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ORIGINAL ARTICLE

Neuroretinal dysfunction revealed by a flicker electroretinogram correlated with peripheral nerve dysfunction and parameters of atherosclerosis in patients with diabetes

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Keywords
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ABSTRACT

Aims/Introduction: Diabetic polyneuropathy (DPN) develops in the early stage of diabetes. However, no common diagnostic protocol has yet been established. Here, to verify that the flicker electroretinogram using a hand-held device can detect the early dysfunction of the peripheral nervous system in patients with diabetes, we investigated the correlation between the progression of DPN and neuroretinal dysfunction.

Materials and Methods: In total, 184 participants with type 1 or 2 diabetes underwent a flicker electroretinogram (ERG) using a hand-held device RETeval™ and nerve conduction study. Participants were also evaluated for intima-media thickness, ankle-brachial index, toe brachial index and brachial-ankle pulse wave velocity. Parameters of the nerve conduction study were used to diagnose the severity according to Baba's classification. A multiple regression analysis was used to examine the associations of ERG parameters with the severity of DPN categorized by Baba's classification. Diagnostic properties of the device in DPN were evaluated using a receiver operating characteristic curve.

Results: A multiple regression model to predict the severity of DPN was generated using ERG. In the model, moderate-to-severe DPN was effectively diagnosed (area under the receiver operating characteristic curve 0.692, sensitivity 56.5%, specificity 78.3%, positive predictive value 70.6%, negative predictive value 66.1%, positive likelihood ratio 2.60, negative likelihood ratio 0.56). In the patients without diabetic retinopathy, the implicit time and amplitude in ERG significantly correlated with the parameters of the nerve conduction study, brachial-ankle pulse wave velocity and intima-media thickness.

Conclusions: Electroretinogram parameters obtained by the hand-held device successfully predict the severity of DPN. The device might be useful to evaluate DPN.

INTRODUCTION

Among chronic diabetic complications, diabetic polyneuropathy (DPN) develops in the early stage of diabetes[1,2]. As more than half of patients with DPN suffer no symptoms at the early stage of DPN[3], DPN develops asymptomatically to an end-stage. The end-stage of DPN increases the risk of ulcer, gangrene and amputation of the foot, which affects the quality of life and prognosis of patients with diabetes. Early detection of DPN is essential to improve the prognosis in patients with diabetes[1,4,5].

A gold standard for the diagnosis of DPN is a nerve conduction study (NCS)[6]. However, as NCS requires expensive equipment and skilled technicians, further diagnostic tools for DPN

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are required. DPN is mainly caused by metabolic stresses induced by a hyperglycemic state, which is an enhancement of polyol pathway, abnormal protein kinase C activities, increased oxidative stress, accumulation of advanced glycation end products and decrease of nitric oxide, resulting in neuronal edema, axonal injury and demyelination[7]. Regarding the other diabetic complication, diabetic retinopathy (DR), which has been conventionally regarded as microangiopathy, it has recently been recognized that retinal neuron and glial cells are dysfunctional from the early stages of the disease. Given the fact that the abnormalities in polyol and protein kinase C pathways could also induce DR[8,9], neurological evaluation could be used to elucidate the pathophysiology of DR. Thus, we hypothesized that evaluation of neuroretinal dysfunction would allow early detection of neurological impairments in patients with diabetes.

The electroretinogram (ERG) is one of the methods for objectively evaluating retinal functions. In the retina, light-stimulated photoreceptors generate electrical impulses, causing potential fluctuations in other retinal cells, such as bipolar cells, horizontal cells, amacrine cells and ganglion cells. The ERG is a comprehensive recording of this potential fluctuation. It is possible to separate the cone or rod responses by changing the intensity and frequency of the light stimulus. Flicker stimuli can isolate the cone response using fast blinking stimuli that trigger no response of rods. Conventional ERG requires contact lens electrodes to be worn after mydriasis, dark adaptation and corneal anesthesia, so that there is a risk of corneal disorder and infection. Additionally, the ERG requires trained examiners. The RETeval™ (LKC Technologies, Gaithersburg, MD, USA) is a flicker ERG device designed to permit a point-of-care testing of ERG. As the amount of light applied by the device is automatically adjusted according to the pupil diameter, mydriasis and dark adaptation are dispensable. The electrode is attached to the skin of the lower eyelid, in contrast to the contact lens electrode. Although the ERG recording using the skin electrodes is prone to background interference, the inspection procedure is simple and can be carried out within 1 min with both eyes. The moderate-to-high reproducibility of RETeval has been reported[10]. The point-of-care device weighs just 240 g, is battery-powered and can be used at the bedside, making it highly versatile.

The waveforms recorded by RETeval are mainly derived from bipolar cells connecting to cones, which are secondary neurons[11]. Decreased amplitude; that is, decreased activity of cones, has been reported in the early stages of DR[12]. Furthermore, the correlation between DR severity and RETeval data has also been reported in patients with diabetes[13].

To evaluate DR, ophthalmologists describe findings of microangiopathy, but not neurological abnormalities. However, the flicker ERG device is good at assessing neurological functions. In the present study, therefore, we investigated whether the progression of DPN and neuroretinal dysfunction are correlated, clarifying the contribution of neuroretinal dysfunction in changes of ERG.

METHODS

Eligibility criteria

From 2014 to 2019, 184 participants who were previously diagnosed with diabetes and hospitalized at Aichi Medical University Hospital to improve their hyperglycemia were invited to participate in the present study. All participants signed a document of consent for the study. Patients were excluded if they had a history of intraocular pressure >22 mmHg. Patient characteristics were assessed by the following items: physical findings, sociodemographic information and laboratory measurements, including serum creatinine, serum urea nitrogen, urinary albumin-to-creatinine ratio, fasting blood glucose, glycoalbumin, glycosylated hemoglobin, high-density lipoprotein, low-density lipoprotein and triglyceride. The estimated glomerular filtration rate (eGFR) was calculated using the equations for eGFR developed by the Japanese Society of Nephrology[14]. Carotid intima-media thickness (IMT), plaque prevalence at the carotid artery, ankle-brachial index, toe brachial index and brachial-ankle pulse wave velocity were evaluated. Ophthalmologic examination to evaluate DR was carried out by ophthalmologists. DR was classified according to the International Clinical Classification System for Diabetic Retinopathy and Diabetic Macular Edema[15], which categorizes DR as no apparent, non-proliferative and proliferative retinopathy. The present study protocol was approved by the ethics committee of Aichi Medical University Hospital (NO. 14-019) and registered with the University Hospital Medical Information Network (UMIN ID: 000021916). The study was carried out in accordance with the Declaration of Helsinki.

ERG

The patients underwent ERG using a flicker ERG testing device, RETeval™, without mydriasis. A sensor firmly attached to the skin just below the lower eyelid was used to record the ERG. The ERG waves were elicited by white light at a frequency of 28.3 Hz and intensity of 8 troland-seconds, which was the default setting of the device. The contralateral eye was covered during the examination of the other eye. During the examination, the patient was instructed not to move the eye. The values of implicit times and amplitudes were automatically displayed on the device.

Coefficient of variation of RR intervals

The coefficient of variation of RR intervals ($CV_{R\text{-}R}$) was measured, as previously reported[16]. To analyze the $CV_{R\text{-}R}$, 1-min electrocardiogram recordings were collected with normal or deep breathing six times per 1 min in the supine position after 5 min of bed rest. The $CV_{R\text{-}R}$ was calculated using the following formula: $CV_{R\text{-}R}$ (%) = (standard deviation of RR intervals) / (mean RR intervals) × 100.

Diagnosis of DPN and classification of DPN severity

Neuropathic symptoms as a result of DPN were determined through a screening questionnaire based on the following

symptoms: bilateral pain, numbness, paresthesia or decreased sensation in the toes and soles of feet. Neurological signs were assessed using physical examinations; for example, ankle tendon reflexes and measuring vibration sensation threshold with a tuning fork. Neurological dysfunction of the peripheral nervous system was screened using the simple diagnostic criteria proposed by the Diabetic Neuropathy Study Group in Japan, as previously described[17]. The criteria consist of two prerequisite items and three neurological examination items. The prerequisite condition includes two items: (i) diagnosed as diabetes; and (ii) other peripheral neuropathies than DPN can be excluded. The criteria require any two or more of the following three items: (i) the presence of symptoms considered to be due to DPN; (ii) the decrease or disappearance of bilateral ankle tendon reflexes; and (iii) decreased vibration sensation threshold in the bilateral medial malleoli. Additionally, the criteria include important references in which, even if the aforementioned criteria are not met, if either one of the following items is met, DPN can be diagnosed: (i) the presence of any abnormality in two or more nerves in the NCS; and (ii) the presence of clinically apparent diabetic autonomic dysfunction. However, in the protocol of the present study, the two reference items were not applied due to the lack of normal limits in each nerve conduction parameter and the lack of definitions of autonomic dysfunction.

The NCS was carried out using an electromyography system (Neuropack X1, MEB-2312; Nihon Kohden, Tokyo, Japan). The skin temperature at the ankle was measured, and, when the temperature was <32°C, the foot was warmed with a hot towel before starting the examination. The NCS was carried out on the ulnar, median, tibial and sural nerves. The clinical status of each participant was concealed from all examiners. Parameters of NCS were used to categorize DPN from stages 0 to 4 based on Baba's classification on the severity of DPN (BC)[18]. In brief, participants were categorized into five stages: (i) normal without any NCS abnormalities (stage 0); (ii) mild neuropathy with the presence of any delay in sural nerve conduction velocity (<40 m/s), tibial motor nerve conduction velocity (<40 m/s) and the presence of A wave or tibial minimal F wave latency (>{12.8 + 0.22 × height (cm)} ms; stage 1); (iii) moderate neuropathy with a decrease in sural sensory nerve action potential (SNAP) amplitude (<5 μV; stage 2); (iv) between moderate-to-severe neuropathy with a decrease in tibial compound muscle action potential (CMAP) amplitude (≥2 to <5 mV) and decrease in sural SNAP amplitude (<5 μV; stage 3); and (v) severe neuropathy with a decrease in tibial CMAP amplitude (<2 mV) and decrease in sural SNAP amplitude (<5 μV; stage 4).

Statistical analysis

SPSS Statistics version 20 for Windows (IBM SPSS, Chicago, IL, USA) was used for data analyses. Characteristics including the mean values of age, sex, data of chemical laboratories, neurophysiological findings, EMG parameters and parameters of NCS were presented as raw data. The χ^2-test with Yates' correction and Student's *t*-test were utilized for analyses of differences in categorical and continuous variables, respectively. Welch's ANOVA and Games-Howell post-hoc test were used for multiple comparisons. The correlation between ERG parameters and other values were analyzed using Spearman's correlation coefficient for non-normally distributed data and Pearson's correlation coefficient for normally distributed data. The implicit time and amplitude in ERG were incorporated into the multiple regression models to create an effective prediction model of DPN. Analyses using a receiver operating characteristic (ROC) curve and the area under the ROC curve (AUROC) were used to evaluate diagnostic validity of the models.

RESULTS

Participant characteristics

The clinical characteristics of the participants are presented in Table 1. In total, 184 patients were included in the analyses (sex: male $n = 119$, female $n = 65$, types of diabetes: type 1 $n = 20$, type 2 $n = 164$). Based on the International Clinical Classification System, 15.8% of the patients ($n = 29$) were classified as non-proliferative DR, and 14.1% of the patients ($n = 26$) were classified as proliferative DR.

Correlation of DR severity and clinical parameters

For correlation analysis, no apparent, non-proliferative and proliferative retinopathies were assigned values of 0, 1 and 2, respectively. DR progression showed significant correlations with the following parameters: duration of diabetes, urinary C-peptides, eGFR, urinary albumin-to-creatinine ratio, the simple diagnostic criteria (0: without DPN, 1: with DPN), the amplitude of the sural SNAP, minimal F wave latency, sural sensory conduction velocity, tibial motor nerve conduction velocity, the amplitude of tibial CMAP, the severity of DPN in BC (stage 0–4), resting and deep breathing $CV_{R\text{-}R}$, baPWV, and the implicit time and amplitude of ERG (Table 2).

Correlation of ERG and clinical parameters in patients without DR

To elucidate the relationships between the dysfunction of neuroretina and DPN, patients without apparent DR were analyzed. In the patients without DR, the implicit time in ERG significantly correlated with duration of diabetes, eGFR, the simple diagnostic criteria (0: without DPN, 1: with DPN), the amplitude of sural SNAP, sural sensory conduction velocity, tibial motor nerve conduction velocity, the severity of DPN in BC (stage 0–4), baPWV and IMT (Table 3). The amplitude in ERG significantly correlated with the duration of diabetes, low-density lipoprotein, eGFR, the simple diagnostic criteria (0: without DPN, 1: with DPN), the amplitude of sural SNAP, the amplitude of tibial CMAP, baPWV and IMT (Table 3).

Estimation of the severity of DPN using ERG data

To examine whether ERG assists a diagnosis of DPN, which is difficult to objectively assess in clinical practice, we evaluated

Table 1 | Participant characteristics

Stage of DR	Total	No apparent	Non-proliferative	Proliferative
n	184	129	29	26
Age (years)	60.0 ± 14.3	58.5 ± 13.9	65.0 ± 14.0	61.2 ± 14.0
Male (%)	64.7%	67.4%	58.6%	57.7%
Duration of diabetes (years)	10.5 ± 11.0	8.4 ± 10.0§§	13.7 ± 11.7	17.5 ± 13.0**
BMI (kg/m^2)	25.0 ± 5.4	24.4 ± 4.8	26.7 ± 6.6	26.1 ± 5.4
sBP (mmHg)	127.4 ± 21.1	124.4 ± 20.3	134.0 ± 20.0	135.4 ± 23.3
dBP (mmHg)	75.6 ± 13.7	75.1 ± 13.2	77.0 ± 15.7	76.3 ± 13.7
Glucose (mg/dL)	212.5 ± 126.5	215.4 ± 138.1	191.6 ± 71.5	221.5 ± 126.5
HbA1c (%)	10.2 ± 2.3	10.2 ± 2.5	9.8 ± 1.7	10.3 ± 2.3
Glycoalbumin (%)	27.5 ± 10.0	27.9 ± 10.5	27.1 ± 8.3	26.3 ± 9.2
Cre (mg/dL)	0.9 ± 1.3	0.9 ± 1.6§	0.7 ± 0.3	1.2 ± 0.7†
eGFR (mL/min/1.73 m^2)	81.6 ± 32.6	83.6 ± 25.5§	88.7 ± 48.1	63.6 ± 38.1*
uACR (mg/g)	219.1 ± 679.0	58.6 ± 162.6§§	175.9 ± 631.9§	1044.9 ± 1378.0**,†
ln (uACR)	1.4 ± 0.8	1.2 ± 0.6§§	1.4 ± 0.6§	2.4 ± 1.0**,†
uCPR (μg/day)	64.1 ± 58.0	70.9 ± 61.8§§§	56.8 ± 52.8	39.2 ± 33.4***
TC (mg/dL)	189.6 ± 44.3	187.6 ± 43.9	180.9 ± 37.0	209.0 ± 49.3
HDL (mg/dL)	46.3 ± 14.2	45.6 ± 13.8	50.1 ± 18.9	45.4 ± 9.4
LDL (mg/dL)	110.4 ± 33.8	111.0 ± 33.4	107.1 ± 32.3	110.5 ± 38.2
TG (mg/dL)	178.3 ± 147.4	175.6 ± 162.0	156.9 ± 87.6	216 ± 114.6
IMT mean (mm)	1.10 ± 0.46	1.07 ± 0.45	1.20 ± 0.54	1.13 ± 0.40
IMT max (mm)	1.66 ± 0.92	1.61 ± 0.92	1.76 ± 1.09	1.92 ± 0.72
ABI	1.13 ± 0.12	1.12 ± 0.12	1.12 ± 0.13	1.17 ± 0.14
TBI	0.72 ± 0.19	0.72 ± 0.21	0.74 ± 0.13	0.71 ± 0.14
baPWV	1578.0 ± 359.7	1508.7 ± 328.6†,§§	1709.2 ± 360.3*	1777.2 ± 404.3**
Tibial nerve				
NCV (m/s)	39,3 ± 10.6	40.7 ± 9.5	35.8 ± 15.0	36.5 ± 8.8
Amplitude (mV)	13.7 ± 7.7	15.7 ± 7.3†††,§§§	9.7 ± 6.4***	8.1 ± 6.3***
F wave latency (ms)	49.5 ± 5.7	48.7 ± 5.3§§§	50.0 ± 6.8	54.0 ± 5.1***
Sural nerve				
NCV (m/s)	46.1 ± 7.5	47.4 ± 5.4§	43.6 ± 10.4	40.4 ± 11.2*
Amplitude (μV)	8.8 ± 6.9	10.0 ± 7.1†,§§§	6.3 ± 5.8*	4.2 ± 2.9***
$CV_{R\text{-}R}$, resting (%)	2.7 ± 1.7	3.0 ± 1.8§§§	2.6 ± 1.0§	1.7 ± 1.3***,†
$CV_{R\text{-}R}$, deep breathing (%)	4.5 ± 2.4	4.9 ± 2.5§§§	4.5 ± 1.6§	2.5 ± 1.1***,†
ERG implicit time (ms)	35.2 ± 4.5	34.5 ± 4.8§§§	35.8 ± 2.7§§§	38.1 ± 3.4***,†††
ERG amplitudes (μV)	4.8 ± 2.1	5.3 ± 2.1§§§	4.5 ± 1.6§§	3.1 ± 1.7***,††

Categorical variables are given as the number (percentage), whereas continuous variables are reported as the mean ± standard deviation.
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ versus no apparent retinopathy. $^{†}P < 0.05$; $^{††}P < 0.01$; $^{†††}P < 0.001$ versus non-proliferative retinopathy.
$^{§}P < 0.05$; $^{§§}P < 0.01$; $^{§§§}P < 0.001$ versus proliferative retinopathy. ABI, ankle-brachial index; baPWV, brachial-ankle pulse wave velocity; BMI, body mass index; $CV_{R\text{-}R}$, coefficient of variation of R-R intervals; dBP, diastolic blood pressure; DR, diabetic retinopathy; eGFR, estimated glomerular filtration rate; ERG, electroretinogram; HbA1c, glycosylated hemoglobin; HDL, high-density lipoprotein; IMT, carotid intima-media thickness; ln, natural logarithm; LDL, low-density lipoprotein; max, maximum; NCV, nerve conduction velocity; sBP, systolic blood pressure; TBI, toe brachial index; TG, triglyceride; uACR, urine albumin-to-creatinine ratio; uCPR, urinary C-peptides.

the correspondence of ERG data to the stages of DPN diagnosed using BC. In a multiple regression analysis, using the stage numbers of BC as the dependent variable, and age, the amplitudes in ERG and the implicit time in ERG as independent variables, the estimated severity in BC (eBC) was obtained as follows: eBC = −1.296 + 0.081 × implicit time (ms) − 0.122 × amplitude (μV) + 0.007 × age (years), $r = 0.374$ (Table 4).

Receiver operating characteristic analysis was used to identify the optimal cut-off on the eBC to categorize participants as having stage 2–4 versus stage 0 or 1 DPN. The eBC showed a mild discriminative power (AUROC 0.692; Figure 1. The cut-off value with maximum accuracy was 1.5216 of the eBC (sensitivity 56.5%, specificity 78.3%, positive predictive value 70.6%, negative predictive value 66.1%, positive likelihood ratio 2.60, negative likelihood ratio 0.56). ROC analyses using the eBC to categorize participants as having nerve conduction dysfunction were also carried out to understand the utility of ERG parameters. As representative parameters, abnormalities in tibial minimal F wave latency (>{12.8 + 0.22 × height (cm)} ms) and

Table 2 | Correlation of diabetic retinopathy severity and clinical parameters

	Correlation coefficient
Duration of diabetes (years)	0.281**
BMI (kg/m^2)	0.125
sBP (mmHg)	0.240**
dBP (mmHg)	0.051
Glucose (mg/dL)	0.049
HbA1c (%)	−0.025
Glycoalbumin (%)	−0.016
Cre (mg/dL)	0.145*
eGFR (mL/min/1.73 m^2)	−0.196**
uACR (mg/g)	0.365**
ln (uACR)	0.362**
uCPR (μg/day)	−0.191**
TC (mg/dL)	0.104
HDL (mg/dL)	0.065
LDL (mg/dL)	−0.028
TG (mg/dL)	0.143
ln (TG)	0.143
IMT mean (mm)	0.124
IMT max (mm)	0.140
ABI	0.060
TBI	0.030
baPWV	0.311**
Tibial nerve	
NCV (m/s)	−0.305**
Amplitude (mV)	−0.406**
F wave latency (ms)	0.278**
Sural nerve	
NCV (m/s)	−0.259**
Amplitude (μV)	−0.324**
CV_{R-R}, resting (%)	−0.265**
CV_{R-R}, deep breathing (%)	−0.306**
Severity of DPN in BC	0.435**
Simple diagnostic criteria	0.315**
ERG implicit time (ms)	0.340**
ERG amplitude (μV)	−0.355**

ABI, ankle-brachial index; baPWV, brachial-ankle pulse wave velocity; BC, Baba's classification on the severity of DPN; BMI, body mass index; CV_{R-R}, coefficient of variation of R-R intervals; dBP, diastolic blood pressure; DR, diabetic retinopathy; eGFR, estimated glomerular filtration rate; ERG, electroretinogram; HbA1c, glycosylated hemoglobin; HDL, high-density lipoprotein; IMT, carotid intima-media thickness; ln, natural logarithm; LDL, low-density lipoprotein; max, maximum; NCV, nerve conduction velocity; sBP, systolic blood pressure; TBI, toe brachial index; TC, total cholesterol; TG, triglyceride; uACR, urine albumin-to-creatinine ratio; uCPR, urinary C-peptides. *$P < 0.05$. **$P < 0.01$.

Table 3 | Correlation of electroretinogram and clinical parameters in patients without diabetic retinopathy

	ERG implicit time	ERG amplitude
Duration of diabetes (years)	0.229**	−0.314**
BMI (kg/m^2)	−0.106	0.103
sBP (mmHg)	−0.004	0.033
dBP (mmHg)	−0.124	0.135
Glucose (mg/dL)	−0.017	0.054
HbA1c (%)	−0.133	0.138
Glycoalbumin (%)	0.072	0.048
Cre (mg/dL)	0.099	−0.192*
eGFR (mL/min/1.73 m^2)	−0.204*	0.235**
uACR (mg/g)	0.114	−0.063
ln (uACR)	0.123	−0.002
uCPR (μg/day)	−0.143	0.150
TC (mg/dL)	−0.077	0.171
HDL (mg/dL)	−0.115	−0.049
LDL (mg/dL)	−0.024	0.196*
TG (mg/dL)	−0.008	0.073
ln (TG)	0.024	0.025
IMT mean (mm)	0.226*	−0.203*
IMT max (mm)	0.115	−0.188*
ABI	−0.054	0.129
TBI	−0.161	0.169
baPWV	0.220*	−0.278**
Tibial nerve		
NCV (m/s)	0.252**	0.158
Amplitude (mV)	−0.158	0.251**
F wave latency (ms)	0.171	−0.030
Sural nerve		
NCV (m/s)	−0.136	0.082
Amplitude (μV)	−0.286**	0.224*
CV_{R-R}, resting (%)	0.023	−0.023
CV_{R-R}, deep breathing (%)	0.258	0.070
Severity of DPN in BC	0.204*	−0.131
Simple diagnostic criteria	0.342**	−0.221*

ABI, ankle-brachial index; baPWV, brachial-ankle pulse wave velocity; BC, Baba's classification on the severity of DPN; BMI, body mass index; CV_{R-R}, coefficient of variation of R-R intervals; dBP, diastolic blood pressure; DR, diabetic retinopathy; eGFR, estimated glomerular filtration rate; ERG, electroretinogram; HbA1c, glycosylated hemoglobin; HDL, high-density lipoprotein; IMT, carotid intima-media thickness; ln, natural logarithm; LDL, low-density lipoprotein; max, maximum; NCV, nerve conduction velocity; sBP, systolic blood pressure; TBI, toe brachial index; TC, total cholesterol; TG, triglyceride; uACR, urine albumin-to-creatinine ratio; uCPR, urinary C-peptides. *$P < 0.05$. **$P < 0.01$.

sural SNAP amplitude (<5 μV) were analyzed. The AUROC for detecting abnormal sural SNAP amplitude was 0.725 (Figure S1a), and that for abnormal tibial minimal F wave latency was 0.600 (Figure S1b).

In patients without apparent retinopathy, multiple regression analysis using the same dependent variable and independent variables as aforementioned resulted in the following eBC:

$$eBC = -1.864 + 0.056 \times \text{implicit time (ms)} + 0.003 \times \text{amplitude (}\mu\text{V)} + 0.017 \times \text{age (years)},\ r = 0.314$$

(Table 5).

Receiver operating characteristic analysis to classify DPN showed that the eBC had a mild discriminative power to predict DPN (AUROC 0.668; Figure 2). The cut-off value with maximum accuracy was 1.146 of the eBC (sensitivity 66.0%, specificity 63.8%, positive predictive value 51.7%, negative predictive value 76.1%, positive likelihood ratio 1.82, negative likelihood ratio 0.534).

Table 4 | Multiple regression analysis to predict the severity of diabetic polyneuropathy using Baba's classification with electroretinogram (in total patients)

	Coefficients		*P*-value	Standardized coefficients
		95% CI		
(Constant)	−1.296	−3.558 to 0.966	0.260	
ERG implicit time (ms)	0.081	0.022 to 0.141	0.007	0.197
ERG amplitude (μV)	−0.122	−0.201 to − 0.043	0.003	−0.225
Age (years)	0.007	−0.004 to − 0.018	0.207	0.093

CI, confidence interval.

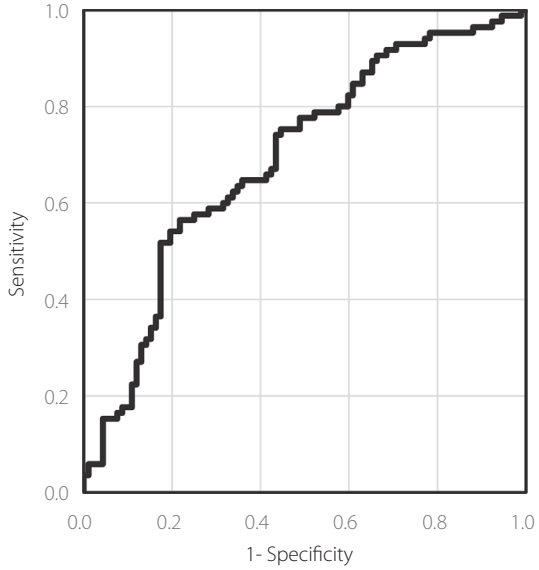

Figure 1 | Receiver operating characteristic curve validating the diagnostic potential of the estimated severity in modified Baba's classification to predict stage 2 or more diabetic polyneuropathy in patients with or without diabetic retinopathy.

DISCUSSION

The current study demonstrated three findings. First, the progression of DR and the dysfunction of neuroretina evaluated using the mydriasis-free flicker ERG showed a significant correlation. Second, even in patients without apparent DR, the ERG data correlated with parameters indicating vascular dysfunction, such as IMT and baPWV, and with parameters showing DPN, such as NCS data . Therefore, the ERG data might reflect the neural and vascular impairments of the retina in patients with diabetes. Third, utilizing multiple regression analysis and ROC analysis, the ERG data might be able to be used to predict the severity of DPN.

Regarding the first finding, the current data of ERG implicit time were comparable to previous reports[13,19,20]. However, the values of ERG amplitude were lower than the previous studies. The mean value of the amplitudes in the present study was 4.8 ± 2.1 μV, but the values in the previous studies were approximately 15 μV. This difference is likely to have been produced by the difference in the devices used in the current study and previous studies. In the current study, the RETeval device stimulated the retina by white light with an intensity of 8 troland-seconds. In contrast, in the previous studies, the device produced white light with intensity of 16 or 32 troland-seconds. Despite the weaker light stimuli and lower amplitudes in the current study, the diagnostic ability of the ERG was comparable to the abilities of the ERG using stronger light stimuli. Because there is, as yet, no study showing whether weaker or stronger light stimuli are better for evaluating neuroretinal functioning in patients with diabetes, we will compare these two devices in future research.

It has been reported that the retina suffers various types of metabolic stresses in diabetes before developing apparent histological changes of DR[21,22]. Hyperglycemia itself has been mentioned to induce the dysfunction of photoreceptors through oxidative stress and hypoxia[23]. In the current study, however, there was no significant correlation between parameters of hyperglycemia and ERG parameters. Given that implicit time and amplitude of ERG significantly correlated with mean IMT, baPWV and amplitudes of sural SNAP, the dysfunction in ERG might be caused by microvascular impairment in the retinal neurons; for example, bipolar, amacrine and horizontal neurons, and glial cells; that is, Müller cells[21]. Additionally, as it has been reported that the elongation of implicit time is caused by the macula ischemia[24], the dysfunction in ERG should be separately analyzed from the effects of metabolic stress and vascular dysfunction. Further research should investigate to verify the influences of this stress and dysfunction of diabetes on neuroretinal dysfunction.

As the correlation between neuroretinal dysfunction and peripheral nerve conduction dysfunction was confirmed, it was determined whether ERG parameters were able to predict the severity of DPN. The ROC analysis showed significant, but mild, discriminative ability of ERG to diagnose moderate-to-severe DPN. As the diagnostic abilities of clinical assessments for DPN, which include Achilles tendon reflex, vibration perception threshold and physical symptoms, have been little investigated, it is difficult to compare the discriminative ability of ERG with other assessments for DPN. However, the

Table 5 | Multiple regression analysis to predict the severity of diabetic polyneuropathy using Baba's classification with electroretinogram (in patients with no apparent retinopathy)

	Coefficients		*P*-value	Standardized coefficients
		95% CI		
(Constant)	−1.864	−4.822 to 1.095	0.215	
ERG implicit time (ms)	0.056	−0.0272 to 0.139	0.185	0.123
ERG amplitude (μV)	0.003	−0.0851 to 0.0912	0.946	0.006
Age (years)	0.017	0.004 to 0.030	0.008	0.257

CI, confidence intervals; ERG, electroretinogram.

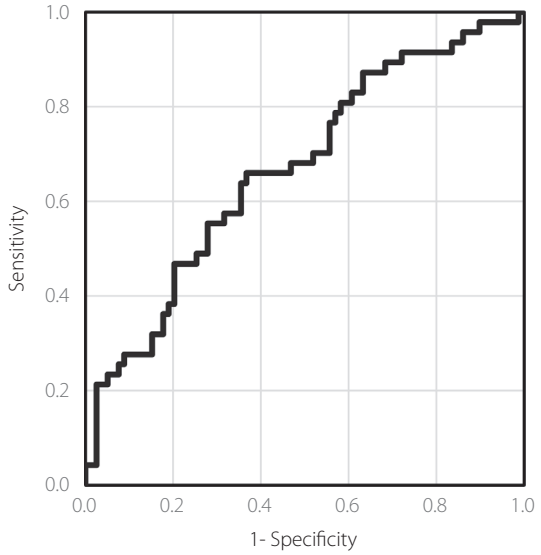

Figure 2 | Receiver operating characteristic curve validating the diagnostic potential of the estimated severity in modified Baba's classification to predict stage 2 or more diabetic polyneuropathy in patients without diabetic retinopathy.

quantitative reproducible examination carried out by the hand-held ERG device would assist the diagnosis of impairments in the peripheral nervous system of patients with diabetes.

There were limitations to the present study. First, as the actual structural changes of the retina were not evaluated in the present study, it is unclear whether the cone dysfunction is caused by functional or structural changes. We believe that this problem can be solved by using optical coherence tomography, which visualizes retinal layers. We will consider further research including optical coherence tomography in the future. Second, as the current study was cross-sectional research, this study was susceptible to bias. Longitudinal research should be designed to confirm the current findings. Third, this study utilized BC to classify the severity of DPN, although the only studies thus far that verify the validity of DPN have been published in Japanese, not in English. As no consensus criteria to evaluate objectively and quantitatively the severity of DPN is available, we believe only the BC could be currently applicable to the evaluation of the severity. Fourth, the participants were under poor glycemic control (10.2% of the average glycosylated hemoglobin) for the reason that they were inpatients aiming to improve glycemic control. It has been reported that delayed implicit time was significantly correlated with increased glycosylated hemoglobin[25]. Therefore, the high levels of blood glucose might affect the ERG parameters. Unfortunately, the participants received only one change of the examination in the current study, further study should be considered to elucidate the influence of blood glucose to the ERG data in the future.

In conclusion, neuroretinal dysfunction that can be evaluated by ERG appears to progress even in patients without DR, and is correlated with the progression of DPN. Although DPN and neuroretinal dysfunction might have similar neuropathological backgrounds, further investigation should be carried out to clarify the relationship in the future.

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DISCLOSURE

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1 | Receiver operating characteristic (ROC) curve.