



## A STUDY ON MALONDIALDEHYDE AS A MARKER OF LIPID PEROXIDATION IN MALE AND FEMALE PATIENTS WITH TYPE 2 DIABETES MELLITUS

\*Ayaz K. Mallick<sup>1</sup> Ravindra Maradi<sup>2</sup> Vivek R. Joshi<sup>1</sup> Gaurav Shorey<sup>3</sup> Marya Ahsan<sup>4</sup>

<sup>1</sup>Department of Biochemistry, Melaka Manipal Medical College, Manipal, Manipal University, India.

<sup>2</sup>Department of Biochemistry, Kasturba Medical College, Manipal, Manipal University, India.

<sup>3</sup>Department of Pathology, Melaka Manipal Medical College, Manipal, Manipal University, India.

<sup>4</sup>Department of Pharmacology, Nalanda Medical College, Patna, Magadh University, India.

\*Corresponding author's E-mail: [ayazmallick@yahoo.com](mailto:ayazmallick@yahoo.com)

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### ABSTRACT

Diabetes mellitus (DM) is associated with hyperglycemia and oxidative stress. It is associated with various life threatening complications like nephropathy, retinopathy, atherosclerosis and coronary artery disease which adds up to the mortality among diabetics. These complications develop due to late presentation of the disease and delayed diagnosis. Oxidative stress which indicates the imbalance between reactive oxygen species (ROS) and the defensive antioxidant system is increased due to prolonged exposure to hyperglycemia. Increased oxidative damage plays an important role in the pathogenesis of diabetic complication. Malondialdehyde (MDA) a marker of lipid peroxidation, is thought to play an important role in development of atherosclerosis and other complications of DM. The study was undertaken to determine the MDA levels in the membrane of erythrocytes of diabetic patients and to study the relationship of lipid peroxidation with severity of diabetes. Fasting plasma glucose (FPG), glycated hemoglobin (HbA1c), erythrocyte malondialdehyde (MDA) levels were determined in 30 normal controls and 50 type 2 diabetic cases. The cases included 27 diabetic males and 23 diabetic females. Diabetic patients, irrespective of their gender, showed an increase in the erythrocyte MDA levels which was statistically significant. The erythrocyte MDA levels showed a significant strong positive correlation with glycated haemoglobin. Increase oxidative stress in Type 2 diabetic subjects contributes to the development of diabetic complications. Therefore, measuring the MDA level during the follow up of diabetic patients would help to early diagnosis of diabetic complication.

**Keywords:** Malondialdehyde, oxidative stress, diabetes mellitus.

### INTRODUCTION

Diabetes mellitus (DM) along with its complications is recognized as a major cause of mortality and morbidity all around the world. DM is a group of metabolic disorders characterised by hyperglycemia and glycosuria and is associated with disturbances in carbohydrate, fat and protein metabolism resulting from either an absolute or relative deficiency of insulin secretion or action.<sup>1</sup> Long term diabetes mellitus, usually associated with a state of chronic hyperglycemia, results in dysfunction and failure of various organs especially the eyes, kidneys, nerves, heart and the blood vessels. This causes dreaded complications which increases the mortality among the diabetics.<sup>2</sup> Microvascular and macrovascular complication in diabetes are predominantly seen due to delayed diagnosis and late presentation of the disease. A link has been established between the oxidative stress and the origin of multi organ complications in poorly controlled diabetes mellitus.<sup>3-6</sup>

Oxidative stress, defined as a measure of steady levels of reactive oxygen species (ROS) or oxygen radical in the biological system, plays an important role in the etiology of diabetic complications as it may be a common pathway linking the diverse mechanism for the pathogenesis of diabetic complications. Increased oxidative stress has been observed in diabetic patients as there is increased production of free radicals like superoxides and hydrogen

peroxides. This increase, in the free radicals, is countered by various antioxidant enzymes like superoxide dismutase, glutathione reductase, catalases, protein thiols etc. Diabetic patients have an increased oxidative damage due to an increase in the production of oxygen free radicals and a deficiency in antioxidant defence mechanisms.<sup>7,8</sup> Lipids are among the primary targets of oxidative stress.<sup>9</sup> Excessive ROS production results in lipid peroxidation of the cellular structures which is thought to play an important role in pathogenesis of many degenerative diseases, such as atherosclerosis, oxidative damage to DNA, aging, carcinogenesis, atherosclerosis and microvascular complications of DM.<sup>10,11,12</sup> Malondialdehyde (MDA), also referred to as TBARS (TBA Reacting Substances), is one of the lipid peroxidation products frequently used to determine the oxidant/antioxidant balance in diabetic patients as they are stable and easily measurable. Although microvascular and macrovascular complications of DM are known to increase with DM severity,<sup>13-15</sup> the association between DM and MDA levels in type 2 DM remains controversial and contradictory as both increases and decrease of MDA have been reported in diabetes mellitus.<sup>16</sup>

The study was undertaken to determine the MDA levels in the erythrocyte membrane of diabetic patients and to evaluate a correlation between lipid peroxidation with severity of diabetes.



## MATERIALS AND METHODS

### Subjects

This cross sectional study was carried out in type 2 diabetes mellitus patients attending the Kasturba Hospital, Manipal. The study included a total of 80 subjects out of which 30 were healthy non diabetic controls (FPG less than 126 mg/dL) and 50 were diabetic cases (FPG <126 mg/dL). 27 diabetic males and 23 diabetic females were included in the study (Table 1). The mean age of normal controls was  $50 \pm 12.21$  years ranging from 35 to 80 years and that of cases was  $53 \pm 9.2$  years ranging from 38 to 75 years. Age and sex matched non diabetic controls were selected for comparison. Informed consent and ethical committee clearance was obtained for the study. Exclusion criteria included patients with complications of diabetes mellitus like retinopathy, nephropathy, patients on antioxidants, smokers and hypertensive patients.

**Table 1:** Diabetic cases and normal controls.

GROUP	GENDER		TOTAL
	Male	Female	
Control	16	14	30
Cases	27	23	50
			80

### Biochemical determination

Auto analyzers were used for determination of various biochemical parameters like fasting plasma glucose levels (FPG) (Glucose oxidase-peroxidase method) and glycated hemoglobin (HbA1c).

### Determination of malondialdehyde

Erythrocyte MDA concentration was determined using the method described by Jain et al.<sup>17</sup>

### Preparation of malondialdehyde standard

Standard Malondialdehyde was prepared from 1, 1, 3, 3-tetramethoxy propane or MDA bisacetal ( $C_{11}H_{24}O_4$ , 99%, Mol Wt 164.2, density 0.997). Fresh standards were prepared. 0.166 ml of MDA bisacetal was made up to 100 ml 1%  $H_2SO_4$ , mixed and kept at room temperature (in dark) for 2 hrs. This gave 10mM MDA solution, which was further diluted with 1%  $H_2SO_4$  to get solutions with different MDA concentrations.

**Table 2:** Comparison of fasting plasma glucose, glycated hemoglobin and erythrocyte MDA levels in controls and diabetic cases (with mean  $\pm$  standard deviation).

	CONTROLS (n = 30)	CASES (n=50)
Age (years)	$50 \pm 12.38$	$52 \pm 9.22$
Fasting plasma glucose (mg %)	$96.47 \pm 14.43$	$184.30 \pm 43.79^{**}$
Glycated haemoglobin (% of Hb)	$6.02 \pm 0.81$	$9.72 \pm 1.8^{**}$
Erythrocyte MDA (nmol/gm Hb)	$3.70 \pm 1.19$	$7.83 \pm 3.26^*$

P value: \* < 0.01; \*\* <0.001

### Procedure

Blood collected in the EDTA (Ethylenediaminetetraacetic acid) tubes was centrifuged at 2000g for 7 minutes. Plasma and the buffy coat were discarded. The cells were washed with cold 0.15M NaCl solution two times after 1 - 10 dilutions. The volume of the packed cell was seen with a graduated centrifuge tube or an ordinary pipette with a wide tip. 0.2 ml of packed cells was suspended in 0.8 ml phosphate buffer saline (PBS) and 0.025 ml butylated hydroxy toluene (BHT). To this 0.5ml of 30% trichloro acetic acid (TCA) was added. The tubes were vortexed and allowed to stand on ice for 2 hours. Tubes were then centrifuged at 2000g for 15 minutes. One ml of the supernatant was transferred into another tube. To this 0.075 ml of 0.1 M EDTA and freshly prepared 0.25ml of 1% thiobarbituric acid (TBA) were added. Contents were mixed and kept in a boiling water bath for 15 minutes. After the tubes were cooled to room temperature, absorbance was read at 532 nm and 600nm using Genesys spectrophotometer. Absorbance at 600nm was subtracted from absorbance at 532 nm. TBARS value was calculated from the standard graph and expressed as nanomoles/gram of hemoglobin.

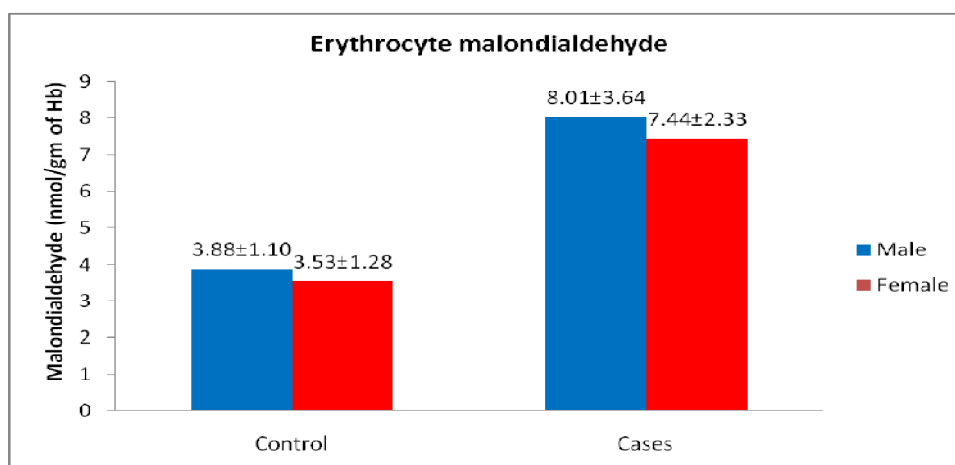
### Statistical analysis

All values of analyzed parameters were expressed as mean  $\pm$  S.D. Statistical analysis was performed using the Statistical Package for Social Science (SPSS/PC; SPSS-11.5, Chicago, USA). As the data showed normal distribution, independent t test was used to compare the mean values in the two groups. Pearson's correlation was applied to correlate between the parameters. A p-value of <0.05 was considered statistically significant.

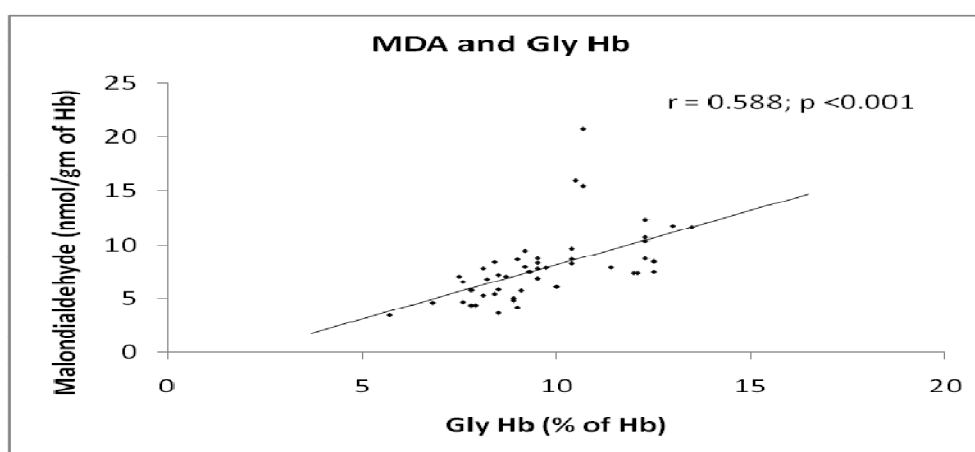
## RESULTS

The study included 27 diabetic males and 23 diabetic females. The results of the study are shown in the table 2. As shown in figure 1, the diabetic patients, irrespective of their gender, showed an increase in the erythrocyte MDA levels which was statistically significant (p value < 0.01). The erythrocyte MDA levels showed a strong positive correlation with glycated hemoglobin ( $r = 0.588$ ) which was also statistically significant ( $p < 0.001$ ) (Figure 2).





**Figure 1:** Graph showing the MDA levels among males and females in diabetic cases and healthy controls.



**Figure 2:** Correlation between Malondialdehyde levels and glycated haemoglobin in diabetic patients.

## DISCUSSION AND CONCLUSION

Diabetes affects the lipid metabolism profoundly and this effect is directly related to the control of their plasma glucose levels. Diabetics who have a poor glycemic control are at more risk of developing micro and macrovascular complication. De Zwart et al proposed that oxidative stress may be associated with the pathogenesis of NIDDM complications, particularly cardiovascular diseases.<sup>18</sup> Hyperglycemia causes the production of free radical in several ways. Firstly, it can lead to auto oxidation of glucose resulting in the formation of  $\alpha$ -hydroxy-aldehyde that can form an enediol and subsequently produce superoxide radicals, hydroxyl radicals and hydrogen peroxide.<sup>19</sup>

Secondly, hyperglycemia leads to formation of advanced glycation end products (AGE) via the non enzymtic Maillard reaction between the reducing sugars and amino acid residues. This is a slow physiological process which occurs in long-lived proteins like collagen and lens. However, in diabetes, hyperglycemia and oxidative stress favours reversible glycosylation of proteins, lipids and nucleic acid with the attachment of glucose to the amino groups forming Schiff bases at a rate proportional to plasma glucose concentration. The early AGEs formed, which are still reversible, undergoes chemical

modification and forms Amadori products. This later undergoes slow complex rearrangement to form irreversible AGEs such as carboxymethyl lysine (CML), pentosidine, imidazolone and pyrroline. These permanent AGEs are highly reactive causing cross link formation and trapping of proteins, lipid peroxidation and inactivation of nitric oxide.<sup>20</sup>

Hyperglycemia also leads to changes in polyol-inositol metabolism. Aldose reductase reduces the aldehydes generated by reactive oxygen species to inactive alcohols, and glucose to sorbitol, using NADH as a cofactor. Sorbitol is then oxidized to fructose. At normal plasma glucose concentrations, metabolism of glucose by this pathway is limited because aldose reductase exerts low affinity for glucose. However in diabetic patients, high intracellular glucose concentration activates aldose reductase, which causes increased conversion of glucose to sorbitol and decreased intracellular NADPH content. Cells with high aldose reductase activity causes depletion of reduced glutathione resulting in increased oxidative stress.<sup>19,20</sup>

Lipid peroxidation is a free radical-related process, which is potentially harmful because its uncontrolled, self-enhancing process causes disruption of membranes, lipids and other cell components. It has been found to be connected with various disease processes and also plays a



very important role in pathogenesis of diabetic complication.

In our study we have used erythrocytes as a model to study oxidative stress. It is a target for oxidative reaction because of high oxygen tension and the presence of hemoglobin and plasma membrane rich in polyunsaturated fatty acids (PUFA). However there are contradictory reports regarding MDA levels in diabetics. Sundaram et al in their study found that lipid peroxidation was significantly increased in plasma and erythrocytes. On the other hand, Bates et al found normal MDA levels.<sup>13</sup> In our study, there was a significant increase in the erythrocyte membrane lipid peroxidation which was shown by increased MDA levels (Table 2). This increase was seen in both male and females indicating that both the groups are equally susceptible to diabetes induced oxidative stress (Fig 1). The study also showed a significant positive correlation between the erythrocytes MDA levels and glycated hemoglobin (fig 2). This is due to auto oxidation of glucose which causes persistent generation of ROS or MDA, pointing towards the fact that prolonged hyperglycemia appears to be a cause for increased oxidative stress which in turn leads to life threatening complication. With our study we can conclude that increase oxidative stress in Type 2 diabetic subjects contributes to the development of micro vascular complications. Therefore, measuring the MDA level during the follow up of diabetic patients would help to early diagnosis of diabetic complication. However a large scale study and a longer follow up of the patients may be required to further establish MDA as a biochemical marker for diabetic complications.

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