

What is the role of oxidative stress in the development of diabetic peripheral neuropathy?

Tekin Yıldırım, Ramazan Önalın

Department of Internal Medical Sciences, Faculty of Medicine, Yozgat Bozok University, Yozgat, Turkey

ORCID ID of the author(s)

TY: 0000-0002-8442-4788
RÖ: 0000-0002-6948-8548

Abstract

Background/Aim: Diabetic peripheral neuropathy (DPN) is a clinical entity affecting approximately half of the patients with diabetes, impairing the quality of life. Patients usually do not receive full welfare despite medical treatment. This study aimed to evaluate the relationship between DPN and oxidative stress.

Methods: A single-center, clinical study was conducted on 80 patients with type 2 diabetes with and without peripheral neuropathy. Glucose, glycosylated hemoglobin (HbA1c), total oxidant capacity (status) (TOS), and total antioxidant capacity (status) (TAS) levels were assessed from blood samples collected from these individuals. Oxidative stress index (OSI) was calculated by the division of TOS to TAS.

Results: TAS levels were within the normal range while TOS and OSI levels were higher compared to individuals without diabetes in both groups. There was no significant difference between the groups in terms of TOS ($P=0.26$), TAS ($P=0.85$), and OSI ($P=0.32$) levels.

Conclusion: In our study, no relationship was found between DPN and oxidative stress. Further studies involving a larger number of diabetic patients with and without DPN are required to clarify the role of oxidative stress in the development of DPN.

Keywords: Diabetic peripheral neuropathy, Total oxidant status, Total antioxidant status, Oxidative stress, Oxidative stress index

Corresponding Author

Tekin Yıldırım

Department of Internal Medical Sciences, Faculty of Medicine, Yozgat Bozok University, Yozgat, Turkey
E-mail: tekindoktor@gmail.com

Ethics Committee Approval

The study was approved by Bozok University Clinical Research Ethics Committee and written consent was obtained from the patients (Research protocol no: 2020-01-07, decision no: 2017-KAEK-189_2020.01.08_16, date: 08.01.2020). All procedures in this study involving human participants were performed in accordance with the 1964 Helsinki Declaration and its later amendments.

Conflict of Interest

No conflict of interest was declared by the authors.

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Introduction

Diabetes is a worldwide metabolic disease with high morbidity and mortality rates due to its macrovascular and microvascular complications. The complex sequence of events leading to cellular failures in response to high glucose levels is not fully explained. Structural and functional disorders of the peripheral nervous system in diabetic patients are described as diabetic peripheral neuropathy (DPN), provided that other etiological factors are excluded. Many factors may contribute to the pathogenesis of DPN such as genetic predisposition, endoneurial hypoxia or ischemia, increased oxidative stress, increased glycosylation end-products, lack of growth factors, or immune mechanisms. The long-term high blood glucose level is the initial inducing factor [1].

Oxidative stress has an important role in the pathogenesis and late complications of diabetes. As a result of having one or more unshared electrons, free radicals are very reactive, and they tend to grab electrons from other atoms or molecules to fill their outer energy levels [2]. In diabetes mellitus, oxidative stress can occur because of the increased production of reactive oxygen species (ROS) including superoxide (O_2^-) radicals, hydroxide radicals (HO^-), and hydrogen peroxide (H_2O_2), and/or inadequacy of antioxidant defense systems [3]. An increase in ROS production is related to protein glycosylation and/or autoxidation of glucose under hyperglycemic conditions. The reason for the inadequacy of neutralization of free radicals is related to the inadequacy of enzymatic and nonenzymatic radical scavengers (antioxidants) [4]. In the literature, the relationship between DPN and oxidative stress in a real-life setting has not been explained clearly. The aim of this study was to evaluate the relationship between DPN and oxidative stress among the patient with type II diabetes.

Materials and methods

Study population

This single-center, prospective clinical study including type 2 diabetes and DPN patients aged between 31-81 years was conducted between January-October 2020. The definition of type II Diabetes Mellitus was made as follows: **a.** At least 8-hour fasting plasma glucose (FPG) ≥ 126 mg/dl **b.** Randomized plasma glucose ≥ 200 mg/dl with diabetes symptoms **c.** In Oral Glucose Tolerance Test (OGTT), 2nd-hour plasma glucose ≥ 200 mg/dl, HbA1c $\geq 6.5\%$ [5]. Exclusion criteria were as follows: The diagnosis of non-diabetic neuropathy, any drug use for neuropathy within the last 6 months, vasculitis, renal failure (glomerular filtration rate less than 90 ml/min), liver failure (liver transaminase levels more than two times of the upper limit), vitamin B12 deficiency, vitamin D deficiency, thyroid pathology, chronic/acute infection findings, cancer diagnosis and/or treatment. A hundred patients with DPN were consecutively assessed in terms of the inclusion and exclusion criteria. Sample size analysis performed at a 5% Margin of Error, 80% Power and a Standard Effect Size of 0.81 revealed that 24 patients were needed in each group. A total of 20 patients were excluded from the study due to the exclusion criteria. Finally, 80 diabetic patients fulfilling the criteria who agreed to participate in the study were included in the analyses. The patients were divided into two groups as the patients with and without DPN. After a detailed

physical examination of all patients, fasting venous blood samples were obtained and routine hematological and biochemical tests were performed accordingly. Body mass index (BMI) was calculated as weight in kilogram divided by the square of height in meter.

Assessment of peripheral neuropathy

Peripheral neuropathy was diagnosed according to EMG findings (nerve conduction velocity, amplitude, and distal latency) and Douleur Neuropathique 4 (DN4) questionnaire. Sensory and motor nerve conduction studies were performed on the median, ulnar, peroneal, and tibial motor nerves, and median, ulnar, and sural sensory nerves with an electromyography (EMG) device (Medelec Synergy; Oxford Instruments; Surrey, UK) by the same neurologist. DN4 survey includes 7 items related to symptoms and 3 items related to neuropathic pain. A total score of 4 or more indicates neuropathic pain. In a developmental study, DN4 showed 83% sensitivity and 90 % specificity in the diagnosis of neuropathic pain [6]. The Turkish version of DN4, used in our study, was validated for the Turkish population [7].

Biochemical analysis

Venous blood samples were collected from the antecubital vein of each patient after a 12-hour overnight fast, and 10 ml samples of venous blood were taken into a biochemistry tube. Their sera were separated with centrifugation at 3000 rpm for 10 min. All material was stored at $-80^\circ C$ until analysis.

Serum TOS and TAS were determined with Rel Assay Diagnostics kit (Mega Tıp, Gaziantep, Turkey, developed by Erel) and Oxidative Stress Index (OSI) values were calculated. Total oxidant status (TOS) was measured as described by the manufacturer's protocol. In this method, the oxidants present in the sample oxidize the ferrous ion-o-dianisidine complex to ferric ion. Ferric ion produces a colored complex with xylenol orange in an acidic medium. The color intensity, which can be measured spectrophotometrically, is related to the total amount of oxidant molecules present in the sample. The assay was calibrated with hydrogen peroxide and the results were expressed in terms of $\mu mol H_2O_2$ equivalent/L of serum.

Total antioxidant status (TAS) was measured in the sera by the generation of 2,2'-azino-di-(3-ethylbenzthiazoline sulphonate) (ATBS) radical cation using the commercial kit according to the manufacturer's manual.

The ratio of TOS to TAS was used as the oxidative stress index (OSI) and calculated as follows: OSI (arbitrary units) = $[(TOS, \mu mol H_2O_2/L) / (TAS, mmol Trolox equiv./L)]$.

Statistical analysis

All analyses were performed using SPSS version 23.0 (IBM Co., NY, USA). The suitability of the data to normal distribution was evaluated by Kolmogorov-Smirnov and Shapiro-Wilk tests. Independent samples t-test and Mann-Whitney U test were used to compare the differences between continuous variables, and chi-square (χ^2) test was used to assess the differences between categorical variables. The relationships between the variables were evaluated by the Pearson Correlation test. One-Sample t-test was used to compare variables with reference values. Descriptive statistics included mean (SD), median 25th-75th percentiles, and percentage. *P*-value <0.05 was considered statistically significant in all tests.

Results

Of 80 patients with type 2 diabetes, 58 were female. The group with DPN had significantly more female patients, higher BMI, bigger waist circumference, and longer duration of diabetes; however, fasting glucose level, creatinine and HbA1c levels of both groups were statistically similar (Table 1).

Table 1: Demographic and laboratory data of diabetic patients with and without diabetic peripheral neuropathy

Variables	Without Peripheral Neuropathy (n=40)	With Peripheral Neuropathy (n=40)	P-value
Age (years)	60.03 (11.78) (31-81)	58.90 (9.31) (41-77)	0.64
Gender (female/ male)	24 (66%) / 16 (40%)	34 (85%) / 6 (15%)	0.012
BMI (kg/m ²)	30.17 (4.35) (22.76-38.96)	33.38 (5.57) (23.51-44.30)	0.005
Waist circumference (cm)	106.35 (7.80) (86-129)	113 (10.15) (97-137)	0.001
Duration of diabetes (years)	8.30 (6.26) (1-30)	11.25 (7.05) (1-35)	0.028
Fasting glucose (mg/ dL)	175.88 (82.33) (85-499)	169.10 (78.98) (84-437)	0.61
Creatinine (mg/ dL)	0.95 (0.31) (0.42-1.89)	0.97 (0.61) (0.39-4.46)	0.36
Glycosylated hemoglobin A1c (%)	7.70 (1.83) (4.8-11.89)	7.55 (1.67) (4.89-12.20)	0.70

Values are expressed as n (%), mean (SD) (minimum-maximum), median (25-75th percentiles), BMI: body mass index

Although TOS levels of both groups were higher than the reference range (5.00 - 8.00 $\mu\text{mol H}_2\text{O}_2$ equivalent/L), there was no statistically significant difference between diabetic patients with and without peripheral neuropathy concerning TOS levels ($P=0.26$). TAS levels of both groups were within reference values (1.4 - 2.0 mmol Trolox equivalent/L). Similarly, no significant difference was found between diabetic patients with and without peripheral neuropathy with respect to TAS levels ($P=0.85$). Although the OSI values in both groups were higher than the reference range (2.50 - 5.72), there was no statistically significant difference between diabetic patients with and without DPN ($P=0.32$). TOS, TAS, and OSI data of diabetic patients with and without peripheral neuropathy are summarized in Table 2.

Table 2: TOS, TAS and OSI measurements of diabetic patients with and without diabetic peripheral neuropathy

Variables	Reference Range	Without Peripheral Neuropathy (n=40)	With Peripheral Neuropathy (n=40)	P-value
TOS ($\mu\text{mol H}_2\text{O}_2$ equivalent / L)	5.00 - 8.00	34.02 (13.43) (10.99-55.57)	37.31 (13.26) (9.28-52.97)	0.26
TAS (mmol Trolox equivalent/L)	1.4 - 2.0	1.78 (0.19) (1.20-2.17)	1.77 (0.19) (1.43-2.26)	0.85
OSI (TOS/TAS)	2.5 - 5.72	19.40 (8.11) (6.03-38.13)	21.20 (7.89) (5.72-35.15)	0.32

Values were expressed as mean (SD) (minimum-maximum). TOS: total oxidant status, TAS: total antioxidant status, OSI: oxidative stress index

In correlation analyses, parameters related to oxidative stress had no significant correlation with fasting glucose, HbA1c, duration of diabetes, BMI, or waist circumference ($P>0.05$ for each).

Discussion

Diabetes is a worldwide metabolic disease with high morbidity and mortality rates due to its macrovascular and microvascular complications. Oxidative stress has a prominent role in the pathogenesis and late complications of diabetes [1]. Structural and functional disorders of the peripheral nervous system in diabetic patients are described as diabetic peripheral neuropathy (DPN), provided that other etiological factors are excluded. Many factors may contribute to the pathogenesis of DPN such as genetic predisposition, endoneurial hypoxia or

ischemia, increased oxidative stress, increased glycosylation end products, lack of growth factors or immune mechanisms.

In a study by Inci et al. [8], the TOS, TAS, and OSI values of healthy subjects were compared with those of diabetic nephropathy patients, and all were significantly greater among the diabetic nephropathy patients compared to controls. However, in this study, diabetic patients with and without diabetic nephropathy were not compared. In another study, serum TOS levels and OSI values of patients with diabetic nephropathy were significantly higher compared to those of healthy subjects. TAS was low in the blood of diabetic nephropathy patients [9]. In our study, we also found that TOS and OSI parameters were significantly higher in patients with diabetes. This data may support the idea that the patients with diabetes have higher oxidative stress, which may play a role in the development of diabetic complications.

In a study by Kasznicki et al. [10], catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPX), and total antioxidant status (TAS) were examined in diabetic patients with and without DPN, and healthy control groups. A significant decrease of SOD, GPX, and nonsignificant decrease of CAT, and TAS status were seen in type II DM patients with neuropathy compared to type II DM patients without neuropathy and the control group. In our study, only serum TAS levels were measured, and similar to this study, no statistically significant difference was found between diabetic patients with and without neuropathy.

Sayin et al. [11] evaluated serum prolidase activity, TAS, malondialdehyde (MDA), and nitric oxide (NO) levels. Serum MDA and NO levels were significantly higher in patients with DPN compared to the control group, while prolidase activity and TAS levels were lower in the patients with DPN. In our study, TAS levels were within the normal range in both groups. In our study, the data of patients with and without diabetic neuropathy were compared, in contrast to Sayin et al.'s study, which cannot provide precise information about the role of oxidative stress in diabetic neuropathy.

In a study which evaluated plasma 8-iso-prostaglandin $F_{2\alpha}$ (8-iso-PGF $_{2\alpha}$), superoxide anion (O_2^-), peroxynitrite (ONOO $^-$), vitamin E to lipid ratio, and vitamin C, only superoxide anion (O_2^-) was significantly higher in diabetic patients with neuropathy compared to those without. Vitamin E to lipid ratio and vitamin C, which are regarded as antioxidants, were significantly lower in those with neuropathy [12].

In the study conducted by Uzar et al. [1], TOS and OSI levels were higher, but TAS levels were lower in diabetic patients with and without DPN compared to healthy subjects. However, they could not find a significant difference between diabetic patients with and without DPN with respect to these parameters. They stated that this may support the role of oxidative stress in the pathogenesis of diabetes mellitus. In our study, TOS and OSI levels in patients with and without DPN were also significantly higher than the reference range, while TAS level was not.

Limitations

One potential limitation is the cross-sectional study design. In addition, the number of diabetic patients with or without DPN was limited. However, this is a preliminary study giving us an idea about oxidative stress in DPN based on TOS, TAS, and OSI.

Conclusion

These data support the increase of oxidative stress in diabetic patients. There was no significant difference between these parameters among diabetic patients with and without DPN in our study, which may yield controversial results in terms of the role of oxidative stress in DPN. However, our study included a small number of diabetic patients. Further studies involving a larger number of diabetic patients with and without DPN are required to clarify the role of oxidative stress in the development of DPN.

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