



## The Risk Factors Associated with the Diabetic Neuropathy Patients from Sensory Functioning and Neurological Symptoms

Li Song<sup>1\*</sup>, Hong Shi<sup>2</sup>, Li Han<sup>1</sup> and Jirui He<sup>2</sup>

<sup>1</sup>Research Center of the Emergency, The Second Hospital of Lanzhou University, P.R. China

<sup>2</sup>Department of Geriatrics, The Second Hospital of Lanzhou University, P.R. China

### Abstract

**Objective:** This clinical study aims to evaluate the risk factors association between the Diabetic Peripheral Neuropathy (DPN) from neuropathic symptoms and nervous sensory signs in patients with DPN.

**Methods:** A total of 81 individuals with DPN participated in this clinical investigation. According to patients' symptoms, the Neuropathy Symptom Scores (NSS) and their Neuropathy Deficit Scores (NDS) category, four symptom clusters were allocated: No symptoms, mild symptoms, moderate symptoms, and severe symptoms. The four neurological function categories were defined as no signs, mild signs, moderate signs and severe signs. We used an enzyme-linked immunosorbent assay kit to measure the levels of urine 8-Hydroxy-2-Deoxyguanosine (8-OHdG). All statistical tests were performed using the Statistical Package for the Social Sciences, version 25.0 (IBM-SPSS, Chicago, IL, USA).

**Results:** Based on the results of the ordinary logistic regression, it can be concluded that neuropathic sensory function deficits were not related to neuropathic symptoms in patients with DPN; age was associated with neuropathic function deficits in DPN patients; and the level of urine 8-OHdG has no correlation with neuropathic symptoms in patients with DPN.

**Conclusion:** In this clinical study, it can be deduced that the age was related to the neuropathic function deficits in DPN patients and the level of urinary 8-OHdG has no association with the development of neuropathic symptoms in patients with DPN.

**Keywords:** Diabetic peripheral neuropathy; Neuropathic symptoms; Neuropathic sensory function; Clinical Study

### Introduction

Diabetic Peripheral Neuropathy (DPN) is one of the most common and serious long-term complications of Diabetes Mellitus (DM) and is estimated to occur in more than half of type 1 and type 2 diabetes mellitus patients [1,2]. As of 2010, there were approximately 132 million people with DN worldwide [3]. In 2030, 366 million (4.4% of the world's population) people suffered from DM [4]. DPN is characterized by the progressive loss of peripheral nerve axons, which may be associated with neuropathic pain, foot ulceration and gangrene, and in severe cases, lower extremity amputation [5]. Nontraumatic lower extremity amputations in DM typically lead to an impaired quality of life and add to the financial burden of healthcare provision for patients with this condition [6].

DPN can be classified according to a combination of pathological, phenomenological, neurophysiological and etiological parameters [7]. Symptoms of DPN can be divided into sensory and motor manifestations. The most common are an abnormal or loss of sensation in the limb, e.g., tingling [8,9]; pain and burning sensations [10,11]; numbness, weakness of the limbs; and hyperalgesia and allodynia in the toes and feet [12]. Changes in sensation and nervous system reflexes, which initially arise in the toes and then extend superiorly, are recognized as the classical "glove and stocking" distribution of numbness, sensory loss, dysesthesia and neuropathic pain at night. Diabetic neuropathic pain can feel like a burning, pricking, achy or dull sensation. Loss of proprioception occurs in the early stages of diabetic neuropathy. Consequently, patients have a high

### OPEN ACCESS

#### \*Correspondence:

Li Song, Research Center of the Emergency, The Second Hospital of Lanzhou University, Cuiyinmen 80, Chengguan District, Lanzhou, Gansu, P.R. China, Tel: 0086-09318942430;

Received Date: 28 May 2024

Accepted Date: 20 Jun 2024

Published Date: 25 Jun 2024

#### Citation:

Li Song, Hong Shi, Li Han, Jirui He. The Risk Factors Associated with the Diabetic Neuropathy Patients from Sensory Functioning and Neurological Symptoms. *Clin Case Rep Int.* 2024; 8: 1694.

**Copyright** © 2024 Li Song. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

risk of developing ulcers and infections on the feet and legs, which can lead to gangrene and even amputation of the lower extremity. Neuropathic pain is prevalent in DPN patients and importantly, causes a detrimental effect on the DPN patient's physical and mental health [13], ultimately leading to a poor quality of life. Amputations in DPN not only have a devastating influence on quality of life but also lead to an increase in mortality [14].

Nerve Conduction Studies (NCS) and questionnaires, such as Neuropathy Symptom Scores (NSS) and Neuropathy Deficit Scores (NDS), are among the most common procedures used for diagnosing and assessing DPN in the clinical literature [12]. Most clinical studies rely on the presence of patient-reported symptoms and physical signs for the diagnosis of DPN. DPN is most likely if the patient experiences neuropathic pain in the legs or feet as described above, so it is important for a physician to estimate the patient's degree of discomfort when performing a clinical assessment. NSS and NDS are typically utilized as diagnostic tools for determining the presence of DPN in DM patients, depending on their symptoms and physical signs. This is important for the prevention of foot ulceration and lower extremity amputation in this patient cohort.

The disease processes underlying the onset and progression of DPN are still not clearly defined [9,15,16]. Although there now exists a long history of research that demonstrates the pathophysiology of DPN, it has recently become widely accepted that the onset and development of DPN is not only a result of hyperglycemia but also associated with several other vascular, metabolic and genetic factors [17]. Among these, hyperglycemia-induced oxidative stress is considered to be a vital driver of diabetic complications and organ dysfunction in patients with DPN [18]. 8-Hydroxy-2-Deoxyguanosine (8-OHdG) as a biomarker for DNA oxidative damage is one of the most widely studied oxidized metabolites [19,20]. The interaction of the hydroxyl radical, the most important oxygen-free radical, with the nucleobases of the DNA strand, such as guanine, leads to the formation of 8-OHdG [21]. It is stable for measuring urinary 8-OHdG [22] and its excretion is likely to reflect the oxidative DNA damage [23].

Thus, a clinical study was performed to assess the association between the risk factors and DPN symptoms and sensory nerve signs in patients with DPN. It would therefore be more effective for approaches to DPN to focus on decelerating DPN development through better glycemic control and pain management, according to neuropathy pain treatment recommendations [24,25]. This would assist DPN patients who are receiving appropriate treatment and care in accordance with recommendations from the clinical literature. It additionally provides special treatment for the early stages of DM to prevent the occurrence of diabetic complications and organ dysfunction.

## Methods

A cross-sectional clinical study was undertaken to evaluate the risk factors associated with sensory functioning and neurological symptoms in patients with DPN. Eighty-one individuals with DPN, with an age range of 18 to 75 years, were recruited to participate in the study from the Geriatrics Department of Lanzhou University Second Hospital.

Inclusion criteria for the study were as follows: (1) All patients were diagnosed according to the guidelines of the American Diabetes Association (ADA) in 2011 (American Diabetes Association. 2011) [26]; (2) patients were asked to provide details of symptoms and

signs at the time of diagnosis; (3) laboratory results of all patients were evaluated; and (4) details of participants' family histories were obtained to exclude maturity-onset diabetes of the young.

Exclusion criteria included liver or kidney failure, malignancy, chronic alcohol abuse, spinal canal stenosis or prolapsed discs, systemic or neurological diseases or medication that could potentially induce polyneuropathy, and any other causes of polyneuropathy. Study procedures were performed in accordance with the guidelines of the Declaration of Helsinki, 2013. Each patient agreed to participate and provided written informed consent. The study procedures were approved by the ethics committee of Lanzhou University Second Hospital (number 2022A-571).

The NSS and NDS were based on the symptoms of patients with DPN, which included pain, numbness, burning, tingling, cramps and weakness. The peripheral nervous system of each patient was assessed using the NSS and NDS; these are frequently utilized techniques for the diagnosis and appraisal of DPN in clinical practice [27,28]. The NSS category was defined in accordance with symptom occurrence: No symptoms, 0 to 2 points; mild symptoms, 3 to 4 points; moderate symptoms, 5 to 6 points, and severe symptoms, 7 to 10 points. Symptom quality and pathological findings were categorized to provide the NDS, *i.e.* no deficit, 0 to 2 points; mild deficit, 3 to 5 points; moderate deficit, 6 to 8 points, and severe deficit, 9 to 10 points. The details of the NSS and NDS categories are shown in Figure 1 [29]. According to patients' symptoms, NSS and their NDS category, four symptom clusters were allocated: No symptoms, mild symptoms, moderate symptoms, and severe symptoms. The four neurological nerve function categories were defined as no signs, mild signs, moderate signs and severe signs, a classification based on the neuropathic signs category.

The medical histories of the study participants were requested and reviewed, after which experienced physicians performed routine physical examinations. The medical history obtained included basic information (age, gender, weight, height), duration of diabetes, diabetic mellitus in the family, previous medical history (hypertension, cardiovascular diseases, malignancy and spinal diseases), smoking and alcohol consumption behaviors, diabetic complications (diabetic retinopathy, nephropathy), drug history (anti-hypertensive agents and statins) and current hypoglycemic treatments (lifestyle and diet interventions, insulin injections, insulin secretagogues, metformin or thiazolidinediones). Somatometric parameters included weight, size, body mass index, waist-hip ratio, Systolic Blood Pressure (SBP) and Diastolic Blood Pressure (DBP), among others.

Blood samples were obtained from each participant in the morning and immediately sent to the Central Laboratory of the Lanzhou University Second Hospital to determine the participants' biochemical indicators. Serum glucose (oxidase method), triglycerides (colorimetry), total cholesterol (cholesterol oxidase technique), Low-Density Lipoprotein (LDL) cholesterol (selective melt method), High-Density Lipoprotein (HDL) cholesterol (chemistry modify enzyme method) and uric acid (uricase-peroxidase assay) were analyzed. The glycosylated Hemoglobin (HbA1c) level was calculated using ionic exchange HPLC in the D-10 hemoglobin analysis system (Bio-Rad). Urinary albumin (mg) was measured using immunoturbidimetry (Image 800, Beckman Coulter). Creatinine (g) titers and the Urinary Albumin: Creatinine Ratio (UACR) were also evaluated. We used an enzyme-linked immunosorbent assay kit (Human 8-hydroxy-2-deoxyguanosine; Cat. # m1057433V, Shanghai Enzyme Union, China)

<b>Symptoms on feet/calves</b>	<b>yes</b>	<b>no</b>		<b>Ankle reflexes</b>	<b>Side</b>	<b>right</b>	<b>left</b>
Burning	<input type="checkbox"/> 2	<input type="checkbox"/> 0		Reflexes:	Normal	<input type="checkbox"/> 0	<input type="checkbox"/> 0
Numbness	<input type="checkbox"/> 2	<input type="checkbox"/> 0			Present with reinforcement	<input type="checkbox"/> 1	<input type="checkbox"/> 1
Paresthasias	<input type="checkbox"/> 2	<input type="checkbox"/> 0	<input type="checkbox"/> Points		Absent	<input type="checkbox"/> 2	<input type="checkbox"/> 2
Fatigue	<input type="checkbox"/> 1	<input type="checkbox"/> 0		<b>Vibration perception threshold (tuning fork)</b>			
Cramps	<input type="checkbox"/> 1	<input type="checkbox"/> 0		Measurement distal on great toe base joint**	<b>right</b>	<b>left</b>	
Pain	<input type="checkbox"/> 1	<input type="checkbox"/> 0	<input type="checkbox"/> Points	Normal	<input type="checkbox"/> 0	<input type="checkbox"/> 0	
<b>Localisation</b>				Reduced or absent	<input type="checkbox"/> 1	<input type="checkbox"/> 1	
Feet	<input type="checkbox"/> 2			<b>Pain sensitivity (pin-prick)</b>			
Calves	<input type="checkbox"/> 1			Measurement on the dorsum of the foot	Normal	<input type="checkbox"/> 0	<input type="checkbox"/> 0
Other	<input type="checkbox"/> 0		<input type="checkbox"/> Points	Reduced or absent	<input type="checkbox"/> 1	<input type="checkbox"/> 1	
<b>Time of appearance</b>				<b>Temperature sensitivity</b>			
Worsening during night	<input type="checkbox"/> 2			Measurement on the dorsum of the foot	Normal	<input type="checkbox"/> 0	<input type="checkbox"/> 0
Day and night	<input type="checkbox"/> 1			Reduced or absent	<input type="checkbox"/> 1	<input type="checkbox"/> 1	
Day only	<input type="checkbox"/> 0						
		add		<b>Total score</b> <input type="checkbox"/> <b>Points</b>			
Woken up from sleep	<input type="checkbox"/> 1		<input type="checkbox"/> Points	<b>NDS:</b>			
<b>Improved by</b>				3-5 = mild deficits			
Walking	<input type="checkbox"/> 2			6-8 = moderate deficits			
Standing	<input type="checkbox"/> 1			9-10 = severe deficits			
Sitting or lying down	<input type="checkbox"/> 0		<input type="checkbox"/> Points	** Age-dependent normal ranges see Table 1			
<b>Total score</b> <input type="checkbox"/> <b>Points</b>							
<b>NSS:</b>							
3-4 = mild symptoms							
5-6 = moderate symptoms							
7-10 = severe symptoms							
* In each section, the maximum number of points can only be assigned once (five times in total)							

**Figure 1:** NSS and NDS.

**Abbreviations:** NSS: Neuropathy Symptom Score; NDS: Neuropathy Deficit Score

according to the manufacturer's instructions. The collected urine samples were stored at  $-80^{\circ}\text{C}$  until just before measurement. First, the urine sample, Enzyme Immunoassay (EIA) buffer, standards, and 8-OHdG-AChE tracer were added to all the wells except for the blank. Then, we added  $100\ \mu\text{L}$  of 8-OHdG antibody labeled with HRP and incubated the plate for 60 min at  $37^{\circ}\text{C}$ . The plate was washed with  $350\ \mu\text{L}$  washing fluid for 1 min five times, and  $50\ \mu\text{L}$  termination fluid was added to each well. The optical density was read at 450 nm, and the levels of 8-OHdG were reported in nanograms per milliliter.

Continuous parameters were summarized as the mean  $\pm$  Standard Error (SE). Categorical parameters were expressed as absolute and relative frequencies. Variance that was equal or uniform was tested by the homogeneity test of variance. The p-value represents the comparison of patients with risk factors in DPN patients. Continuous parameters were analyzed using one-way Analysis of Variance (ANOVA) for normally distributed values and the Kruskal-Wallis test for non-normally distributed values. The Pearson Chi-square test was used when 0 cells had an expected count of less than 5. Where this criterion was not fulfilled, Fisher's exact test for categorical variables was utilized. Ordinal logistic regression models were deployed to quantify the association between the risk factors and DPN sensory symptoms and signs in study participants. Multivariate logistic regression was used if the p-value of the parallel line test was  $<0.05$  following ordinal logistic regression. All statistical tests were performed using the Statistical Package for the Social Sciences, version 25.0 (IBM-SPSS, Chicago, IL, USA). A p-value of  $<0.05$  was assumed to be significant.

## Results

### Baseline clinical characteristics of patients with type 2 diabetes

Eighty-one patients with DPN were recruited to take part in the study. Table 1 compares the participants' clinical characteristics according to the neuropathic symptoms and NSS and the NDS category in patients with DPN. The 11 individuals with no symptoms among the DPN patients were aged  $60.00 \pm 3.95$  years; the 38 subjects with mild symptoms among the DPN patients were aged  $58.39 \pm 1.68$  years; the moderate symptoms among the DPN patients were 25 individuals who were aged  $60.44 \pm 2.18$  years; and there were 10 subjects with severe symptoms among the DPN patients who were aged  $60.00 \pm 2.87$  years. Regarding the most important DNA damage indicators, there were no significant differences in 8-OHdG in the DPN patients according to the sensory symptom classification (Table 1 and Figure 2).

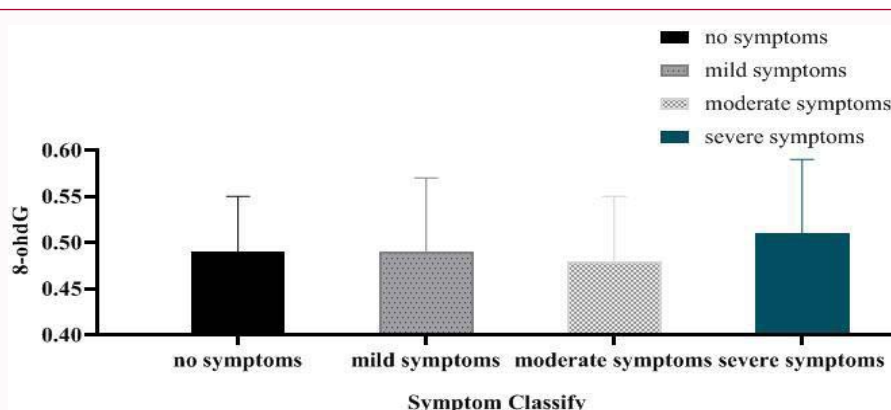
The four neurological nerve function categories were defined as no signs, mild signs, moderate signs and severe signs, a classification based on the neuropathic signs category. There were 2 subjects with no signs, aged  $35.50 \pm 4.50$  years; 13 individuals with mild signs aged  $54.00 \pm 2.76$  years; 34 with moderate signs, aged  $57.41 \pm 1.38$  years; and 32 subjects with mild symptoms among the patients with DPN aged  $65.91 \pm 1.71$  years. Age was significantly different between the four categories of DPN patients in relation to the assessment of nerve function on the left foot (Table 2 and Figure 3). On the right foot, the 5 individuals with no symptoms among the DPN patients were aged  $44.00 \pm 4.80$  years; the 14 subjects with mild symptoms among the DPN patients were aged  $54.86 \pm 2.52$  years; the moderate symptoms

**Table 1:** Symptom classify and the clinical parameters.

	No (n=11)	Mild (n=38)	Moderate (n=25)	Severe (n=7)	P
Age (year)	60.00 ± 3.95	58.39 ± 1.68	60.44 ± 2.18	60.00 ± 2.87	0.889
Diabetes (year)	9.95 ± 2.71	8.98 ± 1.09	10.63 ± 1.74	9.86 ± 1.98	0.862
Hypertension (year)	9.20 ± 5.93	1.700.62	5.42 ± 1.67	6.14 ± 4.10	0.085
Weight (kg)	71.33 ± 2.73	70.00 ± 2.80	68.93 ± 2.88	66.67 ± 5.04	0.917
Hemoglobin (g/l)	148.80 ± 8.21	148.16 ± 3.80	151.72 ± 4.28	153.14.5.79	0.906
FBG (mmol/l)	10.53 ± 1.00	9.94 ± 0.73	9.41 ± 0.67	9.33 ± 1.15	0.864
HbA1C (%)	9.61 ± 0.77	8.54 ± 0.38	9.21 ± 0.39	9.09 ± 0.83	0.471
Insulin (mU/dL)	13.78 ± 5.61	13.13 ± 2.01	10.35 ± 1.49	10.78 ± 2.31	0.760
Urea (mmol/l)	7.09 ± 0.74	6.11 ± 0.25	7.20 ± 0.49	5.60 ± 0.53	0.080
Creatinine (umol/l)	70.55 ± 9.88	62.17 ± 2.15	75.60 ± 7.01	59.41 ± 4.23	0.151
Uric Acid (umol/l)	333.90 ± 32.88	311.27 ± 11.15	341.80 ± 23.06	312.00 ± 24.80	0.580
Albumin (g/l)	41.96 ± 0.94	41.39 ± 1.13	42.02 ± 0.82	43.36 ± 1.33	0.848
Cholesterol (mmol/l)	3.81 ± 0.36	4.21 ± 0.21	4.43 ± 0.20	4.57 ± 0.40	0.458
Triglycerides (mmol/l)	2.37 ± 0.61	1.650.20	1.55 ± 0.15	1.55 ± 0.21	0.295
HDL (mmol/l)	0.99 ± 0.06	1.15 ± 0.03	1.12 ± 0.48	1.27 ± 0.09	0.086
LDL (mmol/l)	2.36 ± 0.22	2.68 ± 0.16	2.88 ± 0.15	3.00 ± 0.30	0.330
UMALB (mg/l)	101.57 ± 37.62	60.11 ± 14.40	85.00 ± 19.92	55.71 ± 41.09	0.552
24 h UTP (g/24 h)	0.56 ± 0.18	0.3340.06	0.96 ± 0.61	0.49 ± 0.29	0.587
8-OhdG (ng/ml)	0.48 ± 0.01	0.49 ± 0.02	0.48 ± 0.02	0.51 ± 0.02	0.803

Data are mean ± SE

**Abbreviations:** FBG: Fasting Blood Glucose; HbA1C: Glycated Hemoglobin; HDL: High Density Lipoprotein; LDL: Low Density Lipoprotein; UMALB: Urine Microalbumin; 24h UTP: 24-hour Urine Protein; 8-OhdG: 8-hydroxy-2-deoxyguanosine



**Figure 2:** There was no correlation between the 8-ohdG level and DPN symptoms classify.

among the DPN patients were 31 individuals aged  $57.55 \pm 1.47$  years; and there were 31 subjects with severe symptoms among the DPN patients aged  $66.52 \pm 1.63$  years. Patients with lower ages were more likely to have no signs among the four categories of DPN patients (Table 3 and Figure 4).

### Risk factors for diabetic peripheral neuropathy with respect to neuropathic sensory function and neurological symptoms

In the ordinary logistic regression analysis, the p-value of the parallel line of the test was 1.000 for the clinical parameters in neuropathic symptoms in patients with DPN. The ordinary logistic regression results showed that 8-OHdG levels (Odds Ratio (OR): 44.12; 95% Confidence Intervals (CI), (-4.760, 12.333);  $P=0.385$ ) were no associated with the nerve function symptoms in patients with DPN (Table 4). However, since the 95% CI was close to 1.00, it

was assumed that the high levels of HDL (OR: 45.51; 95% CI, (1.090, 6.545);  $P=0.006$ ) also had no association with neuropathic sensory symptoms in patients with DPN.

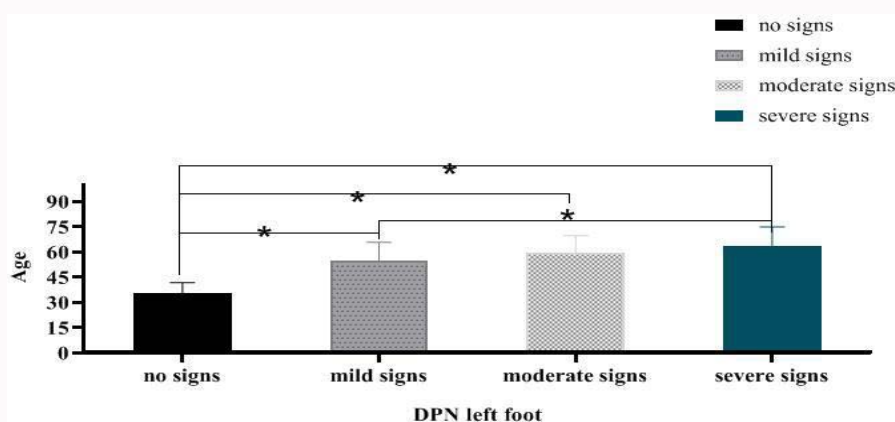
In relation to the nerve function signs on the left foot in subjects with DPN, the p-value of the parallel line of the test was 0.128. The duration of diabetes mellitus, age, Hb and UREA were included in this ordinary logistic regression analysis. The results revealed that older patients with DPN were more likely to develop severe neuropathic sensory function (OR: 1.11; 95% CI (0.049, 0.167);  $P=0.000$ ) (Table 5). The p-value of the parallel line of the test was 0.115 in the ordinary logistic regression analysis for clinical parameters in neuropathic nerve function on the right foot in patients with DPN. The clinical variables included were age, the time of diabetes mellitus, urea, Hb and UMALB. The ordinary logistic regression analysis showed that high age (OR: 1.12; 95% CI (0.054, 0.173);  $P=0.000$ ) was linked with

**Table 2:** DPN neuropathic sensory function of left foot and the clinical parameters.

	No (n=2)	Mild (n=13)	Moderate (n=34)	Severe (n=32)	P
Age (year)	35.50 ± 4.50	54.00 ± 2.76	57.41 ± 1.38	65.91 ± 1.71	0.001
Diabetes (year)	3.00 ± 1.005	10.54 ± 2.75	7.34 ± 0.96	12.24 ± 1.26	0.037
Hypertension (year)	0.00 ± 0.00	8.24 ± 3.22	2.10 ± 0.71	5.42 ± 2.05	0.157
Weight (kg)	70.18 ± 1.45	70.06 ± 2.36	70.92 ± 4.25	68.02 ± 2.12	0.746
Hemoglobin (g/l)	167.50 ± 6.50	153.00 ± 4.21	156.15 ± 3.78	140.25 ± 3.93	0.014
FBG (mmol/l)	5.62 ± 0.15	11.15 ± 1.55	9.18 ± 0.53	10.19 ± 0.64	0.155
HbA1C (%)	9.40 ± 2.20	8.86 ± 0.62	8.54 ± 0.40	9.35 ± 0.36	0.501
Insulin (mU/dL)	4.24 ± 0.52	15.28 ± 4.79	9.04 ± 0.97	14.46 ± 2.16	0.120
Urea (mmol/l)	6.39 ± 0.49	6.08 ± 0.47	5.99 ± 0.23	7.31 ± 0.44	0.043
Creatinine (umol/l)	55.80 ± 9.20	65.01 ± 4.24	63.39 ± 1.95	73.53 ± 6.35	0.339
Uric Acid (umol/l)	331.00 ± 68.00	363.15 ± 34.73	316.36 ± 12.36	313.91 ± 15.57	0.370
Albumin (g/l)	44.55 ± 3.45	42.86 ± 0.96	42.151.22	40.87 ± 0.70	0.566
Cholesterol (mmol/l)	4.30 ± 0.47	4.29 ± 0.30	4.18 ± 0.20	4.27 ± 0.22	0.984
Triglycerides (mmol/l)	2.77 ± 1.49	1.62 ± 0.30	1.52 ± 0.13	1.83 ± 0.26	0.421
HDL (mmol/l)	1.04 ± 0.13	1.13 ± 0.05	1.11 ± 0.04	1.16 ± 0.05	0.813
LDL (mmol/l)	2.84 ± 0.42	2.77 ± 0.21	2.65 ± 0.16	2.75 ± 0.16	0.952
UMALB (mg/l)	6.55 ± 1.55	96.75 ± 35.26	60.31 ± 14.07	80.45 ± 18.54	0.495
24 h UTP (g/24 h)	0.13 ± 0.01	0.57 ± 0.19	0.33 ± 0.05	0.84 ± 0.48	0.666
8-OhdG (ng/ml)	0.48 ± 0.02	0.51 ± 0.02	0.47 ± 0.01	0.49 ± 0.01	0.300

Data are mean ± SE

**Abbreviations:** FBG: Fasting Blood Glucose; HbA1C: Glycated Hemoglobin; HDL: High Density Lipoprotein; LDL: Low Density Lipoprotein; UMALB: Urine Microalbumin; 24h UTP: 24-hour Urine Protein; 8-OhdG: 8-hydroxy-2-deoxyguanosine



**Figure 3:** The age have a significant differences between the four categories of DPN neuropathic sensory function of left foot (DPN: Diabetic Peripheral Neuropathy).

an improvement in neuropathic function of the right foot in patients with DPN (Table 6).

## Discussion

The aim of this clinical study was to evaluate the risk factors association with the neurological symptoms and signs of patients with DPN. According to the clinical study results, the following conclusions could be drawn: 1) the level of 8-OhdG has no relationship with the development of neuropathic symptoms in patients with DPN; 2) age was associated with the development of neuropathic function deficits in DPN patients; and 3) neuropathic sensory function deficits were not related to neuropathic symptoms in patients with DPN.

From this study, it can be demonstrated that neuropathic sensory function was not linked with neuropathic symptoms in diabetic

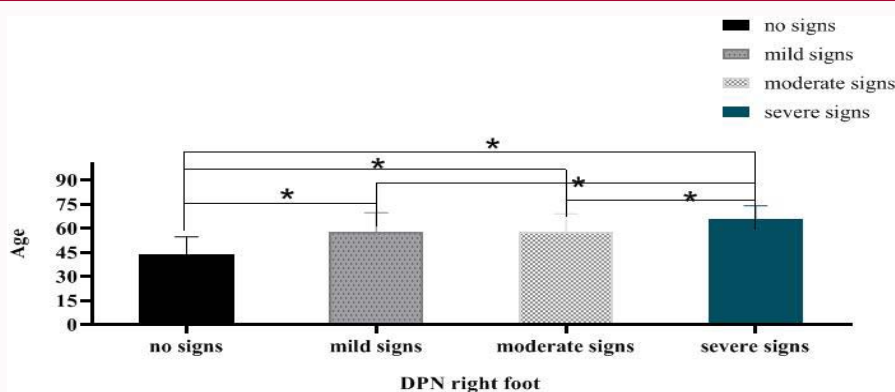
neuropathy patients. Neuropathic symptoms and signs normally do not serve as the only diagnostic criteria for diagnosing diabetic peripheral neuropathy. Kanji documented the useful and rational effects of a clinical examination in the diagnosis of DPN [30]. Studies have found that nerve conduction tests and NSS may also seldom correlate with the severity of diabetic peripheral neuropathy [31]. Moreover, in the early stages of type 2 diabetes mellitus, the symptoms and signs were not very obvious in DPN patients; in particular, there were no neuropathic symptoms or signs, such as neuropathic pain, numbness, tingling, and burning, in patients with sDPN. However, there are already nerve sensory function abnormalities [32] in patients with sDPN. Consequently, it is necessary to take essential steps to assess neuropathic symptoms and measure neuropathic physical signs for diagnosing diabetic peripheral neuropathy.

**Table 3:** DPN neuropathic sensory function of right foot and the clinical parameter.

	No (n=5)	Mild (n=14)	Moderate (n=31)	Severe (n=31)	P
Age (year)	44.00 ± 4.80	54.86 ± 2.52	57.55 ± 1.47	66.52 ± 1.63	0.000
Diabetes (year)	6.67 ± 2.03	5.41 ± 0.96	9.66 ± 1.26	12.22 ± 1.48	0.026
Hypertension (year)	2.20 ± 2.20	1.21 ± 0.55	5.54 ± 1.56	4.92 ± 2.12	0.479
Weight (kg)	65.64 ± 22.01	73.86 ± 6.89	67.87 ± 2.07	68.75 ± 2.10	0.466
Hemoglobin (g/l)	164.60 ± 2.46	155.43 ± 5.98	152.16 ± 4.17	142.10 ± 3.72	0.062
FBG (mmol/l)	8.06 ± 1.34	9.43 ± 0.90	9.74 ± 0.80	10.33 ± 0.64	0.633
HbA1C (%)	9.50 ± 0.94	8.45 ± 0.67	8.54 ± 0.38	9.43 ± 0.38	0.303
Insulin (mU/dL)	8.30 ± 4.07	7.88 ± 0.89	13.48 ± 2.37	13.26 ± 2.13	0.366
Urea (mmol/l)	5.92 ± 0.53	6.08 ± 0.41	6.09 ± 0.37	7.28 ± 0.37	0.065
Creatinine (umol/l)	59.26 ± 5.82	61.79 ± 2.70	66.91 ± 5.46	71.92 ± 4.42	0.521
Uric Acid (umol/l)	357.20 ± 45.34	310.71 ± 16.68	319.16 ± 18.38	327.55 ± 15.37	0.774
Albumin (g/l)	45.64 ± 1.19	42.51 ± 0.60	41.07 ± 1.41	41.62 ± 0.60	0.353
Cholesterol (mmol/l)	4.55 ± 0.28	4.50 ± 0.35	4.10 ± 0.20	4.21 ± 0.22	0.677
Triglycerides (mmol/l)	2.23 ± 0.52	1.28 ± 0.11	1.91 ± 0.25	1.56 ± 0.21	0.247
HDL (mmol/l)	1.08 ± 0.48	1.15 ± 0.06	1.10 ± 0.04	1.16 ± 0.04	0.638
LDL (mmol/l)	3.01 ± 0.24	2.93 ± 0.28	2.56 ± 0.14	2.71 ± 0.16	0.514
UMALB (mg/l)	8.56 ± 1.77	32.21 ± 8.96	96.90 ± 19.68	77.85 ± 19.13	0.076
24 h UTP (g/24 h)	0.13 ± 0.01	0.30 ± 0.06	0.92 ± 0.47	0.39 ± 0.10	0.512
8-OhdG (ng/ml)	0.48 ± 0.02	0.50 ± 0.01	0.47 ± 0.01	0.50 ± 0.01	0.230

Data are mean ± SE

**Abbreviations:** FBG: Fasting Blood Glucose; HbA1C: Glycated Hemoglobin; HDL: High Density lipoprotein; LDL: Low Density Lipoprotein; UMALB: Urine Microalbumin; 24h UTP: 24-hour Urine Protein; 8-OhdG: 8-hydroxy-2-deoxyguanosine



**Figure 4:** The age were related to the four categories of DPN neuropathic sensory function on right foot (DPN: Diabetic Peripheral Neuropathy).

In this clinical study, we identified that the clinical parameters of age were associated with neuropathic function deficits on the left and right feet in DPN patients. The mechanism of diabetic peripheral neuropathy may be associated with the interaction of metabolic disorders such as hyperglycemia toxicity, the aldose reductase-polyol pathway, production of glycosylation end products, oxidative stress, abnormal lipid metabolism, microvascular injury, neurotrophic factor deficiency and abnormal release of cytokines and autoimmune factors. Previous studies have also elaborated that age is correlated with an increase in the prevalence of DPN [33]. Although the molecular mechanics of ageing and age-related damage in cells and organs is still not clearly understood in patients with DPN. Several studies have indicated that the age and duration of diabetes are risk factors in the progression of DPN [34,35]. Aging and the duration of diabetes were reported to be risk factors for DPN, owing to the prolonged exposure of the peripheral nervous system in each case.

Hence, DPN occurred to a greater extent in these contexts.

Our results showed that the level of 8-OHdG had no relationship with the neuropathic symptoms of DPN according to the sensory symptom classification. 8-OHdG as the product of tissue cell injury under oxidative stress also increase due to smoking, aging, or occupational exposure to physical, chemical, or biological substances [36,37]. In recent years, an increasing number of studies have shown that oxidative stress is the common mechanism of diabetic peripheral neuropathy [38,39]. Peripheral nerve damage is the cause of obstruction in the structural and functional nervous responses in the somatosensory system of DPN patients. Persistent hyperglycemia in diabetic patients increases the damage of oxidative stress, which in turn results in the excessive production of Reactive Oxygen Species (ROS), reducing the body's antioxidant capacity and inhibiting cytokine and cytochrome oxidase release. When nervous

**Table 4:** Ordinary logistic regression analysis of parameters associated with symptoms classify in DPN patients.

	Exp(B)	95% CI	P
Hypertension (year)	1.03	(-0.026, 0.078)	0.324
Urea Nitrogen (mmol/l)	1.36	(-0.083, 0.700)	0.122
Creatinine (umol/l)	1.01	(-0.025, 0.047)	0.539
HDL (mmol/l)	45.51	(1.090, 6.545)	0.006
8-OHdG (ng/ml)	44.12	(-4.760, 12.333)	0.385
Glucose (mmol/l)	1.021	(-0.224, 0.267)	0.865
2 h Glucose (mmol/l)	1.029	(-0.110, 0.1687)	0.679
HbA1C (%)	0.904	(-0.443, 0.242)	0.564

**Abbreviations:** 8-OHdG: 8-Hydroxy-2-Deoxyguanosine; HDL: High Density Lipoprotein

**Table 5:** Ordinary logistic regression analysis of parameters associated with DPN left foot in DPN patients.

	Exp(B)	95% CI	P
Age (year)	1.11	(0.049, 0.167)	0.000
Diabetes (year)	1.01	(-0.058, 0.081)	0.744
Hemoglobin (g/l)	0.99	(-0.030, 0.018)	0.606
Urea (mmol/l)	1.22	(-0.060, 0.455)	0.132

**Table 6:** Ordinary logistic regression analysis of parameters associated with DPN right foot in DPN patients.

	Exp(B)	95% CI	P
Age (year)	1.12	(0.054, 0.173)	0.000
Diabetes (year)	1.05	(-0.024, 0.117)	0.196
Hemoglobin (g/l)	0.99	(-0.028, 0.019)	0.703
Urea (mmol/l)	1.07	(-0.189, 0.325)	0.603
UMALB (mg/l)	1.00	(-0.001, 0.009)	0.123

damage occurs in sensory neurons, it will lead to positive symptoms and negative symptoms. The distal symmetric polyneuropathy and distal-to-proximal gradient of severity is the most common pattern in patients with DPN, which is not only predominantly of the feet in the clinic but also of the hands [40]. Sensory loss, ongoing spontaneous pain, allodynia, hyperalgesia and evoked pain compose the neuropathic clinical signs in DPN patients [41]. An imbalance between the oxidation and antioxidant systems *in vivo* induced different types of tissue stress damage. Neurodegeneration disrupts the connection between the peripheral nerve system and the central nerve system and ultimately leads to sensory loss after peripheral nerve damage. Hence, there are several different types of peripheral diabetic neuropathy, and the neuropathic symptoms and signs may differ from each other depending on the diagnostic tools or measurement criteria [42].

## Limitations

This clinical observational trial had a small sample size and was also carried out with Chinese participants, which largely impacts the generalizability of the findings. Moreover, the research participants were assessed for DPN through a clinical examination and measurement depending on the neuropathic symptoms and sensory function, and their symptoms and signs could be nonspecific. Therefore, future researchers should consider examining the topic using more accurate assessment criteria and measurement methods and larger sample sizes to fully understand the pathology in patients

with diabetic peripheral neuropathy.

## Funding

This work was supported by the Gansu Youth Science and Technology Project [21JR1RA156].

## References

- Vinik AI, Maser R, Mitchell B, Freeman R. Diabetic autonomic neuropathy. *Diabetes Care*. 2003;26(5):1553-79.
- Dyck PJ, Davies JL, Wilson DM, Service FJ, Melton 3<sup>rd</sup> LJ, O'Brien PC. Risk factors for severity of diabetic polyneuropathy: Intensive longitudinal assessment of the Rochester diabetic neuropathy study cohort. *Diabetes Care*. 1999;22(9):1479-86.
- Vos T, Flaxman AD, Naghavi M, Lozano R, Michaud C, Ezzati M, et al. Years Lived with Disability (YLDs) for 1160 sequelae of 289 diseases and injuries 1990–2010: A systematic analysis for the Global Burden of Disease Study 2010. *Lancet*. 2012;380 (9859):2163-96.
- Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: Estimates for the year 2000 and projections for 2030. *Diabetes Care*. 2004;27:1047-53.
- Vinik AI, Park TS, Stansberry KB, Pittenger GL. Diabetic neuropathies. *Diabetologia*. 2000;43(8):957-73.
- Ebata-Kogure N, Nozawa K, Murakami A, Toyoda T, Haga Y, Fujii K. Clinical and economic burdens experienced by patients with painful diabetic peripheral neuropathy: An observational study using a Japanese claims database. *PLoS One*. 2017;12(10):e0187250.
- Martyn CN, Hughes RA. Epidemiology of peripheral neuropathy. *J Neurol Neurosurg Psychiatry*. 1997;62(4):310-18.
- Valensi P, Le Devehat C, Richard JL, Farez C, Khodabandehlou T, Rosenbloom RA, et al. A multicenter, double-blind, safety study of QR-333 for the treatment of symptomatic diabetic peripheral neuropathy. A preliminary report. *J Diabetes Complications*. 2005;19:247-53.
- Yuen KC, Baker NR, Rayman G. Treatment of chronic painful diabetic neuropathy with isosorbide dinitrate spray: A double-blind placebo-controlled cross-over study. *Diabetes Care*. 2002;25:1699-703.
- Jensen MP, Friedman M, Bonzo D, Richards P. The validity of the neuropathic pain scale for assessing diabetic neuropathic pain in a clinical trial. *Clin J Pain*. 2006;22:97-103.
- Weintraub MI, Wolfe GI, Barohn RA, Cole SP, Parry GJ, Hayat G, et al. Static magnetic field therapy for symptomatic diabetic neuropathy: A randomized, double-blind, placebo-controlled trial. *Arch Phys Med Rehabil*. 2003;84:736-46.
- Rauk R, Makumi CW, Schwartz S, Graff O, Meno-Tetang G, Bell CF, et al. A randomized, controlled trial of gabapentin enacarbil in subjects with neuropathic pain associated with diabetic peripheral neuropathy. *Pain Pract*. 2013;13:485-96.
- Zis P, Daskalaki A, Bountouni I, Sykioti P, Varrassi G, Paladini A. Depression and chronic pain in the elderly: Links and management challenges. *Clin Interv Aging*. 2017;12:709-20.
- Icks A, Scheer M, Morbach S, Genz J, Haastert B, Giani G, et al. Time-dependent impact of diabetes on mortality in patients after major lower extremity amputation: Survival in a population-based 5-year cohort in Germany. *Diabetes Care*. 2011;34:1350-4.
- Gorst C, Kwok CS, Aslam S, Buchan I, Kontopantelis E, Myint PK, et al. Long-term glycemic variability and risk of adverse outcomes: A systematic review and meta-analysis. *Diabetes Care*. 2015;38(12):2354-69.
- Jun JE, Jin SM, Baek J, Oh S, Hur KY, Lee MS, et al. The association between glycemic variability and diabetic cardiovascular autonomic neuropathy in patients with type 2 diabetes. *Cardiovasc Diabetol*. 2015;14:70.

17. Allen C, Shen G, Palta M, Lotz B, Jacobson R, D'Alessio D. Long-term hyperglycemia is related to peripheral nerve changes at a diabetes duration of 4 years. *Diabetes Care*. 1997;20:1154-8.
18. Giacco F, Brownlee M. Oxidative stress and diabetic complications. *Circ Res*. 2010;107(9):1058-70.
19. Kasai H. Analysis of a form of oxidative DNA damage, 8-hydroxy-2'-deoxyguanosine, as a marker of cellular oxidative stress during carcinogenesis. *Mutat Res*. 1997;387:147-63.
20. Beckman KB, Ames BN. Oxidative decay of DNA. *J Biol Chem*. 1997;272:19633-6.
21. Valavanidis A, Vlachogianni T, Fiotakis C. 8-hydroxy-2'-deoxyguanosine (8-ohdg): A critical biomarker of oxidative stress and carcinogenesis. *J Environ Sci Health C Environ Carcinog Ecotoxicol Rev*. 2009;27:120-39.
22. Barregard L, Moller P, Henriksen T, Mistry V, Koppen G, Rossner P, Jr, et al. Human and methodological sources of variability in the measurement of urinary 8-oxo-7,8-dihydro-2'-deoxyguanosine. *Antioxid Redox Signal*. 2013;18:2377-91.
23. Zanolini ME, Girardi P, Degan P, Rava M, Olivieri M, Di Gennaro G, et al. Measurement of a urinary marker (8-hydroxydeoxy-guanosine, 8-ohdg) of DNA oxidative stress in epidemiological surveys: A pilot study. *Int J Biol Markers*. 2015;30:e341-5.
24. Dobrota VD, Hrabac P, Skegrod D, Smiljanic R, Dobrota S, Prkacin I, et al. The impact of neuropathic pain and other comorbidities on the quality of life in patients with diabetes. *Health Qual Life Outcomes*. 2014;3;12:171.
25. Kim SS, Won JC, Kwon HS, Kim CH, Lee JH, Park TS, et al. Prevalence and clinical implications of painful diabetic peripheral neuropathy in type 2 diabetes: Results from a nationwide hospital-based study of diabetic neuropathy in Korea. *Diabetes Res Clin Pract*. 2014;103(3):522-9.
26. American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care*. 2011;34(Suppl 1):S62-9.
27. Young MJ, Boulton AJ, MacLeod AF, Williams DR, Sonksen PH. A multicentre study of the prevalence of diabetic peripheral neuropathy in the United Kingdom hospital clinic population. *Diabetologia*. 1993;36:150-4.
28. Ziegler D, Keller J, Maier C, Pannek J; German Diabetes Association. Diabetic neuropathy. *Exp Clin Endocrinol Diabetes*. 2014;122(7):406-15.
29. Ziegler D, Siekierka-Kleiser E, Meyer B, Schweers M. Validation of a novel screening device (NeuroQuick) for quantitative assessment of small nerve fiber dysfunction as an early feature of diabetic polyneuropathy. *Diabetes Care*. 2005;28:1169-74.
30. Kanji JN, Anglin RE, Hunt DL, Panju A. Does this patient with diabetes have large-fiber peripheral neuropathy? *JAMA*. 2010;30(15):1526-32.
31. King SA. Diabetic peripheral neuropathic pain: Effective management. *Consultant*. 2008;48(11).
32. Dyck PJ, Albers JW, Andersen H. Diabetic polyneuropathies: Update on research definition, diagnostic criteria and estimation of severity. *Diabetes Metab Res Rev*. 2011;27(7):620-8.
33. Moulin D, Boulanger A, Clark AJ, Clarke H, Dao T, Finley GA, et al. Pharmacological management of chronic neuropathic pain: Revised consensus statement from the Canadian Pain Society. *Pain Res Manag*. 2014;19:328-35.
34. Bansal D, Gudala K, Muthyala H, Esam HP, Nayakallu R, Bhansali A. Prevalence and risk factors of development of peripheral diabetic neuropathy in type 2 diabetes mellitus in a tertiary care setting. *J Diabetes Investig*. 2014;5(6):714-21.
35. Erbas T, Ertas M, Yucel A, Keskinaslan A, Senocak M; TURNEP Study Group. Prevalence of peripheral neuropathy and painful peripheral neuropathy in Turkish diabetic patients. *J Clin Neurophysiol*. 2011;28(1):51-5.
36. Sztanek F, Molnár Á, Balogh Z. The role of oxidative stress in the development of diabetic neuropathy. *Orv Hetil*. 2016;157(49):1939-46.
37. Himeno T, Kamiya H, Naruse K, Harada N, Ozaki N, Seino Y, et al. Beneficial effects of exendin-4 on experimental polyneuropathy in diabetic mice. *Diabetes*. 2011;60(9):2397-406.
38. Abusoglu S, Celik HT, Tutkun E, Yilmaz H, Serdar MA, Bal CD, et al. 8-hydroxydeoxyguanosine as a useful marker for determining the severity of trichloroethylene exposure. *Arch Environ Occup Health*. 2014;69:180-6.
39. Sakano N, Wang DH, Takahashi N, Wang B, Sauriasari R, Kanbara S, et al. Oxidative stress biomarkers and lifestyles in Japanese healthy people. *J Clin Biochem Nutr*. 2009;44:185-95.
40. Callaghan BC, Cheng HLT, Stables CL, Smith AL, Feldman EL. Diabetic neuropathy: Clinical manifestations and current treatments. *Lancet Neurol*. 2012;11:521-34.
41. Jensen TS, Finnerup NB. Allodynia and hyperalgesia in neuropathic pain: Clinical manifestations and mechanisms. *Lancet Neurol*. 2014;13:924-935.
42. Nawroth PP, Bendszus M, Pham M, Jende J, Heiland S, Ries S, et al. The quest for more research on painful diabetic neuropathy. *Neuroscience*. 2018;387:28-37.