

Relationship of Vascular Factors on Electrophysiologic Severity of Diabetic Neuropathy

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Objective To investigate the impact of vascular factors on the electrophysiologic severity of diabetic neuropathy (DPN).

Methods Total 530 patients with type 2 diabetes were enrolled retrospectively. We rated severity of DPN from 1 (normal) to 4 (severe) based on electrophysiologic findings. We collected the data concerning vascular factors (including brachial-ankle pulse wave velocity [PWV], ankle brachial index, ultrasound of carotid artery, lipid profile from the blood test, and microalbuminuria [MU] within 24 hours urine), and metabolic factors of diabetes (such as glycated hemoglobin [HbA1c]). We analyzed the differences among the four subgroups using χ^2 test and ANOVA, and ordinal logistic regression analysis was performed to investigate the relationship between significant variables and severity of DPN.

Results The severity of DPN was significantly associated with duration of diabetes, HbA1c, existence of diabetic retinopathy and nephropathy, PWV, presence of plaque, low density lipoprotein-cholesterol and MU ($p < 0.05$). Among these variables, HbA1c and presence of plaque were more significantly related with severity of DPN in logistic regression analysis ($p < 0.001$), and presence of plaque showed the highest odds ratio (OR=2.52).

Conclusion Our results suggest that markers for vascular wall properties, such as PWV and presence of plaque, are significantly associated with the severity of DPN. The presence of plaque was more strongly associated with the severity of DPN than other variables.

Keywords Diabetic neuropathies, Electromyography, Diabetic angiopathies, Pulse wave analysis, Carotid stenosis

INTRODUCTION

Diabetes mellitus type 2 is a chronic disease. Years after the initial diagnosis, microvascular complications such as diabetic retinopathy, nephropathy and neuropathy can arise [1]. Since microvascular complications have adverse effects on the quality of life of diabetic patients, early detection and care are important.

Till date, the inadequate regulation of blood glucose and the duration of the disease have a high correlation

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with diabetic neuropathy (DPN). The most acceptable treatment was to maintain the blood glucose within the normal range [2]. Previous studies have reported that in a 5-year follow-up period, the intensive blood glucose control group reduced the development of confirmed clinical DPN by 64% in the combined cohorts in diabetic patients [3]. However, the cumulative incidence of DPN in this conducted patient group was considerably high. The results suggested that despite thorough blood glucose regulation, other factors influence the occurrence of DPN, and these factors may play a significant role in the development and maintenance of DPN.

According to several studies [4,5], it was reported that not only metabolic disorders due to hyperglycemia are involved, but vascular impairment is also involved with the DPN pathophysiology. It is being suggested that the angiopathies of a diabetic patient influences the peripheral nerve axonal injury [6]. Primary mechanism of DPN development is the structural hindrance of the blood vessels, like structure change in the endoneurium of microvessels, which can induce chronic ischemia. The fact chronic ischemia aggravates DPN was not only proved by the diabetic rat models, but research on human subjects are also being reported [7,8]. In experiments conducted by Cameron et al. [6], the biopsies taken from mild to severe diabetic neuropathic patients revealed the following: basement thickening, pericyte degeneration and nerve microvasculature with endothelial cell hyperplasia. Cardiovascular risk factors (e.g., hypertension, hyperlipidemia, weight increase, high von Willebrand factor level and microalbuminuria) have a considerable relationship with development of DPN [4]. Thus, we can conclude that vascular change has a strong relation with clinical deficiencies and neuropathology [6,9,10].

However, till date, it has not been fully determined on how angiopathies affect nerve ischemia [11,12]. In addition, there are few studies has been conducted by objective electrophysiologic findings.

This study was designed to find the correlation between angiopathies and DPN, and to determine the influence of vascular factors on the severity of the DPN by electrophysiologic findings.

MATERIALS AND METHODS

Participants

From January 2008 to May 2014, 530 hospitalized patients diagnosed with diabetes mellitus type 2 at the endocrinology department of Korea University Medical Center were retrospectively recruited for the study.

Patients who were pregnant or diagnosed with underlying diseases such as diabetes mellitus type 1, liver or kidney-related diseases, or those who had a medical history that might influence the electrodiagnostic study, were excluded.

Diagnosis of DPN

Patients with confirmed clinical DPN underwent electrophysiologic studies to classify the severity of the disease. Clinical DPN was diagnosed by examination based on the presence of at least two of the findings: physical symptoms, abnormalities on sensory examination, and absent or decreased deep-tendon reflexes. These were suggested by the Diabetes Control and Complications Trial [3,13].

Electrophysiologic study

All subjects underwent a nerve conduction study (NCS) and needle electromyography (EMG). The NCS was performed in the unilateral upper and lower extremities. Motor NCS were performed in the median, ulnar, peroneal, and tibial nerves, and F-waves were recorded for each nerve. Sensory NCS were obtained from the median, ulnar, and sural nerves. H-reflex studies were also performed. Needle EMG was carried out in 3 muscles in the upper (brachioradialis, pronator teres, first dorsal interosseous muscles) and lower extremities (vastus lateralis, tibialis anterior, gastrocnemius medialis muscles).

Electrophysiologic diagnostic criteria of DPN was modified from the suggestions of the Diabetes Control and Complications Trial [3,13]. On the basis of the proposed version, the established diagnostic criteria of Korea University Medical Center's rehabilitation department were used in this study [14,15]. The following criteria were included: the amplitude of the sural nerve is less than 5 μ V, the amplitude of median sensory nerve is less than 10 μ V, the peroneal motor nerve's amplitude is less than 1 mV, latency more than 6 ms or conduction velocity less than 40 m/s, the F-wave latency is absent or more than 55 ms,

the H-reflex is absent, fibrillations to be observed in lower extremity muscles. Classification of the disease was as follows: 'mild' if the amplitude of sural nerve is less than 5 μ V plus one of the remaining criteria, or sural nerve is more than 5 μ V, but including at least two of above criteria; 'moderate' if the amplitude of sural nerve is less than 5 μ V degree plus two to four criteria from the list; 'severe' if the sural nerve is less than 5 μ V plus more than five of the criteria (Table 1).

Evaluation of risk factors

We compared the general characteristics of four groups by collecting demographic factors: age, gender, body mass index (BMI), diabetic parameters, the duration of diabetes, and the glycated hemoglobin (HbA1c) level. In order to evaluate the patients' cardiovascular medical history, we examined the patients' chart to verify the medical history of hypertension, angina pectoris, myocardial infarction, arrhythmia, and stroke.

To assess whether or not there were diabetes-related complications, a 24-hour urine test and ophthalmological examination were carried out. The early stages of the diabetic nephropathy were based on 30–300 mg/day of microalbuminuria (MU) within 24 hours urine. Patients who had apparent albuminuria (over 300 mg of MU, overt proteinuria) were also taken into account.

Atherosclerosis and other similar diseases, which lead to blood vessel shrinkage and clogged arteries, can influ-

ence the pathophysiology of the nerves. The pulse wave velocity (PWV) and the ankle brachial index test (ABI) which are non-invasive methods that are being widely used, are also commonly used for diagnosis of peripheral vascular diseases (PVD) [16-18]. In addition, carotid ultrasound, which is commonly used for predicting risk of myocardial infarction and cerebrovascular disease, were used to measure intima-media thickness (IMT) and the detection of stenosis and plaque [19]. Vascular functions, including arterial stiffness, occlusion, blood vessel elasticity and degree of stenosis, could be evaluated through these non-invasive methods. PWV was recorded on both sides of the artery when the pulse wave acted within the artery. The distance between both pulse waves were divided by the time difference. The stiffness of arteries were more noticeable with higher pulse wave propagation velocity. The results was divided into four subgroups of stiffness, and compared to healthy control reference values of the same age and gender [20]: 'normal' if the PWV value was less than the value of average plus 1 standard deviation (SD) of healthy controls; 'slightly harder' if the PWV value was between 1 to 2 SD higher than the average; 'harder' if the PWV value was larger than average plus 2 SD. Vascular stiffness cannot be evaluated when vascular occlusion is suspected, because then it is considered as occlusion. ABI is the measured systolic blood pressure of the patient's brachial artery on both sides followed by the posterior tibial artery of both the lower limbs in a supine position. It is the ratio of the systolic blood pressure measured at the ankle to that measured at the brachial artery. On the basis of the standard diagnosis for PVD, if the ABI is less than 0.9, it is considered abnormal [21]. If the ABI is between 0.8 and 0.9, the patient has some narrowing of the arteries in the ankle and may have the beginnings of PVD. With range of ABI between 0.5 and 0.8 indicates more significant blockage of ankle and leg arteries and suggests moderate PVD. Similarly, ABI <0.5 suggests severe PVD. If the IMT is more than 0.90 mm, it was classified as abnormal. In conditions where a plaque or stenosis was present, it was categorized as an abnormal finding.

In addition, the lipid profile, including total cholesterol, high-density lipoprotein cholesterol (HDL-cholesterol), low-density lipoprotein cholesterol (LDL-cholesterol) and triglycerides, which influences angiopathies, were examined from the blood test.

Table 1. Diagnostic criteria of diabetic neuropathy

Sural sensory amplitude	$\leq 5 \mu$ V
Median sensory amplitude	$\leq 10 \mu$ V
Peroneal motor amplitude	<1 mV
Peroneal motor velocity	Distal latency (≥ 6 ms) or NCV (<40 m/s)
Peroneal F latency	Absent or >55 ms
H-reflex	Absent
Needle EMG	Fibrillations in lower extremity muscles (tibialis anterior, gastrocnemius etc.)

Disease severity level: mild degree, sural $\leq 5 \mu$ V plus one of above list or sural $>5 \mu$ V, but including at least two of above list; moderate degree, sural $\leq 5 \mu$ V plus at least two to four of above list; severe degree, sural $\leq 5 \mu$ V plus more than five of above list.

NCV, nerve conduction study; EMG, electromyography.

Statistical analysis

SPSS ver. 20 (IBM, Armonk, NY, USA) and SAS ver. 9.1 (SAS Institute Inc., Cary, NC, USA) software packages were used for statistical analysis. We analyzed the differences of risk factors among the four subgroups; the χ^2 test was used for categorical variables. The ANOVA test was used for continuous variables and Tukey's post hoc study was also performed to see whether there are significant differences in the variables between each groups. In order to identify the significant variables in this analysis, the trend test was added to observe the trend according to the severity of DPN. Variables showing a significant trend in the trend test were obtained odds ratio by analyzing ordinal logistic regression in order to affect the degree on the severity of DPN. A p-value of less than 0.05 was considered significant.

RESULTS

According to the severity of DPN, out of the 530 subjects evaluated, 291 cases were of group 1 (no DPN), 82 cases were of group 2 (mild DPN), 84 cases were of group 3 (moderate DPN), and 73 cases were of group 4 (severe DPN).

General characteristics

The average age was 62 from a range of 20 to 88 years old. In addition, the average period of diagnosis with diabetes was 7.5 years, ranging from 0 to 40 years. From the study of DPN, significant differences and trend were shown between the age and the duration of diabetes in the four groups (age, $p < 0.001$; duration, $p < 0.001$) (Table 2). In the trend test, the HbA1c value showed a significant trend according to the severity of DPN ($p = 0.008$).

In other diabetic complications such as nephropathy and the retinopathy, the comparison between the four groups showed significant differences (nephropathy, $p < 0.001$; retinopathy, $p < 0.001$). Also, significant trend was shown from the trend test (retinopathy, $p < 0.001$; nephropathy, $p = 0.001$).

DPN and risk factors of angiopathies

Amongst all the subjects, 381 cases had an abnormality in PWV. Of the 381 subjects, 186 cases were diagnosed with neuropathy and 195 cases were negative. Evaluation of DPN showed that the PWV of the four groups had sig-

nificant differences ($p = 0.005$), and a significant trend was also observed from the trend test ($p < 0.001$) (Table 3).

ABI under 0.9 was seen in 22 cases, of which 13 cases had DPN and 9 cases did not. Although there was a significant difference between the ABI ($p = 0.043$) out of the four groups, a significant trend was not apparent from the trend test ($p = 0.079$).

Carotid artery plaque was diagnosed in 302 cases of which 167 cases had DPN and 135 cases did not. There were significant differences whether or not the carotid artery plaque was present in the four groups ($p < 0.001$), and a significant trend was similarly seen from the trend test ($p < 0.001$). However, with respect to the IMT of the carotid artery ($p = 0.154$) and the presence of stenosis ($p = 0.051$), there were no significant differences.

From the study on severity of DPN and the trend test, the LDL-cholesterol and MU out of the four groups showed significant differences and trend.

DPN and odds ratio of risk factors

The variables of the significant trend according to the severity of DPN were analyzed by ordinal logistic regression analysis: duration of diabetes, HbA1c, BMI, diabetic nephropathy and retinopathy, PWV, the presence or absence of carotid artery plaque, LDL-cholesterol and MU (Table 4).

HbA1c ($p < 0.001$), diabetic nephropathy ($p = 0.0224$) and retinopathy ($p = 0.0481$), presence of plaque ($p < 0.001$) and MU ($p = 0.0264$) showed a significant value. The PWV was examined by normality and abnormality divisions. A significant result of the PWV ($p = 0.2507$) and LDL-cholesterol ($p = 0.1643$) were not obtainable.

Each time the HbA1c value increased by 1%, the risk of DPN would increase by 1.19-fold ($p < 0.001$). In contrast to patients who were diagnosed with carotid artery plaque and with patients who are not, the risk of increasing severity of DPN was 2.52-fold ($p < 0.001$). Amongst all the risk factors, the odds ratio of the presence of plaque was the highest.

DISCUSSION

The aim of this study was to determine the impact of angiopathies on the severity of the DPN by using electrophysiologic findings. Although a number of clinical scales to classify the severity of DPN have been developed, there

Table 2. Demographic and clinical characteristics of 4 subgroups (n=530)

Characteristic	Group 1 (n=291)	Group 2 (n=82)	Group 3 (n=84)	Group 4 (n=73)	p-value
Age (yr)	56.93±15.6 ^{a)}	60.45±15.6 ^{a,b)}	65.26±12.1 ^{b)}	60.30±14.4 ^{a,b)}	<0.001 ^{**e)} 0.018 ^{**f)}
Sex					
Male	129 (44.3)	48 (58.5)	43 (51.2)	47 (64.4)	0.007 ^{**c)}
Female	162 (55.7)	34 (41.5)	41 (48.8)	26 (35.6)	0.003 ^{**d)}
Height (cm)	160.45±9.9	161.60±9.1	159.88±8.4	163.20±9.5	0.098
BMI (kg/m ²)	25.22±4.1 ^{a)}	24.45±4.6 ^{a)}	24.83±4.3 ^{a)}	22.81±3.8 ^{b)}	<0.001 ^{**e)} <0.001 ^{**f)}
Alcohol					
No	188 (64.6)	41 (50.0)	48 (57.1)	40 (54.8)	
Yes	103 (35.4)	41 (50.0)	36 (42.9)	33 (45.2)	
Smoking					
No	194 (66.7)	43 (52.4)	61 (72.6)	44 (60.3)	0.032 ^{**c)}
Yes	97 (33.3)	39 (47.6)	23 (27.4)	29 (39.7)	0.647 ^{d)}
Diabetes duration (yr)	6.62±7.7	13.52±37.3 ^{a)}	13.33±9.9 ^{a)}	14.38±8.9 ^{a)}	<0.001 ^{**e)} 0.001 ^{**f)}
HbA1c (%)	9.86±2.4 ^{a)}	10.86±2.5 ^{b)}	10.15±2.3 ^{a,b)}	11.01±2.7 ^{b)}	<0.001 ^{**e)} 0.008 ^{**f)}
Diabetic retinopathy					
No	208 (71.5)	54 (65.9)	32 (38.1)	21 (28.8)	<0.001 ^{**c)}
Yes	83 (28.5)	28 (34.1)	52 (61.9)	52 (71.2)	<0.001 ^{**d)}
Diabetic nephropathy					
No	276 (95.2)	69 (84.1)	61 (72.6)	47 (65.3)	<0.001 ^{**c)}
Yes	14 (4.8)	13 (15.9)	23 (27.4)	25 (34.7)	0.010 ^{**d)}
Hypertension					
No	161 (55.3)	43 (52.4)	44 (52.4)	36 (49.3)	
Yes	130 (44.7)	39 (47.6)	40 (47.6)	37 (50.7)	0.806
Angina pectoris					
No	262 (90.0)	74 (90.2)	73 (86.9)	68 (93.2)	
Yes	29 (10.0)	8 (9.8)	11 (13.1)	5 (6.8)	0.636
Myocardial infarction					
No	279 (95.9)	77 (93.9)	77 (91.7)	68 (93.2)	0.443
Yes	12 (4.1)	5 (6.1)	7 (8.3)	5 (6.8)	
Arrhythmia					
No	281 (96.6)	79 (96.3)	82 (97.6)	70 (95.9)	0.941
Yes	10 (3.4)	3 (3.7)	2 (2.4)	3 (4.1)	
Stroke					
No	274 (94.2)	77 (93.9)	76 (90.5)	63 (86.3)	0.117
Yes	17 (5.8)	5 (6.1)	8 (9.5)	10 (13.7)	

Values are presented as mean±standard deviation or number (%).

BMI, body mass index.

^{a,b)} Same letters indicate statistically non-significant with Tukey's multiple comparison.

^{c)} p-value of χ^2 test, ^{d)} p-value of χ^2 trend test (between group 1-4) was used when the variables were significant difference in χ^2 test.

^{e)} p-value of ANOVA, ^{f)} p-value of ANOVA trend test (between group 1-4) was used when the variables were significant difference in ANOVA test.

*p<0.05, **p<0.01, statistically significant.

Table 3. Comparison of vascular variables between 4 subgroups (n=530)

Variable	Group 1 (n=291)	Group 2 (n=82)	Group 3 (n=84)	Group 4 (n=73)	p-value
PWV					
Normal	96 (33.0)	22 (26.8)	15 (17.9)	16 (21.9)	
Slightly harder	88 (30.2)	23 (28.0)	21 (25.0)	17 (23.3)	0.005** ^{c)}
Harder	106 (36.4)	33 (40.2)	45 (53.6)	38 (52.1)	<0.001** ^{d)}
Occlusion	1 (0.3)	4 (4.9)	3 (3.6)	2 (2.7)	
ABI					
≥0.9	280 (96.9)	76 (92.7)	80 (96.4)	66 (94.3)	
<0.9 and ≥0.8	6 (2.1)	3 (3.7)	1 (1.2)	1 (1.4)	0.043** ^{c)}
<0.8 and ≥0.5	3 (1.0)	3 (3.7)	2 (2.4)	1 (1.4)	0.079 ^{d)}
<0.5	0 (0.0)	0 (0.0)	0 (0.0)	2 (2.9)	
Carotid artery ultrasound					
IMT					0.154
Normal	262 (90.0)	72 (87.8)	78 (92.9)	71 (97.3)	
Abnormal	29 (10.0)	10 (12.2)	6 (7.1)	2 (2.7)	
Plaque					
No	155 (53.4)	32 (39.0)	24 (28.6)	16 (21.9)	<0.001** ^{c)}
Yes	135 (46.6)	50 (61.0)	60 (71.4)	57 (78.1)	<0.001** ^{d)}
Stenosis					
No	261 (90.3)	67 (81.7)	69 (82.1)	60 (82.2)	0.051 ^{c)}
Yes	28 (9.7)	15 (18.3)	15 (17.9)	13 (17.8)	0.017 ^{d)}
Laboratory parameters					
Total cholesterol (mg/dL)	172.2±47.7	172.3±57.2	160.5±41.6	159.5±45.6	0.069
HDL-cholesterol (mg/dL)	43.8±12.6	45.9±12.5	42.6±12.5	44.9±14.4	0.371
LDL-cholesterol (mg/dL)	93.4±30.9 ^{a,b)}	94.3±37.4 ^{a)}	88.6±29.8 ^{a,b)}	82.5±28.6 ^{b)}	0.043** ^{e)} 0.004** ^{f)}
Triglycerides (mg/dL)	211.3±382.1	199.6±246.2	144.0±68.6	157.5±121.8	0.690
Microalbuminuria (mg/24 hr)	58.0±239.8 ^{a)}	106.5±286.4 ^{a)}	353.7±917.5 ^{b)}	569.8±1103.9 ^{b)}	<0.001** ^{e)} <0.001** ^{f)}

Values are presented as number (%) or mean±standard deviation.

PWV, pulse wave velocity; ABI, ankle brachial index; IMT, intima-media thickness; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

^{a,b)}Same letters indicate statistically non-significant with Tukey’s multiple comparison.

^{c)}p-value of χ^2 test, ^{d)}p-value of χ^2 trend test (between group 1-4) was used when the variables were significant difference in χ^2 test.

^{e)}p-value of ANOVA, ^{f)}p-value of ANOVA trend test (between group 1-4) was used when the variables were significant difference in ANOVA test.

*p<0.05, **p<0.01, statistically significant.

is no uniform criteria, and it is difficult to exclude all other neurogenic disease [22]. Electrophysiologic study has been known as one of the most objective and sensitive tools to detect peripheral nerve dysfunctions [23]. These are widely used for the assessment of DPN, not only to evaluate the degree of abnormality but also to document

serial changes in clinical [24]. Therefore, we use electrophysiologic findings to subgroup the severity.

Severity of DPN was significantly associated with the duration of diabetes, HbA1c, existence of diabetic nephropathy and retinopathy, PWV, presence of plaque, LDL-cholesterol and MU. Among these variables, HbA1c

Table 4. Ordinal logistic regression analysis by severity of diabetic neuropathy

	OR	95% CI	p-value
Age (yr)	1.01	0.9960-1.0320	0.1182
Sex (female vs. male)	1.00	0.9990-1.0150	0.0777
Diabetes duration (yr)	1.01	0.9990-1.0210	0.0714
HbA1c (%)	1.19	1.0930-1.3010	<0.001**
BMI	0.96	0.9120-1.0160	0.1613
Diabetic retinopathy (yes vs. no)	1.68	1.0760-2.6140	0.0224*
Diabetic nephropathy (yes vs. no)	2.04	1.0060-4.1500	0.0481*
PWV (abnormal vs. normal)	1.33	0.8200-2.1440	0.2507
Plaque (yes vs. no)	2.52	1.5300-4.1380	<0.001**
LDL-cholesterol (mg/dL)	0.99	0.9880-1.0020	0.1643
Microalbuminuria (mg/24 hr)	1.00	1.0000-1.0010	0.0264*

BMI, body mass index; PWV, pulse wave velocity; LDL, low-density lipoprotein; OR, odds ratio; CI, confidence interval.

* $p < 0.05$, ** $p < 0.01$, statistically significant.

and presence of plaque were more significantly related to the severity of DPN in logistic regression analysis, and the presence of plaque showed the highest odds ratio. In accordance to this, we suggested that not only the metabolic factors, but also vascular factors such as PWV and presence of plaque, could have a significant influence on the severity of DPN. Additionally, the presence of plaque was more strongly associated with the severity of DPN than other variables.

Until now, a lot of research [6,7] has been done on DPN based on pathological mechanisms. While the acceptance hypothesis was based on the metabolic hypothesis and the recent interest of development of neuropathy involving neural tissue ischemia increased, more research was conducted in experimental animals [7,8,25]. Endoneurium microvessel structural abnormalities, accompanied by hemostatic function abnormalities, nitrogen oxide compounds that originates from the blood vessel endothelium, and prostacyclin that originates from prostaglandin metabolism, are known as a representative cause of neuro-ischemic hypothesis that induce DPN [7,26]. However, it is still controversial which metabolic dysfunctions or angiopathies affect the development of DPN more [27].

Arterial stiffness and/or thickness are sensitive markers indicative of decreased arterial elasticity and vascular wall injury [28]. Kim et al. [29] revealed that arterial stiffness and thickness increase in diabetic patients with peripheral neuropathy, as compared without peripheral

neuropathy. However, the underlying mechanism linking peripheral neuropathy in diabetic patients to arterial stiffness and thickness, is not well understood. One possible explanation is that large artery stiffness may cause microvascular damage via high pulse pressure, leading to diminished blood flow to nerve tissues vulnerable to hypoxic damage, and thereby to the development of neuropathy [29]. Atherosclerosis is a multifactorial disease frequently involving the entire arterial system. Dysfunction of the arterial system would affect impaired blood flow and endoneurial hypoxia, which are considered to play a major role in causing DPN in human and animal models [6,25].

Duration of diabetes and HbA1c level showed significant differences and trend among the four subgroups. Depending on the metabolic hypothesis, the occurrence of DPN is related to metabolic disorders in accordance to hyperglycemia; this was confirmed by the fact that it influences the DPN aggravation [2,30]. In addition, other diabetic complications, such as nephropathy and retinopathy, also showed significant differences and trend. Because Tesfaye et al. [31] had an assertion that DPN had a close relationship with other diabetic complications, it supported the existence of a common pathological mechanism as being the cause of development of diabetic complications.

The PWV and ABI are considered to be the indicators that reflect the arterial stiffness of the central and peripheral artery [16,18]. The PWV and ABI had significant

differences among the four subgroups. Although PWV showed an apparent trend according to the severity of DPN, ABI did not. With increasing arterial stiffness, arterial vessel wall also thickens, in turn leading to the increased occurrence of plaque outbreak, that contributes to the acceleration of angiopathies and ischemic changes of nerves [32]. Compared to other previous study [33], the duration of diabetes was only 7.5 years in our study which was not long enough to find abnormalities in ABI. In this study, therefore, because the number of abnormality in ABI is noticeably small, the accurate relationship according to severity of DPN cannot be seen. In relation to angiopathies, it can be assumed that chronic ischemia applies to the progression and aggravation of neuropathy [26]. The relationship would have been more evident if the morbidity duration of diabetes was longer.

The presence of plaque in carotid artery showed significant differences and trend. However, with respect to the carotid IMT and existence of stenosis, the results were insignificant. The measurement of carotid IMT is being used as an index for the early detection of atherosclerosis, which is the risk factor for cardiovascular disease [19,34]. Although outbreaks of atherosclerosis occur in intima limitedly, the measurement of IMT measures the blood vessel's overall thickness, and primarily measures the changes of the media, a drawback is still present [35]. In addition, Aminbakhsh et al. [36] reported that the measurement of carotid plaque existence is a better method to diagnose the early stages of atherosclerosis rather than the measurement of the carotid IMT. In another study [37], which assessed the association of risk factors with the occurrence of cardiovascular disease, the presence of plaque had higher correlation than the IMT. It was shown that there was borderline difference in the existence of carotid artery stenosis. It was not based on the stenosis percentage, but based on whether or not stenosis was present; however, an accurate relationship could not be seen.

The MU showed significant differences and trend. As in the previous studies, by analyzing the MU seen in early diabetic nephropathy, the correlation between DPN and diabetic nephropathy was confirmed [38]. In addition, because MU is being reflected in systemic blood vessel dysfunctions and signifies subclinical vascular damage in the kidneys or vascular beds, it can be one of the risk factors of cardiovascular disease [39].

Being a means of the blood LDL-cholesterol's primary cholesterol transmittance, it is an affiliated index of cardiovascular risk. For this reason, it is strictly adjusted to a normal value in diabetic patients. In this study, only LDL-cholesterol of cholesterol factors showed significant differences and trend. Because the occurrence of cardiovascular disease is due to the delivery of lipoproteins rather than simply the increase in triglyceride or cholesterol levels [40], it can be predicted with combined index of lipoproteins involved in delivering lipid. Therefore, it cannot be precisely said that there is a relationship between the DPN and simple cholesterol level.

In ordinal logistic regression, it was verified that factors such as HbA1c, diabetic nephropathy and retinopathy, presence of plaque and MU have a higher possibility of increasing the DPN severity, as compared to other factors. Tests to identify the presence of plaque revealed the correlation with atherosclerosis, and it is thus a sensitive method for diagnosing early atherosclerosis [36,37]. The MU reflects the dysfunction of the blood vessels throughout the body and systemic endothelial dysfunction, since it points to subclinical vascular damage in the kidneys or other vascular beds [39]. Accordingly, these two variables are seen to reflect the vascular disorder of the whole body. In particular, in terms of patients who have carotid plaque in comparison to patients who do not, it was shown that the risk of increasing severity of DPN was 2.52 times higher than other factors. With the highest odds ratio, it can be assumed that the existence of carotid plaque in comparison to other factors greatly contributes to the DPN's severity. From this, it is suggested that vascular factors, along with metabolic factors, can influence the DPN pathophysiology. Therefore, patients having DPN must actively manage not only the metabolic factors, but also the vascular factors, in the early stages of DPN.

The patients had a diverse medical history and were taking different medications in this study. Through the limitations of retrospective studies, this cannot fully reflect the patients' medical history and medications. Also, when chronic ischemia occurs in the DPN patients' nerve tissue, further studies, including consistency and change in the index of electrophysiologic findings by severity, are required.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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REFERENCES

1. Orchard TJ, Dorman JS, Maser RE, Becker DJ, Drash AL, Ellis D, et al. Prevalence of complications in IDDM by sex and duration: Pittsburgh Epidemiology of Diabetes Complications Study II. *Diabetes* 1990;39:1116-24.
2. Maser RE, Steenkiste AR, Dorman JS, Nielsen VK, Bass EB, Manjoo Q, et al. Epidemiological correlates of diabetic neuropathy: report from Pittsburgh Epidemiology of Diabetes Complications Study. *Diabetes* 1989;38:1456-61.
3. The Diabetes Control and Complications Trial Research Group. The effect of intensive diabetes therapy on the development and progression of neuropathy. *Ann Intern Med* 1995;122:561-8.
4. Park GY, Park JH, Lee SE, Kang HK, Chung ME, Seong NS. The effect of peripheral vascular disease on diabetic neuropathy. *J Korean Acad Rehabil Med* 2006;30:25-32.
5. Pyorala K, Laakso M, Uusitupa M. Diabetes and atherosclerosis: an epidemiologic view. *Diabetes Metab Rev* 1987;3:463-524.
6. Cameron NE, Eaton SE, Cotter MA, Tesfaye S. Vascular factors and metabolic interactions in the pathogenesis of diabetic neuropathy. *Diabetologia* 2001;44:1973-88.
7. Nukada H. Mild ischemia causes severe pathological changes in experimental diabetic nerve. *Muscle Nerve* 1992;15:1116-22.
8. Nukada H, Dyck PJ, Low PA, Lais AC, Sparks MF. Axonal caliber and neurofilaments are proportionately decreased in galactose neuropathy. *J Neuropathol Exp Neurol* 1986;45:140-50.
9. Malik RA, Masson EA, Sharma AK, Lye RH, Ah-See AK, Compton AM, et al. Hypoxic neuropathy: relevance to human diabetic neuropathy. *Diabetologia* 1990;33:311-8.
10. Teunissen LL, Notermans NC, Wokke JH. Relationship between ischemia and neuropathy. *Eur Neurol* 2000;44:1-7.
11. Cameron NE, Cotter MA. Metabolic and vascular factors in the pathogenesis of diabetic neuropathy. *Diabetes* 1997;46 Suppl 2:S31-7.
12. Stevens MJ, Feldman EL, Greene DA. The aetiology of diabetic neuropathy: the combined roles of metabolic and vascular defects. *Diabet Med* 1995;12:566-79.
13. Dyck PJ, Thomas PK. *Diabetic neuropathy*. 2nd ed. Philadelphia: Saunders; 1999.
14. Kwon HK, Kim L, Park YK, Lee HJ. Frequency of carpal tunnel syndrome according to the severity of diabetic neuropathy. *J Korean Acad Rehabil Med* 2005;29:272-5.
15. Park EM, Kim SJ, Yoon JS. Correlation between the severity of neuropathy and microalbuminuria in patients with diabetes mellitus. *J Korean Acad Rehabil Med* 2002;26:555-61.
16. Murphy TP, Dhangana R, Pencina MJ, D'Agostino RB Sr. Ankle-brachial index and cardiovascular risk prediction: an analysis of 11,594 individuals with 10-year follow-up. *Atherosclerosis* 2012;220:160-7.
17. Potier L, Abi Khalil C, Mohammedi K, Roussel R. Use and utility of ankle brachial index in patients with diabetes. *Eur J Vasc Endovasc Surg* 2011;41:110-6.
18. Munakata M. Brachial-ankle pulse wave velocity in the measurement of arterial stiffness: recent evidence and clinical applications. *Curr Hypertens Rev* 2014;10:49-57.
19. Johnsen SH, Mathiesen EB. Carotid plaque compared with intima-media thickness as a predictor of coronary and cerebrovascular disease. *Curr Cardiol Rep* 2009;11:21-7.
20. Tomiyama H, Yamashina A, Arai T, Hirose K, Koji Y, Chikamori T, et al. Influences of age and gender on results of noninvasive brachial-ankle pulse wave velocity measurement: a survey of 12517 subjects. *Atherosclerosis* 2003;166:303-9.
21. Xu D, Zou L, Xing Y, Hou L, Wei Y, Zhang J, et al. Diagnostic value of ankle-brachial index in peripheral arterial disease: a meta-analysis. *Can J Cardiol* 2013;29:492-8.
22. Kakrani AL, Gokhale VS, Vohra KV, Chaudhary N.

- Clinical and nerve conduction study correlation in patients of diabetic neuropathy. *J Assoc Physicians India* 2014;62:24-7.
23. Chaudhry V, Corse AM, Freimer ML, Glass JD, Mellits ED, Kuncel RW, et al. Inter- and intraexaminer reliability of nerve conduction measurements in patients with diabetic neuropathy. *Neurology* 1994;44:1459-62.
 24. Bertora P, Valla P, Dezuanni E, Osio M, Mantica D, Bevilacqua M, et al. Prevalence of subclinical neuropathy in diabetic patients: assessment by study of conduction velocity distribution within motor and sensory nerve fibres. *J Neurol* 1998;245:81-6.
 25. Hendriksen PH, Oey PL, Wieneke GH, van Huffelen AC, Gispen WH. Hypoxic neuropathy versus diabetic neuropathy: an electrophysiological study in rats. *J Neurol Sci* 1992;110:99-106.
 26. Tesfaye S, Malik R, Ward JD. Vascular factors in diabetic neuropathy. *Diabetologia* 1994;37:847-54.
 27. Kato Y, Kamiya H, Nakamura J. Diabetic neuropathy. *Nihon Rinsho* 2015;73:495-500.
 28. Yokoyama H, Yokota Y, Tada J, Kanno S. Diabetic neuropathy is closely associated with arterial stiffening and thickness in type 2 diabetes. *Diabet Med* 2007;24:1329-35.
 29. Kim ES, Moon SD, Kim HS, Lim DJ, Cho JH, Kwon HS, et al. Diabetic peripheral neuropathy is associated with increased arterial stiffness without changes in carotid intima-media thickness in type 2 diabetes. *Diabetes Care* 2011;34:1403-5.
 30. Simmons Z, Feldman EL. Update on diabetic neuropathy. *Curr Opin Neurol* 2002;15:595-603.
 31. Tesfaye S, Stevens LK, Stephenson JM, Fuller JH, Plater M, Ionescu-Tirgoviste C, et al. Prevalence of diabetic peripheral neuropathy and its relation to glycaemic control and potential risk factors: the EURODIAB IDDM Complications Study. *Diabetologia* 1996;39:1377-84.
 32. Yambe M, Tomiyama H, Hirayama Y, Gulniza Z, Takata Y, Koji Y, et al. Arterial stiffening as a possible risk factor for both atherosclerosis and diastolic heart failure. *Hypertens Res* 2004;27:625-31.
 33. Li Q, Zeng H, Liu F, Shen J, Li L, Zhao J, et al. High ankle-brachial index indicates cardiovascular and peripheral arterial disease in patients with type 2 diabetes. *Angiology* 2015;66:918-24.
 34. Cardoso CR, Marques CE, Leite NC, Salles GF. Factors associated with carotid intima-media thickness and carotid plaques in type 2 diabetic patients. *J Hypertens* 2012;30:940-7.
 35. Adams MR, Nakagomi A, Keech A, Robinson J, McCredie R, Bailey BP, et al. Carotid intima-media thickness is only weakly correlated with the extent and severity of coronary artery disease. *Circulation* 1995;92:2127-34.
 36. Aminbakhsh A, Frohlich J, Mancini GB. Detection of early atherosclerosis with B mode carotid ultrasonography: assessment of a new quantitative approach. *Clin Invest Med* 1999;22:265-74.
 37. Martinsson A, Ostling G, Persson M, Sundquist K, Andersson C, Melander O, et al. Carotid plaque, intima-media thickness, and incident aortic stenosis: a prospective cohort study. *Arterioscler Thromb Vasc Biol* 2014;34:2343-8.
 38. Patel KL, Mhetras SB, Varthakavi PK, Merchant PC, Nihalani KD. Microalbuminuria in non-insulin dependent diabetes mellitus. *J Assoc Physicians India* 1999;47:596-601.
 39. Weir MR. Microalbuminuria and cardiovascular disease. *Clin J Am Soc Nephrol* 2007;2:581-90.
 40. Birtcher KK, Ballantyne CM. Measurement of cholesterol: a patient perspective. *Circulation* 2004;110:e296-7.