Study of Total Antioxidant Capacity in Patients with Diabetic Peripheral Neuropathy

Zahid M. Mohyee Al-Deen¹  Ihsan A. Ajeena²

1 College of Medicine, Babylon University
Email: zahid2ali@yahoo.com
2 College of Medicine, Kufa University
Email: ihsan.ajeena@uokufa.edu.iq

Received 8 September 2014  Accepted 21 September 2014

Abstract
Diabetic peripheral neuropathy (DPN) is the most common microvascular complication of diabetes mellitus (DM). The study included 138 type 2 diabetic patients with DPN with age group 45-65 years matched to 50 diabetic without neuropathy and 50 normal healthy control. The study found that there is lower level of total antioxidant capacity (TAC) in patients with DPN compared to those without and healthy controls. Also the study found that TAC level is correlated with duration of DM and not correlate with glycosylated Hb level. Hyperglycaemia and accompanying consequences result in depletion of antioxidant defense which expose the body to the injurious effects of reactive oxygen species.

Key words: Diabetic peripheral neuropathy, total antioxidant capacity, nerve conduction study.

Introduction
Diabetic neuropathy is the most common type of neuropathies worldwide (1) and it is the most common microvascular complication of diabetic mellitus (DM). Diabetic neuropathy associated with an increased incidence of foot ulcers, Charcot’s neuroarthropathy and account for more than 50% of all limb amputations (2).

There is little agreement about the likely pathogenesis of diabetic neuropathy, however, there is a growing consensus that it is multi-factorial and that various pathogenic factors are interrelated and together contribute to its development and progression. The actual process of neuropathic progression is dynamic, with nerve degeneration and regeneration occurring spontaneously and simultaneously. The net balance between these two processes determines whether the neuropathy progresses, regresses, or stabilizes (3).
Enhanced oxidative stress resulting from imbalance between production and neutralization of reactive oxygen species (ROS) is a well recognized mechanism in the pathogenesis of diabetic neuropathy and other diabetic complications i.e., endothelial dysfunction, cataract, retinopathy, and nephropathy (4). Hyperglycemia and associated increase in aldose reductase activity, activations of non-enzymatic glycation/ glycoxidation and protein kinase C (the latter, in vasa nervorum only) as well as other mechanisms lead to oxidative stress in diabetic peripheral nerve (5).

This study aims to evaluate the total antioxidant capacity in patients with and without diabetic peripheral neuropathy and in normal healthy control and correlating it with duration of diabetes and glycemic control.

**Materials and Methods**

One hundred thirty eight patients (72 males and 66 females) with type 2 diabetes mellitus (DM) were included as the patient group. These were selected to have a complaint of pain, paresthesia and/or a sense of weakness at their extremities, especially lower limbs. Their age ranged from 40 to 65 years.

Additional 100 adults (42 males and 58 females) were also included as the control group whose their ages were consistent with those of the patient group (40-65 years old). This group was subdivided into two subgroups; half of them were diabetic patients without neuropathy (no signs and symptoms and their nerve conduction study were normal) and the rest were normal healthy adults.

All the patients and control undergo measurement of fasting blood, glycosylated hemoglobin, total antioxidant capacity and electrophysiological testing.

**Fasting blood sugar**

Glucose is oxidized by glucose-oxidase to gluconate and hydrogen peroxide according to the following equation.

\[
\text{Glucose} + \text{O}_2 + \text{H}_2\text{O} \xrightarrow{\text{GOD}} \text{H}_2\text{O}_2 + \text{Gluconate}.
\]

The absorbance of standard and sample are measured against reagent blank at 546 nm according to the procedure recommended by the company (Human, Germany) (6).

**Glycosylated hemoglobin measurement**

Whole blood preparation was mixed with a weakly binding cation-exchange resin and the non-glycosylated hemoglobin was bound to the resin, leaving HbA1c. The percent of HbA1c was determined by measuring the absorbance values at 415nm of the HbA1c fraction to the total Hb fraction, according to the procedure explained by the company (Stanbio lab., USA)(7).

**Total antioxidant capacity assay**

The assay procedure included:

1. Adding 100 µl Cu2+ working solution to all standard and sample wells.

2. Covering the plate and incubating at room temperature for 1.5 hours.

3. Reading the absorbance at 570 nm using the plate reader.

The conclusion had passed through the following steps:

1. Plot standard curve: Plot absorbance at 570 nm as a function of Trolox concentration.

2. Determine sample antioxidant Trolox equivalent concentrations: Sample antioxidant capacity = Sa/Sv = nmol/µl or mM Trolox equivalent where Sa is the sample amount (in nmol) read from the standard curve, and Sv is the undiluted sample volume added to the wells (8).

**Electrophysiological testing**
The electrophysiological test was done in the neurophysiology department in Merjan Medical City.

**Conventional nerve conduction study (NCS)**

Each participant had at least four motor nerves tested (median, ulnar, tibial and peroneal), and three sensory nerves (median, ulnar and sural nerves). Limb temperatures were measured using adhesive skin patch and were maintained between 33-36°C by exposing the patient to radiant heater when needed, and the skin was prepared when necessary using abrasive skin cleanser. Maximal responses were obtained using electrical stimuli. Multiple parameters were assessed for each nerve including distal latency, conduction velocity and waveform amplitude, duration and shape were measured and recorded for each nerve at each stimulus site (9). For sensory nerves, the supramaximal stimulating current was kept below threshold for motor fibers especially in the mixed nerves, since sensory fibers generally had lower threshold of stimulation than that of motor fibers (10).

For motor nerve conduction studies, the nerve was stimulated at multiple points along its course, by applying stimuli at distal and proximal sites of the nerve and recording from the muscle innervated by that nerve. The stimulus intensity must be high enough to activate all nerve fibers during stimulation (11).

**Statistical analysis**

This case control study involves arithmetic mean and the standard deviation of distribution of each set of the data is calculated for each of the studied variables. Demographic, clinical, biochemical and electrophysiological data were compared between patients and control groups using a t-test. A P-value of 0.05 or less was considered significant (12). Also, correlation coefficient was used to test the significance of correlation between total antioxidant capacity and some biochemical parameters.

**Results**

**Demographic data and biochemical data**

The study found significant differences between patients with DPN on one hand and patients without DPN and normal healthy control on the other hand regarding age, duration of DM, fasting blood sugar, glycated Hb and total antioxidant capacity. These findings are shown in table 1.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patients with DPN</th>
<th>Patients without DPN</th>
<th>Healthy control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>57.1±1.3</td>
<td>51.9±3.3*</td>
<td>55±4</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>72</td>
<td>22</td>
<td>20</td>
</tr>
<tr>
<td>Female</td>
<td>66</td>
<td>28</td>
<td>30</td>
</tr>
<tr>
<td>Duration of DM (years)</td>
<td>7.3±1</td>
<td>2.2±2*</td>
<td></td>
</tr>
<tr>
<td>FBS (mmol/L)</td>
<td>12.6±1</td>
<td>6.4±2*</td>
<td>5±1†</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>10.1±1.9</td>
<td>5.1±2*</td>
<td>4.5±2†</td>
</tr>
<tr>
<td>TAC (mmol/L)</td>
<td>1.09±0.3</td>
<td>1.4±0.8*</td>
<td>1.52±0.3†</td>
</tr>
</tbody>
</table>

DPN= diabetic peripheral neuropathy, DM= Diabetes mellitus  
FBS= fasting blood sugar, HbA1c= glycated hemoglobin, TAC= total antioxidant capacity.  
Values are expressed in mean ± standard deviation  
* Significant differences between patient with DPN and those without DPN at P<0.05  
† Significant differences between patient with DPN and control subjects at P<0.001
Correlation with duration of diabetes mellitus
There was a perfect indirect correlation between TAC and duration of DM (R=0.74) as shown in figure 1. Also there was a moderately direct correlation between AGE and duration of diabetes (R=0.6) as shown in figure 2.

**Figure 1:** Correlation between total antioxidant status and duration of diabetes in patients with DPN.

**Figure 2:** Correlations between total antioxidant capacity and HbA1c in patients with DPN.

**Electrophysiological assessment**
The results of nerve conduction study (NCS) of motor and sensory nerves of upper and lower extremities are shown below.

**Sensory nerve conduction study**
The results of compound sensory action potential (CSAP) amplitude (Amp) and conduction velocity (CV) in the three study groups along with their significance value are shown in Table (2).
**Table 2:** The comparison of sensory nerve conduction parameters of median, ulnar and sural nerves between patients and control subjects.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Amp (µV)</th>
<th>CV (m/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>median</td>
<td>Ulnar</td>
</tr>
<tr>
<td>patients with DPN</td>
<td>7±1</td>
<td>13±3</td>
</tr>
<tr>
<td>patients without DPN</td>
<td>29±13 *</td>
<td>41±18 *</td>
</tr>
<tr>
<td>Control subject</td>
<td>69±11 †</td>
<td>66±10 †</td>
</tr>
</tbody>
</table>

DPN= diabetic peripheral neuropathy, Amp = Amplitude, CV= conduction velocity,
Values are expressed in mean ± standard deviation
* Significant differences between patient with DPN and those without DPN at P<0.05
† Significant differences between patient with DPN and control subjects at P<0.001

**Motor nerve conduction study**

The comparisons of the compound muscle action potential (CMAP) parameters of the median, ulnar, posterior tibial and common peroneal nerves in the three study groups were shown in Tables (3and 4). All of the distal latency (DML), amplitude (Amp) and conduction velocity (CV) were considered.

**Table 3:** The comparison of the motor nerve conduction parameters of upper limbs between patients and control subjects.

<table>
<thead>
<tr>
<th>Variable</th>
<th>DML (ms)</th>
<th>Amp (mV)</th>
<th>CV (m/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median</td>
<td>Ulnar</td>
<td>Median</td>
</tr>
<tr>
<td>Patients with DPN</td>
<td>4.4±0.3</td>
<td>3.3±0.2</td>
<td>3±1</td>
</tr>
<tr>
<td>Patients without DPN</td>
<td>2.8±1 *</td>
<td>2.6±0.6 *</td>
<td>6±2 *</td>
</tr>
<tr>
<td>Control subject</td>
<td>2.3±3 †</td>
<td>2±0.1 †</td>
<td>12±4 †</td>
</tr>
</tbody>
</table>

DPN= diabetic peripheral neuropathy, DML= Distal motor latency, Amp= Amplitude, CV= conduction velocity, Values are expressed in mean ± standard error
* Significant differences between patient with DPN and those without DPN at P<0.05
† Significant differences between patient with DPN and control subjects at P<0.001

**Table 4:** Comparison of the motor nerve conduction parameters of lower limbs between patients and control subjects.

<table>
<thead>
<tr>
<th>Variable</th>
<th>DML (ms)</th>
<th>Amp (mV)</th>
<th>CV (m/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Peroneal</td>
<td>Tibial</td>
<td>Peroneal</td>
</tr>
<tr>
<td>Patients with DPN</td>
<td>7.3±0.5</td>
<td>6.5±3</td>
<td>1±1</td>
</tr>
<tr>
<td>Patients without DPN</td>
<td>4.7±1 *</td>
<td>4.3±2 *</td>
<td>3±2 *</td>
</tr>
<tr>
<td>Control subject</td>
<td>3.2±1 †</td>
<td>3.4±1 †</td>
<td>6±70 †</td>
</tr>
</tbody>
</table>

DPN= diabetic peripheral neuropathy, DML= Distal motor latency, Amp= Amplitude, CV= conduction velocity, Values are expressed in mean ± standard error
* Significant differences between patient with DPN and those without DPN at P<0.05
† Significant differences between patient with DPN and control subjects at P<0.001
Discussion
The results of this study showed a significant effect of age on the function of peripheral nerves and on disease duration within the patient group (Table 1). Although it is not so clear why advancing age has such impact, these findings could be explained by the association of progressing patients' age with longer duration of the DM that usually resulted in accumulation of deleterious effects on their peripheral nerves' function (13). Also, with increasing age the possibility of macro-vascular disease (even if subclinical) increases which might further augment microvascular complications (14). Furthermore, older age is usually associated with a deficiency of some neuroprotecting hormones and neuroactive steroids (sex steroids) which play important role in maintenance and regeneration of nervous system. This deficiency will be aggravated during DM (15).

These were in agreement with the findings of other studies that showed that older diabetics is at higher risk for developing peripheral neuropathy (16; 17 and 18). On contrary, other studies denied any effect of age on the development of DPN (19 and 20). This discrepancy in assessing the effect of advancing age in diabetic patients on peripheral nerves can be accounted for patients sampling and the choice of diagnostic test used for DPN (21).

On another hand, the duration of DM by itself is related to the prevalence of the DPN (Table 1). This could be elucidated by the hypothesis that longer duration of DM is associated with poorer control of blood glucose level and the cumulative effect of the consequent injurious factors of poor metabolic control along with the atherosclerosis usually lead to and accelerates neuropathy (17 and 22). A finding that was proved by many other studies (23 and 24).

Fasting blood sugar (FBS) and glycated hemoglobin (HbA1c) (Table 1)

Chronic hyperglycemia represents the main causative factor involved in the pathogenesis of diabetic neuropathy. It has been assumed that nerve damage may be directly induced by accumulation of intracellular glucose with its consequences like the generation of glycating sugars and advanced glycation end-products (AGE), enhanced oxidative damage and protein kinase C activation and others (17 and 24).

These finding agrees with that of other studies (24; 25; 26 and 27) who came up with same findings although it disagreed with others (28 and 29).

The inconsistency of these studies' results indicates that the pathogenesis of DPN in type 2 DM is much more complex and not simply related to the hyperglycemia as in type 1 (13 and 18). That is why the UK Prospective Diabetes Study (UKPDS) Group (1998) (30) could not demonstrate a lower progression of neuropathy in intensively treated type 2 diabetic subjects. Additionally, Damci and his co-workers (1999) (31) examined the changes in vibration perception threshold before and after modification of blood glucose levels within the same patients and found no significant difference in vibration perception threshold with instantaneous change in blood glucose levels.

On contrary, the Diabetes Control and Complications Trial (DCCT) in 1995 (32) have established that lowering of glycated hemoglobin (HbA1c) in patients with type 1 diabetes was associated with a reduction in subsequent development of clinical neuropathy.

Total antioxidant capacity (TAC) (Table 1)

The decreased antioxidants seen in patients with DPN could be due to the increase in their consumption during the process of combating excessive free radicals generated in DM. As a result there will be depletion of antioxidant reserves like vitamins C, E, trace elements and others. Furthermore, factors
like low intake of antioxidant rich foods (particularly fruits and vegetables), poor health status, cigarette smoking, and low physical activity could be associated with low plasma antioxidant levels (33). Such findings were consistent with other studies (34; 35 and 36).

Rahbani-Nobar and his co-workers (1999) (37) examined TAC in type 2 DM and compared it to HbA1c, FBS, duration of DM and age. They found that the level of TAC is lower in diabetic patients compared to normal controls and that its level is lower in patients with poorer glycemic control. They also found that TAC in diabetic patients correlated with glycemic control reflected by both FBS and HbA1c, duration of DM and age.

Depending on these findings and assumptions, many attempts was done to treat DPN using antioxidants; like Resveratrol, α-Lipoic acid, Vitamin E, Herbal medicines and others, and many of them succeeded in reduction various symptoms of severe peripheral neuropathy in patients with type 2 diabetes. However, no objective measures of neuropathy, such as nerve conduction studies were recorded in these patients (44).

Seghrouchni and his co-workers in 2002 (38) studied certain oxidant/antioxidant parameters including TAC in type 1 and 2 DM. They assumed that oxidative stress parameters are enhanced in diabetic subjects with significant differences between the two main types of DM (oxidative stress parameters are increased in type 2 compared to type 1). Also, long time insulin treatment improves some of these parameters in type 2 DM and not in type 1 suggesting entirely different pathologies concerning oxidative processes for each type of DM (39).

**Advanced glycation end products correlations with different parameters**

The results of this study showed perfect indirect correlation between TAC levels with duration of diabetes (Figures 1 and 2). These finding can be illustrated by the association of the longer duration of DM with cumulative damaging effect of chronic hyperglycemia and the low TAC were parts of its reflections (39).

On contrary, a no correlation between TAC was seen with HbA1c measurement as shown in figures 4.4 and 5. These can be explained by the presence of patients with fair glycemic control although they had a long history of bad control that resulted in their complications. Furthermore, this finding may reflect that the pathogenesis of type 2 DM is complex and in addition to hyperglycemia, oxidative/nitrosative stress, neurovascular injury, deficiency of neuroprotective factors, accumulation of AGE and more others have important role in the pathogenesis of DPN (3).

**References**


(22) Abougalambou SSI and Abougalambou AS. (2012): Explorative study on diabetes neuropathy among type
II diabetic patients in Universiti Sains Malaysia Hospital. Diabetes & Metabolic Syndrome. Clinical Research and Reviews; 6: 167–72


