

Ferric Reducing Ability of Plasma with Lipid Peroxidation in type 2 diabetes

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ABSTRACT

The FRAP assay offers a putative index of antioxidant, or reducing, potential of biological fluids within the technological reach of every laboratory and researcher interested in oxidative stress and its effects. We have evaluated the importance of this marker in Indian subjects, we have investigated total antioxidant capacity as ferric reducing ability of plasma (FRAP) status in type 2 diabetics and healthy controls. Statistically significant difference confirms that there is an increased oxidative stress in diabetics compared to non diabetic counterparts and emphasize the importance of assessing these markers for early diagnosis and therapeutic interventions.

KEYWORDS: antioxidant activity, FRAP assay, malondialdehyde, total antioxidant capacity (TAC)

INTRODUCTION

Antioxidants play very important role in the prevention of degenerative illness in various diseases along with diabetes. Foods of plant origin not only provide us with important antioxidant vitamins (e.g. vitamin C, vitamin E or provitamin A) but also a complex mixture of other natural substances with antioxidant capacity. It is possible to measure all of the antioxidant components in a sample individually, but this is expensive and time-consuming. Several methods are known to measure the total antioxidant capacity (TAC) of biological samples, but we tried the FRAP assay, which depends upon the reduction of ferric tripyridyltriazine (Fe(III)-TPTZ) complex to the ferrous tripyridyltriazine (Fe(II)-TPTZ) by a reductant at low pH. Fe(II)-TPTZ has an intensive blue colour and can be monitored at 593 nm. [1]

ROS are a byproduct in type 2 diabetes, generated during protein glycation and as a consequence of advanced glycation end-products-receptor binding; they impair insulin Signalling pathways and induce cytotoxicity in pancreatic beta cells. Neutralisation of oxidants by increased antioxidant availability may mitigate these effects. Several human intervention studies have been undertaken to

determine whether dietary antioxidants exert beneficial effects for type 2 diabetes patients. [2] Oxidative stress can be assessed by measuring lipid peroxidation in the body. MDA is a stable product of lipid peroxidation and marker of oxidative stress which occur when there is an imbalance between production and scavenging. Therefore the present study was aimed to determine the ferric reducing ability of plasma (FRAP) status in diabetic patients with lipid peroxidation.

PATIENTS AND METHODS

After clinical examination and confirmed diagnosis by physician, 30 known cases of type 2 diabetes without any micro or macro vascular complications were selected at ACPM Medical College and Hospital, Dhule. These patients were compared age wise & sex wise with non diabetic healthy subjects. Using standard protocols, fasting blood samples were collected from both controls and patients, for measure the MDA level and total antioxidant capacity as ferric reducing ability of plasma (FRAP) status.

Serum lipid peroxide level was measured by thiaobarbituric acid assay. [3,4] TAC was determined by FRAP method 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. [5] Briefly, 3 ml of working FRAP reagent (25 ml 0.3 M sodium acetate buffer, pH 3.6; 2.5 ml 0.01 M TPTZ in 0.04 M HCl ; 2.5 ml 0.02 M FeCl₃. 6 H₂O ; preheated to 37° C) was mixed with 100 µL of supernatant or plasma and the absorbance at 593 nm was recorded after a 5

min incubation at 37° C. The absorption of the blue Fe II-complex was measured at 593 nm using spectrophotometer. FeSO₄.7H₂O solutions from 0.2 to 1 mM were used for calibration. FRAP value in plasma was expressed as mM/l.

Statistical Analysis: The data obtained in our study was analyzed for its statistical significance using 'z' test. P value less than 0.05 was considered the level of significance.

RESULTS

A significant increase in MDA levels were found ($p < 0.05$) when compared to controls. There was a significant decrease ($p < 0.05$) in FRAP levels when compared to the control levels.

Parameter	T2D patients (n= 30)	Control (n= 30)
Age (Yr)	42.50 $\pm$ 8.70	41.65 $\pm$ 7.62
Sex (Male/Female)	17/ 13	17 / 13
Fasting glucose (mg %)	168.14 $\pm$ 62.06	89.16 $\pm$ 17.59 [©]
Serum MDA (nmoles/ml)	5.06 $\pm$ 1.05	2.69 $\pm$ 1.10 [©]
FRAP (mM/l)	539.29 $\pm$ 84.39	893.22 $\pm$ 85.07 [©]

© Significant difference compared with controls ($p < 0.05$)

DISCUSSION

Antioxidants play a vital role as preventive factors in the pathogenesis of vascular complications in diabetics. [6,7,8] The measurement of the total antioxidant capacity in body fluids proved to be an important prognostic or diagnostic guide in patients with diabetes as well as atherosclerosis, septic shock etc for implementation of antioxidant therapy. [9,10] Living organism have developed complex antioxidant systems to counteract ROS and to reduce their damage. These antioxidant systems include enzymes such as superoxide dismutase, catalase, glutathione peroxidase; macromolecules such as albumin, ceruloplasmin and ferritin; and an array of small molecules including ascorbic acid, β carotene, reduced glutathione, uric acid and bilirubin. The sum of endogenous and food derived antioxidants represent the total antioxidant activity of the system. The cooperation among different antioxidants

provides greater protection against attack by ROS than any single compound alone. Thus the overall antioxidant capacity may provide more relevant biological information compared to that obtained by the measurement of individual components, as it considers the cumulative effect of all antioxidants present in plasma and body fluids.

In this present study, significant decreased FRAP levels was observed as compared to controls[11] observed unexpected positive correlation between FRAP values and oxidative DNA damage in diabetic patients; moreover, a positive correlation was found between FRAP and glucose level or HbA(1c) in patients with poor glycemic control. [11] Similar results are also found in patients with type 1 diabetes, [12] however Syed Ibrahim Rizvi and Neeti Srivastava(2009) reported that decreased plasma antioxidant capacity in first degree relatives of T2DM patients.[13]

Diabetic complications are developed with increased activity of free radical-induced lipid peroxidation and accumulation of lipid peroxidation products. Free radicals react with PUFA to form peroxides, thus degrading lipids and releasing malondialdehyde as products. MDA is a stable product of lipid peroxidation which is measured index of lipid peroxidation.[14] Many researchers have found positive relationship between serum lipids, lipoproteins, lipid peroxides and erythrocyte antioxidant enzymes (catalase; glutathione peroxidase and superoxide dismutase) in type 2 diabetes. In our study, it was observed that, mean value of lipid peroxide (MDA) was significantly increased in diabetic group as compared to nondiabetics. Similar results were reported by Uzel et al (1987), Gallow et al (1993), Ayden (2001) and Seghrouchni et al (2002) in type 2 diabetic patients. They observed hyperlipidemia, increased lipid peroxide concentrations and increased thiobarbituric acid reactive substances (TBARS) in both type 2 diabetes groups compared to controls.[15,16]

Free radicals are being continuously produced by the body by different mechanisms which can oxidize multiple fatty acid side chains to lipid peroxides. These peroxidation reactions are countered by antioxidants present in plasma and interstitial fluid and α tocopherol (vitamin E) is the most important compound that inhibit lipid peroxidation in the presence of vitamin C and give protection against cardiovascular disease.[17,18]

The activities of antioxidant Superoxide dismutase, Glutathione peroxidase, Catalase, Glutathione reductase, Glutathione and vitamins A, E and C are usually measured to assess the antioxidant stress in the blood. A single test which denotes the antioxidant power of blood was established & estimated as the ferric reducing ability of plasma (FRAP) was found to give more biologically relevant information than the measurement of individual antioxidants. 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. In recent years several methods have been developed to assess the TAC of human serum (or) plasma. More biologically relevant information can be obtained by assessing FRAP than that obtained by measuring the concentration of individual antioxidants and may more closely describe the dynamic equilibrium between pro oxidant and anti-oxidants accuring in the plasma compartment. FRAP may be considered as an easy, cost-effective method to measure the antioxidant power and it might be incorporated into risk prediction in diabetes and CVD.

Study done by Ufuk Cakatay & Refik Kayali on diabetic rats also found decrease plasma FRAP levels of chronic diabetic rats when compared to those of controls in a significant manner.[19] Similar study done by Opera et al also found depleted total antioxidant capacity (TAC) which is more severe in patients with type 2 DM compared with controls. Significant correlation between the antioxidant capacity and glycemic control of the patients was noticed.[20] In diabetic patients increased levels of lipid peroxidation and decreased value of plasma TAC were observed.

CONCLUSION

FRAP assay, is presented as novel method for assessing "antioxidant power." This study indicates that increased oxidative stress and depleted antioxidant status in patients with type 2 diabetes which sets the stage for further disease progression. Early identification of at-risk individuals using simple screening test like estimation of MDA and ferric reducing ability of plasma (FRAP level) would greatly help in preventing or postponing the onset of diabetes.

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