Research Article (Open access)

Comparative Study of Malondialdehyde and Vitamin C in Type 2 Diabetes Mellitus and Non Diabetic Individuals

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ABSTRACT- Oxidative stress resulting from enhances free-radical formation and/or a defect in antioxidant defences has been implicated in the pathogenesis of diabetes and its associated complications. Oxidative stress (OS) has been implicated in the initiation, progression and pathology of type 2 diabetes mellitus (DM). A cross sectional study conducted during the period from January 2015 to June 2015 to assess the serum levels of malondialdehyde (as a marker of lipid peroxidation), antioxidant vitamin (C) in Sudanese with type 2 diabetes mellitus compared to normotensive persons. In all subjects were Men and Women, age fall between the 25-74 years individuals. Exclusion criteria were chronic disease, alcohol consumer, obesity, smoking/tobacco consumer and current use of any medication. Antioxidant enzymes activity and lipid peroxidation (malondialdehyde) were determined in serum samples. The aim was to determine the oxidative stress status and plasma vitamin antioxidant (Vitamin C) level in diabetes and apparently healthy indviduals. In134 subjects out 67 were found as controls normotensive individuals and the cases 67 diabetic patients. Serum MDA levels were highly significantly elevated in diabetic patients and normotensive individuals (4.36±1.17 µmol/l vs 1.72±0.68 µmol/l and $p < 0.0001$). Vitamin C acts as an antioxidant was highly significantly decrease in diabetic patients and normotensive individuals (0.29± 0.15 mg/dl vs 0.37±0.18 mg/dl and $p <0.0001$). These findings demonstrate a strong association MDA and Vitamin C level, antioxidant level decrease and increase level of MDA in diabetic patients. Supplementary vitamin C may be helpful in decreasing blood glucose in type 2 diabetes and thus reducing the risk of complications, should be considered in further research.

Key-words: Diabetes, Oxidative stress, Dietary antioxidants, Malondialdehyde

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INTRODUCTION

Diabetes mellitus refers to a group of common metabolic disorder that shares the phenotype of hyperglycemia. [1] Prolonged exposure of hyperglycaemia increases the generation of free radicals and reduces capacities of antioxidant defence system. [2] Hyperglycaemia generates reactive oxygen species (ROS), which in turn cause damage to the cells in many ways. Damage to the cells ultimately results in secondary complications in diabetes mellitus. [3]

An imbalance in the oxidant/antioxidant equilibrium leads to a condition called oxidative stress, which is known to be responsible for molecular and cellular tissue damage mechanisms in a wide range of human diseases including diabetes. It has been suggested that free radical activity is high in diabetes leading to increased oxidative stress. Various pathways which lead to oxidative stress include increased non-enzymatic glycosylation, auto-oxidative glycosylation, metabolic stress resulting from changes in energy metabolism, alterations in sorbitol pathway, changes in the level of inflammatory mediators and the status of antioxidant defence systems. [4]

Malondialdehyde is an organic compound with the formula

Received: 17 November 2015/Revised: 16 December 2015/Accepted: 28 December 2015

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$CH_2(CHO)_2$. This reactive species occurs naturally and is a marker for oxidative stress. Reactive oxygen species degrade polyunsaturated lipids present on cell membrane forming malondialdehyde. This aldehyde product is used as a biomarker to measure the level of oxidative stress in an organism. [5] Antioxidant depletion or deficiency may contribute to oxidative stress. Antioxidants not only protect against the direct injurious effects of oxidants, but also alter the inflammatory events that play an important role in the pathogenesis of oxidative stress related diseases.

Vitamin C is a water soluble free radical scavenger, can directly scavenge O_2 and OH^-radicals and help to neutralize physiological oxidant burden created by both exogenous and endogenous sources. [6] Vitamin C is an important antioxidant in human, capable of scavenging oxygen derived free radicals. Several studies showed decreased basal vitamin C level in diabetic patients [7] and also it is suggested that oxidative stress is increased in diabetes. [8] The present study is conducted with an objective to evaluate the oxidative status and serum vitamin antioxidant levels in diabetes. Free radicals are formed disproportionately in diabetes by glucose autoxidation, polyol pathway and non-enzymatic glycation of proteins. [9] Abnormally high levels of free radicals and the simultaneous decline of antioxidant defense systems can lead to the damage of cellular organelles and enzymes, increased lipid peroxidation and development of complications of diabetes mellitus. [10]. These Exists an involvement in between antioxidant nutrient intake and reduction in the improvement of Diabetic complications. [11] Oxidative stress results from an imbalance between radical-generating and radical-scavenging systems, i.e. increased free radical production or reduced activity of antioxidant defences or both lipid peroxides formation and decreased ascorbic acid levels. [12]

MATERIALS AND METHODS

The criteria used for selection of both diabetes mellitus and normotensive controls were performed by well-established diagnostic criteria as recommended by World Health Organization. The present study was conducted on cases of 67 diabetes patients as well as controls 67 normotensive persons. The study was approved by the Institute Ethics Committee, Integral Institute of Medical Sciences & Research Lucknow, India and informed consent was obtained from all the cases and control subjects. A venous blood sample was collected after overnight fasting by using disposable syringes. A volume of 4 ml of blood is collected by venipuncture under aseptic conditions in a sterile clot activator/ plain vial and fluoride vial from selected subjects by the investigator. Blood in the tube was allowed to clot at room temperature for 10-15 minutes and then centrifuged at (3000 rpm) for approx. 2-3 minutes. After centrifugation, serum which was collected and split into2 micro tubes for the study of MDA, Vitamin C and Blood sugar.

The serum/plasma samples were used for the analysis of various parameters:

Estimation of Glucose by GOD/POD Method [13]

Add 0.01 ml of serum/plasma in 1.0 ml of working solution. Incubate mixture at 37^0C for 15 minutes. After completion of incubation measure the absorbance at 505 nm.

Glucose in mg %= Absorbance of sample/ Absorbance of standard x 100

Estimation of MDA by Satoh k. Method [14]

0.8 ml of serum + 1.2 ml of TCA +TBA+HCL (in equal volume) mixed immediately ,keep in boiling water bath for 10 min, cool and add 2 ml of 1 NaOH, mix and read optical density at 532 nm.

MDA (micro mol/l) = OD_{532} X 1.75/0.156

OD 532 (λ) = 532 nm and excitation = 1.56 x$10^5 M^{-1}$ cm^{-1}

Estimation of Vitamin C by DNPH (Dinitro Phenyl Hydrazine) Method [15]

Add 0.5 mL of serum to 2 ml of freshly prepared metaphosphoric acid in a test tube and mix well in a vortex mixer. Centrifuge at 2500 rpm for 10 minutes. Pipette 1.2 ml of the clear supernatant into Teflon lined screw capped test tubes. Add 1.2 mL of each concentration of working calibrator into screw capped test tubes. Prepare calibrators in duplicate. Add 1.2 ml of metaphosphoric acid to two tubes for use as blanks. Added 0.4 mL of DTCS reagent to all tubes. Cap tubes, mix contents and incubate tubes in water-bath at 37^0C for 3 hours. Remove tubes and chill for 10 minutes in an ice-bath. While mixing slowly add to all tubes 2 mL of cold sulphuric acid (12 mol/L), cap and mix in the vortex. The temperature of the mixture should not exceed room tempera-

ture. Adjust the spectrophotometer with the blank to read zero absorbance at 520 nm and read the calibrators and unknowns. Plot the concentration of each working calibrators versus absorbance values. The calibration curve obeys Beer's law up to an ascorbic acid concentration of 2 mg/dl.

The concentration of the samples is obtained from the calibration curve and is multiplied by 5 (to correct for dilution of plasma by metaphosphoric acid) to give the concentration of ascorbic acid of plasma.

STATISTICAL ANALYSIS

The results are presented in mean±SD. The glucose level, MDA and vitamin C level were compared by using unpaired t- test between cases and controls. The Pearson correlation coefficients were calculated among the study parameters. The p-value <0.05 was considered significant. All the analysis was carried out by using Statistical Package for Social Sciences (SPSS) version 22.

Table 1: Comparison of MDA levels between cases and controls

MDA (µmol/l)				
Group	**N**	**Mean**	**Standard deviation**	**Significance**
Control	67	1.72	0.68	t value = 17.27
Case	67	4.36	1.17	p <0.0001

Table 1 shows the comparison of MDA level between cases and controls. MDA was significantly (p=0.0001) higher among cases (4.36± 1.17) than controls (1.72±0.68).

Table 2: Comparison of Vitamin C levels between cases and controls

Vitamin C (mg/dl)				
Group	**N**	**Mean**	**Standard deviation**	**Significance**
Control	67	0.37	0.18	t value = 2.79
Case	67	0.29	0.15	p <0.0001

Table 2 shows the comparison of Vitamin C level between cases and controls. Vitamin C was significantly (p=0.0001) higher among cases (0.29± 0.15) than controls (0.37±0.18).

Table 3: Comparison of Fasting blood sugar levels between cases and controls

Fasting Glucose (mg/dl)				
Group	**N**	**Mean**	**Standard deviation**	**Significance**
Control	67	79.43	9.22	t value = 37.51
Case	67	161.88	15.45	p <0.0001

Table 3 shows the comparison of Fasting glucose level between cases and controls. Fasting glucose was significantly (p=0.0001) higher among cases (161.88±15.45) than controls (79.43±9.22).

Table 4: Comparison of Postprandial blood sugar levels between cases and controls

Postprandial Glucose (mg/dl)				
	N	**Mean**	**Standard deviation**	**Significance**
Control	67	107	8.49	t value = 36.20
Case	67	240.51	28.97	p <0.0001

Table 4 shows the comparison of Postprandial Glucose level between cases and controls. Postprandial Glucose was significantly (p=0.0001) higher among cases (240.51.88± 28.97) than controls (107±8.49).

Table 5: Correlation Coefficient among the Biochemical Parameters in Cases

		MDA	Vit C	Fasting	Postprandial glucose
MDA	Pearson Correlation	1	-.106	.331**	.080
	Sig. (2-tailed)	-	.395	.006	.519
	N	67	67	67	67
Vitamin C	Pearson Correlation	-.106	1	-.034	-.028
	Sig. (2-tailed)	.395	-	.783	.821
	N	67	67	67	67
Fasting Glucose	Pearson Correlation	.331**	-.034	1	.347**
	Sig. (2-tailed)	.006	.783	-	.004
	N	67	67	67	67
Postprandial glucose	Pearson Correlation	.080	-.028	.347**	1
	Sig. (2-tailed)	.519	.821	.004	-
	N	67	67	67	67

***. Correlation is significant at the 0.05 level (2-tailed).**

****. Correlation is significant at the 0.01 level (2-tailed).**

Table 6: Correlation coefficient among the biochemical parameters in controls

		MDA	Vit C	Fasting	Postprandial glucose
MDA	Pearson Correlation	1	-.078	-.305*	-.086
	Sig. (2-tailed)	-	.531	.012	.487
	N	67	67	67	67
Vitamin C	Pearson Correlation	-.078	1	-.072	-.072
	Sig. (2-tailed)	.531	-	.564	.561
	N	67	67	67	67
Fasting Glucose	Pearson Correlation	-.305*	-.072	1	.181
	Sig. (2-tailed)	.012	.564	-	.143
	N	67	67	67	67
Postprandial glucose	Pearson Correlation	-.086	-.072	.181	1
	Sig. (2-tailed)	.487	.561	.143	-
	N		67	67	67

***. Correlation is significant at the 0.05 level (2-tailed).**

DISCUSSION

In this study, we evaluated that the plasma MDA level and its relationship with other biochemical findings i.e., blood sugar fasting, postprandial & vitamin C. The values were compared between diabetic group and control group along with clinical correlation in both groups. The fasting and postprandial plasma glucose along with vitamin C between the study groups differed significantly. The mean plasma level of MDA in the case group was 4.39±1.17 µmol/L and in the control group it was 1.79±0.68 µmol/L, this difference was statistically significant. The mean plasma level of vitamin C in the case group was 0.29±0.15 mg/dl; in the control group it was 0.37± 0.18 mg/dl, this difference was statistically significant. In a study conducted and observed that MDA level was increased and also found that the level of vitamin C was decreased in type 2 diabetic patients. In this study, diabetes was associated with increased oxidative stress, which result is higher plasma concentration of lipid peroxidation products such as MDA in serum [16] and in other think it stated that antioxidant defences were lower depending on the type of diabetes. For example, glutathione was lower in both types of diabetes [17], but ascorbate was lower only in T2DM. [18] Our study confirmed that there is an increased oxidative stress in diabetics compared to non diabetic counterparts and emphasizes the importance of assessing these markers for easily diagnosed and therapeutic interventions. Our findings strongly confirmed the evidence that diabetic patients were susceptible to oxidative stress and higher blood glucose level had an association with free radical mediated lipid peroxidation. It was suggested that in the early stage of type-2 diabetes, the antioxidant defence system counters the effects of increased free radicals, but by the advanced stage the balance between generation of free radicals and antioxidant defence is impaired as a result of decreased antioxidant level or activity. The increase in lipid peroxidation is also an indication of decline in defence mechanisms of enzymatic and non-enzymatic antioxidants. [19] Oxidized lipids are able to produce MDA as a decomposition product Increased MDA level in serum, and many others tissues have been reported in diabetic patients. [20,21]

CONCLUSIONS

In this study, we concluded that the level of non-enzymatic antioxidant vitamin C in type 2 diabetes mellitus is significantly decreased as well as we observed that the MDA level was increased and they also found that the level of vitamin C was decreased in type 2 diabetic patients as well as serum levels of MDA was significantly increased in type 2 diabetes mellitus. Finally, thus it is concluded that MDA can use a marker of oxidative stress in type 2 DM. More extensive study is required to evaluate molecular level the association between diabetes and oxidative stress.

ACKNOWLEDGEMENT

I express my deep sense of gratitude to my guide Dr. Roshan Alam, Associate Professor, Department of Biochemistry, Integral Institute of Medical Sciences and Research, Integral University, Lucknow, who has always been a guiding force for me. His continuous encouragement, suggestion, constrive, critism and moral inspiration were key success.

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