Original Article

Malondialdehyde, Blood Lipids and Antioxidant Activity in Newly Diagnosed Type 2 Diabetics

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ABSTRACT

OBJECTIVE: **To determine malondialdehyde, blood lipids and anti oxidants in newly diagnosed type 2 diabetic patients.**

STUDY DESIGN: **Observational, comparative study**

PLACE & DURATION: **Department of Medicine, Liaquat University of Medical and Health Sciences Hospital from February 2013- May 2014.**

SUBJECTS & METHODS: **97 newly diagnosed type 2 diabetics and 50 healthy controls were selected through non-probability purposive sampling according to inclusion and exclusion criteria. Blood samples were collected after 8-12 hours of fasting. Fasting blood glucose level, lipid and lipoprotein levels were measured by Cobas e411 analyzer. Malondialdehyde (MDA), Superoxide dismutase (SOD) and Glutathione peroxidase (GPX) were measured by Diagnostics kit. Zinc and ascorbic acid were measured by using Centronic GmbH-Germany Kit. Albumin, bilirubin, and uric acid were measured on Hitachi Chemistry analyzer. Data was analyzed on SPSS version 21.0. The significant p-value was taken at ≤ 0.05.**

RESULTS: **MDA, blood lipids and anti oxidant mechanisms showed significant differences between diabetics and healthy controls. Total blood lipids and lipid sub fractions were elevated in diabetics compared to controls. MDA was raised 5.16±0.91 vs. 2.16±0.62 µmol/l in diabetics and controls respectively (p=0.0001). The SOD, GPX, Ascorbic acid, Zinc, albumin, uric acid and bilirubin were reduced in diabetics (p<0.001).**

CONCLUSION: **Diabetes mellitus is characterized by free radical formation, lipid peroxidation, altered blood lipids and reduced anti oxidant mechanisms. An increase in malondialdehyde and reduction of antioxidant mechanisms may contribute to secondary complications.**

KEY WORDS: **Malondialdehyde, Anti oxidants, Diabetes mellitus.**

This article may be cited as: Humaira M, Raqeeb A, Memon A, Khoharo HK. Malondialdehyde, Blood Lipids and Antioxidant Activity in Newly Diagnosed Type 2 Diabetics. J Liaquat Uni Med Health Sci. 2016;15(02):78-82.

INTRODUCTION

Reactive oxygen species (ROS) are by-product in type 2 diabetes mellitus, generated during protein glycation and as a consequence of advanced glycation end-products-receptor binding; they impair insulin signaling pathways and induce cytotoxicity in β-cells of Pancreas. Oxidants neutralization by increased antioxidants utilization may mitigate these effects.[1,2]

Some complications of diabetes are associated with increased activity of free radical-induced lipid peroxidation and accumulation of lipid peroxidation products.[3] Lipid peroxidation is a free radical- mediated process, and carries a harmful potential due to self enhanced and uncontrolled disruption of membrane lipids.

It is also involved in oxidative stress, which in turn exaggerates diabetes associated complications. Beta cells of the pancreas and vascular endothelium are highly susceptible to oxidative stress. This is due to the fact that many biochemical pathways strictly associated with hyperglycemia increase the production of free radicals. Exposure of endothelial cells to high glucose leads to increased production of superoxide radicals.[4]

Antioxidants reverse many of the effects of hyperglycemia on endothelial functions such as reduced endothelial dependent relaxation and delayed cell replication.[5] Naturally existing anti oxidant enzyme and non enzyme systems play a protective role against lipid peroxidation by scavenging the ROS. A balance between peroxidative damage and anti oxidants systems is vital for protection and susceptibility of an organism's body against pathogenesis which might do eruption. Pathology, elevated lipid peroxidation products and the simultaneous decline of antioxidant defense systems may lead to cell damage and a vicious cycle may settle. Different studies have given a lot of

evidence of increased oxidative stress with depleted antioxidant enzymes and vitamins, in both type 1 and type 2 diabetes.[4-6] The human body has a naturally in -built antioxidant system which acts synergistically to protect tissue against free radical mediated injury and the onset of disease.

Levels of zinc, ascorbic acid, albumin, uric acid and bilirubin are often used as major non-enzymatic antioxidant biomarkers. They prevent free radical reaction by sequestering transition metal ions by chelation in plasma, providing the primary extracellular defense against oxidative stress. Therefore, the study was planned to assess these plasma antioxidants along with routine investigations in type 2 diabetes.

The rationale of present study was to determine circulating levels of malondialdehyde, blood lipids, and antioxidant systems in newly diagnosed type 2 diabetic subjects.

SUBJECTS AND METHODS

A prospective comparative study was conducted at the Department of Medicine, Liaquat University of Medical and Health Sciences Hospital Hyderabad/ Jamshoro from February 2013 - May 2014. 97 diagnosed cases of Diabetes mellitus were selected by non probability purposive sampling.

Inclusion criteria: Newly diagnosed cases of DM defined as per American Diabetes Association criteria were selected from age raging between 20- 50 years.

Exclusion criteria: DM subjects with chronic complications of diabetic nephropathy, proteinuria, systemic hypertension and concomitant chronic viral hepatitis. Newly diagnosed diabetics taking metformin, lipid lowering agents and taking vitamin supplements were also excluded.

Blood samples were collected after 8-12 hours of fasting. Fasting blood glucose level, lipid and lipoprotein levels were measured by Cobas e411 analyzer; Roche Diagnosis GmbH, Mannheim, Germany. Obtained serum was pipette into a clean blood sample bottle and analyzed on the day of collection for blood glucose and lipid profile tests. Serum total cholesterol was determined by an enzymatic (CHOD-PAP) colorimetric method and triglycerides were determined by an enzymatic (GPO-PAP) method. HDL-Cholesterol was estimated by a precipitant method and LDL-Cholesterol by then estimated by using Friedewald's formula as; LDL-C = TC - HDL-C – (TG/5).[7] Serum glucose was determined by the glucose oxidase method.

Malondialdehyde (MDA) was detected by TBARS assay kit (Cayman Chemical, USA).[8] Superoxide dismutase (SOD) and Glutathione peroxidase (GPX) were measured by Fortress Diagnostics kit (Fortress Diagnostics, UK). Zinc level was measured by using Centronic GmbH-Germany Kit.[9] Ascorbic acid level was measured by the method as mentioned.[10] Albumin, Bilirubin, Uric acid were measured by using Hitachi Chemistry analyzer. Data was analyzed on SPSS version 21.0. Continuous and categorical variables were analyzed by student's t-test and chi square test respectively. The significant p-value was taken at ≤ 0.05.

RESULTS

The demographic characteristics are shown in (Table I). The study subjects were age and gender matched with statistically insignificant difference. BMI, obesity, hypertension, smoking habits, blood glucose, blood urea and serum Creatinine showed statistically significant differences between cases and controls. The malondialdehyde, blood lipids and anti oxidant mechanisms showed major differences between cases and controls. Total blood lipids and lipid sub fractions were elevated in diabetics compared to controls as shown in (Table II). MDA was raised to 5.16±0.91 vs. 2.16±0.62 in diabetics and controls respectively (p=0.0001). Plasma anti oxidants i.e. the SOD, GPX, Ascorbic acid, Zinc, albumin, uric acid and bilirubin were reduced in diabetics compared to controls as shown in (Table III).

TABLE I: CHARACTERISTICS OF TYPE 2 DIABETICS AND CONTROLS

	Cases (n=97)	Controls (n=50)	P-value
Age (years)	48±7.5	47±7.1	0.53
Male	78 (%)	35 (%)	0.19
Female	19 (%)	15 (%)	0.06
BMI (kg/m^2)	27±6.1	25±1.3	0.01
Obesity	41 (%)	(%)	0.00
Hypertension	56 (%)	16 (%)	0.0001
Smokers	27 (%)	19 (%)	0.002
Blood glucose (mg/dl)	253±61.0	113±51.1	0.0001
BUN (mg/dl)	11±4.5	9±2.7	0.02
Serum creatinine (mg/dl)	1.2±0.4	0.9±0.3	0.07

TABLE II: LIPID PROFILE OF TYPE 2 DIABETICS AND CONTROLS

	Cases (n=97)	Controls (n=50)	P-value
Triglycerides (mg/dl)	252.1±10.9	132.1±47.0	0.001
Cholesterol-Total (mg/dl)	221.1±43.9	118.3±21.9	0.0001
HDLc (mg/dl)	31.9±7.2	39.7±9.4	0.02
LDLc (mg/dl)	129.5±16.2	95.2±17.4	0.001
VLDL (mg/dl)	41 ± 11.2	29.3 ± 8.1	0.001

TABLE III: ANTI OXIDANTS AND LIPID PEROXIDANT LEVELS IN TYPE 2 DIABETICS AND CONTROLS

	Cases (n=97)	Controls (n=50)	P-value
Superoxide dismutase (U/ml)	142.1±24.12	177.3±45.7	0.0001
Glutathione peroxidase (U/ml)	7619.5±2134.0	8177.9±1119.0	0.0001
Ascorbic acid (mg/dl)	0.56±0.23	0.90±0.21	0.0001
Zinc (µg/dl)	49.1± 1.3	58.7±9.1	0.0001
Albumin (g/dl)	3.01±0.4	3.98±0.7	0.0001
Bilirubin (mg/dl)	0.4±0.12	0.57±0.36	0.0001
Malondialdehyde (µmol/ml)	5.16±0.91	2.16±0.62	0.001
Uric acid	2.14±0.78	3.79±1.9	0.0001

DISCUSSION

The malondialdehyde, blood lipids and antioxidant status of newly diagnosed type 2 diabetics is being reported for the first time from our tertiary care hospital of Liaquat University. The study showed comparable results to previous studies mentioned in literature, however, derangement of anti oxidants and lipid peroxidant was more pronounced. An imbalance of anti oxidants status in type 2 diabetics has been linked to chronic hyperglycemia and insulin resistance. The link between chronic hyperglycemia and ROS is supported by findings of present study. An association of hyperglycemia, SOD, GPX and malondialdehyde is found in present study. Plasma MDA as an indicator of oxidative stress in type 2 diabetics is confirmed in present study. The findings of present study are comparable to a previous study.[2]

It is reported that the ROS production is increased with a concomitant reduction in enzymatic and non enzymatic anti oxidants. The ROS increases the chances of vascular endothelial dysfunction which has been implicated in the micro vascular complications.[11] It is reported that some of these derangements are operating simultaneously in a synergistic way.[2] A previous study has reported that the non enzymatic anti oxidant factors such as uric acid, albumin, β-carotene, retinol and retinal, and α-tocopherol delay and inhibit the oxidative agents.[12] The present study also detected low levels of above non enzymatic anti oxidants, hence indirectly it may be concluded that the diabetics are carrying high oxidative load. Our findings are comparable to above mentioned study.

Zinc was also reduced in diabetics which is also an anti oxidant agent. It is reported that the Zinc may act as anti oxidant in various ways; such as by normalizing glycaemia, enhancing insulin release, as co factor for enzymes. In this way Zinc is said to exert anti oxidant activity.[13] The finding of low zinc is in agreement to previous studies which had reported similar results.[14,15] An explanation for the hypozincemia is its loss in urine.[2] Possible explanation of increased ROS in diabetics is the increased production of free oxygen species, especially from lipid peroxidation, glycosylation and auto oxidation of glucose.[16]

Zinc supplementation is reported to ameliorate glycemic control and appears to be a beneficial factor in decreasing lipid peroxidation. Lipid peroxidation helps to delay the vascular complications of diabetic subjects. Hence hypozincemia may be associated with tissue damage in diabetics. The findings of low zinc and ascorbic acid of present study are in agreement with previously mentioned studies.[2, 17, 18]

Other studies had reported a low ascorbic acid in plasma of diabetics.[19,20] Low serum ascorbic acid may be due to increased oxidative load. Another possibility is of competitive inhibition of ascorbic acid and glucose as both shares a common membrane transporter. It is reported that the defect is overcome by supplementing high doses of ascorbic acid.[2, 17, 18] A previous study reported that supplementation of diabetic diet with 2 grams of ascorbic acid daily, brings an improvement in the glycemic control.[21,22] Uric acid is capable of scavenging singlet oxygen, super oxide and hydroxyl radical free radicals. Blood uric acid concentration is ten times higher than that of ascorbic acid; hence it provides a large anti oxidant system.[23-25] Bilirubin and albumin were also altered in diabetics in present study similar to reported previously.[25-26] The present study observed a significant increase in malondialdehyde, an indicator of lipid peroxidation, with a concomitant reduction in anti oxidant systems.

CONCLUSION

The present study concludes that the diabetes mellitus is characterized by free radical formation, lipid peroxidation, altered blood lipids and reduced anti oxidant mechanisms. An increase in malondialdehyde and reduction of antioxidant mechanisms contributes to secondary complications. Hence it is suggested that the diabetics should be routinely investigated for the lipids, lipid peroxidant and anti oxidant mechanism for better glycemic control to reduce the diabetic complications.

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