Association Of Poor Glycemic Control With Increased Lipid Peroxidation And Reduced Antioxidant Vitamin Status In Diabetic Neuropathy

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Citation

J Sawant, U Vhora, N Moulick. *Association Of Poor Glycemic Control With Increased Lipid Peroxidation And Reduced Antioxidant Vitamin Status In Diabetic Neuropathy*. The Internet Journal of Endocrinology. 2006 Volume 3 Number 2.

Abstract

Oxidative stress resulting from enhanced free-radical formation and/or a defect in antioxidant defenses has been implicated in the pathogenesis of experimental diabetic neuropathy. Hence, the aim of the present study was to assess the lipid peroxidation and the antioxidant vitamin status in patients with diabetic neuropathy and to correlate these with glycemic control. Thirty five patients with diabetic neuropathy and thirty age matched healthy controls were included in the study. Fasting blood glucose and glycosylated hemoglobin (HbA1c) were estimated to assess the severity of diabetes and the glycemic control respectively. Serum malondialdehyde (MDA) levels were assessed as a marker of lipid peroxidation and hence oxidative stress. As vitamin C and vitamin E are the major contributors to serum total antioxidant activity, the antioxidant vitamin status was assessed by estimating plasma vitamin C and E levels. 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 HbA1c ($p<0.001$), while statistically significant negative correlations of serum MDA were found with vitamins C ($p<0.001$) and E ($p<0.005$). A highly significant negative correlation was also noted between plasma vitamin C and HbA1c ($p<0.001$). These findings indicate in diabetic neuropathy oxidative stress is inversely related to the glycemic control. This could be the unifying mechanism that leads to nervous system damage causing diabetic neuropathy.

INTRODUCTION

Patients with Non-Insulin Dependent Diabetes Mellitus (NIDDM) have an increased mortality and morbidity compared to non-diabetics due to various associated complications such as neuropathy, nephropathy, cardiovascular, ocular etc. Diabetic neuropathy resulting from chronically high blood sugar is one of the most life threatening disorders ([1]). It is 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], [3], [4], [5]). The protection against such damage can be offered by free radical scavenging antioxidants ([6]).

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 ([7]). 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 these parameters.

MATERIALS AND METHODS

To assess oxidative stress and antioxidant status in patients with diabetic neuropathy as well as in normal healthy individuals, fasting blood samples were collected from thirty five patients with long term diabetic neuropathy and thirty age-matched healthy controls. The diagnosis of diabetic neuropathy was based on the clinical examination of the patients. None of the subjects were receiving any antioxidant vitamin therapy.

Blood glucose was estimated by glucose oxidase – peroxidase method ([8]). Glycosylated hemoglobin (HbA1c) was estimated by the formation thiobarbituric acid – 5-hydroxy methyl furfural adduct, which is measured at 443 nm ([9]). Serum malondialdehyde, a marker of lipid peroxidation, was estimated by thiobarbituric acid method ([10]). Plasma vitamin C and serum vitamin E were estimated as the markers of antioxidant status. Vitamin C was estimated by 2, 6 dichlorophenol indophenol dye reduction

method ([11]). Serum vitamin E was estimated by Emmerie-Engel reaction using ''-'' dipyridyl ([12]).

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. Lipid peroxidation and 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 (FBG) and glycosylated hemoglobin levels (HbA1c) were estimated in patients suffering from diabetic neuropathy to assess the severity of the disease and the glycemic control respectively. The patients had poor glycaemic control (Table 1).

Figure 1

Table 1 : Fasting Blood Glucose (FBG), Glycosylated Hemoglobin (HbA1c), Serum MDA, Plasma Vitamin C and Serum Vitamin E levels in patients with Diabetic Neuropathy and Control group

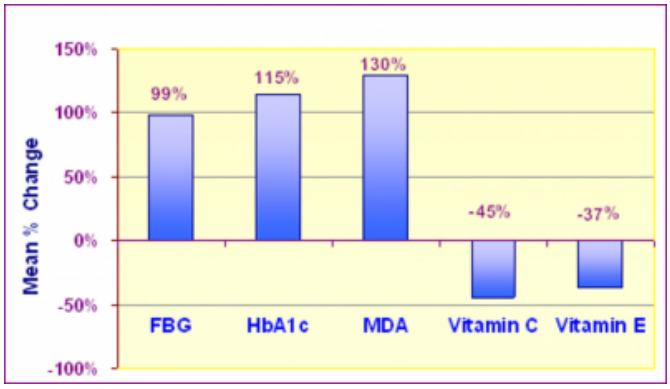
	Controls (n=30)	Diabetic Neuropathy (n=35)
FBG (mg/dl)	96 ± 10.21	206.4 ± 68.19*
HbA1c (gm %)	3.77 ± 0.15	7.74 ± 1.48*
Serum MDA (µmol/L)	1.62 ± 0.69	3.74 ± 0.48*
Plasma Vitamin C (mg/dl)	1.05 ± 0.18	0.58 ± 0.15*
Serum Vitamin E (mg/L)	11.53 ± 1.23	7.3 ± 3.67*

Values are expressed as mean ± SD

* P < 0.001

Figure 2

Figure 1 : Mean Percentage Changes in FBG, HbA1c, Serum MDA, Plasma Vitamin C and Serum Vitamin E levels in Diabetic Neuropathy as compared to controls



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 130% (Figure 1). A highly significant positive correlation was found between serum MDA levels and HbA1c ($r = 0.95$, $p < 0.001$) in these patients. Further, serum MDA levels were inversely correlated with plasma vitamin C ($r = - 0.81$, $p < 0.001$) and vitamin E ($r = - 0.46$, $p < 0.005$) levels.

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 45% (Figure 1). Moreover, a highly significant inverse correlation was found between plasma vitamin C and HbA1c levels ($r = - 0.96$, $p < 0.001$).

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 37% (Figure 1). An inverse correlation was found between plasma vitamin E and HbA1c levels ($r = - 0.18$) which was statistically non-significant.

DISCUSSION

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

of 130 % indicates increased oxidative stress in these patients. This is in agreement with the studies of Ziegler D. et al ([13]) who reported increased oxidative stress in diabetic neuropathy patients in terms of other markers of oxidative stress viz. plasma 8-iso-prostaglandin F(2alpha) (8-iso-PGF(2alpha)), superoxide anion (O2(.-)) generation and lag phase to peroxidation by peroxynitrite (ONOO(-). Abou-Srif M. A. et al ([14]), Martin-Gallan P. et al ([15]), Neri S. et al ([16]) Atli T. et al ([17]) and Altomare E. et al ([18]) reported significant rise in plasma MDA levels in NIDDM patients. A highly significant positive correlation between serum MDA and HbA1c ($r = 0.95$, $p < 0.001$) found in the present study is consistent with the findings of Altomare E. et al ([18]) and is suggestive of accelerated oxidative damage in these patients which is inversely related to the glycemic control.

The increased production of damaging free radicals in these patients may be due to auto-oxidation of glucose and glycosylated proteins ([3]). 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, self-pertctuating cycle of metabolic imbalances.

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 ([19]). Vitamin E is the generic description for all tocopherol and tocotrienol derivatives that comprise the major lipophilic antioxidant of exogenous origin in tissues ([20]). Hence, plasma vitamin C and serum vitamin E were estimated as the markers of antioxidant status.

Plasma vitamin C and vitamin E levels were significantly reduced in our patients with diabetic neuropathy. Our findings are similar to those of Ziegler D. ([13]) et al who reported reduced plasma vitamin C and vitamin E levels in diabetic polyneuropathy. Sundaram et al ([21]) reported significant reductions in vitamin C and vitamin E levels in NIDDM. However, Merzouk S. et al ([22]) found significant lowering in only plasma vitamin E levels without any significant change in plasma vitamin C levels in type II diabetic subjects with and without complications as compared to controls. Hiroshi Yamada et al ([23]), Maxwell SR et al ([24]) reported significant reduction in vitamin C levels in NIDDM patients. 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.

A highly significant inverse correlation was found between plasma vitamin C and HbA1c levels ($r = - 0.96$, $p < 0.001$), which is similar to the findings of Sargeant LA ([25]). This indicates that poor diabetic control is associated with reduced serum free radical scavenging (antioxidant) activity in non-insulin-dependent diabetes mellitus.

An increased loss of water soluble vitamin C in urine of diabetic patient ([26], [27]) 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 ([28], [29]) may be contributing to decreased levels of plasma vitamin C levels observed in these patients. Vitamin C exists in two major forms: the charged form, ascorbic acid (AA), 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 AA 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 AA uptake. Diabetic neuropathies, nephropathies and retinopathies develop in part by exclusion of DHA uptake by GLUT transporters when blood glucose levels rise above normal. AA plays a central role in the antioxidant defense system. Exclusion of DHA from cells by hyperglycemia would deprive the cells of the central antioxidant, worsening the hyperglycemia-induced oxidative stress level ([30]).

Moreover, AA 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 ([30]). 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 ([30]).

As vitamin C and vitamin E are the major contributors to serum total antioxidant activity ([31]), 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. This is further supported by statistically significant strong negative correlations of serum MDA with vitamin C ($r = - 0.81$, $p < 0.001$) and vitamin E ($r = - 0.46$, $p < 0.005$) levels found in the present study.

CONCLUSION

The results of the present study suggest that oxidative stress is greatly increased in patients suffering from diabetic neuropathy and is inversely related to glycemic control. This may be due to depressed antioxidant vitamin levels and may also be responsible for further depletion of antioxidant vitamins C and E. This worsens the oxidative stress creating a vicious cycle of imbalance of free radical generation and deficit of antioxidant status in these patients which may lead to nervous system damage causing diabetic neuropathy. A good glycemic control is essential for prevention of diabetic neuropathy. Further, antioxidant vitamin therapy may help in reducing oxidative stress and hence the incidence of diabetic neuropathy.

ACKNOWLEDGEMENTS

We wish to thank the Department of Biochemistry, Lokmanya Tilak Municipal Medical College and Hospital, Sion, Mumbai for providing all the necessary facilities to carry out this work.

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