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EVALUATION OF ANTIOXIDANT BIOMARKERS IN UNCONTROLLED TYPE 2 DIABETICS

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ABSTRACT

Hyperglycemia influences the aetiopathogenesis of diabetes in more than one way. Oxidative stress and antioxidant status are also linked with glycaemic control and probably contribute to the development of diabetic complications. Our data suggest that increased level of MDA and alterations in antioxidant biomarkers could be high risk for complications in patients with poorly controlled diabetes. Thus, evaluation of these markers are simply done by monitoring MDA , FRAP with Albumin, Uric acid and Total bilirubin. It may be helpful in treatment, relieving symptoms and complications.

KEYWORDS: *antioxidant biomarkers, FRAP, MDA, type 2 diabetics.*

INTRODUCTION

Type 2 or non insulin dependent diabetes mellitus (NIDDM) accounts for about 90%–95% of all diagnosed cases of diabetes.[1] Hyperglycemia alone does not cause of diabetic complications. It is rather the detrimental effect of glucose toxicity due to chronic hyperglycemia mediated and complicated through augmented oxidative stress.[2] Hyperglycemia increases the production of reactive oxygen species (ROS) inside the aerotic endothelial cells. ROS induced activation of protein kinase-C isoforms, increased formation of glucose-derived advanced glycation end products, increased glucose flux through aldose reductase pathways and activation of cytokines are some of the known biochemical mechanisms of hyperglycemia-induced tissue and cell damage.[3] Several assays have been described to assess the involvement of oxidative stress in pathological conditions. In addition to micronutrients, several components of the antioxidant system can be measured in the serum.[4] Because of interactions among different antioxidants, methods which assess the total antioxidant capacity (TAC) of human blood provide an integrated index of the redox status.[5] Levels of Uric acid, Albumin and Bilirubin are often used as major non-enzymatic antioxidant biomarkers.[6,7] They prevent free radical reaction by sequestering transition metal ions by chelation in plasma, providing the primary extracellular defense against oxidative stress.[8] Hence, the present study was evaluate antioxidant biomarkers in uncontrolled type 2 diabetic patients.

PATIENTS AND METHODS

This study included 120 type 2 diagnosed patients, at ACPM Medical College and Hospital, Dhule-424001 (MS). These patients were compared age wise & sex wise with non diabetic healthy subjects. Here none of the any patients and control subjects was taking dietary supplements such as vitamins or minerals.

Using standard protocols, fasting blood samples were collected from both controls and patients. Fasting BGL, Albumin, Uric acid, Total bilirubin were measured by using ERBA diagnostics Mannheim GmbH reagent kits. Serum malondialdehyde (MDA) was assayed as a marker of lipid peroxidation by thiobarbituric acid method.[9] The FRAP assay was performed according to the method of Benzie and Strain in

which a colourless ferric tripyridyltriazine complex is reduced to a blue ferrous complex by the antioxidants in the plasma. The change in absorbance at 593 nm is directly related to the total reducing power of electron donating antioxidants present in the plasma.[10] FRAP value in plasma was expressed as mM/l.

Statistical analysis: Data were expressed as means ± SD. The data obtained in our study was analyzed for its statistical significance using 'z' test of proportion and independent 't' test. P value less than 0.05 was considered the level of significance.

RESULTS

As depicted in following Table-

	Uncontrolled DM (n=53)	Controlled DM (n=67)	Non DM (n=60)
Age (yrs)	44.74 ± 8.95	43.45 ± 9.10	46.11 ± 8.70
Sex (M/F)	32/21	40/27	34/26
BGL-F (mg/dl)	218.69 ± 59.68 ##	118.14 ± 21.50 ** ##	83.16 ± 17.59
Albumin(g/dl)	2.98 ± 0.93 ##	3.19 ± 1.04 ##	4.01 ± 0.75
Uric acid(mg/dl)	2.76 ± 0.69 ##	3.72 ± 0.88 ** #	4.25±1.69
Total Bilirubin(mg/dl)	0.27 ± 0.15 ##	0.33 ± 0.18 ##	0.58 ± 0.27
Serum MDA(nmoles/ml)	5.35 ± 1.06 ##	4.84 ± 0.99 * ##	2.69 ± 1.10
FRAP (mM/l)	442.36 ±25.12 ##	587.17±20.27 ** ##	692.22± 17.25

* p < 0.05 vs. Uncontrolled DM ** p < 0.001 vs. Uncontrolled DM
# p < 0.05 vs. Non DM ## p < 0.001 vs. Non DM

DISCUSSION

The study has made interesting observations on the effect of control of diabetes on oxidative stress. Glucose has been shown to be a direct source of free radicals. In addition, a number of changes in energy metabolism, alterations in sorbitol pathway activity, and changes in the levels of inflammatory mediators, altered antioxidant defense system and localized tissue damage resulting in hypoxia contribute to free radicals stress in diabetes mellitus. [11]

In the present study diabetic patients are divided into two categories controlled and uncontrolled. The category for uncontrolled diabetes included fasting venous blood glucose ≥ 160 mg% These uncontrolled diabetic patients are compared with controlled diabetic patients. Then it was observed that, difference between fasting blood glucose level were highly significant. ($p < 0.001$)

Mean serum MDA levels was significantly higher in uncontrolled diabetic patients as compared with controlled diabetic patients ($p < 0.05$). This clearly shows association between lipid peroxide and glucose concentration. Increase MDA may be due to the increased glycation of protein in diabetes. The glycated protein might themselves act as a source of free radicals, which play a role in increased lipid peroxidation in diabetes. In uncontrolled diabetes, glucose leads to excessive formation of NADPH, which in turn can promote lipid peroxidation in the presence of cytochrome P-450 system. Oxyhaemoglobin in erythrocytes

may act like the cytochrome P-450 system in the presence of NADPH and thus bring about increased lipid peroxidation. Alternatively, inactivation or inhibition of antioxidant enzymes by glycation in poorly controlled diabetes may give rise to increased lipid peroxidation. Evidence of lipid peroxidation has also been reported in a number of diabetic complications.[12] This will prevent to good metabolic control of hyperglycemia.[13]

In this present study, significant decreased FRAP levels was observed as compared to controls. FRAP summarizes the overall activity of antioxidant vitamins and enzymes. Because of the difficulty in measuring each antioxidant component of plasma separately and of the interactions that take place among different components. FRAP is being used as a single test to estimate total antioxidant capacity (TAC) of blood.

Hyperglycaemia in diabetes may increase ROS production via changes in the redox potential of glutathione and decreased antioxidant defences due to reduction in total antioxidant capacity in plasma.[14] The capacity of the antioxidant system cope with or trap the free radicals generated under normal or pathological conditions was evaluated by measuring the level of total antioxidant status. It reflects the status of extracellular antioxidants.[15]. These antioxidants biomarkers delay or inhibit the oxidative process. In this present study we have evaluated the importance of these markers in uncontrolled stage of diabetes.

We observed that, antioxidant biomarkers like albumin, uric acid and total bilirubin were lower in uncontrolled diabetic patients as compared to controlled diabetic patients. Both these parameters indicated an oxidative stress. The oxidative stress is more during uncontrolled stage of diabetes. Our findings are in accordance with the observations made earlier.[16]

In type 2 diabetes, chronic exposure to hyperglycemia and insulin resistance has been implicated in altered oxidative metabolism. Excessive plasma and tissue glucose can exert pathological effects through nonenzymatic glycosylation, which lead to the production of superoxide and hydrogen peroxide.[17] A reduced insulin action and hyperglycemia influence several oxido-reductive pathways including pentose, glycolytic and sorbital pathways. The activities of two major insulin-induced enzymes in the hexose monophosphate shunt, glucose-6-phosphate-dehydrogenase (G-6PD) and 6-phosphogluconate dehydrogenase (6-PG) are impaired, leading to reduced NADPH availability. These negatively influence other enzymes and systems involved in defensive processes against oxidative agents, such as the glutathione system, thus increasing oxidative stress. This led us to think that antioxidants might be playing a significant role during uncontrolled stage of diabetes.

CONCLUSION

It is concluded that good metabolic control of hyperglycemia will prevent alterations in peroxidation and antioxidant defence mechanism. Significantly lower FRAP values denoting maximum oxidative injury. Albumin, Uric acid and Total bilirubin are important contributors of TAC, evaluation of these markers may be useful in the prevention of the diabetic complications.

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