Relationship between time in range and corneal nerve fiber loss in asymptomatic patients with type 2 diabetes

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Abstract

Background: Corneal confocal microscopy (CCM) is a noninvasive technique to detect early nerve damage of diabetic sensorimotor polyneuropathy (DSPN). Time in range (TIR) is an emerging metric of glycemic control which was reported to be associated with diabetic complications. We sought to explore the relationship between TIR and corneal nerve parameters in asymptomatic patients with type 2 diabetes (T2DM).

Methods: In this cross-sectional study, 206 asymptomatic inpatients with T2DM were recruited. After 7 days of continuous glucose monitoring, the TIR was calculated as the percentage of time in the glucose range of 3.9 to 10.0 mmol/L. CCM was performed to determine corneal nerve fiber density, corneal nerve branch density, and corneal nerve fiber length (CNFL). Abnormal CNFL was defined as <15.30 mm/mm².

Results: Abnormal CNFL was found in 30.6% (63/206) of asymptomatic subjects. Linear regression analyses revealed that TIR was positively correlated with CCM parameters both in the crude and adjusted models (all P < 0.05). Each 10% increase in TIR was associated with a 28.2% (95% CI: 0.595–0.866, P = 0.001) decreased risk of abnormal CNFL after adjusting for covariates. With the increase of TIR quartiles, corneal nerve fiber parameters increased significantly (all P for trend <0.01). The receiver operating characteristic curve indicated that the optimal cutoff point of TIR was 77.5% for predicting abnormal CNFL in asymptomatic patients.

Conclusions: There is a significant independent correlation between TIR and corneal nerve fiber loss in asymptomatic T2DM patients. TIR may be a useful surrogate marker for early diagnosis of DSPN.

Keywords: Continuous glucose monitoring; Corneal confocal microscopy; Time in range; Type 2 diabetes

Introduction

Diabetic sensorimotor polyneuropathy (DSPN) is one of the most common complications of diabetes affecting up to 50% of all patients and is associated with high morbidity and a high risk of lower limb amputation.[1] Importantly, up to 50% of DSPN may be asymptomatic. Therefore, the early recognition and appropriate intervention treatment of DSPN in patients with diabetes is critical.[2]

Corneal confocal microscopy (CCM) is a noninvasive ophthalmic application, which is objective and reproducible for quantifying small nerve fibers. It has been employed to detect early subclinical small nerve fiber loss and stratify the severity of DSPN, and has comparable diagnostic utility to intraepidermal nerve fiber density (IENFD) in skin biopsy specimens, which is the gold standard for assessing small fiber damage in the early diagnosis of DSPN.[3-7]

During recent years, time in range (TIR) has emerged as a simple and intuitive glycemic marker that denotes the proportion of time that a person’s glucose level is within a desired target range (3.9–10.0 mmol/L). Recent studies have indicated that TIR is significantly associated with DSPN, diabetic retinopathy, microalbuminuria, carotid intima-media thickness, and all-cause and cardiovascular mortality in type 2 diabetes (T2DM) patients.[8-12] As a result, it was recommended as the preferred metric for determining the outcome of clinical studies.[12,13]

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However, to our knowledge, the relationship between TIR and corneal nerve fiber loss has not been described in T2DM patients. Therefore, in this context, our study aimed to clarify the possible link between TIR and nerve fiber loss in asymptomatic patients with T2DM.

Methods

Ethical approval
The study was approved by the Ethics Committee of Shanghai Jiao Tong University Affiliated Sixth People’s Hospital (No. 2020-KY-024). It was designed in accordance with the principles of the Helsinki Declaration. Written informed consent to participate in this cross-sectional study was obtained from all participants.

Patients
We recruited 206 inpatients (85 women and 121 men) with previously diagnosed T2DM at the Department of Endocrinology and Metabolism, Shanghai Jiao Tong University Affiliated Sixth People’s Hospital from September 2020 to July 2021. T2DM was diagnosed according to the 1999 World Health Organization criteria. Inclusion criteria were age ≥18 years, with the presence of T2DM, without neurological symptoms, a stable glucose-lowering regimen for the previous 3 months, and valid data on both 7 days continuous glucose monitoring (CGM) and CCM. Exclusion criteria included diabetic ketoacidosis or diabetic foot, acute infectious disease or a history of other illnesses which is known to be associated with neuropathy, the use of drugs that affect neurological function, vitamin B12 or folic acid deficiency, malignant tumor, mental disorders, pregnancy, or severe liver or kidney dysfunction. Participants were also excluded from this study if they previously had ocular trauma or surgery, corneal pathology, contact lens use history, or any other disease affecting the cornea. Participants who had cataract surgery in the last year were also excluded.

Anthropometric and laboratory measurement
A clinical examination of each participant was performed by trained researchers to assess demographic and anthropometric parameters, including height, weight, blood pressure (BP), and waist and hip circumference. Body mass index (BMI) was calculated as weight (kilograms) divided by squared height (meters). BP was measured three times in the sitting position after a rest period of >5 min using a standard mercury sphygmomanometer, and then the average value was taken. Diabetes duration, alcohol consumption, smoking status, hypertension history, and history of other diseases were also collected in the hospital using a standardized questionnaire. A person who had smoked continuously or cumulatively for ≥6 months was defined as a smoker. Alcohol consumption was defined as the amount of alcohol consumed >100 mL/week. Each patient underwent the assessment of neurological symptoms and signs, which was based on the Toronto Clinical Scoring System. Any pain, numbness, tingling, foot weakness, ataxia, or upper-limb symptoms were considered positive symptoms. In addition, reflexes of the ankle and knee tendons were also assessed by the same physician.

Venous blood was drawn from all subjects after an overnight fast to assess the following laboratory parameters: fasting plasma glucose, fasting C-peptide (FCP), total cholesterol, triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), glycosylated hemoglobin A1c (HbA1c), glycated albumin (GA), alanine amino-transferase (ALT), aspartate amino-transferase, urea nitrogen (BUN), serum creatinine (Scr), and uric acid (UA) levels. Lipid profiles, BUN, Scr, and UA concentrations were analyzed by applying standard enzymatic methods using a biochemical analyzer (7600-120; Hitachi, Tokyo, Japan). Plasma glucose was measured using the Glamour 2000 autoanalyzer (Molecular Devices, Sunnyvale, CA, USA) and the glucose oxidase method (Glucose Kit; Shanghai Kehua Bio-engineering, Shanghai, China). HbA1c was measured by using high performance liquid chromatography (Bio-Rad Variant II; Bio-Rad Laboratories, Hercules, CA, USA). The GA value was determined using an enzyme-based assay (Lucica GA-L; Asahi Kasei Pharma, Tokyo, Japan) and the Glamour 2000 autoanalyzer.

Assessment of CGM parameters
The iPro2 system (Medtronic Inc, Northridge, CA, USA) was used for subcutaneous interstitial glucose monitoring. The sensor (Enlite, Medtronic Inc) recorded glucose levels every 5 min for seven consecutive days, and was inserted on the first day and removed after 7 days, generating a daily record of 288 continuous sensor values. At least two capillary blood glucose readings per day were measured by using a Sure Step blood glucose meter (LifeScan, Milpitas, CA, USA) to calibrate the CGM system. TIR was defined as the percentage of time in the target glucose range of 3.9 to 10.0 mmol/L during the 7 days. Intraday glycemic variability (GV) parameters included the standard deviation (SD) of sensor glucose values, glucose coefficient of variation (CV), and mean amplitude of glycemic excursions (MAGE). CV was determined as SD divided by the mean glucose level. In addition, the arithmetic mean of the differences between consecutive nadirs and peaks was computed to determine the MAGE value. During the 7-day CGM period, all subjects adhered to the original therapy regimen and standard diet.

Assessment of CCM parameters
CCM (Heidelberg Retinal Tomograph III with Rostock Cornea Module; Heidelberg Engineering GmbH, Heidelberg, Germany) was performed by one trained examiner on the right eye of each subject to image the central corneal subbasal nerve plexus. First, a drop of gel (Genteal; Alcon, Fort Worth, TX, USA) was applied between the newly opened TomoCap (Heidelberg Engineering GmbH) and the tip of the objective lens. After informing the patient of the steps and operation precautions, the right eye was anesthetized with a drop of 0.5% proparacaine hydrochloride (Alcon). Approximately 50 single images were obtained during each examination. The images had a size of 384 μm × 384 μm. Two to five clearest images from each subject were selected for analysis using the validated and fully automated nerve analysis software ACCMetrics (Corneal Nerve Fiber Analyzer V.2, University of Man...
The linear regression model was used to explore the potential associations between TIR and CCM parameters (CNFD, CNBD, and CNFL) as continuous variables after controlling for confounding factors. The restricted cubic spline model was conducted to test whether there was nonlinear association of TIR as a continuous variable with the risk of abnormal CNFL. Binary logistic regression analysis was performed to investigate the effect of TIR on the risk of abnormal CNFL. In some comparisons, the patients were also grouped by quartiles. Receiver operating characteristic (ROC) curves analyses were performed to find the cutoff points and performance of TIR for predicting abnormal CNFL in asymptomatic adult T2DM patients. A two-sided P value of <0.05 was considered statistically significant.

**Results**

A total of 206 patients were enrolled in this study, including 121 men and 85 women aged 19 to 86 years, with the mean diabetes duration of 11 years. Among them, abnormal CNFL was found in 63 (30.6%) subjects. The clinical characteristics of patients categorized by the quartiles of CNFL (quartile 1 [Q1]: ≤14.58 mm/mm²; quartile 2 [Q2]: 14.59–16.37 mm/mm²; quartile 3 [Q3]: 16.38–18.11 mm/mm²; quartile 4 [Q4]: ≥18.12 mm/mm²) were shown in Table 1. There were significant differences in CNFD, CNBD, SD, MAGE, TIR (all

**Table 1: Comparisons of clinical characteristics in CNFL quartile groups of asymptomatic adult T2DM patients.**

| Variables | All subjects | Q1 (≤14.58) | Q2 (14.59–16.37) | Q3 (16.38–18.11) | Q4 (≥18.12) | P for trend |
|-----------|--------------|-------------|-----------------|------------------|-------------|------------|
| Male sex (%) | 58.74 | 59.62 | 59.62 | 54.90 | 60.78 | 0.934 |
| Age (years) | 61 (47, 66) | 61 (51, 69) | 61 (48, 64) | 60 (47, 66) | 60 (38, 67) | 0.140 |
| Diabetes duration (years) | 11.0 (2.0, 17.0) | 12.5 (2.7, 20.0) | 10.0 (3.0, 15.7) | 12.0 (2.0, 17.0) | 9.0 (1.2, 15.0) | 0.082 |
| Male sex (%) | 58.74 | 59.62 | 59.62 | 54.90 | 60.78 | 0.934 |
| BMI (kg/m²) | 24.79 (22.82, 26.98) | 24.99 (22.57, 27.28) | 24.17 (23.51, 27.02) | 24.95 (23.51, 27.02) | 24.71 (22.02, 26.87) | 0.838 |
| TC (mmol/L) | 4.64 ± 1.19 | 4.60 ± 1.15 | 4.85 ± 1.11 | 4.66 ± 1.18 | 4.45 ± 1.34 | 0.412 |
| TG (mmol/L) | 1.26 (0.89, 1.96) | 1.09 (0.79, 1.83) | 1.29 (0.94, 1.84) | 1.50 (0.96, 2.35) | 1.13 (0.83, 1.64) | 0.369 |
| HDL-C (mmol/L) | 1.13 (0.93, 1.31) | 1.17 (0.99, 1.45) | 1.12 (0.87, 1.32) | 1.07 (0.89, 1.27) | 1.12 (0.94, 1.30) | 0.131 |
| LDL-C (mmol/L) | 2.68 ± 0.90 | 2.59 ± 0.81 | 2.79 ± 0.90 | 2.75 ± 0.87 | 2.57 ± 0.83 | 0.866 |
| FPG (mmol/L) | 7.06 (5.66, 8.63) | 7.40 (5.77, 8.65) | 7.18 (5.15, 8.42) | 7.51 (5.54, 8.81) | 6.38 (5.47, 8.63) | 0.541 |
| HbA1c (%) | 19.90 (16.80, 25.90) | 23.90 (19.03, 27.58) | 19.20 (15.70, 23.90) | 20.40 (16.83, 25.90) | 18.55 (16.10, 23.05) | 0.004 |
| BUN (mmol/L) | 68.70 (54.45, 90.00) | 71.00 (62.15, 90.00) | 60.00 (52.00, 79.00) | 67.00 (53.05, 90.00) | 63.00 (48.00, 81.50) | 0.176 |
| HbA1c (%) | 23.17 (17.33, 25.00) | 23.17 (17.33, 25.00) | 23.17 (17.33, 25.00) | 23.17 (17.33, 25.00) | 23.17 (17.33, 25.00) | 0.368 |
| ALT (U/L) | 328 (272, 375) | 323 (264, 364) | 319 (279, 359) | 349 (285, 392) | 316 (250, 379) | 0.779 |
| AST (U/L) | 26.70 | 25.00 | 32.69 | 29.41 | 19.61 | 0.469 |
| Cr (μmol/L) | 82.5 | 83.46 | 83.79 | 83.92 | 84.15 | 0.384 |
| CNFD (No./mm²) | 24.65 (2.08, 0.89) | 2.68 (0.98, 3.75) | 2.33 (0.75, 3.85) | 2.33 (0.81, 3.75) | 2.21 ± 0.89 | <0.001 |
| FPG (mmol/L) | 7.06 (4.96, 10.74) | 7.40 (5.77, 8.65) | 7.18 (5.15, 8.42) | 7.51 (5.54, 8.81) | 6.38 (5.47, 8.63) | 0.541 |
| CNBD (No./mm²) | 43.75 (31.25, 56.25) | 43.75 (31.25, 56.25) | 43.75 (31.25, 56.25) | 43.75 (31.25, 56.25) | 43.75 (31.25, 56.25) | 0.001 |
| TIR (%) | 72.49 ± 18.15 | 61.65 ± 20.53 | 74.68 ± 23.78 | 71.69 ± 20.88 | 80.27 ± 14.30 | <0.001 |

Data were mean ± SD and median (Q2, Q1–Q3), or as percentage unless otherwise indicated. ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; BMI: Body mass index; BUN: Urea nitrogen; CNBD: Corneal nerve branch density; CNFL: Corneal nerve fiber length; CV: Coefficient of variation; DBP: Diastolic blood pressure; FC: Fasting C-peptide; FPG: Fasting plasma glucose; GA: Glycated albumin; HbA1c: Glycosylated hemoglobin A1c; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol; MAGE: Mean amplitude of glycemic excursions; SBP: Systolic blood pressure; Scr: Serum creatinine; SD: Standard deviation; T2DM: Type 2 diabetes; TG: Total cholesterol; TIR: Time in range; UA: Uric acid.
levels are shown in Figure 1. With the increase of TIR quartiles, corneal nerve fiber parameters increased significantly (all P for trend < 0.01) [Figure 2A]. However, there were no significant differences in corneal nerve fiber parameters among the quartiles of HbA1c ([Q1]: ≤7.08%; [Q2]: 7.09–8.20%; [Q3]: 8.21–9.70%; [Q4]: ≥9.71%) (all P for trend > 0.05, Figure 2B).

Next, all patients were stratified by TIR quartiles ([Q1]: ≤59%; [Q2]: 60–76%; [Q3]: 77–86%; [Q4]: ≥87%). The representative CCM images of subjects with varying TIR levels are shown in Figure 1. With the increase of TIR quartiles, corneal nerve fiber parameters increased significantly (all P for trend < 0.01) [Figure 2A]. However, there were no significant differences in corneal nerve fiber parameters among the quartiles of HbA1c ([Q1]: ≤7.08%; [Q2]: 7.09–8.20%; [Q3]: 8.21–9.70%; [Q4]: ≥9.71%) (all P for trend > 0.05, Figure 2B).

Figure 1: Representative images of CCM according to TIR quartiles. (a) atypical image from TIR quartile 1 [Q1]; (b) a typical image from TIR quartile 2 [Q2]; (c) atypical image from TIR quartile 3 [Q3]; (d) a typical image from TIR quartile 4 [Q4]. The images (a, b, c, d) were analyzed by automatic neural analysis software to obtain the images (A, B, C, D): (A) (CNFD = 28.8, CNBD = 31.3, CNFL = 13.1); (B) (CNFD = 25.0, CNBD = 37.5, CNFL = 15.7); (C) (CNFD = 37.5, CNBD = 68.8, CNFL = 17.2); (D) (CNFD = 37.5, CNBD = 62.5, CNFL = 22.1). CNFD and CNBD were expressed in units of nerve per square millimeter (No./mm²) and CNFL in units of mm/mm². All images were 384 μm × 384 μm. Blue lines showed corneal nerve branches and red lines showed corneal nerve fibers. CCM: Corneal confocal microscopy; CNBD: Corneal nerve branch density; CNFD: Corneal nerve fiber density; CNFL: Corneal nerve fiber length; TIR: Time in range.

Figure 2: (A) Box diagram plots demonstrating the distribution of CNFD, CNBD, and CNFL according to TIR quartiles. There were significant differences in corneal nerve fiber parameters (CNFD, P for trend = 0.006; CNBD, P for trend = 0.001; CNFL, P for trend = 0.004) among the quartiles of TIR. (B) Box diagram plots demonstrating the distribution of CNFD, CNBD, and CNFL according to HbA1c quartiles. There were no significant differences in corneal nerve fiber parameters (CNFD, P for trend = 0.773; CNBD, P for trend = 0.189; CNFL, P for trend = 0.079) among the quartiles of HbA1c. CNBD: Corneal nerve branch density; CNFD: Corneal nerve fiber density; CNFL: Corneal nerve fiber length; HbA1c: Glycosylated hemoglobin A1c; TIR: Time in range.
As shown in the restricted cubic spline model, the linear relationship of TIR with abnormal CNFL was observed ($P$ for nonlinearity = 0.711) [Figure 3]. Compared with the reference point (TIR = 70%), patients with TIR <70% had significantly higher risk of abnormal CNFL [Figure 3]. Linear regression analyses revealed that TIR was positively correlated with all corneal nerve fiber parameters (CNFD, CNBD, and CNFL) both in the crude and adjusted models including age, sex, BMI, diabetes duration, systolic blood pressure (SBP), TG, HDL-C, and LDL-C as covariates (all $P$ < 0.05, Table 2). Each 10% increase in TIR was associated with a 28.2% (95% CI: 0.595–0.866, $P$ = 0.001) decreased risk of abnormal CNFL [Table 2, Model 1], and the statistical significance remained ($P$ < 0.001) even after adjustment of HbA1c [Table 2, Model 2]. In contrast, less consistent results regarding the relationship between corneal nerve fiber parameters and HbA1c were observed [Table 3]. FCP was correlated with CNBD ($P$ = 0.042) and the risk of abnormal CNFL ($P$ = 0.001) in the adjusted models including age, sex, BMI, diabetes duration, SBP, TG, HDL-C, LDL-C, and HbA1c as covariates [Table 3, Model 5].

The ROC curve of TIR for the identification of abnormal CNFL indicated that the optimal cutoff point of TIR was 77.5% for predicting abnormal CNFL in asymptomatic patients (area under curve = 0.673; 95% CI, 0.591–0.755; $P$ < 0.001; Youden index = 0.307; sensitivity, 75.8%; specificity, 54.9%).

**Discussion**

The diagnosis of DSPN is mainly a clinical diagnosis. As up to half of the patients may be asymptomatic, early diagnosis is difficult in many cases, leading to delayed treatment and therefore higher risk of foot ulceration and increased mortality.[2,17] Quantitative evaluation of small fiber injury is the key to early diagnosis of DSPN.

Conventional methods for assessing DSPN, such as the clinical neurological assessment and quantitative sensory testing, are limited by either poor reproducibility[18] or subjectivity. Of note, IENFD in skin biopsy specimens is widely used as a gold standard to evaluate small fiber injury, but it is invasive. Ideally, a non-invasive, non-subjective, cost-effective test should be developed for the early diagnosis of DSPN.

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is the current gold standard of small fiber damage, but this method is invasive and requires specialist judgment. CCM is an in vivo ophthalmic imaging modality which can identify early small nerve fiber damage and accurately quantify the severity of DSPN. Perkins et al. demonstrated that corneal sensitivity gradually decreased and corneal nerve degeneration increased with the increase of the severity of DSNP in a large cohort of 9988 patients, and the diagnostic validity and diagnostic thresholds of CNFL in type 1 diabetes (T1DM) and T2DM were established. A previous study showed that the CNFL best discriminated DSPN cases from control among CCM parameters. Moreover, CCM had been proved to have comparable diagnostic efficiency with IENFD. In our study population, which was composed of asymptomatic patients, abnormal CNFL was observed in 30.6% of participants, indicating that a large portion of subjects were affected by small fiber neuropathy at the preclinical stage.

There is convincing evidence that glucose control is tightly linked to diabetic neuropathy. With the rapid growth of CGM usage, TIR has been considered as a promising glycemic marker of glucose control, as this metric was reported to be associated with microvascular complications, cardiovascular disease, pregnancy-related outcomes, and mortality. Our study showed that among the parameters of CGM, TIR was significantly related to parameters of corneal nerve fiber loss. Interestingly, in our study the relationship between HbA1C and corneal nerve fiber loss did not reach statistical significance in most analyses, which was consistent with a previous study. A possible explanation for this observation could be that many factors such as anemia, kidney function, and ethnicity could interfere with the measurement of HbA1C. Indeed, TIR, but not HbA1C, was reported to be significantly associated with DSPN ascertained by the Michigan Neuropathy Screening Instrument questionnaire in 105 patients with T2DM and chronic kidney disease. Besides, it is noteworthy that TIR and HbA1C reflect different aspects of glycemia. HbA1C is an indirect measure of average glucose, and it provides no indication of hyperglycemia, hypoglycemia, and GV. Indeed, significant associations of TIR with CCM parameters were noted after further adjustment of HbA1C, suggesting that TIR may provide additional information beyond HbA1C. Taken together, our findings implied that, as compared with HbA1C, TIR may be a more sensitive and valid marker for assessing the risk of early stage DSPN, which needs to be validated in the future.

In addition to TIR, a significant association of C-peptide with CCM parameters was noted in this study. It is obvious that higher C-peptide is related to better glucose control and therefore lower risk of DSPN. Besides, there is evidence that C-peptide is an endogenous peptide with physiological effects of its own. C-peptide levels were related to fewer and slower development of diabetic microvascular complications, consistent with antioxidant protection by C-peptide. Several clinical trials investigating C-peptide replacement therapy effects had indicated potential therapeutic effects in T1DM patients, and positive effects on nerve and kidney function. In addition to directly reflecting the endogenous islet cell function, C peptide can also bind to G protein coupled receptor on cell membrane, causing calcium influx, to activate Na+-K+-adenosine triphosphate pump and vascular endothelial nitric oxide synthase activity, so as to improve neuronal energy metabolism, endothelial cell function, stimulate the release of nerve growth factor, and play a neuroprotective role.

The strength of this analysis is that it is a rare study to explore the potential relationships between CGM parameters and early stage DSPN ascertained by CCM in asymptomatic patients with T2DM. The present study
also has some limitations that need to be noted. First, due to the cross-sectional design of the study, the temporal relationship between TIR and DSPN could only be inferred. The second limitation of this study is that each patient underwent 7 days of CGM for the evaluation of TIR and GV metrics, while 14 days of monitoring may be needed to optimally assess the glucose status. In addition, only hospitalized patients with T2DM were enrolled in the study. Therefore, our results may not be generalizable to patients in the ambulatory setting or those with T1DM. Finally, due to the limited data on CCM in Chinese subjects, the cut-off point for defining abnormal CNFL in the current study was derived from a large multicenter consortium study in predominantly Caucasians. Therefore, the possibility of misclassification could not be fully excluded.

In conclusion, small nerve fiber damage assessed by CCM was found in around 30% of asymptomatic subjects with T2DM. This significant association between TIR and CNFL supported TIR as a sensitive glycemic marker for the identification of early-stage DSPN.

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Conflicts of interest

None.

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