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Original Research Paper

Biochemistry

OXIDATIVE STRESS IN DIABETES MELLITUS PATIENTS : A STUDY OF MALONDIALDEHYDE (MDA) AND ISCHEMIA MODIFIED ALBUMIN (IMA) AS INDICATORS OF OXIDATIVE STRESS.

Neerja Agrawal — Associate Professor, Department of Obstetrics and Gynaecology, Raipur Institute of Medical Sciences, Raipur, CG.

Swati Hiwale* — Professor and Head, Department of Biochemistry, Raipur Institute of Medical Sciences, Raipur, CG. *Corresponding Author

ABSTRACT

Background: Hyperglycaemia in diabetic condition is metabolic dysfunction which can lead to increased free radical production causing oxidative damage and stress. The present study aims to evaluate malondialdehyde (MDA), ischemia modified albumin (IMA) concentrations in diabetics, as a marker of oxidative stress.

Methodology: A total of 60 diabetic subjects with 45 age, sex matched healthy controls. MDA and IMA were assessed from serum using standardised method and were compared amongst diabetic subjects and control subjects.

Results: Two groups were found to be matched for general characteristics. The concentration of MDA and IMA was found to be increased significantly in diabetics compared to normal controls. Further MDA and IMA were also found to be significantly higher in male diabetic subjects compared to female diabetic subjects when differences in non diabetic male and female subjects failed to reach statistical significance.

Conclusion: Diabetic subjects show high levels of MDA and IMA those can be result of oxidative stress of the hyperglycaemic state.

KEYWORDS : diabetes mellitus, malondialdehyde (MDA), ischemia modified albumin (IMA), Oxidative stress.

Background

Diabetes mellitus (DM) represents a group of chronic diseases characterized by high levels of glucose in the blood that results from defects in insulin production, insulin action, or both. Symptoms of marked hyperglycemia include polyuria, polydipsia, weight loss, sometimes with polyphagia, and blurred vision. Impairment of growth and susceptibility to certain infections may also accompany chronic hyperglycemia. Acute, life-threatening consequences of uncontrolled diabetes are hyperglycemia with ketoacidosis or the nonketotic hyperosmolar syndrome.[1] Patients with diabetes show an increased incidence of atherosclerotic cardiovascular, peripheral arterial, cerebrovascular disease, hypertension and abnormalities of lipoprotein metabolism.[2] As per estimate of the International Diabetes Federation (IDF), the total number of people in India with diabetes which was around 50.8 million in 2010 would be 87.0 million by 2030.[3]

Oxidative stress plays a major role in cellular injury from hyperglycemia. High glucose level can lead to free radical production. Weak defence system of the body becomes unable to counteract the enhanced ROS generation and as a result condition of imbalance between ROS and their protection occurs which leads to domination of the condition of oxidative stress.[4]

Oxidative stress is defined as a state in which oxidation exceeds the capacity of antioxidant systems in the body secondary to a loss of the balance between them. It not only causes hazardous events such as lipid peroxidation and oxidative DNA damage, but also physiologic adaptation phenomena and regulation of intracellular signal transduction.[5] Studies have demonstrated that hyperglycemia-induced oxidative stress led to the activation of mitogen-activated protein kinase (MAPK), which may have contributed to neuronal pathogenesis.[6,7]

Increased oxidative stress could be related to the complications in patients with diabetes such as oxidative DNA damage and insulin resistance. Due to this complications of diabetes increase which include cardiovascular disease, nerve damage, blindness, and nephropathy. Thus, the increasing incidence of diabetes is a significant health concern beyond the disease itself.[8]

Oxidative stress-induced injury in the cells include lipid peroxidation, protein oxidation. Out of all these processes, lipid peroxidation is one of the highly toxic mechanisms of generating ROS. Assessment of the extent of oxidative stress using biomarkers is interesting from a clinical standpoint. The markers found in blood, urine, and other biological fluids may provide information of diagnostic value.[9]

Taking into consideration the above points, the present study has been undertaken to determine the oxidative stress by measuring the levels of malondialdehyde (MDA), ischemia modified albumin (IMA) in serum of diabetic patients and comparing it with normal subjects.

MATERIALS AND METHODS

Place of study

The present study was carried out in Department of Biochemistry, Raipur Institute of Medical Sciences & department of Obstretrics and Gynaecology Raipur and approved by the institutional ethical committee the institute. The study was carried out in accordance with declaration of Helsinki. The purpose of study was clearly explained to every patient before enrolling them and proper informed written consent was obtained.

Study population

A total of 60 patients who were diagnosed cases for DM II reporting to RIMS Hospital for their regular OPD checkup were recruited for the study. Another 45 volunteers were recruited from healthy blood donors and the ones who came for medical fitness tests. Pregnant ladies, cancer patients and patients taking antioxidant supplements were excluded from the study.

The patients

The study subjects included both male and female sexes of various age groups ranging from 30 to 65 years age group. In total an average of 105 patients were studied. The personal information, physical characteristics, habits and other relevant information of the patients were obtained.

Collection of samples

Blood sample was collected by expert technicians of clinical biochemistry sample collection laboratory of RIMS Hospital . 5.0 ml of venous blood was drawn in vacutainers and allowed to clot at room temperature for 20 min, centrifuged at 3000 rpm for 10 minutes and the sera was then divided into aliquots and stored frozen at (-20° C) for determination of lipid peroxidation product MDA and IMA.

Biochemical estimation

Lipid peroxidation product MDA was estimated by method of

Cynamon et al. [10]. The Albumin Cobalt-Binding Test was used to detect IMA.[11]

The data was presented as mean ± SD. Student's *t* test was used to evaluate the significant of differences between the parameters measured. Chi square test was used to assess the difference in frequency distribution. SPSS© for windows™ Vs 17, IBM™ Corp NY and Microsoft excel™ 2007, Microsoft® Inc USA was used perform the statistical analysis.

Results

Out of total 105 subjects 60 were diabetic patients and 45 were normal age and sex matched controls Overall the study group comprised of 31 females (52%), 29 males (48%) and the control group comprises of 26 (58%) females and19 (42%) males (p=0.03). The participants were between 30 and 65 years with a mean of 45.75 years of age. The mean BMI of control and diabetic subjects were measured as 24.89 and 27.81 Kg/m^2 respectively. The physical characteristics of healthy control and diabetic subjects have been shown in table 1. Two study groups were found to be matched for Age, Height, Weight and BMI.

Table 1: General characteristics of control and diabetic subjects.

Characteristics/ parameters	Control (n=45) Mean (±SD)	Case(n=60) Mean (±SD)
Age (years)	46.21 (±2.32)	52.6 (±5.29)
Height (m)	1.59 (±0.93)	1.59 (±0.025)
Weight (Kg)	62.49 (±3.68)	70.45 (±6.25)
BMI (Kg/m2)	24.89 (±3.4)	27.81 (±5.21)

*p<0.05

The mean serum MDA concentration of control male and female subjects was determined as 202.64 and 247.48 nmol/100 ml where as in case of the male and female belonging to DM II individuals the mean MDA content were 281.049 and 266.820 nmol/100ml respectively. An increase of 39% in MDA content of diabetic male was observed as compared to the control males where as in diabetic female the increase was only 8% as compared to the control females.

The mean IMA content of males and females of control subjects was 0.42 and 0.34 absorbance units; while the mean concentrations of IMA in diabetic male and female subjects were calculate to be 0.61 and 0.46 absorbance units respectively. The IMA content of both male and diabetic subjects increased significantly compared to that of control subjects of respective sex. IMA content in male diabetic subjects increased by 45% where as in female subjects the IMA content increased about 35% over the control females.

Table 2: MDA and IMA content in male and female subjects of control and diabetic subjects.

Parameters	Control		Case	
	Male Mean (±SD)	Female Mean (±SD)	Male Mean (±SD)	Female Mean (±SD)
Postprandial sugar (mg/dl)	112.52(±4.89)	264.87(±8.56)		
MDA (nmol/100 mL)	202.64(±13.21)	247.48(±10.54)	281.049*(±7.26)	266.820(±8.46)
IMA (absorbance units)	0.42(±0.013)	0.34(±0.021)	0.61 (±0.09) *	0.46(±0.021)

* P<0.05

Discussion

Diabetes mellitus is characterized by hyperglycemia resulting from defects in insulin secretion and insulin action or both. Hyperglycaemic condition is known to generate ROS, which in turn cause damage to the cells in many ways that leads to secondary complications in diabetes mellitus.[12] The lipid peroxidation product, MDA has been recognized as a primary biomarker of free radical mediated lipid damage and oxidative stress. [13] In the present study, the MDA levels have been measured in diabetic and healthy control subjects. Higher level of MDA in diabetic subjects observed in this study could be attributed to higher ROS.

The study shows that, there is significant elevation in MDA concentration of diabetics subjects compared to that of control healthy individuals. Increased MDA level in plasma, serum, and many others tissues have been reported in diabetic patients. [14] Increased level of MDA in diabetics suggests that peroxidative injury may be involved in the development of diabetic complications. The increase in lipid peroxidation is also an indication of decline in defense mechanisms of enzymatic and non-enzymatic antioxidants. There occurs decreases in the activity of non-enzymatic antioxidants vitamin E and vitamin C in diabetic subjects as Vitamin E acts as lipid peroxidation chain breaking antioxidant as well as quenches the free radicals. Vitamin C reacts directly with superoxide and hydroxyl ions and acts synergistically with vitamin E as chain breaking antioxidant by regenerating the reduced form of vitamin E from tocoperoxy radical.

Clinical studies have reported that significantly higher lipid peroxidation is associated with high glucose levels as observed by the fasting glucose and HbA1c levels. [15] The plasma antioxidant level is significantly lower in diabetic subjects with poor glycaemic controll than healthy subjects, while patients with good glycaemic control had plasma antioxidative values similar to controls. Another study has reported significant reduction in biological antioxidant potential in sciatic nerve homogenates of diabetic animals.[16] Thus, oxidative stress event in diabetics coexists with a decrease in the antioxidant status could further increase the deleterious effects of free radicals in type II patients.

Ischemia-modified albumin (IMA) is a promising biomarker for evaluating patients with ischemic events. Some previous studies have reported that compared to healthy controls, patients with type II diabetes and chronic microvascular complications have higher serum levels of IMA.[17] One of the major findings of the present study is significant increase in serum IMA content of both male and female type II patients. Some studies have reported that compared to healthy controls, patients with type II diabetes and chronic microvascular complications have higher serum levels of IMA.[18] The findings of the present study further substantiate IMA as a biomarker of ischemic associated diseases including diabetics.

The increased level of oxidative stress as shown from the byproduct biomarkers such as MDA and IMA in diabetic patients observed in the present work could cause complications in patients causing damage to proteins, lipid and nucleic acids. This may also cause insulin resistance in type II diabetic patients.[19] This may further lead to more complications in diabetes like cardiovascular diseases, nerve damage, and blindness etc.

Conclusion:

Markers of oxidative stress such and MDA and IMA are significantly increased in subjects with diabetes mellitus. They pose increased risk of oxidative damage and the risk in extended in male subjects beyond that in otherwise healthy males.

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