Estimation Of Plasma Levels Of Lipid Peroxidation And Erythrocyte Enzymatic Antioxidants In Type II Diabetes Without Complications

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Abstract: Background & Objective: Diabetes being a chronic disorder is associated with increased oxidative stress by glucose oxidation and non enzymatic protein glycation. Imbalance between oxidants and antioxidants lead to oxidative stress. Increased levels of malondialdehyde (MDA) end product of lipid peroxidation have been observed in type II diabetes but results regarding status of enzymatic antioxidants, Glutathione peroxidase (GPx) and Glutathione reductase (GR) are conflicting. Therefore this study was conducted to estimate levels of MDA, GPX & GR and to find the correlation of these to glycaemic control if any in diabetics without complications. Methodology: Type II diabetics (n=40) diagnosed not more than 5 yrs back, without any complications were cases and age & sex matched normal subjects (n=40) were controls in this study. Blood levels of glucose, glycosylated Hemoglobin (HbA1C), MDA and erythrocytic activity of GPX and GR were estimated. Results: Blood MDA level was found to be significantly increased and erythrocyte GPx & GR activity was decreased significantly. A strong positive correlation was seen between MDA and HbA1C. Conclusion: Thus imbalance between MDA and antioxidant activity indicate that the increased oxidative stress is present even in early period of diabetes without any complications and glycaemic control may help in reducing oxidative stress and further complications. [Ghugare B NJIRM 2015; 6(5):17-21]

Key Words: diabetes, oxidative stress, antioxidants, MDA.

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Introduction: Diabetes mellitus, a fastest growing metabolic disease in the world and India continues to be the "diabetes capital" of the world, and by 2030. [1] Type 2diabetes is the commonest form of diabetes contributing to 90% diabetic population. Chronic hyperglycemia can result in tissue damage by glycation of tissue proteins and other macromolecules and excess production of polyol compounds from glucose. [2] Elevated extracellular and intracellular glucose concentration in diabetes mellitus results in oxidative stress. [3] Diabetic patients are having risk of increased oxidative stress by several mechanisms including glucose oxidation and non enzymatic protein glycation. [4]

All major classes of biomolecules can be attacked by free radicals but lipids are most susceptible, especially polyunsaturated fatty acids (PUFA) in cell membranes which undergo oxidative destruction called as 'lipid peroxidation'. Oxidative deterioration of lipids results in generation of peroxy radicals, intermediate and short chain aldehydes, like malondialdehyde (MDA) as an end product. [5] Free radical scavenging mechanism includes various enzymatic antioxidants like superoxide dismutase enzyme, (catalyzing the dismutase reaction), glutathione peroxidase (GPx) and catalase disposing of hydrogen peroxide at low and high concentration respectively.

Various studies showed increased in levels of MDA in type 2 diabetic patients with [6] or without complications. Turk TM 2002 found its positive correlation with the HbA1c levels. [7] Nakhjanvi et al studied the effect of duration by measuring MDA in patients with DM since 10 years and with less than 10 years and concluded that the duration is independently associated with increased levels of lipid peroxidation. [8] Conflicting results are available regarding status of GPx and glutathione reductase (GR) in diabetic patients with and without complications. [9, 10, 11, 12, 13] With above background present study aimed to estimate the levels of serum MDA and erythrocyte GPx, and GR in type 2 diabetics without any complications to evaluate balance of oxidants and antioxidants during early stage, within 5 yrs of clinical diagnosis and to find the correlation of MDA verses HbA_1C.

Material and Methods: The present study was a case-control study conducted in J.J.Group of Hospitals, Mumbai, to evaluate oxidative stress in

type 2 diabetes mellitus patients. It was approved by institutional ethical committee and the informed consent was taken before the study. In this study cases were type 2 diabetes mellitus patients (n=40) attending the diabetic clinic of J.J. group of Hospital without complication, out of which 23 were males and 17 were females. All patients were non-alcoholic, non-smokers, on oral antidiabetic medicines for not more than 5 years, not taking any antioxidant supplements and showing normal hepatic and renal function on clinical examination. The patients with known history of coronary heart disease, hypertension, kidney disorder, and retinopathy were excluded. Control group was age and sex matched healthy adults from hospital staff.

The fasting and postprandial plasma glucose were done by glucose oxidase and peroxidase method [14] and total HbA_1C was done by Thiobarbituric acid method. [15] Blood concentration MDA, end product of lipid peroxidation was estimated by thiobarbituric acid (TBA) method. [16] Enzymatic antioxidants GPX activity and GR activity were estimated in red blood cell by preparing hemolysate. [17, 18]

Results: Statistics: Statistical analysis was done by unpaired't' test at 95 % confidence interval for comparison of diabetic and control group. P value less than 0.05 was considered statistically significant. Observations: Total 40 diabetics without complication along with the same number of controls were included in the study whose clinical and anthropometric data is shown in table I.

Table 1: Anthropometric and clinical characteristics of diabetic and control group

Parameters	Control group (n=40) Mean ±S.D.	Diabetic group (n=40) Mean ±S.D.
Age(yrs)	53.27±5.26	52.57±6.08
Height (cm)	161.55±7.70	160.52±8.98
Weight (Kg)	59.65±6.93	60.82±8.65
BMI(Kg/m^2)	22.83±1.67	23.62±3.00
Pulse(bpm)	82.65±5.99	83.1±6.10
Systolic B.P. (mm Hg)	127.9±5.72	137.97±15.17
Diastolic B.P. (mm Hg)	84.7±5.15	86.1±7.45

A Significant ($p<0.0001$) increase in systolic blood pressure of diabetics was seen compared with control subject whereas there was no difference in diastolic blood pressure.

On biochemical estimation of blood sugar level it was observed that plasma fasting glucose as well as Postprandial glucose level of diabetic group were was significantly increased ($p < 0.0001$) as compared to control group as shown in table 2. There was significant increase in level of Glycosylated hemoglobin of diabetics as compared to control.

Table 2: Blood sugar levels (fasting and postprandial) and HbA1c in control and diabetes

Parameters	Control group (n=40) Mean ±S.D.	Diabetic group (n=40) Mean ±S.D.
BSL Fasting(mg/dl)	84.6±11.61	148.3±59.90
BSL Post prandial (mg/ dl)	128.02±9.25	225.75±85.28
HbA1c (%)	4.73±0.74	7.86±0.87

It was revealed that MDA level was significantly raised in diabetics compared to control, as shown in fig I. Whereas activity of enzymatic antioxidants like erythrocyte GPx and GR was decreased significantly in diabetics compared to control group as depicted in fig II.

Figure 1: Comparison of MDA levels in control and diabetic group

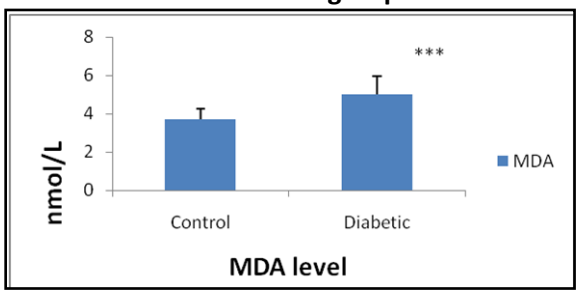
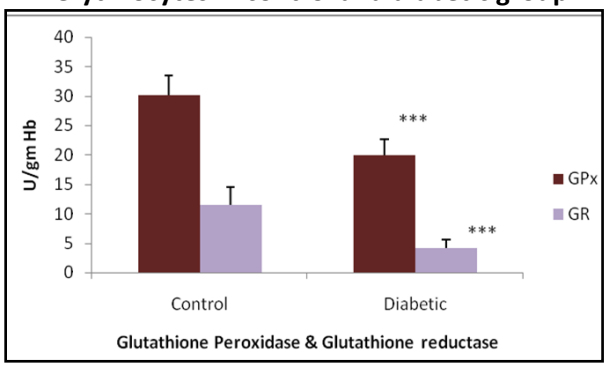


Figure 2: Comparison of levels of glutathione peroxides and glutathione reductase activity in erythrocytes in control and diabetic group



When levels of HbA_1C was correlated verses MDA and GPx, it showed strong positive correlation with MDA r=0.6258 (p<0.001) and significant negative correlation with glutathione peroxidase, (r= -0.3582 and p<0.02). HbA_1C showed negative correlation with GR although it was not significant, (r =-0.0900, p >0.05).

When MDA levels were correlated verses GPx, strong negative correlation was observed (r= -0.5938 and p<0.001) but MDA had non-significant negative correlation with GR, (r = -0.2104, p >0.05). When GPx was correlated vs. GR, there was strong positive correlation between GPx and GR (r = 0.6081 and 'p' value was <0.001).

Discussion and Conclusion: There was significant increase in fasting and postprandial blood glucose levels as well as statistically significant increased in HbA_1C levels in the study group. It is due to increased glucosylation of hemoglobin. [19] MDA showed significant increase in diabetic patient as compared to healthy control. In diabetes free radical formation is increased by glucose auto oxidation, non-enzymatic glycation of proteins, polyol pathway and this increase generation of free radicals leads to lipid peroxidation and formation of its end product MDA. [20] Hyperglycemia in insulin resistance state lead to oxidative DNA damage and may contribute to pancreatic β cell dysfunction, insulin resistance and more prominent hyperglycemia and a vicious cycle can continue predominance of oxidative stress over antioxidant defense system. [21] Plasma MDA was found elevated significantly in type 2 DM with complications.[6] We have observed significant increased in MDA levels even in patients with DM diagnosed before 5 yrs and on antidiabetic treatment.

In 2002, Turk TM et al found significantly elevated (Thiobarbituric acid substances) TBARS levels in diabetic patients but did not observe any correlation between TBARS levels and blood glucose and HbA_1C levels. [7] In 2004 Philips M et al measured a new set of markers of oxidative stress, the breath methylated alkane contour (BAMC) using gas chromatography and mass spectroscopy. By this method oxidative stress was increased in patients with type 1 and type 2 DM but was not related with blood glucose concentration or HbA_1C levels. [22]

But in our results showed a strong significant correlation of MDA with HbA_1C which indicate that control of glucose level may affect MDA levels and similarly can contribute in combating with the oxidative stress. The glycaemic control, measure by HbA1c (<7%) as indicated by the American Diabetes Association (ADA) if implemented soon after diagnosis, the microvascular complications can be reduced. [23] But in our study HbA1c > 7% indicate poor glycaemic control in the newly diagnosed diabetics which may be responsible for increased oxidative stress in these subjects and thus they are more prone for developing early complications.

Enzymatic antioxidants : Glutathione Peroxidase catalyzes reduction of H_2O_2 and hydroperoxides formed from fatty acids, thereby effectively removing toxic peroxides from living cells. [24] Glutathione Reductase catalyzes the reduction of oxidized glutathione (GSSG) to reduced glutathione (GSH).

Previously no change in the activity of glutathione peroxidase and glutathione reductase was reported in types 2 diabetes but decreased activity in type 1 diabetes was reported making it more prone for complications such as nephropathy. [10, 11] Some authors found increased in erythrocyte GPx & GR levels in type 2 diabetics with and without complications. [13-14, 25]

In the present study GPx activity in red blood cells was significantly decreased in diabetic patients than healthy controls. It may be attributed to decrease in blood glutathione content since it is a substrate and cofactor for glutathione. A possible cause of decreased activity of GPx may be glycation of enzyme subsequently leading to inactivation of enzyme. [26] The decrease in GPx in diabetics without complication could be to counteract the oxidative stress. [27] Although GPx is relatively stable enzyme, in vitro studies have shown that it may also be inactivated in conditions of severe oxidative stress. [26]

In present study results revealed decreased GR activity as reported by some other authors and it may due to glycation of the enzyme. [29-30] Thus decreased levels of both the enzymatic

antioxidants, erythrocyte GPx and GR, decreases the antioxidant defense in early stage of diabetes and strong positive correlation between Gx & GR indicate deficiency of one enzyme affect other and ultimately antioxidant effect of these enzymes Strong negative correlation of GPx with MDA suggest, further increase production end products of lipid peroxidation with decrease GPx and the oxidative stress. Positive correlation of MDA with HbAc1 may indicate glycaemic control to be a major defense against oxidative stress.

Limitation of the study is that we have not estimated other antioxidant like SOD, vitamin C and vitamin E levels. Thus we can conclude that the oxidative stress is occurring even in early period of diabetes without complications and it can be counteracted by glycaemic control. By achieving the target glycemic control we can reduce the oxidative damage and subsequently prevent complications.

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