Continuous Glucose Monitoring Reveals a Novel Association between Duration and Severity of Hypoglycemia and Small Nerve Fiber Injury in Patients with Diabetes

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Received: 06-Jul-2022, Manuscript No. jdm-22-18241; **Editor assigned:** 08-July-2022, PreQC No: jdm-22-18241(PQ); **Reviewed:** 22-Jul-2022, QC No. jdm-22-18241; **Revised:** 27-Jul-2022, Manuscript No. jdm-22-18241(R); **Published:** 03-Aug-2022, DOI: 10.35248/2155-6156.1000943

Abstract

Background/Aim: Continuous glucose monitoring (CGM) has revealed that glycemic variability (GV) and low time in range (TIR) are associated with albuminuria and retinopathy. We have investigated the relationship between CGM metrics and highly sensitive markers of nerve pathology using corneal confocal microscopy (CCM) in participants with type 1 and type-2 diabetes.

Methods: A total of 40 participants with diabetes and 28 healthy controls underwent quantification of corneal nerve fiber density (CNFD), corneal nerve branch density (CNBD), corneal nerve fiber length (CNFL) and inferior whorl length (IWL). Participants with diabetes underwent CGM for 4 consecutive days.

Results: CNBD was significantly lower in patients with high GV compared to low GV median (range) (25.0(19.0-37.5) vs. 38.6(29.2-46.9); P=0.007); in patients who spent >4% compared to <4% time below range (54-69 mg/dl) (25.0(22.9-37.5) vs. 37.5(29.2-46.9); P=0.045) and in patients who spent >1% compared to <1% time in severe hypoglycemia (<54 mg/dl) (25.0 (19.8-41.7) vs. 35.4 (28.1-44.8); P=0.04). Duration in hyperglycemia and severe hyperglycemia did not correlate with CNFD (P>0.05), CNBD (P>0.05), CNFL (P>0.05) or IWL (P>0.05), but duration in hypoglycemia correlated with CNBD (r=-0.342, P=0.031).

Conclusions: Greater glucose variability and duration in hypoglycemia, rather than hyperglycemia are associated with nerve fiber loss in diabetes.

Keywords: CCM • Diabetes • Hypoglycemia • Peripheral Neuropathy • CGM • TIR

Introduction

Diabetic peripheral neuropathy (DPN) affects ~50% of people with T1DM and T2DM [1, 2]. It has an insidious onset which can lead to painful diabetic neuropathy, erectile dysfunction, foot ulceration and lower limb amputation [1]. Recognized risk factors for DPN include poor glycemic control, obesity, hypertension, and dyslipidemia [3, 4]. However, HbA1c provides limited insight in the short-term variations in blood glucose which may affect nerve fibers [5]. Continuous glucose monitoring (CGM) provides time in range (TIR) which is directly related to HbA1c, but also additional measures in relation to high and low blood glucose levels [6, 7].

Increased glycemic variability and low time in range (TIR) were associated with albuminuria and retinopathy, whilst neuropathy was associated with the standard deviation of blood glucose levels (SD) and mean amplitude of glycemic excursions (MAGE) [5]. In a small proof of principle study, a higher mean glucose, M-value and greater glycemic excursions were demonstrated in patients with painful compared to painless diabetic neuropathy [8]. A recent systematic review evaluating the relationship between CGM-derived TIR, and diabetic peripheral neuropathy (DPN) demonstrated that a 10% increase in TIR was associated with a reduction in the prevalence of DPN and cardiac autonomic neuropathy [9].

Severe iatrogenic hypoglycaemia can lead to neurological sequelae including cerebral dysfunction, seizures, and death and recurrent hypoglycemia is associated with hypoglycaemia-associated autonomic failure (HAAF), reduced sympathetic neural responses and autonomic neuropathy [10-12]. In a recent study higher MAGE and CV and especially nocturnal hypoglycemia were associated with an increased risk of DPN [13]. In a study of 80 adults with T1DM the standard deviation, coefficient of variation, mean amplitude of glycaemic excursion, percent time in level 1 (glucose 54-69 mg/dL) and level 2 (glucose < 54 mg/dL) hypoglycaemia, low blood glucose index and high blood glucose index were independently associated with cardiac autonomic neuropathy (CAN) [14]. Sudomotor dysfunction, a measure of peripheral autonomic dysfunction [15] has been independently associated with TIR in T1DM [16] and T2DM [17].

Corneal confocal microscopy (CCM) is a rapid non-invasive ophthalmic imaging technique that can identify early small nerve fiber loss in patients with DPN [18] and has demonstrated comparable diagnostic utility to intra-epidermal nerve fiber density (IENFD) [4, 19, 20]. A recent meta-analysis has confirmed the diagnostic utility of CCM in sub-clinical and clinical DPN [21]. In the current study we have investigated the relationship between different glucose metrics obtained using CGM and corneal nerve pathology using CCM in patients with type-1 and type-2 diabetes.

Methods

Patients

We recruited 68 participants (20 T1DM, 20 T2DM, and 28 healthy volunteers) between June 2021 to October 2021. Inclusion criteria were age ≥ 18 years and treatment with insulin. Exclusion criteria included vitamin B12 or folic acid deficiency, cancer, pregnancy, breast-feeding, or cardiac, liver, or renal dysfunction. Participants were also excluded if they had corneal pathology, allergy to eye-drops or previous ocular trauma or surgery in the past six months. The study was approved by the Ethics Committee of Weill Cornell Medicine-Qatar, Hamad Medical Corporation, and Qatar University and was designed in accordance with the principles of the Helsinki Declaration. Written informed consent was obtained from all participants.

Basic and clinical demographics

Participants' height, weight, BMI, and blood pressure (BP) were measured. The lipid profile: total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and glycosylated hemoglobin A1c (HbA1c) were assessed.

Continuous Glucose Monitoring (CGM)

The Freestyle Libre 1 system (Abbott) was used for subcutaneous interstitial continuous glucose monitoring. The sensor recorded glucose levels every 5 minutes for 4 consecutive days. The sensor was placed on the upper back part of the arm. Time in range 70-180mg/dL (TIR) was defined as >70% of the glucose readings (~16h 48min), time below range <70 mg/dL (TBR) <4% of the reading (~58min), TBR <54 mg/dL <1% of readings (~14min), time above range >180 mg/dL (TAR) <25% of the readings (~6h), TAR >250 mg/dL <5% of the readings (1h 12min). Glucose variability was defined as percent coefficient of variation (%CV) with a target ≤36%.

Corneal Confocal Microscopy (CCM)

Corneal confocal microscopy was undertaken using the Heidelberg Retina Tomograph Cornea Module (Heidelberg Engineering, Germany). Both eyes were anaesthetized with 2 drops of Bausch & Lomb Minims ® (Oxybuprocaine hydrochloride 0.4% w/v). A drop of hypotears gel (Carbomer 0.2% eye gel) was placed on the tip of the objective lens and a sterile disposable TomoCap was placed over the lens, allowing optical coupling of the objective lens to the cornea. Six images were selected from the sub basal nerve plexus (SBNP) in the central cornea and corneal nerve fiber density (CNFD) (fibers/mm^2) corneal nerve branch density (CNBD) (branches/mm2) and corneal nerve fiber length (CNFL) (mm/mm^2) were quantified manually using CCMetrics. Six images centered on the inferior whorl and immediately adjacent area were selected and inferior whorl length (IWL) (mm/mm^2) was quantified manually using the manual CNFL mode in CCMetrics. The investigator was blind to the study group when performing CCM and analyzing CCM images.

Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics software Version 27 and P<0.05 was considered statistically significant. Normality of the data was assessed using the Shapiro-Wilk test and by visual inspection of the histogram and a normal Q-Q plot. Data are expressed as mean and SD for the normally distributed variables and as median and range for the skewed variables. Inferential analyses were conducted for the corneal nerve parameters and clinical demographics using both parametric (T-test) and non-parametric (Mann-Whitney U) tests, with post-hoc adjustment. To investigate the association between corneal nerve parameters and clinical and CGM variables, Pearson and Spearman correlation were performed as appropriate. Graph prism version 9 was used to build dot plots.

Results

A total of 40 participants with diabetes aged 37-48 years and 28 healthy controls aged 24-49 years were enrolled in the study. Participants with diabetes and controls had comparable systolic blood pressure (SBP) mmHg (P=0.45), diastolic blood pressure (DBP) mmHg (P=0.45) and BMI kg/m^2 (P=0.20) Table 1. Interstitial glucose was in range for 60% of patients with diabetes, 32% were above range and 8% were very high (Figure 1). CNFD fiber/mm2 (25.79±5.96 vs. 29.97±6.02; P=0.006), CNBD branch/mm^2 (31(26.0-40.60) vs. 56.25(46.87-68.75); P<0.001), CNFL mm/mm2 (16.78±4.10 vs. 22.55±3.57; P<0.001) and IWL mm/mm2 (15.35±6.2 vs. 20.82±5.07; P<0.001) were significantly lower in participants with diabetes compared to controls (Table 1). Participants with diabetes spent an average time in range of 719.4±322.7 minutes, 388.62±223.57 minutes above range and 73.77±76.70 minutes below range with an average of 2.9 hypoglycemic events over a period of 4 days (Table 2). (Tables 1 and 2) (Figure 1)

Corneal confocal microscopy (CCM) based on CGM

CNFD (P=0.50), CNBD (P=0.68), CNFL (P=0.71) and IWL (P=0.10) did not differ between patients who had diabetes duration for <10 years, >10 years or more than 20 years (Table 3). There was no difference in CNFD (P=0.67), CNBD (P=0.89), CNFL (P=0.85), and IWL (P=0.47) between participants with an HbA1c <8% or >8%. CNBD was significantly lower in patients with high GV compared to low GV (25.0(19.0-37.5) vs. 38.6(29.2-46.9); P=0.007). There was no difference in CNFD (P=0.62), CNFL (P=0.09) and IWL (P=0.73) between patients with high GV compared to low GV. There was no significant difference in CNFD (P=0.64), CNBD (P=0.75), CNFL (P=0.91) and IWL (P=0.59) between participants with diabetes who spent >70% time in range (TIR) (70-180 mg/dl) and <70% TIR. CNBD was significantly lower (25.0(22.9-37.5) vs. 37.5(29.2-46.9); P=0.045), with no difference in CNFD (P=0.38), CNFL (P=0.51) and IWL (P=0.35) in patients who spent >4% time below range (54-69 mg/dl) compared to <4% time below range. CNBD (25.0 (19.8-41.7) vs. 35.4 (28.1-44.8); P=0.04) was significantly lower, whilst CNFD (P=0.79), CNFL (P=0.36) and IWL (P=0.62) did not differ between patients who spent >1% compared to <1% time in severe hypoglycemia (<54 mg/dl). CNFD (P=0.71), CNBD (P=0.09), CNFL (P=0.43) and IWL (P=0.37) did not differ between patients who had >1 hypoglycemic event compared to those who had no hypoglycemic events. CNFD (P=0.61), CNBD (P=0.44), CNFL (P=0.83) and IWL (P=0.62) did not differ between

Table 1: Demographics of diabetic patients and controls.

Demographics	Healthy volunteers	Diabetes	P-Value
Subjects, n	28	40 T1DM, 20 T2DM, 20	-
M:F ratio	22:6	28:12	-
Age (years)	36(24-49)	41(37-48)	0.11
Diabetes duration (years) <10years 10-20years 21-40yeas	-	9(22.5) 23(56.5) 8(20)	-
SBP (mmHg)	122(120-136)	123(119-127)	0.45
DBP (mmHg)	79.04±10.58	77.03±10.39	0.45
BMI (kg/m^2)	27.05±5.48	28.84±5.69	0.20
TC (mmol/L)	<5.2	4.38(3.90-4.95)	-
TG (mmol/L)	<1.7	1.86±2.33	-
HDL-C (mmol/L)	>1.0	1.30±0.36	-
LDL-C (mmol/L)	<2.59	2.30(0.90-3.60)	-
Interstitial glucose (mg/dL)	<100	178.77±47.38	-
HbA1c (%)	<5.7	8.85±1.70	-
CNFD (fiber/mm^2)	29.97±6.02	25.79±5.96	**0.006***
CNBD (branch/mm^2)	56.25(46.87-68.75)	31(26.0-40.60)	**<0.001***
CNFL (mm/mm^2)	22.55±3.57	16.78±4.10	**<0.001***
IWL (mm/mm^2)	20.82±5.07	15.35±6.21	**<0.001***

M: male, F: female, SBP: systolic blood pressure, DBP: diastolic blood pressure, BMI: body mass index, TC: total cholesterol, TG: triglycerides, HDL-C: high density lipoprotein-cholesterol, LDL-C: low-density lipoprotein cholesterol, HbA1c: glycated hemoglobin, CNFD: corneal nerve fiber density, CNBD: corneal nerve branch density, CNFL: corneal nerve fiber length, IWL: inferior whorl length.

Table 2: CGM time in different ranges and hypoglycemic events in patients with diabetes.

TIR (min) (70-180 mg/dL)	TAR (min) (181-250 mg/dL)	TBR (min) (54-69 mg/dL)	No. of hypoglycemic events
719.4±322.7	388.62±223.57	73.77±76.70	2.90±3.43
Data is expressed as mean ± SD. CGM: continuous glucose monitoring, TIR: time in range, TAR: time above range, TBR: time below range.			

Table 3: Changes in corneal nerve morphology in relation to different glucose metrics on CGM.

Glycemic control indicators	CNFD	CNBD	CNFL	IWL
Duration of diabetes				
<10years	27.21±5.99	35.40(25.0-50.0)	17.28±2.17	15.54±6.96
10-20years	24.83±5.64	29.2(25.0-39.6)	16.32±4.19	13.89±5.57
21-40years	26.96±7.10	39.6(18.7-55.2)	17.55±5.62	19.31±6.08
P-value	0.50	0.68	0.71	0.10
HbA1c%				
<8%	26.49±6.30	33.3(25.0-43.7)	17.0±2.44	16.60±5.57
>8%	25.55±6.30	30.2(25.0-40.6)	16.71±4.56	14.93±6.44
P-Value	0.67	0.89	0.85	0.47
GV (%CV)				
Low <36%	26.17±6.26	38.6(29.2-46.9)	17.68±4.64	5.63±6.82
High>36%	25.21±5.65	25.0(19.0-37.5)	15.43±2.75	14.92±5.34
P-Value	0.62	**0.007***	0.09	0.73
TIR (70-180 mg/dL)				
In range >70%	26.70±4.66	31.25(26.0-44.8)	16.94±3.0	14.29±6.26
In range <70%	25.56±6.29	31.20(25.0-40.6)	16.74±4.37	15.61±6.27
P-Value	0.64	0.75	0.91	0.59
TBR (54-69 mg/dL)				
Below range >4%	27.0±5.08	25.0(22.9-37.5)	16.15±2.31	14.02±6.45
Below range <4%	25.20±6.35	37.5(29.2-46.9)	17.08±4.75	15.99±6.11
P-Value	0.38	**0.045***	0.51	0.35
TBR (<54 mg/dL)				
Severely below range >1%	26.37±6.6	25.0(19.8-41.7)	15.66±2.93	14.42±6.63
Severely below range<1%	25.65±5.86	35.4(28.1-44.8)	17.11±4.38	15.62±6.17
P-Value	0.79	**0.040***	0.36	0.62
Hypoglycemic events				
>1 event	26.1±5.38	28.1(25.0-39.6)	16.33±3.51	14.59±5.77
No events	25.37±6.83	37.5(29.2-51.0)	17.39±4.84	16.38±6.78
P-Value	0.71	0.08	0.43	0.37
TAR (181-250 mg/dL)				
Above range >25%	25.34±6.92	36.5(25.0-50.0)	16.90±5.23	15.80±6.18
Above range <25%	26.33±4.67	30.2(25.0-37.5)	16.63±2.19	14.79±6.38
P-Value	0.61	0.44	0.83	0.62
TAR (>250 mg/dL)				
Severely above range >5%	25.51±6.33	31.20(25.0-41.70)	16.74±4.46	16.13±6.48
Severely above range <5%	26.74±4.72	31.20(27.10-43.70)	16.94±2.75	12.53±4.44
P-Value	0.59	0.97	0.89	0.14
CNFD: corneal nerve fiber density, CNBD: corneal nerve branch density, CNFL: corneal nerve fiber length, IWL: inferior whorl length, HbA1c: glycated hemoglobin, GV: glycemic variability, TIR: time in range, TBR: time below range, TAR: time above range.				

patients who spent >25% time in hyperglycemia (181-250 mg/dl) compared to those who spent <25% time in hyperglycemia. CNFD (P=0.59), CNBD (P=0.97), CNFL (P=0.89) and IWL (P=0.14) did not differ between patients who spent >5% time in severe hyperglycemia (>250 mg/dl) compared to patients who spent <5% time in severe hyperglycemia (Table 2). CNFD (P=0.11) did not differ significantly between patients in TIR, TAR or TBR compared to healthy controls. CNBD (P<0.0001) and CNFL (P<0.0001) were significantly lower in participants with diabetes in TIR, TAR and TBR compared to healthy controls (Figure 2A-G). (Table 3) (Figure 2A-G)

Correlation between corneal nerve parameters and CGM indicators of glycemia

Duration of diabetes (years), plasma glucose, average interstitial glucose, and HbA1c did not correlate with CNFD (P>0.05), CNBD (P>0.05), CNFL (P>0.05) and IWL (P>0.05) (Table 4). Glucose variability correlated significantly with CNBD (r=-0.398, P=0.011) (Figure 3A), but did not correlate with CNFD (P>0.05), CNFL (P>0.05), or IWL (P>0.05). Duration in hyperglycemia and severe hyperglycemia did not correlate with CNFD (P>0.05), CNBD (P>0.05), CNFL (P>0.05) and IWL (P>0.05). However, the duration in hypoglycemia

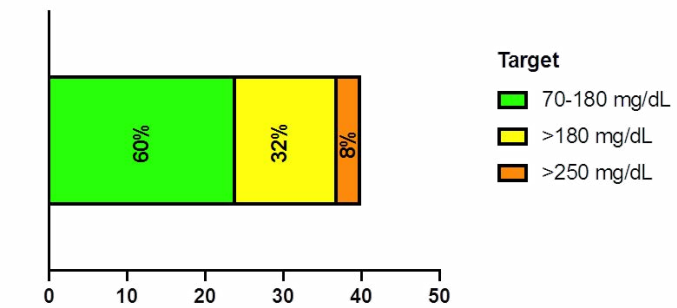
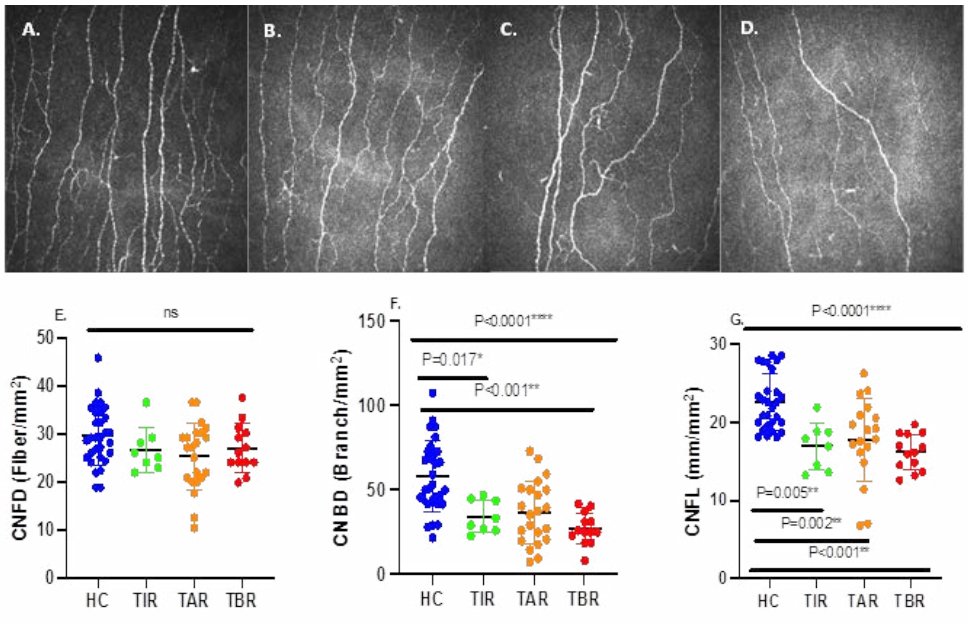


Figure 1: Distribution of diabetic patients based on their interstitial glucose targets.

correlated significantly with CNBD (r=-0.342, P=0.031) (Figure 3B), but not with CNFD (P>0.05), CNFL (P>0.05) and IWL (P>0.05). The number of hypoglycemic events did not correlate with CNFD (P>0.05), CNBD (P>0.05), CNFL (P>0.05) or IWL (P>0.05). (Table 4) (Figure 3A, 3B)



CNFD: corneal nerve fiber density, CNBD: corneal nerve branch density, CNFL: corneal nerve fiber length, HC: healthy control, TIR: time in range, TAR: time above range, TBR: time below range

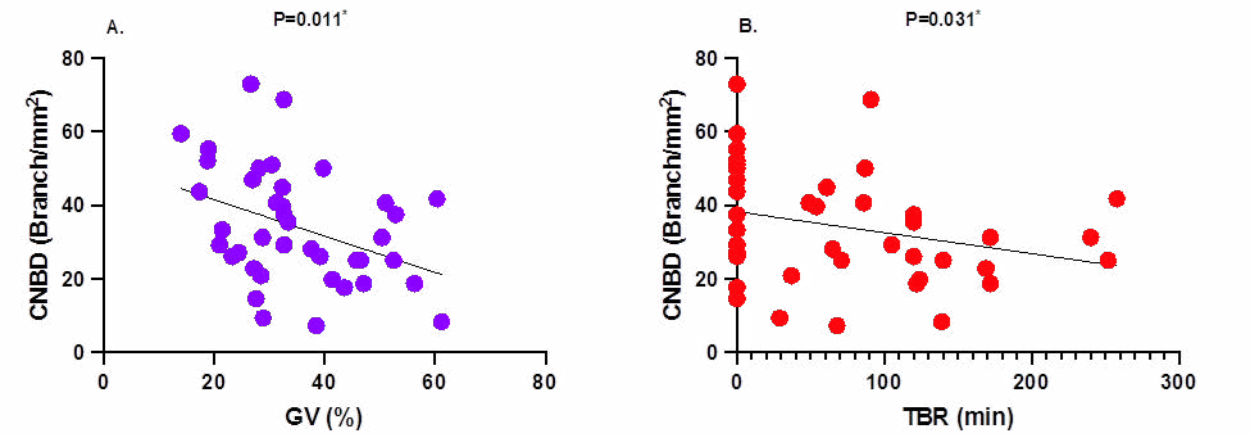
A. HC
B. Diabetic with TIR
C. Diabetic with TAR
D. Diabetic with TBR
E. CNFD in HC vs. diabetic in TIR, TAR, TBR
F. CNFD in HC vs. diabetic in TIR, TAR, TBR
G. CNFL in HC vs. diabetic in TIR, TAR, TBR

Figure 2: Corneal nerve fiber morphology and CCM parameters in diabetic patients compared to healthy controls based on glycemic targets.

Table 4: Correlation between CCM parameters and glycemic variables using CGM.

Glycemic indicators	CNFD (fiber/mm²)	CNBD (branch/mm²)	CNFL (mm/mm²)	IWL (mm/mm²)
Duration of diabetes (years)	0.112(0.49)	0.101(0.53)	0.008(0.96)	0.004(0.98)
Plasma Glucose (mmol/L)	0.195(0.25)	-0.051(0.77)	0.075(0.66)	0.080(0.64)
Average interstitial glucose (mg/dl)	-0.097(0.55)	0.042(0.79)	0.071(0.66)	0.093(0.57)
HbA1c (%)	-0.079(0.63)	0.068(0.67)	-0.046(0.78)	-0.004(0.98)
GV (%)	0.043(0.79)	**-0.398(0.011)***	-0.281(0.08)	-0.050(0.76)
Duration in high glucose (min)	0.152(0.35)	0.246(0.12)	0.266(0.09)	0.156(0.337)
Duration in very high glucose (min)	-0.114(0.48)	0.024(0.88)	0.053(0.74)	0.152(0.35)
Duration in low glucose (min)	0.121(0.46)	**-0.342(0.031)***	-0.232(0.15)	-0.088(0.59)
Number of hypoglycemic events (no)	0.072(0.66)	-0.258(0.11)	-0.187(0.25)	-0.074(0.65)

HbA1c: glycated hemoglobin, GV: glucose variability, CNFD: corneal nerve fiber density, CNBD: corneal nerve branch density, CNFL: corneal nerve fiber length



CNBD: corneal nerve branch density, GV: glucose variability, TBR: time below range

Figure 3: Correlation between CNBD vs. GV (%) and TBR (min) in patients with diabetes.

Discussion

We have demonstrated that increased glucose variability and asymptomatic hypoglycemia detected using CGM are associated with lower corneal nerve branch density in patients with type 1 and type 2 diabetes. Several previous studies have reported an association between corneal nerve measures with the duration of diabetes [22, 23] and HbA1c [24, 25] in type 1 and type 2 diabetes. Several large clinical trials have shown that improved glycemic control can prevent the development and progression of diabetic neuropathy in type 1 diabetes [26], but not type-2 diabetes [27-30]. However, smaller interventional studies utilizing CCM in type 1 and type 2 diabetes have demonstrated that lowering HbA1c is associated with an increase in corneal nerve parameters [31-34]. Although, in patients with type 2 diabetes, we have recently shown that despite an improvement in HbA1c, in patients taking glucose lowering therapies associated with weight gain and hypoglycemia there was a reduction in corneal nerve branch density [35].

Therefore, the relationship between glycemic control and complications is complex and whilst HbA1c is an important measure of overall glucose control, it fails to capture the magnitude and frequency of glucose variations and indeed the contribution of hypoglycemia. Indeed, intensive glycemic control is associated with an increased incidence of hypoglycemia and adverse cardiovascular outcomes [36]. Hence, there has been an increasing emphasis on defining the role of optimal glucose range and glucose variability in the development of diabetic complications [37]. Diabetic neuropathy has been associated with an increase in the standard deviation of blood glucose (SD) and mean amplitude of glycemic excursions (MAGE) [5] and a recent study also demonstrated that TIR was associated with DPN symptoms [38]. A systematic review showed that a 10% increase in TIR was associated with a reduction in the prevalence of DPN and cardiac autonomic neuropathy [9]. Whilst higher MAGE and CV were associated with an increased risk of DPN, there was also a significant association with the occurrence of nocturnal hypoglycemia [13]. Furthermore, in adults with T1DM a range of indices of hypoglycaemia have been independently associated with cardiac autonomic neuropathy (CAN) [14].

Sudomotor dysfunction has also been independently related to nocturnal TIR in T1DM [16] and T2DM [17]. In a recent study, glucose variability assessed by calculating the continuous overall net glycemic action (CONGA) and the percentage of time in normal and high range glucose was associated with nerve excitability and inferior whorl length but not corneal nerve fiber density or length in a cohort of patients with T1DM [39]. We now show that increased glucose variability and time below range (TBR), but not overt episodes of symptomatic hypoglycemia, was associated with small nerve fiber damage evidenced by lower corneal nerve branch density in patients with type 1 and type 2 diabetes. We believe the underlying mechanisms of nerve damage here are very different from the severe insulin induced experimental hypoglycemic neuropathy characterized by reduced motor and sensory nerve conduction velocities and a distal dying back axonal degeneration affecting motor more than sensory axons [40] and axonal degeneration [41] in the proximal sciatic rather than distal plantar nerves [42], with myelinated nerve fiber damage [43] affecting motor rather than sensory roots [44]. Indeed, in a study of diabetic BB rats with insulin implants to induce moderate hypoglycemia there was evidence of shorter and thinner intraepidermal nerve fibers [45]. Thus, in the current study we show sensory small nerve fiber pathology characterized by a lower corneal nerve branch density in patients with level 1 (54-69 mg/dl) and level 2 (<54 mg/dl) hypoglycemia. In a case report of a 26-year-old female with T1DM, frequent silent hypoglycemia (average <60mg/dl) was associated with numbness and tingling in both hands and feet, which resolved with resolution of hypoglycemia [46]. Several mechanisms may underlie hypoglycemia-induced nerve injury, including reduced blood flow and nerve hypoxia [47-49] and a slowing of axonal transport [50].

We acknowledge limitations of the current study include the relatively small cohort size and short duration of CGM monitoring. Nevertheless, CCM shows small nerve fiber damage in participants with diabetes with higher glucose variability and in those who spent a longer duration in hypoglycemia. CGM, alongside CCM are highly sensitive technologies to explore the relationship between glycemic variability and nerve damage and provide novel insights into the development of diabetic neuropathy.

Authorship contributions

Rayaz Malik: has full access to all the data in the study and takes responsibility for the integrity of the data and accuracy of the analysis.

Study concept and design

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Data acquisition

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Conflict of Interest

None declared.

Funding

This work was supported by Qatar National Research Fund under the grant no. NPRP11S-0110-180247.

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Cite this article: Hoda Gad, Einas Elgassim, Ibrahim Mohammed, Ahmad Yaser Alhaddad, Hussein Ahmed Hussein Zaky Aly, John-John Cabibihan. Continuous Glucose Monitoring Reveals a Novel Association between Duration and Severity of Hypoglycemia and Small Nerve Fiber Injury in Patients with Diabetes. J Diabetes Metab, 2022, 13(7): 943.