Original article:

The levels of oxidative stress and antioxidants in diabetes mellitus before and after diabetic treatment with or without antioxidants

***Sarita A Shinde, Anita D. Deshmukh, Adinath N. Suryakar, Umesh K. More , Mona A. Tilak.**

Department of Biochemistry ; Pad. Dr. D.Y. Patil Medical College, Hospital And Research centre; Pimpri (DPU) , India

Corresponding Author : *Sarita A. Shinde **; Email ID** : snc_unc@yahoo.com

Abstract:

Background: Hyperglycemia which is associated with increased oxidative stress and decreased antioxidant status because of imbalance between oxidative stress and antioxidant status is considered as primary cause of diabetic micro as well as macrovascular complications.

Material and Methods: The present study has been undertaken to evaluate oxidative stress and antioxidants in diabetes mellitus before and after diabetic treatment with and without antioxidants. In all 90 subjects were enrolled, 30 subjects were age (35-55years) and sex matched controls. Test group comprised of clinically diagnosed cases of Type 2 (n=60) diabetic patients. Biochemical parameters like serum Malon-di-aldehyde (MDA), nitric oxide (NO), Superoxide dismutase (SOD), Reduced Glutathione (GSH) and total antioxidant status (TAS) were analyzed in control and diabetic patients. The diabetic group was further categorized as Group I (n=30) had only diabetic treatment and Group II (n=30) had received A-Z multiantioxidant tablet (vitamins & minerals) along with diabetic treatment for a period of 3 months. All above parameters reassessed after 3 months.

Results : The results shows significant increased concentration of MDA and NO and significant decreased concentration of SOD, GSH and TAS in diabetes mellitus as compare to controls. But group II showed significant reduction in oxidative stress and significant rise in antioxidant status as compare to group I who were treated without antioxidants.

Conclusion : Hence the present study concluded that the supplementation of multiantioxidants along with diabetic treatment improve antioxidant status and decreased oxidative stress which can help to minimize further micro as well as macrovascular complication in diabetes mellitus.

Keywords: Oxidative stress, Antioxidants, type 2 Diabetes mellitus

Introduction

Diabetes mellitus (DM) is the most rapidly growing chronic disease in the world. It is characterized by absolute or relative deficiencies in insulin secretion and /or insulin action associated with chronic hyperglycemia and disturbances in carbohydrate, lipid and protein metabolism. Prolonged exposure of hyperglycemia increases the generation of free radicals and reduces capacities of antioxidant defense system [1]. It has been also reported that diabetic patients have significant defects of antioxidant protection and generation of reactive oxygen species (oxidative stress) which may play an important role in the etiology of diabetic complications [2]. Long-term vascular complications represent a major cause of morbidity and mortality in patients with diabetes mellitus. Understanding the role of free radical in the pathogenesis of diabetes will be useful for the treatment and prevention of diabetes and its complications.

Alteration in the plasma concentrations of several trace elements have been suspected in diabetic

patients and may be involved in some of the metabolic dysfunctions in diabetes mellitus. Interconnecting systems of antioxidant micron-utrients (minerals) and enzymes also accomplish the body defense against oxidative stress. Therefore the aim of present study was to study the role oxidative stress and antioxidants in pathogenesis of diabetic complications and determination of efficacy of antioxidant supplementations.

Material and Methods

Selection of Subjects

In all 90 subjects were enrolled in the present study. Control group comprising of 30 healthy sex and age (35-55 years) matched subjects. Test group comprising of total 60 patients of type 2 DM of age group (35-55 years). The patients were diagnosed on the clinical basis and laboratory data. They were stabilized with hypoglycemic drugs. None were current users of antioxidants. The patients suffering from hepatic disease, cardiovascular disease, chronic or acute inflammatory illness, cancer of all types, alcoholics and smokers were excluded from the study. All enrolled subject in the study were volunteered after proper consent and reported for follow –up at right time. The study was approved by institutional ethical committee. The type 2 DM patients are treated by hypoglycemic drugs (sulfonylurea and metformin).

All DM patients along with routine treatment were received A-Z multiantioxidant tablets (10 vitamin + 5 minerals) for a period of three months.

Collection of Specimen

After 12hrs fast, venous blood sample were collected in different bulbs under aseptic conditions. Fluoride bulb was used for fasting blood glucose (BSL) estimation by Glucose Oxidase Peroxidase enzymatic test kit. The EDTA bulb was used for glycated hemoglobin (HbA1C) estimation by resin binding kit method. Plain bulb was used for estimations of serum superoxide dismutase (SOD) by Kakker et al [3], nitric oxide (NO) by Cortas and Wakid [4] , malondialdehyde (MDA) by Erdincler et al method [5] and total antioxidant status(TAS) by FRAP method [6] .

Acid citrate bulb was used for erythrocyte reduced glutathione (GSH) measurement by method of Beutler et al [7]. Baseline level of all of the above biochemical parameters were measured at the time of enrollment in the study for all subjects. But only the test group reassessed for the same parameters after follow-up of 3 months period of multiantioxidant supplementation. The glycated hemoglobin (HbA1C) levels were used as an index of metabolic control.

Statistical Analysis

The results were expressed as mean ± SD. Comparison of control group and test group was done by unpaired‘t’ tests. The change in parameters between baseline and after 3 months follow up was studied by paired‘t’ tests.

Observations and Results

Table 1 shows the characteristics of enrolled subject. It also shows BSL(F) and HBA1C is significantly decreased after 3 months follow of A-Z tablets in DM patients. Table 2 depicts, significant rise ($p<0.001$) in concentration of serum MDA and NO in both type of DM as compared to controls. Significant fall ($p<0.001$) was observed in antioxidants like serum SOD, erythrocyte GSH and total antioxidant binding capacity i.e.TAS in both type of DM as compared to controls.

www.ijbamr.com P ISSN: 2250-284X , E ISSN :2250-2858

In Table 3, it was observed that serum MDA and NO is significantly ($p<0.001$) decreased and significantly ($p<0.001$) increased concentration of serum SOD, reduced GSH and total antioxidant status after 3 months supplementation of A-Z tablets in type 2 DM as compared to respective baseline levels. But in group I the concentration of serum SOD, reduced GSH and TAS is significantly ($p<0.001$) decreased and MDA and NO is significantly increased ($p<0.001$) in DM after receiving only hypoglycemic drugs for period 3 months as compared to respective baseline levels.

Table 1. Clinical Characterization of study subjects

Subjects	BSL(F) mg/dl		HbA1c %	
	Baselines	After 3months	Baselines	After 3months
Controls	74.35 ± 8.7	--	4.5 ± 0.90	--
Type 2 DM (Group I)	162.7 ± 8.73	145.1 ± 7.66**	7.87 ± 0.18	6.40 ± 0.25**
Type 2 DM (Group II)	164.1±7.86	144.3 ± 6.96**	7.80 ± 0.18	6.57 ± 0.21**

Table 2. Levels of oxidative stress and antioxidants in Controls and DM

Parameters	Controls	Type 2 DM
Serum MDA (nmole/ml)	3.85±0.61	7.77±0.28**
Serum NO (µmole/l)	51.71±7.10	62.63±3.13**
Serum SOD (units/ml)	4.21±0.58	3.07±0.67**
Erythrocyte GSH (µmole/gm of Hb)	5.40±0.52	4.56±0.36**
Serum TAS (µmole/l)	828.27±36.38	662.3±40.51**

Values are expressed as Mean ± SD ; **indicates $p<0.001$

www.ijbamr.com P ISSN: 2250-284X , E ISSN :2250-2858

Table 3. Comparison of biochemical parameter before and after 3 months in DM

Parameters	Group I (Only Drugs)		Group II (Drugs+A-Z)	
	Before	After 3 months	Before	After 3 months
Serum MDA (nmole/ml)	7.19±0.44	8.08±0.27*	7.77±0.28	5.40±0.79**
Serum NO (µmole/l)	61.67±3.50	65.97±2.35**	64.63±3.33	54.3±2.08**
Serum SOD (units/ml)	3.34±0.44	2.70±0.38**	3.17±0.67	4.19±0.79**
Erythrocyte GSH (µmole/gm of Hb)	4.66±0.34	3.74±0.41**	4.64±0.36	5.39±0.34**
Serum TAS (µmole/l)	668.6±28.18	651.6±25.19*	672.3±45.51	725.3±40.4**

[Values are expressed as Mean ± SD ; * p< 0.05;**indicates p<0.001]

Discussion

The disturbances of micronutrients and antioxidants in DM have been extensively described in many laboratories, and there is good evidence to suggest that oxidative stress may play a role in the pathogenesis of both type 2 DM [8]. In present study serum MDA and NO is significantly increased in DM patient, this could probably attribute to increased oxidative stress which may be a one of the cause of complications DM.

Mechanisms involved in the increased oxidative stress in diabetes not only oxygen free radical generation due to nonezymatic glycation, autooxidation of glycation end products but also changes in the tissue content and activity of antioxidant defense systems [9]. In present study it is reflected in form of decreased concentrations of serum SOD, reduced GSH and TAS in DM. There are two interconnected micronutrient and enzymatic antioxidant defence system against free radical damage. The non-enzymatic micronutrient system involves small molecular weight molecules e.g. glutathione and vitamins like vitamin E, C and previtamin A (β- carotene). The enzymatic antioxidants include several metalloenzymes, such as the selenium containing glutathione peroxidase, the iron containing catalase and SOD with different isoenzyme containing copper, zinc or manganese [10]. Since enzymes are proteins which are generally prone to degradation by enteric enzymes, unmodified enzymes are not usually recommended for oral ingestion [11].

In present study antioxidants are significantly increased and oxidative stress is significantly decreased in type 2 DM after 3 months of A-Z tablets i.e. multivitamin and trace elements supplementation. Only three essential nutrients can directly scavenge free radicals i.e. vitamin E lipid soluble antioxidant

www.ijbamr.com P ISSN: 2250-284X , E ISSN :2250-2858

protects against lipid peroxidation, vitamin C and β-carotene water soluble antioxidants along with vitamin E quench free radicals as well as singlet oxygen, superoxide & hydroxyl radicals [12,13]. In addition, several nutritionally essential minerals incorporated into protective antioxidant enzymes. Zinc, copper and manganese are required for the activity of SOD. Selenium, an essential component of glutathione peroxidase, is important in the decomposition of hydrogen peroxide and lipid peroxides. Catalase also decomposes hydrogen peroxide [14]. The antioxidants are working synergistically, antioxidant vitamins are required to quenching free radicals directly and trace elements are required for activation of enzymatic antioxidants. In diabetes deficiency of trace elements and vitamins are observed which is responsible for increased oxidative stress which further causes late macro as well as micro vascular complications. High doses of single antioxidant supplements may perturb the antioxidant –prooxidant balance of cell systems. In addition, the activity of endogenous antioxidant enzymes can only be altered to a limited extent by ingestion of supra physiological amounts of essential trace elements, the activity of exogenous micronutrient antioxidants depends largely on intake [15].

Hence the present study concluded that the use of multiantioxidants as an adjuvant therapy with antidiabetic drugs is better therapeutic option to minimize micro as well as macrovascular complications of type 2 diabetes mellitus. Thus, additional clinical trials with larger patient populations are needed to replicate the results of this study.

References

1. Lingjie Zhao. Effects of free radicals in diabetes, Free Radical and radiation biology graduate program 2001; May2:1-24.
2. E C Opara . Oxidative stress, micronutrients, diabetes mellitus and its complications. J.R. Soc Health 2002: 122(1):28-34.
3. Kakker P, Das B, Viswanathan PN . A modified spectrophotometric assay of superoxide dismutase. Indian J Biochem Biophys 1984: 21:130–2.
4. Cortas NK, Wakid NW . Determination of inorganic nitrate in serum and urine by a kinetic cadmium reduction method. Clin Chem 1990:36 (8):1440–3.
5. Erdincler DS, Seven A, Inci F, Beger T, Canden G. Lipid peroxidation and antioxidant status in experimental animals: effects of aging and hypercholesterolemic diet. Clin Chim Acta1997: 265:77–84.
6. Iris FF, Benzie and strain J J . The ferric reducing ability of plasma (FRAP) assay. Analytical Biochem 1996: 239: 70-76.
7. Fairbanks V, Klee G. Biochemical aspects of hematology. In: Burtis C, Ashwood E, editors. Tietz text book of clinical chemistry 3rd ed. Philadelphia, PA: WB Saunders Co; 1999:1652-1653.
8. Paolisso G and Giugliano D . Oxidative stress and insulin action: is there a relationship?Diabetologia1996: 39:357-363.
9. Ceriello A. A hyperglycemia : the bridge between non enzymatic glycation and oxidative stress in the

www.ijbamr.com P ISSN: 2250-284X , E ISSN :2250-2858

pathogenesis of diabetic complications. Diabetes Nutr Metab1999:12(1):42-46.

10. Macehlin LJ and Bendich A . Free radical tissue damage: protective role of antioxidant nutrients. FASEB J1987: 1:441-445.
11. Rice-Evans C A and Diplock A T . Current status of antioxidant therapy. Free Rad Biol Med 1993: 15:77-96.
12. McCay, P.B. Vitamin E: interactions with free radicals and ascorbate. Annu. Rev. Nutr 1985: 5: 323- 340.
13. Bielski, B.H. Chemistry of ascorbic acid radicals. Ascorbic acid: chemistry, metabolism and uses. Adv.Chem.Ser 1982: 200 : 81-100.
14. Combs J.F.,Jr . Protective roles of minerals against free radicals tissue damage. Nutrition. Bethesda: Am. Inst. Nutr 1987.
15. Biesalskin H K, Bohles H, Esterbauer H, Furst P, Gey F et al . Antioxidant vitamins in prevention. Clin Nutr 1997:16:151-155.

Date of submission: 29 December 2013 Date of Provisional acceptance: 08 January 2014
Date of Final acceptance: 12 February 2014 Date of Publication: 04 March 2014
Source of support: Nil; Conflict of Interest: Nil

www.ijbamr.com P ISSN: 2250-284X , E ISSN :2250-2858