Evaluation of Oxidative Stress Before and After Control of Glycemia and After Vitamin E Supplementation in Diabetic Patients

Anjali Sharma, Simmi Kharb, S.N. Chugh, Rajesh Kakkar, and G.P. Singh

The present study evaluates the presence of oxidative stress in the uncontrolled diabetic state. Glycemic control reduced the oxidative stress, but total normalization of the parameters of oxidative stress was not achieved, indicating continued oxidant injury despite optimal control of the diabetes. Vitamin E supplementation for 4 weeks in these patients further reduced the oxidative stress, suggesting that vitamin E supplementation might be helpful in reducing free-radical–induced oxidant injury.

MICROVASCULAR complications in diabetes result from the interaction of multiple metabolic, genetic, and other factors and are an important cause of morbidity and mortality. There is immense variability in the susceptibility to microvascular disease which, in individual patients, cannot be predicted from the glycemia pattern.

Recently, many reports have indicated that oxidative stress may have a significant role in diabetic complications.[1,2] In diabetes mellitus, the oxidative stress may be due to many factors such as nonenzymatic glycosylation, the polyol pathway, hypoxia-reperfusion injury, a reduced antioxidant defense system, etc.[3] Under certain conditions, glucose molecules can induce free-radical production.[4] Also, hyperglycemia enhances reduced glutathione (GSH) depletion and the polyol pathway. It is known that oxidative stress occurs when prooxidant stress overwhelms the antioxidant defenses. There are several reports of increased levels of free-radical reaction products in serum, plasma, or other tissue cells of diabetic patients.[1,2] Many studies have shown that the levels of these oxidation products are higher in diabetic patients with chronic complications than in patients without complications.[4,5] The antioxidant defense system appears to be compromised in diabetic patients, and more so in those with chronic complications.[6] Some investigators have shown a decrease in GSH[7] while others[8] have found normal levels in diabetes mellitus. The vitamin E status in diabetes mellitus also is not clear.[3,6]

It appears that oxidative stress occurs in diabetes mellitus irrespective of the type and complications, but its relation to parameters like glycemic control in diabetes and the status of oxidative stress after vitamin E supplementation has not been defined. Thus, the present study was designed to evaluate oxidative stress in diabetes before and after glycemic control and to study the effect of vitamin E supplementation on oxidative stress.

SUBJECTS AND METHODS

The study was performed with 30 diabetic patients (19 men and 11 women) with a mean age of 52.03 ± 13.12 years (>1 year diabetes duration) admitted to the Medical Ward of Pt. B.D. Sharma Postgraduate Institute of Medical Sciences, Rohtak, India. Fifteen age-matched healthy individuals (9 men and 6 women) with a mean age of 44.27 ± 18.71 years served as controls. Chronic smokers, alcoholics, and patients with acute infection, ischemic heart disease, or end-stage diabetic nephropathy were excluded from the study. Total and differential leukocyte counts were within the normal range in all patients; red blood cell counts were also obtained.

Informed consent was provided by the patients, and a detailed history, physical examination, and routine investigations were performed. Serial blood glucose, fasting and postprandial[9] and glycosylated hemoglobin ([GHb] by Stangen's [India] HbA_{1c} estimation kit) were estimated. Whole blood (collected with EDTA) was mixed with lysing reagent to prepare a hemolysate. This is then mixed with cation-exchange resin. The nonglycosylated Hb binds to the resin, leaving GHb free in the supernatant. The GHb level (percent) is determined by measuring the absorbance of the GHb fraction and total Hb at 415 nm. Each sample was analyzed for GHb in triplicate, and samples with more than 5% variation and/or with hemolysis were not included. Evidence of nephropathy was confirmed by a positive urine examination for blood cells, albumin, and pus cells and positive dipstick test for albuminuria.

Fasting venous blood samples were obtained from each patient and divided into 2 parts. In 1 part, serum was separated by centrifugation and malondialdehyde (MDA) was estimated by thiobarbituric acid reaction.[10] In the second part, EDTA was added and samples were processed immediately to avoid glutathione oxidation, and glutathione levels were estimated by the reduction of Ellman reagent DTNB (5,5-dithio-bis-2-nitrobenzoic acid) to nitro-5-thiobenzoic acid, and the yellow compound thus obtained was read at 412 nm.[11] Vitamin E was estimated spectrofluorometrically in the serum.[12]

The patients were then placed under appropriate antidiabetic treatment (glipizide or glyburide) and optimal glycemic control was achieved per recommendations of the European NIDDM Policy Group.[13] Repeat samples were taken (when control was achieved) for measurement of thiobarbituric acid–reactive substances (TBARS), GSH, and vitamin E levels to evaluate the effect of diabetes control on oxidative stress. These patients were then administered vitamin E 400 mg daily for 4 weeks and were evaluated after every week in the diabetic clinic. After 4 weeks of vitamin E supplementation, samples were repeated for the above-mentioned parameters. To better ascertain the vitamin E status, the lipid-standardized vitamin E concentration was used (computed as the ratio of vitamin E to serum total cholesterol plus triglyceride, micromoles per gram). Also, the mean ratio of lipid peroxide to corrected vitamin E (vitamin E to total cholesterol ratio) was calculated. Serum TBARS levels were normalized with lipid levels by calculating the TBARS to total cholesterol ratio and TBARS to triglyceride ratio. Results were analyzed statistically using Student's *t* test.

RESULTS

Of 30 patients, only 4 had insulin-dependent diabetes mellitus, while 26 had non–insulin-dependent diabetes mellitus. No marked variation in the red blood cell count was observed

From the Postgraduate Institute of Medical Sciences, Rohtak, Haryana, India.
Submitted May 19, 1998; accepted August 13, 1999.
Address reprint requests to Dr Simmi Kharb, 1447, Sector 1, Urban Estate, Rohtak—124001, Haryana, India.

0026-0495/00/4902-0014$10.00/0

Table 1. Parameters of Metabolic Control in Diabetic Patients (mean ± SD)

Parameter	Before Control	After Control
Body mass index (kg/m²)	26.45 ± 0.97	25.92 ± 0.96
Urine glucose (g/dL)	1.55 ± 0.45	0.25 ± 0.25
Blood glucose (mmol/L)		
Fasting	10.28 ± 2.061	5.94 ± 0.99
Postprandial	16.50 ± 3.42	8.98 ± 1.24
GHb (%)	10.71 ± 0.94	7.57 ± 0.92

(5.0 ± 0.62 *v* 5.1 ± 0.54 × 10^6/mm^3 before and after metabolic control). After enrollment in the study, appropriate treatment was started and diabetic control was achieved in the patients in 6 to 10 weeks. The parameters of metabolic control are shown in Table 1.

Serum TBARS were significantly higher ($P < .001$) in uncontrolled versus controlled diabetes, but the values remained higher than the control values, indicating only a partial reduction in lipid peroxidation. Also, no significant variation for the TBARS to cholesterol ratio and TBARS to triglyceride ratio was observed in the study or control groups (Table 2).

Mean GSH levels were lower ($P < .001$) in patients before diabetic control as compared with healthy subjects. GSH increased in a significant manner ($P < .001$) after control of the diabetes, but remained significantly lower ($P < .001$) than the healthy control levels. Mean vitamin E levels were lower in patients with an uncontrolled diabetic state than in the healthy controls ($P < .001$). After achieving diabetic control, the levels increased significantly ($P < .001$) but, remained lower than the healthy control values (Table 2).

After vitamin E supplementation in patients with a controlled state of diabetes, a 3-fold increase in serum vitamin E was achieved (Table 3). Mean fasting and postprandial venous blood glucose levels showed no significant change following vitamin E supplementation ($P > .05$). However, TBARS levels decreased further ($P < .001$) and GSH levels improved significantly ($P < .001$) following vitamin E supplementation.

DISCUSSION

Apart from glucose, a number of mechanisms such as a change in energy metabolites,[3] an alteration in the polyol pathway, changes in the level of inflammatory mediators, altered antioxidant defense systems, and localized tissue damage resulting from hypoxia and reperfusion injury lead to oxidant injury. Oxidative stress is widely believed to be a major factor in the development of diabetic complications.[3] Many investigators have demonstrated increased MDA levels in diabetes mellitus,[1,5] while few have denied finding any increase in MDA levels in uncontrolled diabetes.[14] In the present study, mean serum TBARS levels were significantly higher ($P < .001$) in the patients compared with the controls. This suggested the presence of free-radical–mediated injury in uncontrolled diabetes. Further, the TBARS to cholesterol and TBARS to triglyceride ratios were found to be unaltered. The increase in MDA may not be relative, but instead absolute.

Inconsistent reports are available in the literature showing the effects of diabetes control on MDA levels. Some groups found total normalization of MDA levels with diabetes control,[7,15] while others[1,5] demonstrated that normal baseline values were not achieved despite a significant decrease in MDA levels after diabetes control as compared with the healthy control group. The finding of higher MDA levels in diabetic patients versus control subjects after optimal glycemic control indicated a persistence of oxidative stress. Our findings are consistent with reports in the literature.[1,5]

However, controversy exists regarding the status of GSH in diabetes. Some groups[7,16,17] have shown a decrease of GSH and others[8,18] have found normal levels in patients with uncontrolled diabetes. In the present study, whole-blood GSH levels were significantly lower ($P < .001$) in patients in an uncontrolled state of diabetes versus the controls. There is a paucity of literature on the status of GSH after achieving control of diabetes. Available reports show an improvement in GSH levels.[19] Control of diabetes is likely to improve GSH levels by truncating the effect of hyperglycemia in the causation of oxidative stress. In our study, GSH levels remained significantly lower in patients after control of diabetes versus the control groups. This suggested the persistence of oxidative stress after achievement of glycemic control, which might possibly have a role in the pathogenesis of diabetic complications. This further suggested that oxidant injury overwhelms the major nonenzymatic antioxidant defense. Variable observations have been made in the literature regarding the vitamin E status in diabetes mellitus. Vitamin E levels have been found to be decreased,[6,20,21] normal,[14] and even increased[21] in patients with diabetes. In the present study, mean serum vitamin E levels were significantly lower in an uncontrolled state of diabetes, suggesting the utilization of vitamin E in the process of scavenging free radicals. In addition to its antioxidant property, vitamin E has actions that may be relevant to vascular reactivity, such as antiplatelet properties, inhibition of vascular smooth muscle cell proliferation, and inhibition of protein kinase C stimulation.[22] Vitamin E levels improved significantly after achieving control of the diabetes, but remained significantly lower as compared with control levels. MDA (TBARS) is an indicator of free-radical production, and an increase in MDA may therefore

Table 2. Parameters of Oxidative Stress Before and After Control of Diabetes Mellitus (mean ± SD)

Group	TBARS (μmol/L)	GSH (mg/dL)	Vitamin E (μg/mL)	Vitamin E to Total Lipid Ratio (μmol/g)	Lipid Peroxide to Corrected E Ratio	TBARS to Cholesterol Ratio (μmol/mmol)	TBARS to Triglyceride Ratio (μmol/g)
Controls	0.69 ± 0.24	22.28 ± 2.22	13.33 ± 2.82	11.98	0.83 ± 0.03	0.26 ± 0.06	0.46 ± 0.03
Diabetic patients							
Before control	3.62 ± 0.49	8.14 ± 0.92	1.92 ± 0.42	1.26	4.11 ± 0.98	0.68 ± 0.16	2.10 ± 0.33
After control	2.44 ± 0.62	18.25 ± 0.74	3.64 ± 0.69	2.40	1.41 ± 0.1	0.44 ± 0.29	1.54 ± 0.52

Table 3. Effect of Vitamin E Supplementation on Blood Glucose and Parameters of Oxidative Stress in Diabetic Patients (mean ± SD)

Parameter	After Control	After Vitamin E Supplement	P
Blood glucose (nmol/L)			
Fasting	5.94 ± 0.99	5.78 ± 0.8	NS
Postprandial	8.98 ± 1.24	8.79 ± 1.28	NS
TBARS (μmol/L)	2.49 ± 0.62	1.76 ± 0.30	<.001
GSH (mg/dL)	10.25 ± 0.74	13.51 ± 0.81	<.001
Vitamin E (μg/mL)	3.64 ± 0.69	13.14 ± 2.35	<.001

Abbreviation: NS, nonsignificant.

be due to oxidative stress. However, increased utilization of antioxidants, including vitamin E, may be expected to combat the stress. Hence, a decrease in the vitamin E level in diabetic patients compared with controls can be due to increased utilization or decreased intake of vitamin E. The latter possibility cannot be tested because of the similar dietary regimen in the region. The significant decrease in TBARS and increase in GSH and vitamin E in a controlled state of diabetes further suggested that there was a tendency for the body to overcome the oxidative stress and that, in addition to hyperglycemia, other mechanisms might play an important role in the pathogenesis of oxidative injury. These observations suggested that some measures are desirable to combat oxidative stress, eg, supplementation of antioxidant vitamins.

In the present study, vitamin E supplementation was used to evaluate its effect in further combating oxidative stress. There was a 3-fold increase in serum vitamin E levels (Table 3). TBARS levels were still significantly higher after the supplementation. Similarly, mean blood GSH levels increased significantly. There was an improvement in oxidative stress after vitamin E supplementation, but normalization of oxidative stress parameters was not achieved. In conclusion, the present study suggests a possible role for vitamin E supplementation in the prevention of chronic complications in diabetes mellitus, along with conventional therapy.

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