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ORIGINAL ARTICLE

Study of Oxidative Stress Status in Relation to Glycemic Index Fluctuations in Type II Diabetes Mellitus Patients

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ABSTRACT

Background: Oxidative stress is characterized by an increased generation of O_2-derived molecules called reactive oxygen species that provoke critical, even irreversible, cell injury.

Aim: To evaluate oxidative stress status through measurement of malondialdehyde (MDA) and to analyze association of changes in MDA status with respect to fluctuations in glycemic control.

Materials and methods: A total of 112 subjects, both males and females, aged above 30 years were enrolled for this study, in which 81 had type II diabetes and 31 were without diabetes. Random blood sugar (RBS) was measured by glucose oxidase and peroxidase method. Serum MDA was measured by thiobarbituric acid reactive substances method. Glycated hemoglobin (HbA1c) was measured by ion exchange resin method.

Results: The MDA and HbA1c levels were increased in diabetics and were statistically significant. In all the studied groups, MDA was positively correlated with RBS and HbA1c.

Conclusion and clinical significance: The study suggests that MDA should be measured along with routine parameters of disease and the use of redox active antioxidants to tone down MDA levels may be evaluated to contribute in early and improvised clinical management of type II diabetes mellitus and also to delay the development of secondary complications of the disease.

Keywords: Diabetes mellitus, Glycated hemoglobin, Malondialdehyde, Reactive oxygen species, Thiobarbituric acid reactive substances, Type II diabetes mellitus.

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INTRODUCTION

Prophetically, Himsworth stated that diabetes mellitus is a disease in which the quintessential lesion is a diminished ability of the tissues to utilize glucose.[1] Globally, diabetes is not regarded as an epidemic anymore. It has turned into a pandemic[2] and has become one of the largest health emergencies of the 21st century. Diabetes not only kills or disables but also has an impact on socioeconomic growth. Diabetes would not have gained so much magnetism if the person would have had only hyperglycemia but it is not so. The longevity of diabetes leads to the development of macrovascular or microvascular complications. Diabetes mellitus is associated with endothelial dysfunction, autooxidation, nonenzymatic protein glycation, and activation of polyol pathway with increase in oxidative stress.[3,4]

Oxidative stress is characterized by an increased generation of O_2-derived molecules called reactive oxygen species (ROS) that provoke critical, even irreversible, cell injury. In diabetes mellitus, both exposure to hyperglycemia and functional limitation of hexose monophosphate shunt pathway lead to oxidative stress. So, there is a need to check the development of these complications in diabetic subjects by early detection of predisposing factors, such as oxidative stress in terms of malondialdehyde (MDA) levels.

AIM

To evaluate oxidative stress status through measurement of MDA and to analyze association of changes in MDA status with respect to fluctuations in glycemic control.

MATERIALS AND METHODS

Study Population

A total of 112 subjects participated in the study. Type II diabetes mellitus (T2DM) patients attending the diabetic outpatient department during May 2015 through 2016 of Hind Institute of Medical Sciences, Barabanki, Uttar Pradesh, India, were encouraged to participate in the study. Participants were categorized according to World Health Organization (WHO) criteria and were classified into three groups, namely nondiabetics ("n" = 31, the negative control group), T2DM patients group showing poor glycemic control ("n" = 55), and the diabetics displaying good glycemic control ("n" = 26, positive control).

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Ethical Clearance

The study was approved by the Scientific and Ethical Committees of Hind Institute of Medical Sciences, Safedabad, Barabanki (Uttar Pradesh, India).

Materials

Materials used were acetic acid (product code A0060), Hydrochloric Acid (product code H0090), and trichloro acetic acid (product code T0160), which were obtained from RANKEM, India; tetra ethoxy propane (product code T9889), Thiobarbituric acid (product code T5500) were purchased from Sigma-Aldrich (USA). Weighing scale, wall-mounted ruler, sphygmomanometer and stethoscope, tourniquet, syringes, fluoride vials (product number REF 83100), ethylene diamine tetraacetic acid vials (product code REF 82150), centrifuge, glycohemoglobin kit for glycated hemoglobin (HbA1c) assay (Asritha Diatech, India Pvt. Ltd; Prasantha Nagar, Kulkatpally, Hyderabad, Telangana, India), autoanalyzer (Turbochem-100, model no. 4600), semi autoanalyzer (Lab India 2001, Optimas), ultraviolet–visible double beam spectrophotometer (Systronics Model 2701) were also arranged for the study.

Methods

The study subjects were selected based on a structured questionnaire. The questionnaire was intended to obtain information on the subject's demographic data, smoking habits, alcohol consumption, and duration of disease (i.e., T2DM), medications, and harboring of any other disease, which was used for inclusion and exclusion criteria. Inclusion criteria included both diabetes and normal controls as per well-established diagnostic criteria as recommended by the WHO, known cases of T2DM undergoing treatment, and patients aged above 30 years. Exclusion criteria included smokers; alcoholics; type I diabetic patients; diabetic emergencies; pregnant women; patients with chronic infections, renal disease, endocrine disease, malignancy, and patients on warfarin, steroids, or hormone replacement therapy; use of regular antioxidant supplements (vitamin C and folic acid) for at least 1 month before the start of the study. Random blood sugar (RBS) was measured by glucose oxidase and peroxidase method. Serum MDA was measured by the thiobarbituric acid reactive substances method. The HbA1c was measured by ion exchange resin method.

Statistical Analysis

All the data were analyzed using Statistical Package for the Social Sciences. Significance of differences was determined using Student's t-test. Pearson's correlation coefficient (r value) was determined within groups. The values were considered statistically significant if $p < 0.05$.

RESULTS

Anthropometric Parameters

The characteristics of the participants are summarized in Table 1; mean ± standard deviation (SD) and range are shown.

Biochemical Parameters

The results of RBS, HbA1c, and MDA determinations are summarized in Table 2. The MDA and HbA1c levels were increased in diabetics and were statistically significant. In

Table 1: Anthropometric parameters in nondiabetic subjects, T2DM subjects with poor glycemic control, and T2DM subjects with good glycemic control

	Nondiabetics (n = 31)		*T2DM subjects with poor glycemic control (n = 55)*		*T2DM subjects with good glycemic control (n = 26)*	
Parameter	*Mean ± SD*	*Range*	*Mean ± SD*	*Range*	*Mean ± SD*	*Range*
Age (years)	45.06 ± 7.48	32–64	53.07 ± 8.37	35–74	47.46 ± 9.15	30–65
BMI (kg/m^2)	24.06 ± 2.7	16.4–28.3	22.78 ± 3.5	15.6–31	24.5 ± 2.4	19.7–29.0
SBP (mm Hg)	116.39 ± 6.94	102–138	121.24 ± 12.68	96–160	120.23 ± 9.27	100–140
DBP (mm Hg)	74.06 ± 6.90	60–86	75.24 ± 9.88	50–92	76.92 ± 9.16	60–90

BMI: Body mass index; SBP: Systolic blood pressure; DBP: Diastolic blood pressure

Table 2: Biochemical characteristics of nondiabetic subjects, T2DM subjects with poor glycemic control, and T2DM subjects with good glycemic control

	Nondiabetics (n = 31)		*T2DM subjects with poor glycemic control (n = 55)*		*T2DM subjects with good glycemic control (n = 26)*	
Parameter	*Mean ± SD*	*Range*	*Mean ± SD*	*Range*	*Mean ± SD*	*Range*
RBS (mg/dL)	102.42 ± 21.17	76–152	277.29 ± 108.74***	140–547	130.35 ± 23.78***	92–184
HbA1c (%)	5.31 ± 0.52	4.3–6.2	8.73 ± 2.28***	5.7–14.6	5.96 ± 0.52***	5.0–6.9
MDA (nmol/dL)	324.75 ± 43.18	260–430	638.44 ± 76.72***	465–763	430.77 ± 31.74***	327–474

Statistically significant, ***<0.001, **<0.01, *<0.05

Table 3: Pearson's correlation table of data on biochemical variables in T2DM subjects with poor glycemic control

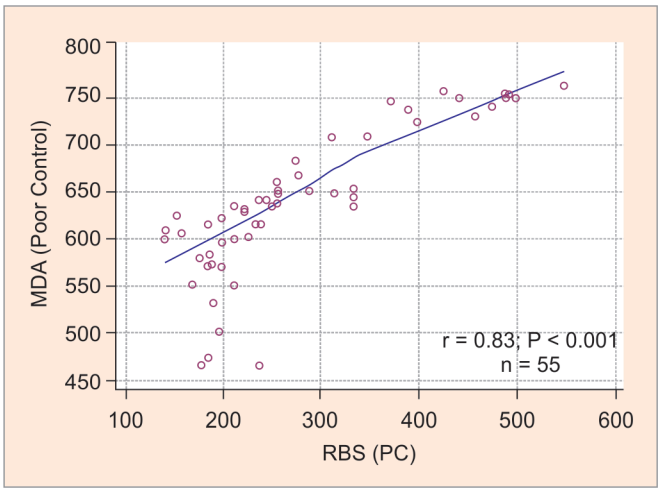
	RBS		*HbA1c*		*MDA*	
	r-value	*p-value*	*r-value*	*p-value*	*r-value*	*p-value*
RBS	–	–	–	–		
HbA1c	0.82	<0.001	–	–		
MDA	0.83	<0.001	0.78	<0.001		

Statistically significant, ***<0.001, **<0.01, *<0.05

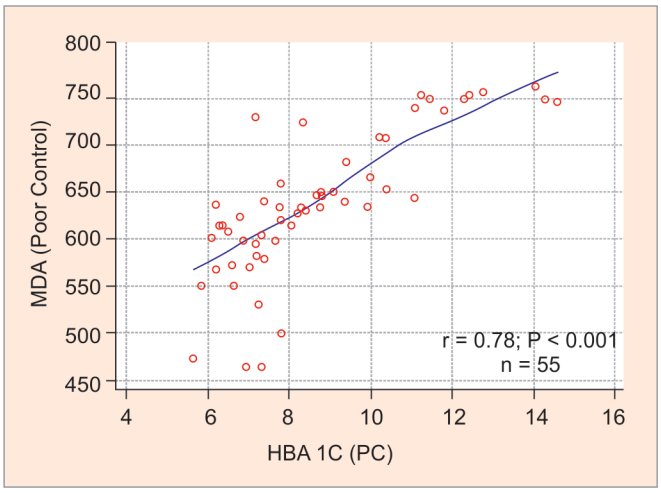
Table 4: Pearson's correlation table of data on biochemical variables in T2DM subjects with good glycemic control

	RBS		*HbA1c*		*MDA*	
	r-value	*p-value*	*r-value*	*p-value*	*r-value*	*p-value*
RBS						
HbA1c	−0.04	0.848				
MDA	0.34	0.086	0.38	0.058		

Statistically significant, ***<0.001, **<0.01, *<0.05



Graph 1: Correlation between RBS and MDA in T2DM subjects with poor glycemic control



Graph 2: Correlation between HbA1c and MDA in T2DM subjects with poor glycemic control

all the studied groups, MDA positively correlated with RBS and HbA1c (Tables 3 and 4).

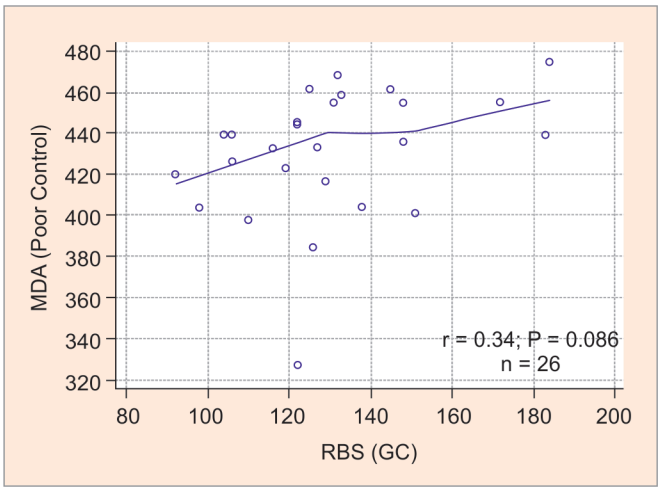
DISCUSSION

The aim of the present study was to investigate the state of oxidative stress as measured by MDA levels in controlled and uncontrolled diabetic patients. We have found an association of increase in systemic oxidative stress with poor glycemic control in T2DM subjects. The decreased tone of stress seen in T2DM subjects with good glycemic control reaffirmed the above observation. In nondiabetic subjects, a lower tone of oxidative stress was observed.

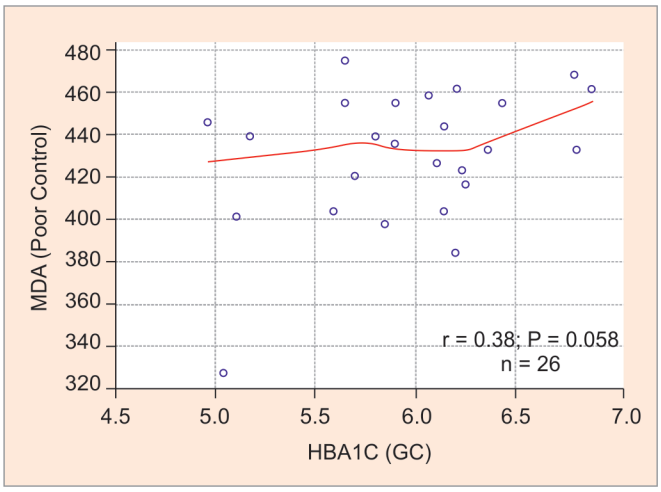
Glycemic status was assessed through the analysis of RBS and HbA1c. The RBS and HbA1c levels characterized T2DM subjects into good or poor control of diabetes. Values of all the parameters were expressed as mean ± SD. A strong correlation between RBS and HbA1c level is described in the literature. Several studies have reported increases in HbA1c level to be directly proportional to the fasting serum glucose levels in T2DM subjects.[5-10]

Lines of evidence have postulated an association between status of oxidative stress and T2DM.[11] We have observed a strong direct correlation between levels of MDA and RBS; MDA and HbA1c in T2DM subjects having poor glycemic control ("r" = 0.83; 0.78, Graphs 1 and 2). This observation indicated the prevalence of oxidative injury to lipids and possibly to other biomolecules also. A loss of such correlation (decrease in respective "r" values, 0.34 and 0.38; Graphs 3 and 4) in T2DM subjects with good glycemic control validated the above results. The observed increase in the MDA levels concurrent with rise in RBS or HbA1c (Table 5) is in consonance with the reports available in the literature. By estimating MDA, several studies have reported increase in oxidative injury to biomolecules in T2DM subjects. In 2007, Meigs et al[12] have found association of systemic oxidative stress with insulin resistance even in individuals at average or elevated risk of diabetes. Maritim et al[13] have reported an increase in oxidative damage (manifested as increase in MDA levels) among patients with T2DM. The observed increase in MDA release in diabetes can be attributed to the increase in peroxidative damage to lipids and setting in of the oxidative stress. In 2014, Prabhakar Reddy et al[14] have reported hyperglycemia-induced oxidative stress by showing increase in the MDA levels among diabetic patients. More studies describing similar outcome are listed in Table 5.

This study encountered with a limited budget, time, and sample size. Due to the limited duration and convenience of subjects, RBS was selected and not fasting blood sugar which might have been a better marker for diabetes. In future study, we can examine this variable with large sample size or with more sensitive indicators in diabetic patients.



Graph 3: Correlation between RBS and MDA in T2DM subjects with good glycemic control



Graph 4: Correlation between HbA1c and MDA in T2DM subjects with good glycemic control

Table 5: Studies with similar outcome

Reference	*Control group Mean ± SD*	*T2DM subjects Mean ± SD*	*Statistical significance**
Jamunarani et al[15]	2.62	4.36	p<0.001
Padalkar et al[16]	6.13 ± 2.3	13.29 ± 0.72	p<0.001
Vivian Samuel[17]	3.62 ± 0.24	5.14 ± 0.68	p<0.001
Kedari[18]	2.41 ± 0.12	6.98 ± 0.13	p<0.001
Shinde et al[19]	3.59 ± 0.97	7.19 ± 0.64	p<0.001
Al-Rawi[20]	1.1 ± 0.35	2.38 ± 0.97	p<0.001
Salem et al[21]	5.81 ± 2.39	11.13 ± 3.13	p<0.001
Nakhjavani et al[22]	2.91 ± 0.59	3.82 ± 0.93	p<0.001
Moussa[23]	1.3 ± 0.3	3.0 ± 0.7	p<0.001

*p < 0.001

CONCLUSION

The study suggests that MDA should be measured along with routine parameters of disease and the use of redox-active antioxidants to tone down MDA levels may be evaluated to contribute in early and improvised clinical management of T2DM and also to delay the development of secondary complications of the disease.

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