insulin resistance and other influencing factors like obesity, increase calorie intake, genetic, sedentary lifestyle, lack of muscular activity, in type-2 DM patients. In our study we concluded that poor glycemic control, dyslipidemia due to insulin resistance contributed to the increased oxidative stress. In our study increased MDA levels indicate the increased oxidative stress. Enzymatic antioxidant like SOD activity is significantly decreased in our study. Non enzymatic antioxifdant vitamin C and E levels are also decreased. From this we can conclude that decreased erythrocyte SOD activity, decreased vitamin C and E levels indicate the reduced antioxidant status in Type-2 DM patients. Hence dietary supplementation of

vitamin C and E may be helpful to improve antioxidant status of the body.

Conflicts of interests: There is no conflict of interest.

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Address for correspondence: ***Miss.P.M.Kamble***

Government Medical College & Hospital, Miraj

E- mail - swee2mkamble@gmail.com

Mobile–+91-9130102187