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CORRELATION OF LIPID PEROXIDE -MALONDIALDEHYDE LEVEL WITH DURATION OF DIABETES MELLITUS (TYPE 2)

DR.A.J.MANJULA DEVI[*1], DR.S.SRIDEVI[2] , DR.SHANTHI.B[3] AND DR.RATHIROOPAVATHY[4]

[1] Professor & HOD , Department of biochemistry, sree balaji medical college and hospital , Bharath university , India.
[2]Post graduate , Department of biochemistry, sree balaji medical college and hospital , Bharath university , India.
[3] Professor ,Department of biochemistry, sree balaji medical college and hospital , Bharath university , India
[4]Post graduate , Department of biochemistry, sree balaji medical college and hospital , Bharath university , India.

ABSTRACT

Type 2 diabetes mellitus (DM) is a metabolic disorder characterized by hyperglycemia and oxidative stress which occurs as a result of imbalance between pro-oxidants and antioxidants. Malondialdehyde (MDA) is a product of the peroxidation and it is considered to be one of the markers of oxidative stress. The association between DM duration and MDA levels in type 2 DM remains controversial. This study was undertaken to evaluate the correlation between MDA levels and DM duration. To compare the oxidative stress in two study groups MDA levels were compared. The mean MDA levels were high in diabetics with duration more than 5 years. The same was compared between the two study groups (Group I-Duration of DM < 5 years & Group II-Duration of DM > 5 years) using student t test. The difference in MDA levels between two diabetic groups was significant (P<.01).But the comparison of long term glycemic control (HbA1C) between two diabetic groups was not statistically significant. This suggests that oxidative stress might be related to duration of DM irrespective of glycemic control. The correlation study of MDA levels with the duration of diabetes and glycemic control (HbA1C) showed that the levels were statistically significant for the duration of DM and not statistically significant for glycemic control.

KEYWORDS: DM - Diabetes mellitus , MDA – Malondialdehyde , TBA - Thiobarbituric acid ALE - Advanced Lipoxidation End products , AGE - Advanced Glycation End products

DR.A.J.MANJULA DEVI
Professor & HOD , Department of biochemistry,
sree balaji medical college and hospital , Bharath university , India.

INTRODUCTION

Diabetes mellitus is a systemic metabolic disorder characterized by hyperglycaemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycaemia of diabetes is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels. There are many pathogenic processes involved in the development of diabetes which range from autoimmune destruction of the beta-cells of the pancreas to abnormalities that result in resistance to insulin action. The two broad categories of diabetes are type 1 and type 2. In type 1 diabetes, there is an absolute deficiency of insulin secretion. In type 2 diabetes, which is the more prevalent category there is a combina tion of resistance to insulin action and an inadequate compensatory insulin secretory response. Type 2 diabetes (accounting for 90–95% of those with diabetes), previously referred to as non–insulin-dependent diabetes or adult onset diabetes, and encompasses individuals ranging from predominantly insulin resistance with relative insulin deficiency to predominantly an insulin secretory defect with insulin resistance. Diabetes mellitus (DM) is associated with oxidative stress which occurs as a result of imbalance between pro-oxidants and antioxidants[1-3] Chronic hyperglycaemia and high fatty acid concentrations can cause damage in different types of cells by a variety of mechanisms collectively known as glucolipotoxicity, and oxidative stress is considered to be the common link.[4,5] Lipid peroxidation of the cellular structures, a consequence of increased oxygen free radicals, is thought to play an important role in atherosclerosis and micro vascular complications of DM.[6] Malondialdehyde (MDA) is a product of the peroxidation of arachidonic, eicosapentaenoic and docosahexaenoic acids.[7,8] Interactions between MDA and LDL results in oxidised-LDL (ox-LDL) which could induce atherosclerosis, atherothrombosis and plaque erosion. Although studies done extensively have shown that micro vascular and macro vascular complications of DM are known to increase with DM duration,[9-11] the association between DM duration and MDA levels in type 2 DM remains controversial. The present study is aimed to evaluate the correlation between MDA levels and DM duration.

MATERIALS AND METHODS

The study was conducted in a sample of 70 Type 2 diabetic patients attending diabetic outpatient department and 35 healthy nondiabetic individuals who came for routine check-up at Sree Balaji Medical College & Hospital.

Study individuals were divided into 3 groups.

Group I - comprises of 35 type-2 diabetic individuals of duration less than 5 years belonging to the age group between 40 and 50 years.

Group II - comprises of 35 type-2 diabetic individuals of duration more than 5 years belonging to the age group between 40 and 50 years.

Group III - 35 age, sex and Body mass index matched healthy controls.

This study was conducted between December 2012 and May 2013. The research and ethics committee of the university approved the study protocol. All participants were provided with written informed consent before enrolment in the study. Demographic data, age, gender, height, weight, DM duration, general history and medications, were recorded. Blood pressure (systolic and diastolic) of the patient was measured in the sitting position after ten minutes of rest, and the average of two measurements (with a 5-minute interval) was used for analysis. Blood samples were collected following overnight fasting. The fasting plasma sugar (FPS), total cholesterol, triglyceride, high-density lipoproteins (HDL), low density lipoprotein(LDL)very low density lipoprotein(VLDL), glycated

haemoglobin (HbA1c), serum urea, creatinine, and MDA levels were measured. Urine was collected for urine albumin creatinine ratio (ACR).

Inclusion criteria:

Cases:

Type 2 Diabetic patients in the age group of 40 - 50 years.

Exclusion criteria:

Smokers
Hypertension
Alcoholics
Patients on antioxidants
Serum creatinine>2mg/dl
Chronic illness

Sample collection:

The study was explained to all three groups and informed consent obtained from them before taking the blood sample. The blood samples were collected by venepuncture under aseptic precautions. Random urine sample was collected from the patients for the estimation of albumin creatinine ratio in urine.

INVESTIGATIONS

1. Estimation of HbA1c by Ion Exchange Resin method :
2. Estimation of Albumin Creatinine Ratio (ACR):
   Method: Turbidimetric immunoassay for microalbumin
3. Estimation of Serum Malondialdehyde (MDA):
   Method: Spectrophotometric assay of Thiobarbituric acid reactive substances (TBARS)
4. Estimation of FBS , PPBS by GOD – POD method ,
   Diatek Kit, Fully Automated Analyser
5. Estimation of Fasting serum lipid profile Total cholesterol by CHOD-POD method
   HDL cholesterol by CHOD-POD method after precipitation ,VLDL cholesterol, calculated value [triglycerides / 5] LDL cholesterol, by friedwald equation [Total cholesterol-(VLDL+HDL)]
6. Estimation of Serum Creatinine By Jaffes Kinetic Method , Erba Biochem

The study population comprised of a total of 105 individuals and of this, 70 were Diabetic study Individuals and 35 were healthy controls. Of this 70 Diabetic individuals, one half were in group I belonging to duration of Diabetes (Type 2) less than 5 years and the other half were in group II belonging to duration of Diabetes (Type 2) more than 5 years. All the study individuals were in the age group between 40 and 50 years. 35 healthy controls (Group III) were age, sex and Body mass index matched individual.

All the biochemical study parameters were analysed using Statistical Product and Service Solutions (SPSS) 17 software. Statistical tests used were Descriptives, Student t test & Pearson's Correlation.

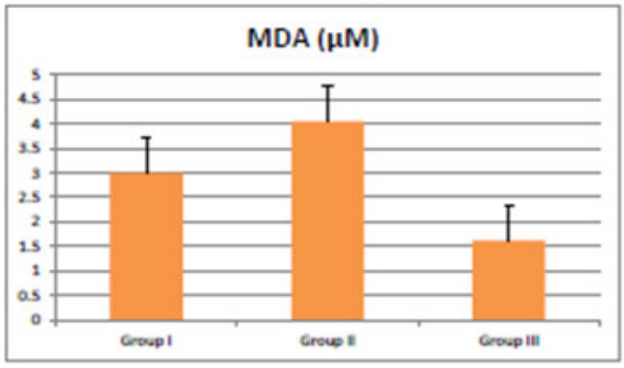
*P < .05 is significant. , **P < .001 is strongly significant.

RESULTS

DESCRIPTIVE STATISTICS

TABLE 1
MDA levels

Graph 11: Comparison of serum Malondialdehyde (MDA) levels between 3 groups



Parameter	Group I (mean ± SD)	Group II (mean ± SD)	Group III (mean± SD)	Significance
MDA (µM/L)	3.00± .73	4.05±.53	-	t=-4.382;P<.01 (between Group I and II)
MDA (µM/L)	-	4.05±.53	1.62±.47	t=15.790;P<.001 (between Group II and III)
MDA (µM/L)	3.00± .73	-	1.62±.47	t=10.036;P<.001 (between Group I and III)

Comparison of MDA levels between 3 groups. MDA levels in Group I and Group II are 3.00± .73 and 4.05±.53 respectively. The value in healthy control Group III is 1.62±.47. The difference in mean values between two study groups is significant (t=-4.382; P<.01).There is also statistically significant difference between each study group and the healthy control group.
Comparison between Group I & III: t=10.036; P<.001
Comparison between Group II & III: t=15.790; P<.001

TABLE 2
Correlation of MDA levels in Diabetic individuals with Duration of Diabetes (Type 2) and HbA1C.

Group	Parameter	Duration of Dm	HbA1C
I	MDA(Pearson correlation) (Significance)	r=.501** P=.002	r=.146 P=.401
II	MDA(Pearson correlation) (Significance)	r=.443** P=.008	r=.205 P=.237

Pearson's correlation of MDA levels of Study Group I and Study Group II with the duration of Diabetes are r=0.501 and r=0.443 respectively. Both the correlations are statistically significant. Correlation coefficient(r) of Group I MDA levels with HbA1C is 0.146(P=.401).Correlation coefficient(r) of Group II MDA levels with HbA1C is 0.205(P=.237)

TABLE 3
Correlation of Glycaemic control in Diabetics with duration of Diabetes

Group	Parameter	Duration of DM
I	HbA1C (Pearson correlation) (Significance)	r=.329 P=.054
II	HbA1C (Pearson correlation) (Significance)	r=.299 P=.081

Correlation of HbA1C levels in both the Study Groups with the duration of diabetes is not statistically significant

DISCUSSION

Our study was done on Type 2 Diabetic patients. Age, Sex and BMI matched healthy individuals were taken as controls. Between the two study groups and the control group, the routine biochemical parameters, fasting plasma sugar (FPS), glycated haemoglobin (HbA1C), serum urea, serum creatinine, lipid profile and urine Albumin creatinine ratio(ACR) differed significantly. Malondialdehyde has been done as the marker of oxidative stress in diabetics and this study was done in the intention of correlating oxidative damage with the duration of diabetes (Type 2). The fasting plasma sugar was done to assess the short term glycaemic control. The FPS values in Study Group I, Study Group II and Control Group III were 131.26±35.508, 151.31±31.535 and 82.74±8.889 respectively. The difference in short term glycaemic control (FPS) values between the two study groups was statistically significant (P< .05). To assess the long term glycaemic control HbA1C levels were measured. Mean HbA1C values in Study Group I, Study Group II and Control Group III were 7.6463±.41920, 8.2563±.61980and 5.2998±.54143 respectively. The difference in mean values between two study groups was not statistically significant (P=0.07). This shows that long term glycaemic control was not significantly different in diabetics with duration more than 5 years when compared to diabetics with duration less than 5 years. This suggests that long term glycaemic control might not be directly related to duration of diabetes. Insulin resistance and type 2 diabetes are associated with a clustering of interrelated plasma lipid and lipoprotein abnormalities, which include reduced HDL cholesterol and elevated triglyceride levels. Increased hepatic secretion of large triglyceride-rich VLDL and impaired clearance of VLDL appears to be of central importance in the pathophysiology of this dyslipidaemia. These changes are also a feature of the insulin resistance syndrome (also known as the metabolic syndrome), which underlies many cases of type 2 diabetes. In fact, pre-diabetic individuals often exhibit an atherogenic pattern of risk factors that includes higher levels of total cholesterol, LDL cholesterol, and triglycerides and lower levels of HDL cholesterol than individuals who do not develop diabetes. This study also observed that there is significant elevation of total cholesterol, LDL, VLDL, triglycerides and significant lowering of HDL in diabetics when compared to healthy controls. But there was no significant difference in lipid profile between two study groups. In this study, the renal parameters- serum urea, creatinine and albumin creatinine ratio (ACR) were included. In study group I, we found that serum urea levels were within normal range (15-40mg/dl). Mean serum urea levels in Group II was 39.37±7.240, which was higher than Group I (30.91±4.7550) and the difference was statistically significant. Similarly, serum creatinine levels were higher in Group II (1.3229±.25447) when compared to Group I (1.0400±.25228) and the difference was significant (P<.05).

In the early 1980s, studies from Europe revealed that small amounts of albumin in the urine, not usually detected by conventional

methods, were predictive of the later development of proteinuria in type 1.[12-14] and type 2 diabetic patients. This stage of renal involvement was termed microalbuminuria or incipient nephropathy. U.K. Prospective Diabetes Study (UKPDS) shows that in patients with type 2 diabetes, the incidence of microalbuminuria was 2.0% per year and the prevalence 10 years after diagnosis is 25%. In this study ACR was done to correlate with the oxidative stress and the glycaemic control. It was found that in this study, ACR level was significantly high in Group II when compared to Group I (P<.01). Two important consequences of hyperglycaemia in DM are oxidative stress and the formation of advanced glycation end products (AGE). In this study, to evaluate the oxidative damage caused by diabetes, malondialdehyde (MDA) has been taken as the marker. A rise in plasma MDA levels in DM reflects oxidative damage to lipids as it is a lipid peroxidation marker. Many studies have shown that increased oxidative stress is present in diabetic subjects. [15-17] There are several reports indicating increased MDA levels in patients with type 2 diabetes Similarly, this study's data also provides further evidence that there is presence of oxidative stress and increased lipid peroxidation (MDA levels) in Type 2 diabetics. This study shows that there is significant increase in mean MDA levels in Group II (DM duration > 5 years) (4.0526±.53155) as compared to Group I (DM duration < 5 years) (3.0023± .73704).The Mean MDA levels in Group III (healthy controls) was 1.6240±.47884.This shows that MDA levels were significantly high in diabetics and this could be attributed to lipid peroxidation secondary to oxidative stress. Our findings strongly confirmed the evidence that diabetic patients were susceptible to oxidative stress.

Pearson's correlation coefficient was applied to find the correlation of MDA level with duration of diabetes and glycaemic control. In a study done on 20 patients with newly diagnosed DM and 20 patients with a mean duration of seven years, patients in the latter group had higher serum MDA concentrations and lower levels of glutathione. A Spanish study by Dominguez C *et al* showed that the level. of estimated MDA was higher with the earlier onset and prolonged duration of diabetes, and these levels continued to rise during the course of the disease. An Iranian study by Nakhjavani M et al observed that elevated MDA levels significantly correlated to duration of diabetics with more than 10 years compared to those with comparatively lesser duration. Similar to the afore mentioned studies, this study also observed that in comparison to those with DM duration less than 5 years there was significantly higher levels of MDA in those with DM duration over 5 years(P < .01) .This study shows that there is positive correlation between MDA levels and duration of diabetes. Some studies have shown a positive correlation between MDA level and glycaemic control, while several others have failed to do so.[18,19]. The results of this study are in support of the latter. We found no significant correlation between MDA levels and glycaemic control. This result is similar to the study done by Pasaoglu H et al Domínguez C et al and Nakhjavani M et al. The data from this study suggests that the increase in MDA with DM duration might not be due to poor glycaemic control. The probable reason might be the effect of time. (duration of diabetes) on lipid peroxidation and MDA production, independent of glycaemic control.

CONCLUSION

The results of this study provide evidence that oxidative stress is present in patients with type 2 diabetes mellitus and is influenced by the duration of diabetes. The present study observed that there is positive correlation of MDA levels with the duration of diabetes and is independent of glycemic control. In conclusion, chronicity of diabetes mellitus promotes lipid peroxidation and MDA production, independent of glycaemic control.These observations suggest that supportive therapy aimed at reducing oxidative stress may help in reducing complications of type 2 diabetes mellitus. Hence, antioxidant therapy to all patients diagnosed with diabetes mellitus might help to reduce oxidative damage induced complications.

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