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Antioxidant Status in Type 2 Diabetic Neuropathy

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Objective: To measure the concentration of total antioxidant status (TAS) in type 2 diabetes mellitus patients complicated with peripheral neuropathy.

Design: Case-control study.

Setting: The College of Medicine and Al-Wafaa Center of Diabetes, Mosul.

Method: Thirty type 2 diabetic patients, having evidence of distal symmetrical polyneuropathy and thirty sex and age-matched healthy volunteers participated in the study. Serum glucose concentration and total antioxidant status (TAS) was measured in both groups.

Result: Mean fasting blood sugar of the patient group (11.31±2.84 mmol/l) was significantly higher ($p<0.001$) than that of the control group (4.97±0.95 mmol/l). Mean TAS of the patient group (1.31±0.42 mmol/l) was significantly lower ($p<0.001$) than that of the control group (1.98±0.16 mmol/l).

Conclusion: The present study demonstrated that type 2 diabetic patients with peripheral neuropathic complications have lower levels of TAS. This low value of TAS may be due to oxidative stress caused by hyperglycemia that reduce the concentration of the antioxidant status of the body.

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Diabetic peripheral neuropathy is the most common complication of long-standing diabetes mellitus which frequently results in clinically significant morbidities (e.g. pain, foot ulcers and amputations)[1].

It is estimated that the prevalence of neuropathy in diabetic patients is approximately 30% in hospital patients and 20% in community patients[2]. A commonly cited study in 1977 reported that approximately 7% of patients had neuropathy upon diagnosis of diabetes, and the incidence approached 50% for patients with diabetes for more than 25 years[3].

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The primary risk factor for diabetic neuropathy is hyperglycemia[2]. The duration of diabetes also increases the risk of neuropathy but the association between duration and prevalence may depend in part upon patient age, which is a risk factor[2,4]. Cigarette smoking, alcohol consumption, hypertension, height and hypercholesterolemia are all considered independent risk factors for diabetic neuropathy[2,4,5].

A unifying hypothesis for the pathogenesis of diabetic neuropathy is difficult to synthesize. The heterogeneity in clinical form of diabetic neuropathy illustrates the difficulty in identifying a singular cause[6]. An increasing body of data supports the role of oxidative stress in the pathogenesis of diabetic neuropathy in animal models[7]. Information from clinical studies confirming the role of oxidative stress in the pathogenesis of diabetic neuropathy is limited. However, benefits have been observed with α-lipoic acid, a powerful antioxidant that scavenges hydroxyl, superoxide, and Peroxyl radicals and regenerates glutathione in many clinical trials[8-10].

Oxidative stress is defined as the excess formation and/or insufficient removal of highly reactive molecules (free radicals) such as reactive oxygen species and reactive nitrogen species[11,12]. It usually occurs when the available supply of the body's antioxidants is insufficient to handle and neutralize free radicals of different types. The result is massive cell damage that can result in cellular mutations, tissue breakdown, and immune compromise[13].

There is a high correlation between oxidative stress in diabetes and the development of complications. In type 1 diabetic patients, oxidative stress is evident within a few years of diagnosis before the onset of complications. As the disease progresses, antioxidant potential decreases, and the plasma lipid peroxidation products increase depending upon the level of glycemic control[14]. Type 2 diabetic patients have increased lipid peroxidation compared with age-matched control subjects, as well as decreased plasma GSH and GSH-metabolizing enzymes and antioxidant potential, all of which relate directly to the rate of development of complications[15,16]. Increase in oxidative stress has clearly been shown to contribute to the pathology of neural and vascular dysfunction in diabetes[17].

Diabetes-associated oxidative stress is clearly evident in the peripheral nerve, dorsal root, and sympathetic ganglia of the peripheral nervous system and endothelial cells; it has implications on nerve blood flow and conduction deficits, impaired neurotrophic support, changes in signal transduction and metabolism, and morphological abnormalities that are characteristic of peripheral diabetic neuropathy[18].

Pathogenesis of diabetic neuropathy is complex. Chronic hyperglycemia is a major factor which induces nerve fibers injury. Chronic hyperglycemia causes oxidative stress in tissues prone to complications in patients with diabetes[19,20]. High levels of glucose stimulate the polyol pathway causing osmotic stress, enhance reactive oxygen species generation, and play an important role in diabetic angiopathy development[21].

Hyperglycemia control remains a major therapeutic target when dealing with diabetic peripheral neuropathy in both types of diabetes, treatment should be supplemented by

aldose reductase inhibition and antioxidant treatment; the progression of diabetic neuropathy is dependent on glycemic control in both type 1 and 2 diabetes patients [2,22,23,24].

Trials dealing with the measurement of total antioxidant status in diabetic patients with peripheral neuropathic complications are limited. The present study was designed to measure the concentration of TAS in a number of type 2 diabetes mellitus patients with peripheral neuropathic complications and to compare the results with those of healthy controls.

METHOD

Thirty type 2 diabetic patients (according to American Diabetes Association Criteria) having evidence of distal symmetrical polyneuropathy with at least moderate severity of one or more of the typical symptoms (pain, burning, numbness and paresthesia) and confirmed by electroneurograph (ENG) participated in the study. Another thirty sex and age-matched healthy volunteers were included in the study as the control group. Each of the control and patient groups consisted of 18 males and 12 females. Participants' age range was 40 to 60 years. All participants completed a consent form, and the study protocol was approved by the Research Ethics Committee.

Pregnant women, lactating women, individuals receiving trace element or antioxidants or vitamin B complex, patients with acute or chronic illness other than diabetes, smokers and alcohol users were excluded from study.

The study was performed during fasting time in the morning. Fasting blood sugar (FBS) was measured by Glucose-oxidase-peroxidase colorimetric method by using a commercially available kit supplied by (Randox, UK)[25]. Total antioxidant status (TAS) was measured in the immunology laboratory using an antioxidant assay kit (Cayman Chemical Company/ U.S.A).

Paired t-tests were used to compare FBS, TAS and ages of the two groups. All values were expressed as Mean ± SD and a p-value of ≤ 0.05 was considered statistically significant.

RESULT

The individuals in the diabetic and control groups were comparable in terms of age (Mean 51.3±6.08 years for the diabetic group and 50.7±7.1 years for the control group, $p>0.5$), and sex (18 males and 12 females in each group).

Mean FBS for the patient group (11.31±2.84 mmol/l) was significantly higher ($p<0.001$) than that for the control group(4.97±0.95 mmol/l).

Mean TAS for the patient group (1.31±0.42 mmol/l) was significantly lower ($p<0.001$) than that for the control group (1.98±0.16 mmol/l).

DISCUSSION

This study indicates that type 2 diabetic patients with peripheral neuropathic complications have lower values of TAS compared to normal healthy individuals.

The increased presence of free radicals has been suggested to be one of the major causes of diabetic complications, having implications on the pathogenesis of type 2 diabetes mellitus[26,27,28]. Several hypotheses have been tested to evaluate the possible causal mechanism of increased free radicals in diabetes[29,30]. Some studies suggest enhanced free radicals due to elevated glucose concentrations. Other studies focus on reduced antioxidant defense in diabetes[28,31].

The current study involved 60 individuals, divided into two sex and age-matched groups. This matching of individual groups may exclude any effect of the difference in sex and age on the study outcome.

The diabetic patients in our study had high FBS values compared with the control individuals, indicating that the patients have poor glycemic control. There is considerable evidence suggesting that hyperglycemia results in the generation of reactive oxygen species, ultimately leading to increased oxidative stress in a variety of tissues[32]. In the absence of an appropriate compensatory response from the endogenous antioxidant network, this will lead to cellular damage and ultimately would be responsible for the complications of diabetes[32].

The implication of hyperglycemia in the development of diabetic neuropathy have been studied by a number of investigators. Rolo and Palmeira, reported that hyperglycemia resulting from uncontrolled glucose regulation is widely recognized as the causal link between diabetes and diabetic complications[33].

Hyperglycemia can induce oxidative stress that contribute in the development of diabetic vascular and neuronal dysfunction[18].

Hyperglycemia has a key role in oxidative stress in diabetic nerve, whereas the contribution of other factors, such as endoneurial hypoxia, transitional metal imbalance, and hyperlipidemia, has not been rigorously proven[34]. Both chronic and acute hyperglycemia cause oxidative stress in the peripheral nervous system that can promote the development of diabetic neuropathy[35].

Many hypotheses have been tested to provide an explanation for the contribution of hyperglycemia in the development of oxidative stress and diabetic complications. Hyperglycemia can induce oxidative stress via glucose autoxidation, non enzymatic glycation of proteins, disruption of the polyol pathway, altered eicosanoid metabolism and decreased antioxidant defenses [36,37]. Rolo and Palmeira, reported that four major molecular mechanisms have implications on hyperglycemia-induced tissue damage: activation of protein C (PKC) isoforms via de novo synthesis of the lipid, second

messenger diacylglycerol (DAG), increased hexosamine pathway flux, increased advanced glycation end product (AGE) formation, and increased polyol pathway flux[33]. Hyperglycemia-induced overproduction of superoxide is the causal link between high glucose and the pathways responsible for hyperglycemic damage.

Yorek and Pop-Busuil showed that the possible sources for the overproduction of reactive oxygen species in hyperglycemia are widespread and include enzymatic pathways, autoxidation of glucose and mitochondrial superoxide production[17,18].

CONCLUSION

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. This low value of TAS may be due to hyperglycemia which causes the development of oxidative stress that reduces the concentration of the antioxidant status of the body.

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