Ind J Clin Biochem (Jan-Mar 2011) 26(1):74–77
DOI 10.1007/s12291-010-0041-y

ORIGINAL ARTICLE

Evaluation of Oxidative Stress in Type 2 Diabetes Mellitus and Follow-up Along with Vitamin E Supplementation

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Received: 11 February 2009 / Accepted: 12 November 2009 / Published online: 22 December 2010

Abstract Increased oxidative stress is a widely accepted participant in the development and progression of diabetes and its complications. The present study has been undertaken to evaluate oxidative stress in type 2 diabetes mellitus and effect of vitamin E supplementation on oxidative stress. In all 120 subjects were enrolled in the present study, 40 subjects are age and sex matched controls. Test group comprised of clinically diagnosed ($n = 80$) type 2 diabetic patients. Biochemical parameters like serum MDA, nitric oxide, superoxide dismutase, erythrocyte reduced glutathione and platelet aggregation were analyzed in control and diabetic group. Test group is further categorized as Group I ($n = 40$) diabetics were treated by only hypoglycemic drugs and Group II ($n = 40$) diabetics were treated by hypoglycemic drugs with vitamin E supplementation. All above biochemical parameters were again reassessed after 3 months follow-up in both group and its values were compared with its respective baseline levels. The study shows, reduction of oxidative stress, improvement in antioxidant enzymes and endothelial dysfunction in group II, those were on treatment of hypoglycemic drugs along with vitamin E supplementation. Hence the present study may conclude that vitamin E supplementation along with hypoglycemic drugs may be beneficial to type 2 DM patients to minimize vascular complications.

Keywords Oxidative stress · Type 2 diabetes mellitus · Endothelial dysfunction · Vitamin E

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Introduction

Diabetes mellitus (DM) is not a single disease entity but rather a multi systemic metabolic disorder showing the common underlying feature of hyperglycemia [1]. Oxidative stress thought to be increased in a system where the rate of free radical production is increased and/or the antioxidant mechanisms are impaired [2].

Increased oxidative stress is a widely accepted participant in the development and progression of diabetes and its complications [3]. A well established correlation exists between development of macro and microvascular disease in diabetes mellitus [4]. Vascular endothelial cells are an important target of hyperglycemic damage, but the mechanisms underlying this damage are not fully understood. Early marker of such damage is the development of an endothelial dysfunction [5].

The chronic complications of DM affect many organ systems and are responsible for the majority of morbidity and mortality associated with the disease [6]. Hence the aim of the present was to evaluate the oxidative stress in type 2 DM and follow-up along with vitamin E supplementation.

Materials and Methods

Selection of Subjects

In all 120 subjects were enrolled in the present study. Control group comprising of 40 healthy age and sex

(40–60 years) matched subjects. Test group comprising of 80 diabetic subjects of age (40–65 years) selected from Dr. V. M. Medical College and Chatrapati Shivaji Maharaj General Hospital; Solapur and Pad. Dr. D.Y. Patil Medical College, Hospital and Research Centre, Pune. All selected diabetic subjects were unaware that they have the disorder. World Health Organization Criteria i.e. symptoms of diabetes plus random blood glucose concentration ≥11.1 mmol/l (200 mg/dl) or fasting plasma glucose ≥7.0 mmol/l (126 mg/dl) at more than one occasion, is applied for a diagnosis type 2 DM. Also they had one or more specific types of micro and macrovascular complications at the time of their diagnosis. No history of any medication and having usual diet.

The exact duration was not known but according to patients history it might be 6 ± 0.7 years.

The test group was further categorized into Group I ($n = 40$) which include 29 macrovascular and 11 microvascular diabetic patients. They were treated only by hypoglycemic drugs (Sulfonylurea and metformin). Group II ($n = 40$) which include 27 macrovascular and 13 microvascular diabetic patients were received both hypoglycemic drugs as well as vitamin E (Evinal—400 mg/day). Both group received the treatment for a period of 3 months. None were current users of vitamin E, and all had avoided aspirin and other platelet active agents but the patients having macrovascular complications were on beta blockers or calcium channel blockers drugs during the study period. The drugs and vitamin E was prescribed by the physician to the subjects.

All the subjects included in the study volunteered after proper consent and reported for follow-up at right time. The study was approved by ethical committee.

Collection of Specimen

After 12 h fast, venous blood sample were collected in different bulbs under aseptic conditions. Fluoride bulb was used for fasting blood glucose estimation by Glucose Oxidase Peroxidase method [7]. EDTA bulb was used for glycated hemoglobin (HbA_{1C}) estimation by resin binding method [8]. Plain bulb was used for estimations of serum superoxide dismutase (SOD) by Marklund and Marklund [9], nitric oxide (NO) by Cortas and Wakid [10] and malondialdehyde (MDA) by Wilbur et al. method [11]. Acid citrate bulb was used for erythrocyte reduced glutathione (GSH) measurement by method of Beutler et al. [12]. Heparin bulb used for estimation of platelet aggregation by ADP induced aggregation method [13].

Baseline level of all of the above biochemical parameters were measured at the time enrollment in the study for all subjects. But test group I and II again reassessed for the same parameters after follow up of 3 months period. The glycated hemoglobin (HbA_{1C}) 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.

Results

Table 1 shows the characteristics of enrolled subject. It also shows BSL(F) and HbA_{1C} is significantly decreased after 3 months follow of vitamin E supplementation in group II.

Table 2 shows, serum MDA and NO are significantly increased in diabetic group as compared with the control group ($P < 0.001$). It is also observed that platelet aggregation is significantly increased ($P < 0.001$) and activity of serum SOD and reduced GSH is significantly decreased ($P < 0.05$) in diabetic group as compared to control group.

In Table 3, it was observed that serum MDA and NO is significantly increased ($P < 0.05$) further in group I after 3 months follow up as compared to its baseline levels which is in contrast to group II where serum MDA and NO is significantly decreased ($P < 0.001$) as compared to its baseline levels after supplementation of vitamin E with hypoglycemic drugs.

There is no significant change in serum SOD activity after 3 months follow up of hypoglycemic drugs in group I as compared to its baseline levels. But serum SOD activity is significantly increased ($P < 0.001$) in group II after 3 months follow up.

Table 1 Characteristics of enrolled subjects in the study

Subjects	Number (*n*)	BSL (F)		HbA_{1C} (%)	
		Baseline	3 Months follow-up	Baseline	3 Months follow-up
Controls	40	74.33 ± 8.7	–	4.5 ± 0.9	–
Diabetics Group I	40	195 ± 12.6	160 ± 10.1*	9.8 ± 1.0	9.07 ± 2.1
Diabetics Group II	40	185 ± 15.6	145 ± 12.3*	10.1 ± 1.5	8.23 ± 1.2*

Values are expressed as mean ± SD

* $P < 0.05$

Table 2 Comparison of biochemical parameter in between control and diabetic group

	Control	Diabetic group
Serum MDA (nmol/ml)	3.59 ± 0.97	7.19 ± 0.64**
Serum NO (μmol/l)	50.00 ± 9.27	65.07 ± 5.33**
Serum SOD (units/ml)	3.50 ± 1.35	2.75 ± 0.98*
Erythrocyte GSH (μmol/g of Hb)	5.03 ± 0.90	4.74 ± 0.35*
Platelet aggregation (%)	28.91 ± 3.59	48.51 ± 3.81**

Values are expressed as mean ± SD

** $P < 0.001$ and * $P < 0.05$

Reduced GSH is significantly decreased ($P < 0.001$) in group I but it is significantly increased ($P < 0.05$) in group II after 3 months as compared to its respective baseline levels.

As shown in Table 3, % of platelet aggregation is significantly decreased ($P < 0.001$) after 3 months follow up in both group as compared to its respective baseline levels.

Discussion

Diabetes is associated with a number of metabolic alterations and principal among these is hyperglycemia. Known sequelae of hyperglycemia such as cellular damage, increased extra cellular matrix production and vascular dysfunction have all been implicated in the pathogenesis of vascular disease in type I and type II diabetes [14].

Free radicals and oxidative stress may act as a common pathway to diabetes itself, as well as to its complications [15].

In present study HbA_{1C} is used as index of metabolic control. It was observed that HbA_{1C} is significantly increased in diabetic group which is one of the causes for increased production of free radical by direct Aamadori reaction. But it significantly decreased in group II as compared to group I after vit E supplementation.

In present study serum MDA and NO is significantly increased in cases of type 2 diabetes with complications as compared with controls. And it is in accordance with previous findings of that hyperglycemia induces overproduction of oxygen free radicals in diabetes [16]. Increased levels of the products of oxidative damage to lipids have been detected in serum of diabetic patients and their presence correlates with the development of vascular complications [3]. Increased NO activity is because of increased nitric oxide synthase expression due to high glucose level [17]. This could probably attribute to increased oxidative stress which may further cause complications of type 2 DM.

In healthy individuals, oxidative damage to tissue is prevented by a system of defense which includes antioxidant enzymes and small molecules with scavenging ability such as antioxidant vitamins.

Mechanisms involved in the increased oxidative stress in diabetes include not only oxygen free radical generation due to nonenzymatic glycation, autooxidation of glycation products, but also changes in the tissue content and activity of antioxidant defense systems [18].

As per Table 2 serum SOD activity and concentration of erythrocyte GSH is significantly decreased in type 2 DM as compared to the control group.

Reduced glutathione functions as a direct free radical scavenger as a cosubstrate for glutathione peroxide (GPx) which explained decreased GSH concentration with increased oxidative stress. In diabetic patients, the autoxidation of glucose results in the formation of hydrogen peroxide which inactivates SOD [19] and this accumulated hydrogen peroxide may be one of the explanations for decreased activity of SOD in type 2 diabetic patients.

In present study platelet aggregation is significantly increased in diabetes mellitus as compared to control group. The two of the most antiaggregants are the eicosanoids, prostacyclins (PGI_2) and nitric oxide. In healthy vessels, PGI_2 and nitric oxide combine to prevent platelet adherence to endothelium and platelet aggregation [20].

Table 3 Comparison of biochemical parameter between before and after vitamin E supplementation in diabetic Group I and II

	Group I		Group II	
	Baseline level	After 3 Months	Baseline level	After 3 Months
Serum MDA (nmol/ml)	7.16 ± 0.71	8.07 ± 1.36*	7.22 ± 0.56	5.78 ± 0.83**
Serum NO (μmol/l)	65.79 ± 5.06	66.89 ± 5.13*	64.37 ± 5.49	57.47 ± 5.38**
Serum SOD (units/ml)	2.76 ± 0.99	2.85 ± 0.72	2.75 ± 0.98	3.77 ± 0.79**
Erythrocyte GSH (μmol/g of Hb)	4.71 ± 0.37	3.99 ± 0.58**	4.77 ± 0.30	5.49 ± 0.30**
Platelet aggregation (%)	49.87 ± 3.02	44.35 ± 3.41**	47.19 ± 4.06	42.13 ± 3.36**

Values are expressed as mean ± SD; ** $P < 0.001$ and * $P < 0.05$

Platelet from diabetic subjects have been reported to have diminished sensitivity to PGI_2 and NO [21] hence platelet aggregation is increased in type 2 DM. Increased platelet aggregation is multifactorial.

Decreased activity of the antioxidant enzymes may increase the susceptibility of diabetic patients to oxidative injury. Appropriate support for enhancing antioxidant supplies may help to prevent clinical complications of diabetes mellitus.

In point of view, in present study, vitamin E as an antioxidant was supplemented to diabetic patients along with hypoglycemic drugs and compared with those patients who were taken only hypoglycemic drugs.

As per Table 3, Serum MDA and NO levels are significantly decreased after 3 months of vitamin E supplementation along with hypoglycemic drugs in group II. Vitamin E is major lipid soluble chain breaking antioxidant has been associated with reduction in lipid peroxidation, has been shown to block the formation of malondialdehyde, an end product of lipid peroxidation [22]. But serum MDA level is significantly increased after 3 months follow up in group those were taken only hypoglycemic drugs. The antioxidant activity is normalized in group II but it worsens in group I so diabetic patients may have elevated requirement for antioxidants. Vitamin E supplementation increases cellular GSH concentration by sparing action [23].

Platelet aggregation significantly reduced in both group I and II after 3 months when compared with its respective baseline levels. Vit E modulates prostaglandin synthesis and hence platelet adhesiveness and thrombosis [24].

Hence the present study suggest that Vitamin E supplementation along with hypoglycemic drugs may be minimize vascular complications in type 2 diabetes mellitus by decreasing oxidative stress and boosting antioxidants and preventing platelet aggregations.

There is controversy regarding vit E supplementation. Some study shows detrimental instead of beneficial effect because of proxidant effect of vit E. But proxidant action is depending on doses of vitamin E, duration of supplementation and also duration of diabetes. The present clinical trial of vitamin E supplementation along with hypoglycemic drugs to type 2 diabetic patients with vascular complications was a short-term study so it given benefit effect to type 2 DM.

Thus, additional clinical trials with larger patient populations are needed to replicate the results of this study.

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