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Oxidative modification in diabetic neuropathy

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ABSTRACT

Oxidative stress and impaired anti-oxidant defense have been suggested as contributory factors for initiation and progression of complications in diabetes mellitus. The aim of the present study was to evaluate the levels of selected oxidation and antioxidation indicators in relevance to diabetic complication, neuropathy and to determine the impact of glycemic control on such parameters. Thirty patients with diabetic neuropathy and thirty age matched healthy controls were included in the study. Fasting blood glucose was estimated to assess the severity of diabetes and the glycemic control. Serum malondialdehyde (MDA) levels were measured as markers of oxidative stress by thiobarbituric acid method. As vitamin C and vitamin E are the major contributors to serum total antioxidant activity, the antioxidant status was assessed by 2,4-dinitrophenyl hydrazine method and Baker and Frank method respectively. The serum MDA levels were found to be increased significantly ($p<0.001$) while plasma vitamin C and E levels were significantly ($p<0.001$) reduced as compared to controls. A highly significant positive correlation was found between serum MDA and FBS ($p<0.001$). A highly significant negative correlation was noted between plasma vitamin C, Vitamin E with FBS ($p<0.001$). These findings indicate in diabetic neuropathy there is oxidative stress and antioxidant deficiency is inversely related to the glycemic control. We conclude that striving for superior antioxidative therapies remains essential for the prevention of complication like neuropathy in diabetic patients.

KEYWORDS

Oxidative stress;
Vit C;
Vit E;
MDA.

INTRODUCTION

Oxidative stress results from a cell or tissue failing to detoxify the free radicals that are produced during metabolic activity. Diabetes is characterized by chronic hyperglycemia that produces deregulation of cellular metabolism. Diabetes overloads glucose metabolic pathways, resulting in excess free radical production and oxidative stress. Toxic oxygen derived products are generated in all aerobic cells which include superoxide radical (O^{-2}), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH^-), the latter being the most lethal[1]. It is

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well established that there is an increased production of damaging free radicals in non-insulin dependent diabetes mellitus (NIDDM) patients which may be due to auto-oxidation of glucose and glycosylated proteins[2]. Proteins that are damaged by oxidative stress have decreased biological activity leading to loss of energy metabolism, cell signaling, transport, and, ultimately, to cell death. Evidence is presented to support the idea that both chronic and acute hyperglycemia cause oxidative stress in the peripheral nervous system that can promote the development of diabetic neuropathy[3]. Examination of the data from animal and cell culture models of diabetes, as well as clinical trials of antioxidants, strongly implicates hyperglycemia-induced oxidative stress in diabetic neuropathy. The protection against such damage can be offered by free radical scavenging antioxidants[4]. An imbalance between the generation and scavenging of these free radicals leads to "oxidative stress", which may be associated with the pathogenesis of the complications of NIDDM including nerve damage leading to diabetic neuropathy[5,6]. Hence, the present study was planned to assess the oxidative stress and antioxidant vitamin status in patients with diabetic neuropathy and also to investigate whether there exists any relationship between glycemic control and antioxidant deficiency.

MATERIALS AND METHODS

The control group comprised of 30 healthy volunteers. The controls were examined and were found to be free from disease. 30 cases of NIDDM with neuropathy were included in the study. The diagnosis of diabetic neuropathy was based on the clinical examination of the patients. Peripheral neuropathy was evaluated clinically and was defined as more than ≥ missing deep tendon reflexes in legs, diminished distal touch (assessed by cotton wool), pinprick or pressure sensations, distal vibratory sensation (assessed by graduated tuning fork of 128 Hz) and joint position sense. Other potential causes of peripheral neuropathy were excluded before attributing peripheral neuropathy to diabetes. The groups as a whole were similar in age and sex distribution. Patients with coronary heart disease or hypertension, chronic renal insufficiency, uncontrolled primary/secondary hypertension, and other life-threatening diseases, such as cancer, were excluded from the study. All the diabetics were on hypoglycemic drugs and none of the study subjects were on antioxidant supplementation or lipid lowering drugs.

Fasting Blood sugar (FBS) was estimated by glucose o-toludine method[7]. Serum malondialdehyde, (MDA) a marker of lipid peroxidation, was estimated by thiobarbituric acid method[8]. Plasma vitamin C and serum vitamin E were estimated as the markers of antioxidant status. Vitamin C was estimated by 2,4-dinitrophenyl hydrazine method[9]. Serum vitamin E was estimated by Baker and Frank method[10].

Statistical analysis

The statistical results are expressed as Mean ± SD. The comparison of the results of patients and healthy controls was done by performing unpaired t-test and the statistical significance was determined from the p value. The antioxidant vitamin status were correlated with glycemic control in patients with diabetic neuropathy by calculating the Pearson's coefficient of correlation (r value) and the statistical significance was determined from the p value.

RESULTS

Fasting blood glucose was estimated in patients suffering from diabetic neuropathy to assess the severity of the disease and the glycemic control respectively. The patients had poor glycemic control (TABLE 1).

The serum levels of MDA, a lipid peroxidation product, were estimated in these patients as a marker of oxidative damage and a highly significant rise ($p < 0.001$) was found in their levels as compared to the controls (TABLE 1). The mean percentage rise in serum MDA levels was 46 % (Figure 1). Plasma vitamin C levels were found to be reduced in patients as compared to controls (TABLE 1) and the decline was statistically highly significant ($p < 0.001$). The mean percentage reduction was 58 % (Figure 1). As compared to controls, serum vitamin E levels were found to be reduced in these patients (TABLE 1) and the decline was statistically highly significant ($p < 0.001$). The mean percentage reduction was 35% (Figure 1). A negative correlation was found between FBS and Vit C, $r = -0.9056$ (Figure 2) and also negative correlation was

found between FBS and Vit E, r = -0.7652 (Figure 3).

DISCUSSION

Oxidative Stress occurs in a cellular system when the production of free radical moieties exceeds the antioxidant capacity of that system. If cellular antioxidants do not remove free radicals, radicals attack and damage proteins, lipids, and nucleic acids[2]. Chronic hyperglycemia causes oxidative stress in tissues prone to complications in patients with diabetes. The microvascular complications of diabetes carry a high morbidity and, when coupled with macrovascular complications, high mortality[1]. The most common microvascular complication is neuropathy. Diabetic neuropathy resulting from chronically high blood sugar is one of the most life threatening disorders[5].

In view of the increased risk of oxidative damage in diabetic patients leading to diabetic neuropathy, this study was planned to assess the oxidative stress as well as antioxidant vitamin status of patients suffering from diabetic neuropathy and to correlate them with glycemic control.

The serum MDA levels were found to be significantly elevated in our patients as compared to controls and the rise indicates increased oxidative stress in these patients. This is in agreement with the studies of Ziegler D. et al.[11] who reported increased oxidative stress in diabetic neuropathy patients in terms of other markers of oxidative stress viz. The increased production of damaging free radicals in these patients may be due to auto-oxidation of glucose and glycosylated proteins[12]. This creates highly toxic by-products called advanced glycosylation end products (AEGs). These themselves cause degenerative changes in human body causing nerve damage. These further generate 50 times more free radicals than non-glycated proteins. Thus, chronic blood sugar imbalances or dysglycemia, may both fuel and be fueled by oxidative stress, creating a vicious cycle of metabolic imbalances[12].

Water-soluble vitamin C and fat-soluble vitamin E together make up an antioxidant system for mammalian cells. Vitamin C, or ascorbic acid, is considered the most important antioxidant in plasma and forms the first line of defense against plasma lipid peroxidation[13]. Vitamin E is the generic description for all tocopherol and tocotrienol derivatives that comprise the major lipophilic antioxidant of exogenous origin in tissues[14]. Hence, plasma vitamin C and serum vitamin E were estimated as the markers of antioxidant status.

TABLE 1 : The oxidative stress and antioxidant vitamin status in patients with diabetic neuropathy and control group

	Controls N=30	Diabetic neuropathy N=30	p value
FBS (mg/dl)	97.7 ± 5.7	249.6 ± 17.2	p < 0.001
MDA (nmol/ml)	3.83 ± 0.26	5.60 ± 0.19	p < 0.001
Vit. C (mg/dl)	1.58 ± 0.10	0.67 ± 0.08	p < 0.001
Vit. E (mg/L)	11.02 ± 1.23	7.2 ± 2.28	p < 0.001

Plasma vitamin C and vitamin E levels were significantly reduced in our patients with diabetic neuropathy. Ziegler D. et al.[11] and Sundaram et al.[15] who reported reduced plasma vitamin C and vitamin E levels in diabetic polyneuropathy. Reduction in plasma vitamin C and vitamin E levels may be the result of rapid depletion of these antioxidant vitamins due to increased oxidative stress. This indicates that poor diabetic control is associated with reduced serum free radical scavenging (antioxidant) activity in non - insulin dependent diabetes mellitus[16].

An increased loss of water soluble vitamin C in urine of diabetic patient may be responsible for fall in plasma vitamin C levels observed in these patients. Also, impaired transport or dietary deficiency of vitamin C and increased demand for vitamin C to relieve increased oxidative stress may be contributing to decreased levels of plasma vitamin C levels observed in these patients[17]. Vitamin C exists in two major forms: the charged form, ascorbic acid, is taken up into cells via sodium-dependent facilitated transport. The uncharged form, dehydroascorbate (DHA), enters cells via glucose transporters (GLUT) and is then converted back to ascorbic acid within these cells. Cell types such as certain endothelial and epithelial cells as well as neurons that are particularly prone to damage during diabetes tend to be those that appear to be dependent on GLUT transport of DHA rather than sodium-dependent uptake. Diabetic neuropathies, nephropathies and retinopathies develop in part by exclusion of DHA uptake by GLUT transporters when blood glucose levels rise above normal. Ascorbic acid plays a central role in the antioxidant defense system. Exclusion of DHA from cells by hyperglycemia would deprive the cells of the

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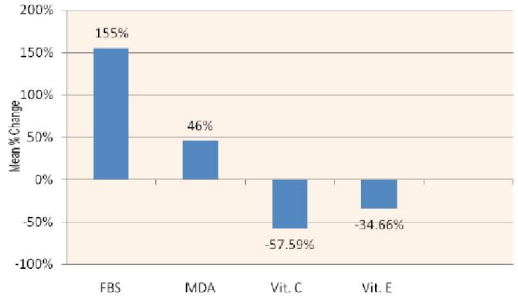

Figure 1 : Mean percentage changes in FBS, serum MDA, vitamin C and vitamin E levels in diabetic neuropathy as compared to controls

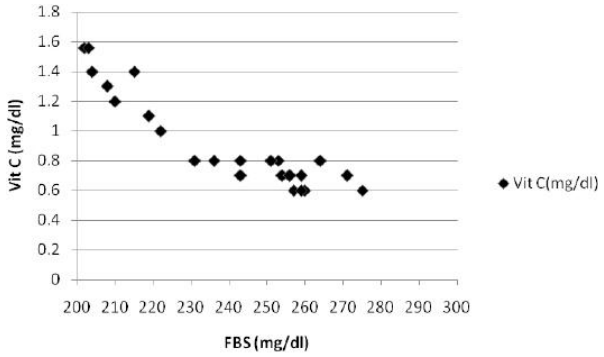

Figure 2 : Correlation between FBS (mg/dl) and vitamin C (mg/dl) in diabetic neuropathy

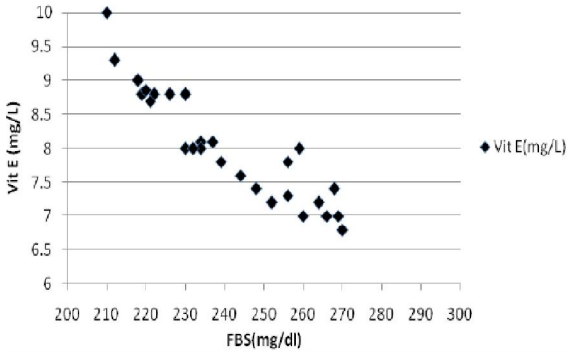

Figure 3 : Correlation between FBS (mg/dl) and vitamin E (mg/L) in diabetic neuropathy

central antioxidant, worsening the hyperglycemia-induced oxidative stress level[18].

Moreover, ascorbic acid participates in many cellular oxidation–reduction reactions including hydroxylation of polypeptide lysine and proline residues and dopamine that are required for collagen production and metabolism and storage of catecholamines in neurons[18]. Increase in the oxidative stress level and metabolic perturbations can be expected in any tissue or cell type that relies exclusively or mainly on GLUT for co-transport of glucose and DHA including neurons, epithelial cells, and vascular tissues. On the other hand, since DHA represents a significant proportion of total serum ascorbate, by increasing total plasma ascorbate concentrations during hyperglycemia, it should be possible to correct the increase in the oxidative stress level and metabolic perturbations, thereby sparing diabetic patients many of their complications[18]. As vitamin C and vitamin E are the major contributors to serum total antioxidant activity our results indicate that diabetic patients have significant defects in antioxidant protection, which may increase the vulnerability to oxidative damage and the development of diabetic complications such as diabetic neuropathy.

CONCLUSION

Diabetic neuropathy probably arises from a combination of microvascular and neuronal deficits. Oxidative stress can contribute significantly to these deficits and may be a direct result of hyperglycemia. Brief postprandial peaks in blood glucose are sufficient to generate hyperglycemic oxidative stress. The present study showed that type 2 diabetic patients complicated with peripheral neuropathy have a low antioxidant status as compared to those of healthy individuals. The results of the present study suggest that oxidative stress is greatly increased in patients suffering from diabetic neuropathy and antioxidant status is inversely related to glycemic control. A good glycemic control is essential for prevention of diabetic neuropathy Therefore, until we can fully control blood glucose levels, therapies such as antioxidants that are targeted against oxidative stress remain our most promising approach to preventing neuropathy as well as other complications in diabetes.

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