

Estimation of Aldose Reductase Activity and Malondialdehyde Levels in patients with Type 2 Diabetes Mellitus

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Type 2 diabetes mellitus (T2DM) is a metabolic disorder characterized by hyperglycemia due to insufficient secretion or action of insulin. Elevated oxidative stress and diminished antioxidants may play an important role to develop T2DM and its complications. Aldose reductase (AR) enzyme plays a key role in the reduction of glucose to sorbitol by Polyol pathway. To estimate the AR activity and malondialdehyde (MDA) levels and in patients with T2DM. In this case-control study, a total number of 60 subjects (30 T2DM and 30 age-matched controls) were recruited. Fasting blood sugar (FBS), Post-Prandial blood sugar (PPBS), AR activity and MDA levels were estimated in all the subjects. The AR activity was estimated by nicotinamide adenine dinucleotide phosphate hydrogen (NADPH) oxidation method. The plasma MDA levels were estimated by the thiobarbituric acid reactive substance (TBARS) method. For Statistical analysis, all the data were compared between the two groups by using unpaired t-test. Pearson correlation coefficient was calculated among T2DM. A P value <0.05 was considered as statistically significant for all data analyzed. The mean of FBS, PPBS, AR activity, and MDA levels were found significantly high in T2DM as compared to controls (P<0.001, P<0.001, P<0.001, P<0.001, respectively). A significant positive correlation was found between FBS and PPBS among T2DM (r=0.71, P<0.01). However, There was no significant correlation found between AR activity and MDA level among T2DM (r=0.002, P>0.05). Results showed that the mean of FBS, PPBS, AR activity, and MDA levels were found significantly higher in T2DM than controls. There was no significant correlation found between AR activity and MDA level among T2DM.

Keywords: Malondialdehyde, Aldose Reductase, Type 2 Diabetes Mellitus, Oxidative stress, Lipid peroxidation, Polyol Pathway.

Type 2 diabetes mellitus (T2DM) is a metabolic disorder with characteristics of hyperglycemia due to insufficient secretion or action of insulin^{1, 2}. In T2DM patients, an imbalance between reactive oxygen species (ROS)

production and antioxidant level has been reported³. Abnormally high levels of lipid peroxidation and the simultaneous decline of antioxidant defense mechanism can lead to damage of cellular organelles like mitochondria, Golgi bodies etc and

lead to increased oxidative stress^{5,6}. It is reported that the chronic rise of plasma glucose causes major complications of diabetes, including retinopathy, neuropathy, nephropathy, and macrovascular and microvascular damage^{2,7}. According to International Diabetes Federation (IDF) report, a total of 425 million adults have diabetes and 1 in 2 remains undiagnosed worldwide⁸. There are more people with diabetes residing in urban (269.7 million) than in rural (145.1 million) areas⁹. In India, there are 72.9 million people living with diabetes mellitus. The overall prevalence of diabetes is 8.8% in Indian adults⁸. Anjana *et al* reported that the prevalence of diabetes is higher in low socio-economical status (SES) groups mainly in city dwellers or slum dwellers residing in the economically developed states¹⁰.

Diabetes and free radicals are strongly associated⁴. Studies reported that T2DM revealed oxidative stress load, oxidative destruction of sub-cellular membrane lipids has been implicated along with other types of intracellular oxidative damage in the normal aging process and in the pathophysiology of a number of chronic illnesses^{11,12}. Glucose oxidation is the main source of free radicals generation. The polyol pathway leads to the reduction of glucose to sorbitol via aldose reductase (AR) enzyme in a nicotinamide adenine dinucleotide phosphate hydrogen (NADPH) dependent manner. Sorbitol is then oxidized to fructose by the enzyme sorbitol dehydrogenase, with nicotinamide adenine dinucleotide (NAD⁺) reduced to nicotinamide adenine dinucleotide hydrogen (NADH). The main function of AR enzyme is to reduce toxic aldehydes formed by ROS or other substrates to inactive alcohols. Under normal conditions, AR has a low affinity for glucose, with a very small percentage of total glucose converted to sorbitol via this pathway. Under hyperglycemic conditions, there is an increase in the enzymatic activity and production of sorbitol, resulting in an overall decrease in NADPH. NADPH is an essential cofactor for the production of glutathione (GSH), a critical intracellular antioxidant^{3,13-16}. Increased glucose pumped by the polyol pathway does not produce ROS directly but contributes greatly to an overall redox imbalance in the cell that leads to oxidative stress¹⁷ summarized in Figure 1. More than 30% of the glucose pumped into the polyol pathway

causing a significant depletion of NADPH and as a result a significant decrease in the GSH level. Thus, AR activity diminishes the cellular antioxidant capacity during hyperglycemic conditions^{3,13,17}. It is reported that lipids are the primary targets of ROS. They may also react with transition metals like iron or copper to form stable aldehydes, such as active aldehydes malondialdehyde (MDA), which damage cell membranes^{16,18}. Peroxidation of lipids produces highly reactive aldehydes, such as MDA^{18,19}. MDA has been an established biomarker of free radical-mediated lipid damage and oxidative stress²⁰.

Elevated oxidative stress and diminished anti-oxidants may increase the risk for T2DM and its complications. Various studies have reported the association of imbalance oxidants and anti-oxidants ratio with T2DM⁴, Retinopathy²¹, Neuropathy²², Nephropathy²³, and CVD²⁴ summarized in Figure 1.

Thus, it is aimed to estimate the AR activity and MDA levels and in patients with T2DM. This study might be helpful for the better management and treatment of T2DM patients and delay and/or reduce its complications.

MATERIAL AND METHODS

Subject Selection

In this case-control study, a total of 60 subjects (30 T2DM and 30 age-matched controls) aged between 30-70 years was enrolled from outpatient Department of Medicine and Diabetes Clinic of IIMS&R, Integral University, Lucknow, Uttar Pradesh, India. The study was conducted out from January 2017 to June 2017. This study is approved by the institutional research and ethical committee and followed the ethical standards with the 1964 Helsinki declaration and its later amendments or comparable ethical standards²⁵. Written informed consent was taken from each subject recruited for the study.

The inclusion of T2DM and control subjects: The person having FBS (≥ 126 mg/dl) and PPBS (≥ 200 mg/dl) considered as cases. The person having FBS (< 125 mg/dl) and PPBS (< 200 mg/dl) was considered as controls²⁶.

Subjects with ischemic heart disease, angina, Myocardial Infarction (MI), electrocardiogram abnormalities, anemia (Hb

of 8.0 g/dl or less), those with other concurrent sicknesses like the chronic liver disease, chronic kidney disease, hypothyroidism or those on drugs like antihypertensive, antioxidants and diuretics, and pregnant women were excluded for both case and control groups. Detailed medical history was taken from each subject.

Laboratory investigations

About 3ml of venous blood was collected in EDTA vial from each subject for estimation of AR activity and MDA levels. The AR activity was estimated by the NADPH oxidation method²⁷. A 10% erythrocyte suspension (hemolysate) was made by adding 50 mM sodium phosphate buffer, pH 7.4, containing 150 mM NaCl. The suspension was lysed by repeated freezing and thawing (three cycles) and centrifuged to remove insoluble material if any. The assay mixture in 1 ml contained 50 μ mol potassium phosphate buffer pH 6.2, 0.4 mmol lithium sulfate, 5 μ mol 2-mercaptoethanol, 10 μ mol DL-glyceraldehyde, 0.1 μ mol NADPH and enzyme preparation (hemolysate). The assay mixture was incubated at 37 °C and initiated by the addition of NADPH at 37°C. The change in the absorbance at 340 nm due to NADPH oxidation. Absorbance was measured at 340nm by UV-Visible double beam spectrophotometer (Systronics-2205,

Systronic India Ltd. Gujarat, India). One unit was defined as micromoles NADPH oxidized/g Hb/min. The plasma MDA levels were estimated by thiobarbituric acid reactive substance (TBARS) method²⁸. Plasma was deproteinized and the precipitate is treated with thiobarbituric acid (TBA) at 90°C 1 hour. The pink color product formed at the end of the reaction which measured at 535 nm by UV-Visible double beam spectrophotometer (Systronics-2205, Systronic India Ltd. Gujarat, India).

Statistical analysis

Data analysis was performed using the IBM SPSS software version 20.0 (Armonk, NY, USA). All the data were compared between the two groups by using analysis of variance (ANOVA) or unpaired t-test. Values were represented as mean \pm SD (Standard Deviation). Pearson correlation coefficient was calculated among T2DM. A P value <0.05 was considered as statistically significant for all data analyzed.

RESULTS

The mean age of T2DM and controls were found 55.7 \pm 5.85 and 57.03 \pm 4.15 years, respectively (P=0.31). The mean level of FBS and

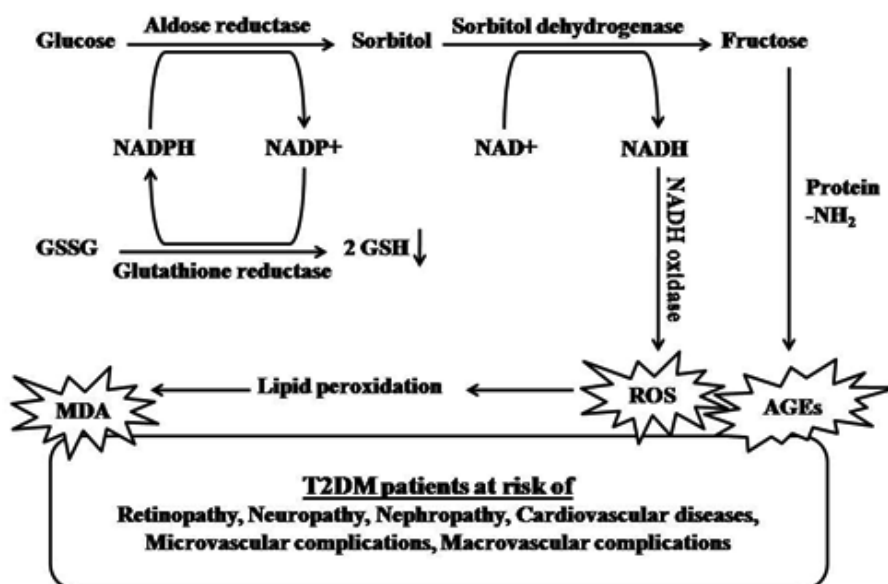


Fig. 1. AR activity and consumption of NADPH: Diagrammatic representation of the production of reactive oxygen species (ROS), advanced glycated products (AGEs) and malondialdehyde (MDA) in T2DM increase the risk for T2DM complications

PPBS were found significantly higher in T2DM than controls ($P<0.001$, $P<0.001$, respectively). Similarly, the mean activity of AR was found significantly high in the T2DM as compared to controls ($P<0.001$). In addition, the mean level of MDA was also found significantly high in the T2DM as compared to controls ($P<0.001$) shown in Table 1.

A significant positive correlation was found between FBS and PPBS among T2DM ($r=0.71$, $P<0.01$). However, There was no significant correlation found between AR activity and MDA level among T2DM ($r=0.002$, $P>0.05$) shown in Table 2.

DISCUSSION

The mean level of FBS and PPBS were found significantly higher in T2DM than controls ($P<0.001$). Previous studies have reported that the mean level of FBS and PPBS have been observed significantly higher in T2DM as compared to controls in all age groups²⁹⁻³¹.

The mean activity of AR was found significantly high in the T2DM as compared to controls ($P<0.001$). It is reported that the AR activity increased in T2DM as compared to controls which are the leading cause of diabetic retinopathy in T2DM^{15, 27, 32}. The study has been reported that when intracellular glucose levels are high polyol pathway for glucose metabolism is activated by AR activity. It is observed that the activity of AR is significantly higher in diabetic patients with complications like retinopathy as compared to controls³³. Increased AR activity has been correlated with cataract formation and retinopathy³².

In addition, the mean level of MDA was also found significantly high in the T2DM as compared to controls ($P<0.001$). Previous studies have reported that the MDA levels have been found significantly increased in T2DM as compared to controls³⁴⁻³⁶.

A significant positive correlation was found between FBS and PPBS among T2DM ($r=0.71$, $P<0.01$). Previous studies have reported that the FBS and PPBS have been shown strong

Table 1. Clinical characteristics of T2DM and control

Parameters	T2DM (n=30)	Control (n=30)	P value
Age (years)	55.7±5.85	57.03±4.15	0.31
FBS (mg/dl)	231.0±82.0	83.0±9.3	<0.0001*
PPBS (mg/dl)	329.0±108	125.0±11.2	<0.0001*
AR (U/g Hb/min)	4.65±1.62	2.22±0.62	<0.0001*
MDA (imol/l)	3.74±0.86	2.09±0.76	<0.0001*

Values are expressed as Mean ± Standard Deviation

*Significant considered as $P<0.05$.

FBS: Fasting Blood Sugar, PPBS: Post-Prandial Blood Sugar, AR: Aldose Reductase,

MDA: Malondialdehyde

Table 2. Correlation between variables in T2DM

	FBS (mg/dl)	PPBS (mg/dl)	AR (U/g Hb/min)	MDA (μ mol/l)
Age (years)	-0.113	-0.035	0.245	-0.085
FBS (mg/dl)	1	0.714**	0.123	0.064
PPBS (mg/dl)	-	1	0.044	0.013
AR (U/g Hb/min)	-	-	1	0.002
MDA (imol/l)	-	-	-	1

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

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

FBS: Fasting Blood Sugar, PPBS: Post-Prandial Blood Sugar, AR: Aldose Reductase,

MDA: Malondialdehyde

positive correlation in T2DM²⁹⁻³¹. However, There was no significant correlation found between AR activity and MDA level among T2DM ($P>0.05$). In the animal model, Lee *et al* reported that the flux of glucose through the polyol pathway is the major cause of hyperglycemic oxidative stress in the tissue³⁷. The decrease in the reduced glutathione (GSH) levels with a rise in the MDA level in the transgenic mice lens, but not in the non-transgenic mice. A study suggested that auto-oxidation of glucose and non-enzymatic glycation do not contribute significantly to oxidative stress in diabetic lenses. AR reduction of glucose to sorbitol probably contributes to oxidative stress by depleting its cofactor NADPH, which is also required for the regeneration of GSH. Sorbitol dehydrogenase, the second enzyme in the polyol pathway that converts sorbitol to fructose, also contributes to oxidative stress, most likely because depletion of its cofactor NAD/ leads to more glucose being channeled through the polyol pathway^{3, 13, 17, 37}. The MDA plays only a minor role in the development of cataract. However, chronic oxidative stress generated by the polyol pathway is likely to be an important contributing factor in the slow-developing diabetic cataract and other diabetic complications^{16, 18, 37}. In hyperglycemia conditions, the rate of reduction of glucose to sorbitol increases with increasing glucose levels in tissues that do not require insulin for glucose uptake mainly in lens due to the AR activation¹⁵. Several studies suggested that ROS, MDA and AR activity is generated in the significant amount in T2DM, which play a key role in the pathogenesis of T2DM and its complications^{3, 4, 17}.

Genetic predisposition may influence the development of T2DM complications. The C(-104) T polymorphism in the regulatory region of the AR gene have observed the risk for diabetic retinopathy in T2DM³⁸. In diabetic mice model, the human AR expression regresses atherosclerosis may be due to interfering in plaque macrophage inflammation reduction³⁹.

In the therapeutic approach, some studies proposed that the AR inhibitors may prevent the glucose flux by the polyol pathway in hyperglycemic conditions. The AR inhibitors may delay or reduce the development of T2DM and its complications^{40, 41}.

Limitations

This study has some limitations: firstly, the sample size is less and this study should be replicated in large sample size. Secondly, we have studied AR activity and MDA levels. Some other enzymes of the polyol pathway such as sorbitol dehydrogenase and glutathione levels should be included to clarify the consumption of NADPH and reduction of GSH simultaneously.

CONCLUSION

Results showed that the mean of FBS, PPBS, AR activity, and MDA levels were found significantly higher in T2DM than controls. There was no significant correlation found between AR activity and MDA level among T2DM. However, elevated AR activity and oxidative stress play a key role in the pathogenesis of T2DM and its complications.

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