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ORIGINAL ARTICLE

Deterioration in ankle reflex is associated with a reduced estimated glomerular filtration rate in patients with type 2 diabetes: A retrospective observational cohort study

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ABSTRACT

Aims/Introduction: We investigated the association between the ankle reflex and the estimated glomerular filtration rate (eGFR) in patients with type 2 diabetes.

Materials and Methods: This was a single-center, retrospective, observational cohort study. A total of 1,387 patients who underwent an ankle reflex examination between January 2005 and December 2015 were included in the analysis for the primary outcome. The findings of the ankle reflex examination were classified into three groups: normal, decreased, or absent. The primary outcome was defined as the incidence of a 40% loss of eGFR from baseline. A survival time analysis using the Kaplan–Meier method and a regression analysis using a Cox proportional hazards model were conducted to evaluate the association between the ankle reflex test results and loss of eGFR.

Results: The ankle reflex test results were as follows: normal, n = 678 (48.9%); decreased, n = 270 (19.5%); and absent, n = 439 (31.6%) patients. The median follow-up period was 5.6 years in the observational period. In the univariate regression analysis, decreased and absent ankle reflexes were significantly associated with loss of eGFR. Moreover, decreased ankle reflex (hazard ratio: 1.83, 95% confidence interval: 1.16–2.87) and absent ankle reflex (hazard ratio: 2.57, 95% confidence interval: 1.76–3.76) were independently associated with loss of eGFR after adjusting for prognostic risk factors.

Conclusions: Decreased and absent ankle reflexes are closely and independently associated with loss of eGFR in patients with type 2 diabetes.

INTRODUCTION

Diabetic kidney disease (DKD) is one of the most common complications of patients with diabetes[1]. Furthermore, DKD is a leading cause of end-stage renal failure and dialysis[2]. A reduced renal function is considered a predictor of cardiovascular disease (CVD)[3]. Therefore, identifying patients with a higher risk of renal function decline is important for improving their prognosis. The diagnostic criteria for DKD include albuminuria and loss of the estimated glomerular filtration rate (eGFR)[4]. Albuminuria is widely accepted as the first clinical sign, and loss of eGFR occurs after overt albuminuria in patients with DKD. However, the rate of eGFR decline in patients with normoalbuminuria is increasing in Japan[5]. Therefore, the eGFR is an important indicator for assessing the grade of renal function in patients with DKD.

Along with DKD, diabetic peripheral neuropathy (DPN) is one of the most common complications in patients with diabetes[6]. The neuropathic pain caused by DPN markedly decreases patients' quality of life (QOL)[7]. In addition, the progression of DPN leads to hypoesthesia, which increases the risk of diabetic foot ulceration and lower-limb amputation[8,9]. Therefore, early diagnosis of DPN is essential to improve their QOL and prognosis. Several scoring systems are used to diagnose DPN[10–13], including the neuropathy disability score (NDS),

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Michigan neuropathy screening instrument (MNSI), clinical neurological examination (CNE), and the Toronto clinical scoring system (TCSS). In Japan, the abbreviated diagnostic criteria proposed by the Diabetic Neuropathy Study Group are widely used in daily clinical practice[14].

The previous study has shown that DPN assessed by MNSI was associated with DKD[15]. Besides, our previous study has suggested that DPN assessed using diagnostic criteria proposed by the diabetic neuropathy study group was associated with eGFR loss[16]. However, this scoring system and diagnostic criteria require multiple examinations such as neuropathic subjective symptoms, vibration perception test, and ankle reflex examination among others. In daily clinical practice, it is difficult to perform all the neurological examinations included in these diagnostic criteria. Among neurological examinations, ankle reflex is more objective than neuropathic symptoms, and is a simple, non-invasive, and powerful screening tool in detection of DPN[17]. Moreover, ankle reflex examination is more widely used than vibration sensation test using a tuning fork, testing of touch, pressure, temperature, and pain sensation[18]. But, to our best knowledge, there are no studies investigating the association between ankle reflex findings and eGFR loss in patients with type 2 diabetes.

Therefore, we investigated the association between ankle reflex examination findings and eGFR decline in patients with type 2 diabetes.

MATERIALS AND METHODS

Study design and ethics

This retrospective observational cohort study was conducted at the Institute for Medical Science, Asahi Life Foundation, Tokyo, Japan. The protocol, which is in accordance with the Declaration of Helsinki, was approved by the Committee of Ethics of this institution on November 11, 2019 (approval number: 08702-6). Confidentiality was safeguarded by the Institute for Medical Science, Asahi Life Foundation. Per the Ethical Guidelines for Medical and Health Research Involving Human Subjects in Japan, and individual informed consent was waived owing to the retrospective nature of the study and opt-out method of inclusion. Information about this study was available to patients on a website, and patients reserve the right to withdraw the registration of their data at any time.

Participants

We recruited 3,406 outpatients with diabetes who initially visited the clinic of the Institute for Medical Science, Asahi Life Foundation, between January 2005 and December 2015. The following inclusion criteria were applied: (1) diagnosis of type 2 diabetes; (2) examination of the ankle reflex between January 2005 and December 2015; (3) measurement of serum creatinine (Cr) level within the period of −84 to 28 days from the date of ankle reflex examination; (4) measurement of Cr level more than once; and (5) follow-up for ≥29 days. Patients diagnosed with kidney diseases other than diabetic nephropathy, and patients with outcomes within 28 days were excluded from the study.

Clinical and biochemical parameters

Sex, age (years), diabetes duration (years), body mass index (BMI; kg/m^2), glycated hemoglobin (HbA1c; %), Cr (mg/dL), eGFR (mL/min/1.73 m^2), urine albumin–creatinine ratio (UACR; mg/gCr), dipstick urine test, blood pressure (systolic blood pressure [SBP; mmHg] and diastolic blood pressure [DBP; mmHg]), lipid profile (total cholesterol [TC; mg/dL], triglyceride [TG; mg/dL], low-density lipoprotein cholesterol [LDL-C; mg/dL], and high-density lipoprotein cholesterol [HDL-C; mg/dL]), uric acid (UA; mg/dL), neuropathic subjective symptoms (pain and numbness), vibration sensation, ankle reflex findings, patellar reflex findings, smoking status, and use of medications were collected as baseline data.

Definitions

The diabetes duration was calculated by subtracting the date of diabetes diagnosis, which is obtained from medical records, from the date of ankle reflex examination. The BMI was calculated using the following equation: weight (kg)/square of height (m). The eGFR was calculated using the Japanese GFR calculation formula proposed by the Japanese Society of Nephrology[19]: $eGFR = 194 \times Cr^{-1.094} \times age^{-0.287}$ (female × 0.739). The stage of albuminuria was defined as normoalbuminuria (UACR < 30 mg/gCr), microalbuminuria (UACR 30–299 mg/gCr), and macroalbuminuria (UACR ≥ 300 mg/gCr)[20]. Proteinuria was defined as a dipstick urine test score of ≥1+ based on a previous study[21]. Decreased vibration sense was defined by the vibration perception threshold in the inner malleolus using a 128 Hz tuning fork ≤10 s. The ankle and patellar reflexes were examined using a percussion hammer. Depending on severity, ankle and patellar reflexes were classified into three groups: normal, decreased, or absent. Regarding the ankle reflex, the patients were placed in a kneeling position and the Achilles tendon was struck with a percussion hammer by the physician. If no reflex was obtained, patients were required to push the wall in front of them to induce reflection. In cases in which the reflex was obtained by induction, the ankle reflex of these patients was defined as decreased. In contrast, if no reflex was obtained by induction, the ankle reflex in these patients was defined as absent. A total of 38 physicians were involved in the ankle reflex examination. Hypertension was defined as SBP ≥140 mmHg and/or DBP ≥90 mmHg and/or the use of antihypertensive drugs. Dyslipidemia was defined as follows: TG ≥ 150 mg/dL, LDL-C ≥ 140 mg/dL, HDL-C < 40 mg/dL, and/or use of antihyperlipidemic drugs.

Renal function outcomes

As described in our previous study[16], the primary outcome was defined as the incidence of a 40% loss of eGFR from baseline. Furthermore, sensitivity analyses were conducted to assess the robustness of the results for the primary outcome. The

secondary outcomes were defined as the incidence of a 30% loss of eGFR from baseline and the onset of eGFR <60 mL/min/1.73 m^2, as a sensitivity analysis. In the analysis for the onset of eGFR <60 mL/min/1.73 m^2, we excluded the patients with eGFR <60 mL/min/1.73 m^2 at baseline. Additionally, exploratory outcomes were set as the onset of microalbuminuria and macroalbuminuria to investigate whether ankle reflex was associated with albuminuria. Microalbuminuria and macroalbuminuria were defined as the first detection of UACR ≥30 mg/gCr and UACR ≥300 mg/gCr, respectively.

Statistical analyses

Statistical analyses were conducted using JMP® Pro 17.2 (SAS Institute Inc., Cary, NC, USA). Continuous variables with normal distribution were compared using an analysis of variance (ANOVA) and are represented as the mean ± standard deviation (SD). Regarding continuous variables with non-normal distributions, the Kruskal–Wallis test was performed and the results were expressed as medians and interquartile ranges (IQRs). Categorical variables were analyzed using Fisher's exact test and are represented as numbers with percentages (%). Moreover, survival time analyses were performed using the Kaplan–Meier method and a Cox proportional hazard model. First, a log-rank test was conducted to compare the difference in survival time among the groups using the Kaplan–Meier method. Next, univariate and multivariate regression analyses using a Cox proportional hazard model were conducted to estimate hazard ratios (HRs) and 95% confidence intervals (CIs). In a univariate analysis, the following 18 variables at baseline were analyzed as potential confounders for eGFR decline: patellar reflex, neuropathic symptoms, vibration sense, sex, age, diabetes duration, BMI, HbA1c, eGFR, proteinuria, SBP, TG, HDL-C, UA, smoking status, use of insulin, use of angiotensin-converting enzyme inhibitors or angiotensin receptor blockers (ACEIs/ARBs), and use of statins[22–25]. Three types of models were used to estimate adjusted HRs and 95% CIs in multivariate analyses. First, Model 1 was adjusted using only the patellar reflex. Second, Model 2 was adjusted for neuropathic symptoms and decreased vibration sensation. Finally, Model 3 was adjusted for other variables in the univariate analysis. Besides, subgroup analyses stratified by mean HbA1c levels, use of ACEIs/ARBs, and use of sodium–glucose cotransporter-2 (SGLT2) inhibitors during follow-up period, including baseline, were conducted to assess whether the association between ankle reflex and eGFR decline was consistent across subgroups. The results of subgroup analyses were presented as HRs and 95% CIs. P values of <0.05 were considered to indicate statistical significance.

RESULTS

Study participants

A flowchart of the patients included in the analyses for primary and secondary outcomes is shown in Figure 1. Among 3,406 outpatients, 1,403 patients met the inclusion criteria. Sixteen patients were excluded from the analysis for the primary outcome. The details were as follows: 13 patients were diagnosed with kidney disease (e.g., kidney infraction and chronic glomerulonephritis) and 3 patients had a 40% loss of eGFR within 28 days. Consequently, 1,387 patients were included in the primary outcome analysis. In the analysis for 30% loss of eGFR from baseline, eight patients who had 30% loss of eGFR within 28 days were excluded from the analysis. As a result, 1,382 patients were included in the analysis. In the analysis for the onset of eGFR <60 mL/min/1.73 m^2, 15 patients who occurred the onset of eGFR <60 mL/min/1.73 m^2 within 28 days, and 135 patients with baseline eGFR <60 mL/min/1.73 m^2 were excluded from the analysis. Accordingly, 1,240 patients were included in the analysis.

The analyses for exploratory outcomes were conducted on a cohort of 631 patients who were assessed for UACR at baseline. Among 631 patients, 95 patients who had UACR measurements less than twice during follow-up period were excluded. Additionally, in the analysis for microalbuminuria, 127 patients with UACR ≥30 mg/gCr at baseline were excluded. In the analysis for macroalbuminuria, 15 patients with UACR ≥300 mg/gCr at baseline were excluded. As a consequence, 409 patients were included in the analysis for microalbuminuria, and 521 patients were included in the analysis for macroalbuminuria.

Comparison of clinical and biochemical parameters

A total of 1,387 patients were included in the analysis of the primary outcome. The clinical and biochemical parameters are shown in Table 1. The number of patients classified according to ankle reflex was as follows: normal ankle reflex, $n = 678$ (48.9%); decreased ankle reflex, $n = 270$ (19.5%); and absent ankle reflex, $n = 439$ (31.6%). Each physician's ankle reflex findings are shown in Table S1. The overall mean age was 56.2 ± 11.2 years and 21.4% of the patients were women. The median duration of diabetes was 2 years (range 0–8), the mean BMI was 25.4 ± 4.3 kg/m^2, and the mean HbA1c level was 8.7 ± 2.0%. In addition, 282 (21.7%) patients had neuropathic symptoms, 359 (37.7%) patients had decreased vibration sensation, and 533 (40.7%) patients had a decreased or absent patellar reflex. The comparison of background data at baseline revealed significant differences among the groups in the following variables: age ($P < 0.0001$); diabetes duration ($P < 0.0001$); HbA1c ($P = 0.01$); Cr ($P = 0.01$); UACR ($P < 0.0001$); albuminuria ($P < 0.0001$); dipstick urine test ($P < 0.0001$); SBP ($P < 0.0001$); neuropathic symptoms ($P < 0.0001$); decreased vibration sense ($P < 0.0001$); patellar reflex ($P < 0.0001$); and hypertension ($P = 0.003$).

Kaplan–Meier analyses for the primary outcome according to neurological findings: ankle reflex, patellar reflex, neuropathic symptoms, and vibration sensation

Figure 2 shows the Kaplan–Meier curves for a 40% loss of eGFR from baseline in patients stratified by neurological findings. Figure 2a shows the Kaplan–Meier curves of 1,387

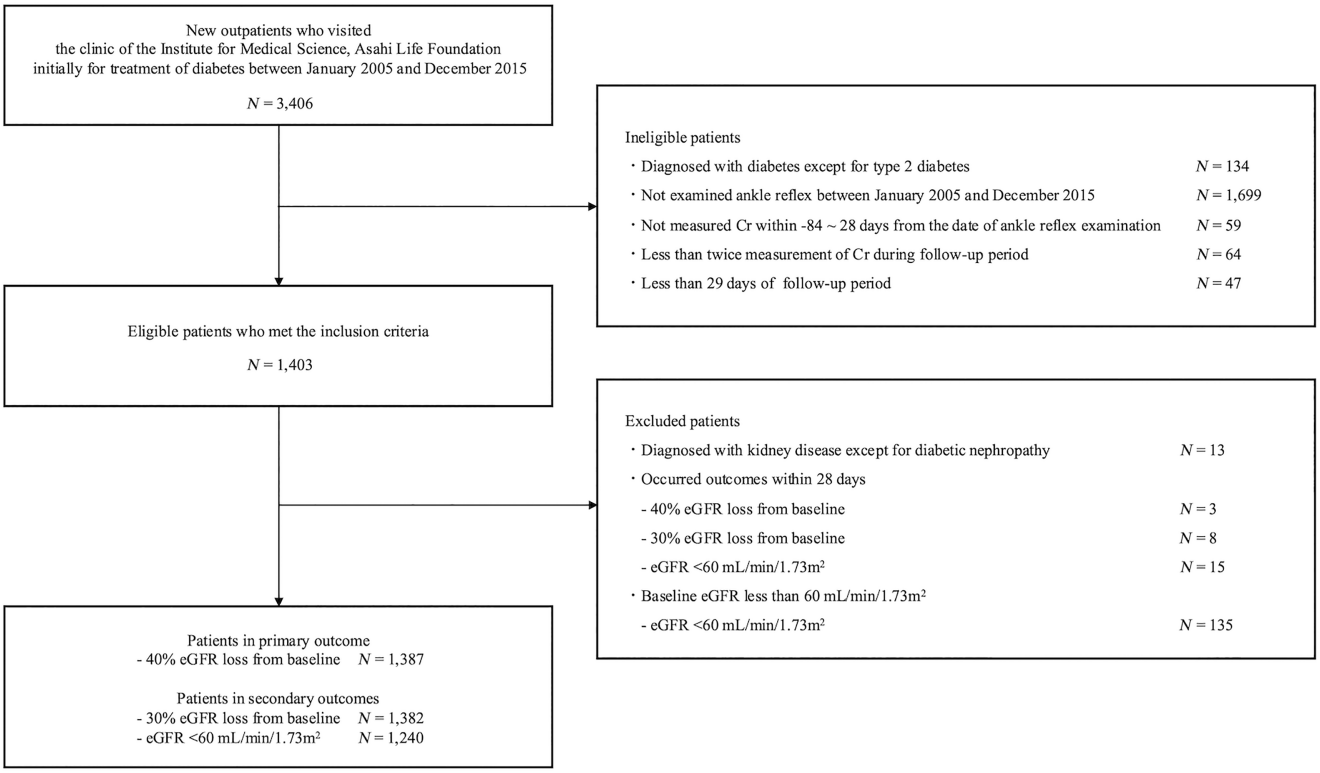

Figure 1 | Flowchart of inclusion and exclusion criteria. A total of 1,403 patients met the inclusion criteria. In the analysis for the primary outcome (40% loss of eGFR from baseline), 1,387 patients were included. In the analyses for secondary outcomes, 1,382 patients were included in the analysis for 30% loss of eGFR from baseline, while 1,240 patients were included in the analysis for eGFR <60 mL/min/1.73 m^2. Cr, serum creatinine.

patients stratified based on the findings of the ankle reflex examination. The median follow-up period was 5.6 years (range 0–13 years). The median follow-up period (months) for each group was as follows: normal ankle reflex, 71 months (IQRs: 39–108 months); decreased ankle reflex, 71 months (IQRs: 43–105 months); and absent ankle reflex, 66 months (IQRs: 33–107 months). One hundred seventy-five (12.6%) patients developed a 40% loss of eGFR from baseline: normal ankle reflex, $n = 44$ (6.5%); decreased ankle reflex, $n = 34$ (12.6%); and absent ankle reflex, $n = 97$ (22.1%), respectively (log-rank: $P < 0.0001$). Figure 2b shows the Kaplan–Meier curves of 1,308 patients stratified by the results of the patellar reflex examination. One hundred sixty-six (12.7%) patients developed a 40% loss of eGFR: normal patellar reflex, $n = 67$ (8.6%); decreased patellar reflex, $n = 49$ (16.1%); and absent patellar reflex, $n = 50$ (21.9%; log-rank: $P < 0.0001$). Figure 2c shows the Kaplan–Meier curves of 1,297 patients stratified according to neuropathic symptoms. One hundred fifty-nine (12.3%) patients developed a 40% loss of eGFR from baseline: patients with neuropathic symptoms (–), $n = 110$ (10.9%); and patients with neuropathic symptoms (+), $n = 49$ (17.4%; log-rank: $P = 0.19$). Figure 2d shows the Kaplan–Meier curves of 953 patients stratified according to the presence of decreased sense of vibration. One of 119 (12.5%) patients developed a 40% loss of eGFR: 57 (9.6%) patients with decreased vibration sense (–) and 62 (17.3%) patients with decreased vibration sense (+) (log-rank: $P = 0.001$).

Univariate and multivariate regression analyses using Cox proportional hazards models

The results of the univariate and multivariate regression analyses using Cox proportional hazards models are shown in Table 2. In the univariate analysis, the following factors were significantly associated with incidence of a 40% loss of eGFR from baseline: decreased and absent ankle reflex, decreased and absent patellar reflex, decreased vibration sense, female sex, diabetes duration, HbA1c, proteinuria, SBP, TG, UA, and the use of insulin. In the multivariate analysis, we used three types of models to assess whether decreased and absent ankle reflexes were independently associated with the onset of a 40% loss of eGFR. In Model 1 (adjusted by patellar reflex), decreased ankle reflex (HR: 1.72, 95%CI: 1.03–2.87) and absent ankle reflex (HR: 3.34, 95%CI: 2.10–5.29) were significantly associated with the onset of a 40% loss of eGFR, while decreased patellar reflex (HR: 1.17, 95%CI: 0.77–1.77) and absent patellar reflex (HR: 1.24, 95%CI: 0.79–1.95) were not. In Model 2 (adjusted for neuropathic symptoms and decreased vibration sense), decreased ankle reflex (HR: 2.21, 95%CI: 1.24–3.95) and absent

Table 1 | Baseline characteristics of 1,387 patients with type 2 diabetes

	Total *N* = 1,387	Normal *N* = 678	Decreased *N* = 270	Absent *N* = 439	*P*-value
Female, *N* (%)	297 (21.4)	131 (19.3)	60 (22.2)	106 (24.1)	0.15
Age (years)	56.2 ± 11.2	54.2 ± 10.5	56.7 ± 11.6	59.1 ± 11.5	<0.0001
Diabetes duration (years)	2 (0, 8)	1 (0, 5)	2 (0, 7)	4 (0, 11)	<0.0001
Body mass index (kg/m^2)	25.4 ± 4.3	25.4 ± 4.1	25.4 ± 4.2	25.4 ± 4.6	0.99
HbA1c	8.7 ± 2.0	8.7 ± 2.0	8.6 ± 2.1	9.0 ± 2.0	0.01
Unknown, *N* (%)	5 (0.4)	2 (0.3)	1 (0.4)	2 (0.5)	
Serum creatinine (mg/dL)	0.77 ± 0.30	0.76 ± 0.17	0.76 ± 0.22	0.81 ± 0.46	0.01
eGFR (mL/min/1.73 m^2)	82.9 ± 20.4	83.8 ± 18.3	83.6 ± 20.1	81.1 ± 23.4	0.08
Urine albumin–creatinine–ratio (mg/gCr)	10.6 (5.9, 27.8)	8.8 (4.9, 19)	9.9 (5.6, 29.7)	15.3 (7.6, 54.9)	<0.0001
Albuminuria					
Normo-/micro-/macroalbuminuria, *N*	484/130/17	268/43/4	80/22/3	136/65/10	<0.0001
Normo-/micro-/macroalbuminuria, %	76.7/20.6/2.7	85.1/13.6/1.3	76.2/21.0/2.8	64.5/30.8/4.7	
Unknown, *N* (%)	756 (54.5)	363 (53.5)	165 (61.1)	228 (51.9)	
Dipstick urine test					
(–)/(±) / (1+, 2+, 3+), *N*	1,129/85/169	587/28/60	225/17/27	317/40/82	<0.0001
(–)/(±) / (1+, 2+, 3+), %	81.6/6.2/12.2	87.0/4.1/8.9	83.6/ 6.3/ 10.1	72.2/9.1/18.7	
Unknown, *N* (%)	4 (0.3)	3 (0.4)	1 (0.4)	–	
Systolic blood pressure (mmHg)	133.0 ± 18.2	130.9 ± 17.6	133.7 ± 16.8	135.8 ± 19.7	<0.0001
Unknown, *N* (%)	9 (0.6)	3 (0.4)	1 (0.4)	5 (1.1)	
Diastolic blood pressure (mmHg)	79.5 ± 11.8	79.5 ± 12.0	79.9 ± 11.5	79.2 ± 11.6	0.74
Unknown, *N* (%)	9 (0.6)	3 (0.4)	1 (0.4)	5 (1.1)	
Total cholesterol (mg/dL)	207.0 ± 44.0	207.7 ± 46.9	207.3 ± 40.6	205.6 ± 41.4	0.74
Unknown, *N* (%)	3 (0.2)	2 (0.3)	–	1 (0.2)	
Triglyceride (mg/dL)	134 (91, 204)	129 (90, 199)	139 (93, 235)	138 (92, 200)	0.34
Unknown, *N* (%)	1 (0.1)	–	–-	1 (0.2)	
LDL cholesterol (mg/dL)	121.8 ± 33.5	123.2 ± 32.9	121.5 ± 33.6	119.5 ± 34.3	0.26
Unknown, *N* (%)	252 (18.2)	103 (15.2)	61 (22.6)	88 (20.0)	
HDL cholesterol (mg/dL)	52.3 ± 14.1	52.0 ± 14.2	52.1 ± 13.0	52.8 ± 14.7	0.66
Unknown, *N* (%)	1 (0.1)	–	–	1 (0.2)	
Uric acid (mg/dL)	5.4 ± 1.4	5.4 ± 1.4	5.4 ± 1.3	5.4 ± 1.5	0.97
Unknown, *N* (%)	3 (0.2)	1 (0.1)	-	2 (0.5)	
Neuropathic symptoms, *n* (%)	282 (21.7)	105 (16.6)	57 (22.0)	120 (29.5)	<0.0001
Unknown, *N* (%)	90 (6.5)	47 (6.9)	11 (4.1)	32 (7.3)	
Decreased vibration sense, *n* (%)	359 (37.7)	141 (30.3)	70 (38.7)	148 (48.4)	<0.0001
Unknown, *N* (%)	434 (31.3)	212 (31.3)	89 (33.0)	133 (30.3)	
Patellar reflex					
Normal/decreased/absent, *N*	775/305/228	571/56/9	116/121/19	88/128/200	<0.0001
Normal/decreased/absent, %	59.3/23.3/17.4	89.8/8.8/1.4	45.3/47.3/7.4	21.2/30.8/48.1	
Unknown, *N* (%)	79 (5.7)	42 (6.2)	14 (5.2)	23 (5.2)	
Smoking status, *N* (%)	808 (58.3)	399 (58.9)	154 (57.0)	255 (58.1)	0.87
Hypertension, *N* (%)	581 (41.9)	253 (37.3)	128 (47.4)	200 (45.6)	0.003
Dyslipidemia, *N* (%)	828 (59.7)	403 (59.4)	160 (59.3)	265 (60.4)	0.94
Use rate of insulin, *N* (%)	104 (7.5)	50 (7.4)	15 (5.6)	39 (8.9)	0.26
Use rate of SGLT2 inhibitors, *N* (%)	6 (0.4)	2 (0.3)	2 (0.7)	2 (0.5)	0.64
Use rate of ACEIs/ARBs, *N* (%)	162 (11.7)	79 (11.7)	24 (8.9)	59 (13.4)	0.18
Use rate of statin, *N* (%)	86 (6.2)	36 (5.3)	17 (6.3)	33 (7.5)	0.33

The baseline characteristics of the 1,387 patients included in the analysis for the primary outcome. Continuous variables with normal and non-normal distributions were represented as the mean ± SD and median with interquartile range, respectively. Categorical variables were presented as numbers with percentages. *P* values of <0.05 were considered to indicate statistical significance. ACEIs/ARBs, angiotensin-converting enzyme inhibitors/angiotensin receptor blockers; eGFR, estimated glomerular filtration rate; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SGLT2, sodium–glucose cotransporter-2.

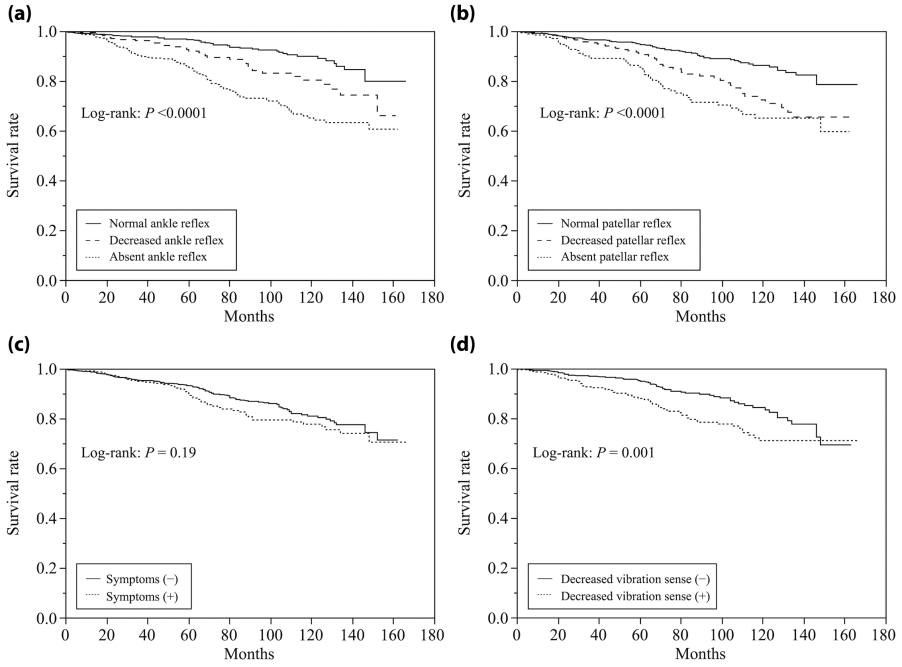

Figure 2 | (a) Kaplan–Meier curves for 1,387 patients stratified by the findings of the ankle reflex examination (*P*-value < 0.0001). (b) Kaplan–Meier curves for 1,308 patients stratified by the findings of the patellar reflex examination (*P*-value < 0.0001). (c) Kaplan–Meier curves for 1,297 patients stratified by the presence of neuropathic symptoms (pain or numbness; *P*-value = 0.19). (d) Kaplan–Meier curves for 953 patients stratified by the presence of vibration sensation abnormality (128-Hz tuning fork ≤ 10 s; *P*-value = 0.001). eGFR, estimated glomerular filtration rate.

ankle reflex (HR: 3.60, 95%CI: 2.22–5.85) were associated with the onset of a 40% loss of eGFR. Consistently, in Model 3 (adjusted for sex, age, diabetes duration, BMI, HbA1c, eGFR, proteinuria, SBP, TG, HDL, UA, smoking status, use of insulin, use of ACEIs/ARBs, and use of statins), decreased ankle reflex (HR: 1.83, 95%CI: 1.16–2.87) and absent ankle reflex (HR: 2.57, 95%CI: 1.76–3.76) were associated with the onset of a 40% loss of eGFR. As a result, decreased and absent ankle reflexes were associated with the onset of a 40% loss of eGFR from baseline, independent of conventional risk factors.

Analyses for secondary outcomes comparing the normal, decreased, and absent ankle reflex groups

The results of secondary outcomes (30% loss of eGFR from baseline, eGFR <60 mL/min/1.73 m^2) are shown in Figure S1. Figure S1a shows a Kaplan–Meier curve and HRs with 95% CIs for a 30% loss of eGFR from baseline in 1,382 patients stratified by the ankle reflex findings. Overall, 365 (26.4%) patients completely developed a 30% eGFR decline: normal ankle reflex, n = 115 (17.0%); decreased ankle reflex, n = 79 (29.4%); and absent ankle reflex, n = 171 (39.2%; log-rank: P < 0.0001). Decreased (HR: 1.81, 95% CI: 1.36–2.41) and absent ankle reflex (HR: 2.67, 95% CI: 2.11–3.39) were associated with a 30% loss of eGFR. Figure S1b shows the Kaplan–Meier curves and HRs with 95% CIs for eGFR <60 mL/min/1.73 m^2 in 1,240 patients stratified by ankle reflex findings. Three hundred ninety-three (31.7%) patients developed the onset of eGFR <60 mL/min/1.73 m^2: normal ankle reflex, n = 170 (27.1%); decreased ankle reflex, n = 86 (35.0%); and absent ankle reflex, n = 137 (37.3%; log-rank: P = 0.01). Decreased (HR:1.32, 95% CI: 1.02–1.71) and absent ankle reflex (HR: 1.38, 95% CI: 1.10–1.73) were associated with the onset of eGFR <60 mL/min/1.73 m^2.

Analyses for exploratory outcomes comparing the normal, decreased, and absent ankle reflex groups

The results of exploratory outcomes (microalbuminuria and macroalbuminuria) are shown in Figure S2. Figure S2a shows a Kaplan–Meier curve and HRs with 95% CIs for microalbuminuria in 409 patients stratified by ankle reflex finding. A total of 153 (37.4%) patients developed microalbuminuria: normal ankle reflex, n = 72 (32.9%); decreased ankle reflex, n = 27 (38.0%); and absent ankle reflex, n = 54 (45.4%; log-rank test = 0.08). Absent ankle reflex (HR: 1.48, 95% CI: 1.04–2.11) was significantly associated with the onset of microalbuminuria, while decreased ankle reflex (HR: 1.28, 95% CI: 0.82–2.00) was not. Figure S2b shows a Kaplan–Meier curve and HRs with 95% CIs for macroalbuminuria in 521 patients stratified by ankle reflex finding. Among 521 patients, 35 (6.7%) patients developed macroalbuminuria: normal ankle reflex, n = 12 (4.7%); decreased ankle reflex, n = 6 (6.9%); and absent ankle reflex, n = 17 (9.6%; log-rank test = 0.12). Similarly to

Table 2 | Univariate and multivariate regression analyses using Cox proportional hazard models

	Univariate Hazard ratio (95%CI)	Multivariate (model 1) Hazard ratio (95%CI)	Multivariate (model 2) Hazard ratio (95%CI)	Multivariate (model 3) Hazard ratio (95%CI)
Ankle reflex (*N* = 1,387)				
Normal	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
Decreased	1.91 (1.22–2.99)**	1.72 (1.03–2.87)*	2.21 (1.24–3.95)**	1.83 (1.16–2.87)**
Absent	3.57 (2.50–5.10)**	3.34 (2.10–5.29)**	3.60 (2.22–5.85)**	2.57 (1.76–3.76)**
Patellar reflex (*N* = 1,308)				
Normal	1.00 (reference)	1.00 (reference)		
Decreased	1.93 (1.34–2.79)**	1.17 (0.77–1.77)		
Absent	2.79 (1.94–4.03)**	1.24 (0.79–1.95)		
Neuropathic symptoms (*N* = 1,297)	1.26 (0.90–1.77)		0.89 (0.58–1.38)	
Decreased vibration sense (*N* = 953)	1.79 (1.25–2.57)**		1.39 (0.94–2.06)	
Sex (Female)	1.54 (1.11–2.13)**			1.95 (1.30–2.93)**
Age	1.00 (0.99–1.02)			1.01 (0.99–1.03)
Diabetes duration (years)	1.03 (1.01–1.05)**			1.02 (0.99–1.04)
Body mass index (kg/m^2)	1.03 (0.99–1.05)			0.98 (0.94–1.02)
HbA1c (%)	1.15 (1.08–1.22)**			1.11 (1.02–1.20)*
eGFR (mL/min/1.73 m^2)	1.00 (0.99–1.01)			1.01 (1.003–1.02)*
Proteinuria	5.10 (3.69–7.06)**			3.67 (2.52–5.34)**
Systolic blood pressure (mmHg)	1.02 (1.01–1.03)**			1.01 (1.006–1.02)**
Triglyceride (mg/dL)	1.002 (1.001–1.003)**			1.002 (1.001–1.003)**
HDL cholesterol (mg/dL)	0.99 (0.98–1.01)			0.98 (0.97–0.99)*
Uric acid (mg/dL)	1.13 (1.01–1.26)*			1.15 (1.01–1.31)*
Smoking status	0.89 (0.66–1.20)			1.12 (0.79–1.58)
Use of insulin	2.28 (1.48–3.50)**			2.43 (1.50–3.92)**
Use of ACEIs/ARBs	1.40 (0.93–2.11)			1.41 (0.90–2.22)
Use of statin	0.68 (0.32–1.45)			0.69 (0.32–1.50)

The results of univariate and multivariate regression analyses using Cox proportional hazard models. In the multivariate analysis, hazard ratios (HRs) with 95% confidence intervals (CIs) adjusted by patellar reflex are shown in Model 1. HRs with 95% CIs adjusted for neuropathic symptoms and decreased vibration sensation are shown in Model 2. HRs with 95%CIs adjusted for the other potential confounding factors are shown in Model 3. ACEIs, angiotensin-converting enzyme inhibitors; ARBs, angiotensin II receptor blockers; eGFR, estimated glomerular filtration rate; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein. *$P < 0.05$. **$P < 0.01$.

microalbuminuria, absent ankle reflex (HR: 2.13, 95% CI: 1.02–4.47) was significantly associated with the onset of macroalbuminuria, but decreased ankle reflex (HR: 1.56, 95% CI: 0.59–4.15) was not.

Subgroup analyses stratified by mean HbA1c levels, use of ACEIs/ARBs, and use of SGLT2 inhibitors during follow-up period

The results of subgroup analyses are shown in Table S2. Decreased ankle reflex was significantly associated with a 40% loss of eGFR in patients with mean HbA1c ≥7.0% (HR: 2.10, 95%CI: 1.26–3.52), ACEIs/ARBs non-users (HR: 4.14, 95%CI: 1.77–9.69), and SGLT2 inhibitors non-users (HR: 1.98, 95%CI: 1.17–3.34). However, decreased ankle reflex was not significantly associated with a 40% loss of eGFR in patients with mean HbA1c <7.0% (HR: 1.62, 95%CI: 0.65–4.02), ACEIs/ARBs users (HR: 1.29, 95%CI: 0.75–2.22), and SGLT2 inhibitors users (HR: 1.65, 95%CI: 0.70–3.93). Absent ankle reflex was significantly associated with a 40% loss of eGFR from baseline in all subgroups.

DISCUSSION

The present study revealed an association between ankle reflex findings and eGFR decline in patients with type 2 diabetes. When the severity of the ankle reflex abnormalities progressed, the risk of eGFR decline increased. Furthermore, independent of potential confounders, including other neurological findings, diabetes history, patient background of diabetes, comorbidities, and medications, ankle reflex deterioration was associated with eGFR decline.

The ankle reflex examination is a screening tool for DPN. The absence of an ankle reflex is a common finding in patients with type 2 diabetes[26]. The prevalence of abnormal ankle reflexes was 51.8% among Japanese outpatients with diabetes[18]. In our study, 51.1% of patients had abnormal ankle reflexes; the prevalence was almost the same as in the previous study. The Achilles tendon is mainly innervated by the S1 nerve root of the tibial nerve[27]. The S1 nerve is injured by atherosclerotic obstruction, nutritional deficiency, or other underlying causes[28]. Therefore, absent ankle reflex findings are also observed in healthy subjects of ≥60 years of age[29]. In the present study, the

association between ankle reflex findings and loss of eGFR remained significant after adjustment for patient age.

The patellar reflex is a deep tendon reflex similar to the ankle reflex. Impaired patellar reflexes are often accompanied by impaired ankle reflexes. Decreased and absent ankle reflexes were significantly associated with a loss of eGFR, independent of the patellar reflex in the present study. In general, the patellar reflex may be a more powerful screening tool than the ankle reflex because the patellar reflex is not affected by age[30]. However, whether the diagnostic efficacy of the patellar reflex is higher than that of the ankle reflex remains to be determined. Since neuropathy progresses from the peripheral side[31], the predictive ability of the patellar tendon reflex is assumed to be dependent on the ankle reflex if a decline in renal function can be predicted at the time of the loss or decline in the ankle reflex.

In Japan, the diagnostic criteria proposed by the diabetic neuropathy study group are used in clinical practice[14]. The criteria consisted of sensory symptoms, sense of vibration, and ankle reflexes. We estimated the effect of sensory symptoms and vibration sensation on eGFR decline. Sensory symptoms were unrelated to renal deterioration, similar to the findings reported by Moorthi *et al.*[32] The positive and negative symptoms of DPN are reportedly independent risk factors, suggesting different underlying mechanisms[33]. Hence, the association between symptoms and decreased renal function may differ from that of the tendon reflexes. Decreased vibration sense was not associated with eGFR decline after adjustment for ankle reflex. Vibration sensation is a neurological finding with a subjective component as well as a symptom because it is detected when the patient no longer senses vibration. In symptoms and vibration perception, the subjective component may make it less sensitive than the ankle reflex in diagnosing neuropathy and predicting renal dysfunction[17].

Previous studies have shown an association between DPN and renal dysfunction in patients with type 2 diabetes[34,35]. A previous study has shown that the presence or absence of foot insensitivity using a monofilament test influences the risk of eGFR decline[34]. In the previous study, it was unclear whether the risk of renal decline increased with the severity of DPN progression. In contrast, our present study demonstrated that the risk of eGFR decline increased with the progression of the deterioration of ankle reflex findings, namely, DPN. In addition, we investigated the relationship between the DNP level and eGFR decline in detail by evaluating the effects of various neurological findings. Moreover, our study had a larger sample size and longer follow-up period in comparison to the previous study. However, the monofilament test may not have been used frequently in our clinic. This is because the monofilament test was not included in the DPN criteria recommended by the diabetic neuropathy study group. Another previous study showed an association between nerve conduction velocity (NCV) and renal decline[35]. NCV has long been used as the gold standard test to confirm the diagnosis of DPN due to its objectivity, reliability, and sensitivity in the measurement of peripheral nerve function[36,37]. However, an NCV is easily affected by the filter setting, type of electrodes, location of recording, limb temperature, and other factors[38]. With regard to the predictive power, taking NCV as the gold standard, ankle reflex yielded high sensitivity and specificity (91.5 and 67.4%, respectively)[17]. Thus, the ankle reflex examination may be an acceptable screening tool for DPN in daily clinical practice.

The pathophysiological mechanisms underlying DPN and DKD involve chronic hyperglycemia-induced microvascular damage. Hyperglycemia can lead to formulation of advanced glycation end products (AGEs), which cause oxidative stress and inflammation[39]. Oxidative stress and inflammation damage the peripheral nerves and kidneys[40,41]. Hence, it is biologically plausible that ankle reflex findings are associated with DKD in patients with type 2 diabetes mellitus. In the present study, decreased and absent ankle reflex was significantly associated with an eGFR decline in both primary and secondary outcomes. Although the association between ankle reflex findings and the prevalence of albuminuria at baseline was observed, the analysis for the onset of albuminuria using the log-rank test revealed no significant difference in survival time among groups. This result may be attributed to the small number of patients included in the analysis, which has reduced the detection power. Therefore, further longitudinal studies with larger sample sizes are warranted to clarify the association between ankle reflex and the onset of albuminuria.

The glycemic target of HbA1c <7.0% is recommended to prevent microvascular complications[42]. Furthermore, ACEIs, ARBs, and SGLT2 inhibitors have demonstrated potential renal protective effects[43–45]. Thus, the glycemic control and use of these drugs may affect the eGFR decline. In the subgroup analyses, absent ankle reflex was consistently associated with a 40% eGFR decline in all subgroups. Regarding decreased ankle reflex, there was no significant association in subgroup of mean HbA1c <7.0%, ACEIs/ARBs users, and SGLT2 inhibitors users. Even considering the result of decreased ankle reflex, decreased and absent ankle reflex was associated with a 40% eGFR decline in most subgroups. Therefore, the results obtained in the present study may be applicable to many patients with type 2 diabetes. The examination of ankle reflex should be performed in daily clinical practice to identify patients at high risk of renal function decline.

The present study was associated with several limitations. First, there is a possibility of selection bias. In our study, 1,699 patients were ineligible for inclusion because the ankle reflex at the first visit of the patient was not examined by physicians between January 2005 and December 2015. The decision to perform an ankle reflex examination was made by the physician; however, the reason for the decision was unclear. Second, the results of the examination might have been influenced by the physician's skill. In the present study, ankle reflex examinations were conducted by experienced board-certified diabetologists of the Japan Diabetes Society or physicians specializing in

diabetes treatment with aim of becoming a diabetologist. Therefore, inaccuracies relying on such techniques are expected to be minimized. Third, we were not able to distinguish whether eGFR decline during follow-up was due to diabetic or non-diabetic nephropathy. A kidney biopsy is necessary to definitively diagnose diabetic nephropathy, but kidney biopsy is an invasive examination and rarely performed in routine clinical practice. In order to exclude patients with non-diabetic nephropathy from the analysis as much as possible, we exclude patients diagnosed with kidney disease other than diabetic nephropathy before baseline. Finally, unknown confounders may have existed due to the retrospective nature of our study. In the multivariate analysis using Cox proportional hazard models, we estimated HRs with adjustment for conceivable confounders.

Deterioration of the ankle reflex was associated with eGFR decline. Furthermore, decreased and absent ankle reflexes were found to be independent risk factors for eGFR decline in patients with type 2 diabetes.

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DISCLOSURE

The authors declare no conflict of interest.

Approval of the research protocol: The present study was approved by the Committee of Ethics of the Institute for Medical Science, Asahi Life Foundation, Tokyo, Japan (approval number: 08702–6), in accordance with the principles of the Declaration of Helsinki; every effort was made to ensure patient anonymity.

Informed consent: N/A

Approval date of Registry and the Registration No. of the study/trial: N/A

Animal studies: N/A

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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. Kaplan-Meier curves and hazard ratios for secondary outcomes (30% eGFR decline from baseline, eGFR < 60 mL/min/1.73 m^2).

Figure S2. Kaplan-Meier curves and hazard ratios for exploratory outcomes (microalbuminuria, macroalbuminuria).

Table S1. The ankle reflex findings in 1,387 patients performed by 38 physicians.

Table S2. Subgroup analyses stratified by mean HbA1c levels, use of ACEIs/ARBs, and use of SGLT2 inhibitors during follow-up period.