DOI: 10.1111/dme.12583

Short Report: Complications

In vivo corneal confocal microscopy as a novel non-invasive tool to investigate cardiac autonomic neuropathy in Type 1 diabetes†

E. Maddaloni[1,*], F. Sabatino[2,*], R. Del Toro[1], S. Crugliano[2], S. Grande[2], A. Lauria Pantano[1], A. R. Maurizi[1], A. Palermo[1], S. Bonini[2], P. Pozzilli[1,3] and S. Manfrini[1]

Departments of [1]Endocrinology and Diabetes, [2]Ophthalmology, University Campus Bio-Medico of Rome, Rome, Italy and [3]Centre for Diabetes, Barts and the London School of Medicine, Queen Mary University of London, London, UK

Accepted 22 August 2014

Abstract

Aims To investigate whether small nerve fibre degeneration detected using corneal confocal microscopy is associated with cardiac autonomic neuropathy in people with Type 1 diabetes.

Methods Thirty-six people with Type 1 diabetes and 20 age- and sex-matched healthy control subjects were enrolled. Tests to determine heart rate response to deep-breathing (expiratory-to-inspiratory ratio), heart rate response to lying-to-stand test (30:15 ratio) and blood pressure response to standing were performed to detect cardiac autonomic neuropathy. Corneal confocal microscopy was performed to assess: corneal nerve density and corneal nerve beadings; branching pattern; and nerve fibre tortuosity.

Results Compared with control participants, participants with Type 1 diabetes had fewer (mean ± SD 45.4 ± 20.2 vs 92.0 ± 22.7 fibres/mm^2; $P < 0.001$) and more tortuous corneal nerve fibres (20 participants with Type 1 diabetes vs four control participants had nerve tortuosity grade 2/3; $P = 0.022$) and fewer beadings (mean ± SD 15.1 ± 3.5 vs 20.6 ± 5.0; $P < 0.001$). Of the participants with Type 1 diabetes, 11 met the criteria for the diagnosis of cardiac autonomic neuropathy. Corneal nerve density was significantly lower in participants with cardiac autonomic neuropathy than in those without (mean ± SD 32.8 ± 16.4 vs 51.7 ± 18.9 fibres/mm^2; $P = 0.008$). This difference remained significant after adjustment for age ($P = 0.02$), gender ($P = 0.04$), disease duration ($P = 0.005$), insulin requirement ($P = 0.02$) and neuropathy disability score ($P = 0.04$).

Conclusion This study suggests that corneal confocal microscopy could represent a new and non-invasive tool to investigate cardiac autonomic neuropathy in people with Type 1 diabetes. Larger studies are required to define the role of corneal confocal microscopy in the assessment of cardiac autonomic neuropathy.

Diabet. Med. 32, 262–266 (2015)

Introduction

Cardiac autonomic neuropathy is a serious diabetes-related complication caused by damage to small nerve fibres (classes Aδ, B and C) [1]. The currently available tests for studying small fibre status are quantitative sensory tests of thermal and pain perception and invasive biopsy methods, such as sural nerve biopsy with electron microscopy and the *ex vivo* confocal microscopy of skin biopsy [2–4].

Although screening for cardiac autonomic neuropathy is recommended at the time of Type 2 diabetes diagnosis and 5 years after diagnosis of Type 1 diabetes [5], the tests that are currently performed are time-consuming, difficult to perform or not sufficiently accurate in non-collaborative patients [6]; therefore, cardiac diabetic autonomic neuropathy remains under-diagnosed [7].

Corneal confocal microscopy is an ophthalmic imaging technique that has been shown to be a marker of diabetic sensorimotor neuropathy [8,9], but its role in autonomic neuropathy has not yet been investigated. It can accurately show the extent of corneal nerve fibre damage in people with diabetes [10] and, because of the structural similarity of corneal nerve fibres with the small autonomic fibres (Aδ and

Correspondence to: Ernesto Maddaloni. E-mail: e.maddaloni@unicampus.it
*These authors contributed equally to the paper.
†Presented at the 73rd Scientific Sessions of the American Diabetes Association, 21–25 June 2013, Chicago, IL, USA.

What's new?

- Cardiac autonomic neuropathy is one of the most overlooked but life-threatening complications of diabetes mellitus.
- It is possible to improve impaired cardiac autonomic function, especially in the earlier stages of cardiac autonomic neuropathy but, unfortunately, tests currently available for the investigation of cardiac autonomic neuropathy are invasive and/or inaccurate, difficult to perform and time-consuming. New techniques that allow easier screening for cardiac autonomic neuropathy are therefore required.
- We show, for the first time, that corneal confocal microscopy can be an effective tool for the clinical diagnosis of cardiac autonomic neuropathy.

C) [11,12], we hypothesized that corneal confocal microscopy could also detect cardiac autonomic neuropathy. The aim of the present study, therefore, was to investigate whether small nerve fibre degeneration detected with corneal confocal microscopy was associated with cardiac autonomic neuropathy.

Patients and methods

A total of 36 white adults (age > 18 years) with Type 1 diabetes (mean ± SD disease duration 20.3 ± 11.2 years) and 20 age- and sex-matched healthy control subjects (mean ± SD age 41.2 ± 12.7 vs 41.7 ± 15.4 years, respectively; $P = 0.885$) were consecutively recruited in the outpatient clinic of the Department of Endocrinology and Diabetes of the University Campus Bio-Medico of Rome. Participants were excluded from the study if they had a previous diagnosis of neuropathy/arrhythmia from any cause other than diabetic neuropathy or if they had any condition potentially changing the anatomy or the function of the corneal nerves (previous or present corneal disease, history of corneal surgery and contact lens wearing).

Cardiac autonomic neuropathy assessment

Participants were asked about symptoms of impaired cardiac autonomic function through the use of validated questions included in the 'orthostatic intolerance' and 'vasomotor' domains of the Compass 31 score [13]. In addition, participants were asked about the presence of resting tachycardia. The following cardiovascular provocative tests [14] were performed to detect cardiac autonomic neuropathy: heart rate response to deep breathing (expiratory-to-inspiratory ratio), heart rate response to lying-to-stand test (30:15 ratio), blood pressure response to standing (Appendix S1). Participants were asked to discontinue any interfering drug (i.e. anti-hypertensive or antidepressant drugs) at least 24 h before the tests. Normal values were adapted for age [6].

Participants were considered to have cardiac autonomic neuropathy if they had one abnormal result on the heart rate test (early cardiac autonomic neuropathy) or two or more abnormal results on heart rate tests (definite cardiac autonomic neuropathy) or if they had orthostatic hypotension (severe cardiac autonomic neuropathy) [15].

Peripheral neuropathy

Peripheral neuropathy was screened by using the neuropathy disability score, a clinical scoring system (0 to 10 scale, diagnostic for scores > 3) depending on vibration, pin prick and temperature perception, as well as the presence or absence of ankle reflexes [16].

Corneal confocal microscopy

Corneal confocal microscopy was performed for each participant using Confoscan4 (Nidek Technologies Co, Ltd, Tokyo, Japan). The quantitative sub-basal corneal nerve measurements, assessed with ImageJ software (1.48v; NIH, USA), were the following: nerve fibre density (fibre/mm^2); nerve fibre length (mm/mm^2); number of nerve beadings (no/100 μm); nerve branch density (branching grade); and nerve fibre tortuosity (tortuosity grade) [11,17,18].

Cardiac autonomic reflex tests and corneal confocal microscopy were performed by trained, blinded investigators (for more details see Appendix S1).

Ethics

The study was conducted in compliance with the Declaration of Helsinki and the International Conference on Harmonization Principles of Good Clinical Practice. The research protocol was approved by the Ethical Committee at the University Campus Bio-Medico of Rome and all participants gave informed consent.

Statistical analysis

Student's *t*-test and ANOVA were used to compare quantitative variables between groups and the chi-squared test was used to compare categorical variables. The non-parametric Kruskal–Wallis test and Fisher's exact test were used when appropriate. One-way ANCOVA was used for adjusted analysis. Based on our preliminary data [19], a sample size of 10 participants per group was considered sufficient to detect a 40% difference in nerve fibre density between groups (significance level 0.05, power 80%). Considering an expected prevalence of cardiac autonomic neuropathy in Type 1 diabetes of 25–30%, 36 people with Type 1 diabetes were enrolled in the study. A P value < 0.05 was considered to indicate statistical significance at

80% power. SPSS 21.0 for Windows was used to compute the analysis.

Results

The participants with Type 1 diabetes and the age-matched control group were homogeneous in terms of BMI (mean ± SD 24.7 ± 4.0 vs 22.6 ± 3.9 kg/m^2, respectively; $P = 0.137$) and gender (females 36.1 vs 55.0%; $P = 0.271$). Eleven (30.6%) participants with Type 1 diabetes met the predefined, age-adjusted, criteria for the diagnosis of cardiac autonomic neuropathy (early, $n = 8$; definite, $n = 3$; severe, $n = 0$), but none reported symptoms. Peripheral neuropathy was diagnosed in four participants with cardiac autonomic neuropathy (none without cardiac autonomic neuropathy). No significant differences in terms of gender, BMI or HbA_{1c} were found between those with cardiac autonomic neuropathy and those without (Table 1).

Participants with Type 1 diabetes had fewer corneal nerve fibres/mm^2 (mean ± SD 45.4 ± 20.2 vs 92.0 ± 22.7; $P < 0.001$) and fewer beadings per 100 μm (mean ± SD 15.1 ± 3.5 vs 20.6 ± 5.0; $P < 0.001$) compared with control participants. No participant had grade 4 fibre tortuosity; 20 participants with Type 1 diabetes (55.6%) had nerve tortuosity grade 2 or 3 [vs four participants (20%) in the control group ($P = 0.022$)], suggesting that corneal nerve fibres are slightly more tortuous in people with Type 1 diabetes. A near significant difference was found in terms of

Table 1 Differences between participants with Type 1 diabetes, with and without cardiac autonomic neuropathy, and control participants

	Study group						
	Participants with Type 1 diabetes		Control participants, $N = 20$				
	with cardiac autonomic neuropathy, $N = 11$	without cardiac autonomic neuropathy, $N = 25$		P (among groups)	P (with cardiac autonomic neuropathy vs without cardiac autonomic neuropathy)	P (with cardiac autonomic neuropathy vs control)	P (without cardiac autonomic neuropathy vs control)
Gender,							
Female, n (%)	6 (54.5)	7 (28.0)	11 (55.0)	0.131	0.153	1.000	0.126
Males n (%)	5 (45.5)	18 (62.0)	9 (45.0)				
Mean ± SD age, years	51.5 ± 10.3	36.7 ± 11.1	41.7 ± 15.4	**0.009**	**0.001**	0.072	0.205
Mean ± SD BMI, kg/m^2	24.5 ± 4.6	24.8 ± 3.7	22.6 ± 3.9	0.326	0.817	0.326	0.123
Mean ± SD disease duration, years	26.4 ± 13.2	17.7 ± 9.2	–	–	**0.029**	–	–
Mean ± SD HbA1c							
mmol/mol	62 ± 13	62 ± 17	–	–	0.999	–	–
%	7.8 ± 1.2	7.8 ± 1.6					
Mean ± SD insulin requirement, UI/kg	0.8 ± 0.3	0.6 ± 0.3	–	–	**0.046**	–	–
Mean ± SD corneal nerve density, fibres/mm^2	32.8 ± 16.4	51.7 ± 18.9	92.0 ± 22.7	**< 0.001**	**0.008***	**< 0.001**	**< 0.001**
Mean ± SD nerve fibre length, mm/mm^2	5.5 ± 2.4	9.2 ± 3.8	10.0 ± 3.3	**0.003**	**0.005†**	**< 0.001**	0.465
Mean ± SD branching pattern, grade	1.4 ± 0.8	1.9 ± 0.7	1.4 ± 0.5	**0.045**	0.066	0.717	**0.030**
Mean ± SD corneal nerve beadings, number/100 μm	14.8 ± 4.2	15.3 ± 3.2	20.6 ± 5.0	**< 0.001**	0.719	**0.003**	**< 0.001**
Tortuosity, n (%)							
Grade 0–1	7 (63.6)	9 (36.0)	16 (80)	**0.011**	0.159	0.450	**0.008**
Grade 2–3	4 (36.4)	16 (64.0)	4 (20)				
Grade 4	0 (0)	0 (0)	0 (0)				

*$P = 0.02$ after adjustment for age; $P = 0.04$ after adjustment for gender, $P = 0.005$ after adjustment for disease duration; $P = 0.04$ after adjustment for insulin requirement; $P = 0.047$ after adjustment for neuropathy disability score.
†$P = 0.097$ after adjustment for age; $P = 0.197$ after adjustment for gender; $P = 0.02$ after adjustment for disease duration, $P = 0.027$ after adjustment for insulin requirement; $P = 0.012$ after adjustment for neuropathy disability score.
Bold is used for significant P-values.

nerve fibre length (mean ± SD 8.0 ± 3.8 vs 10.0 ± 3.3 mm/mm^2; $P = 0.06$) while no differences were found in terms of branching grade (mean ± SD 1.7 ± 0.8 vs 1.4 ± 0.5; $P = 0.167$).

The participants with a diagnosis of cardiac autonomic neuropathy were significantly older (mean ± SD age 51.5 ± 10.3 vs 36.7 ± 11.1 years; $P < 0.001$) than those without cardiac autonomic neuropathy. Participants with cardiac autonomic neuropathy had longer disease duration (mean ± SD 26.4 ± 13.2 vs 17.7 ± 9.2 years; $P = 0.029$) and a greater insulin requirement (mean ± SD 0.8 ± 0.3 vs 0.6 ± 0.3 UI/kg).

Corneal nerve density was significantly lower in the participants with cardiac autonomic neuropathy than in those without (mean ± SD 32.8 ± 16.38 vs 51.7 ± 18.9 fibres/mm^2; $P = 0.008$). This difference remained significant after adjustment for age ($P = 0.02$), gender ($P = 0.04$), disease duration ($P = 0.005$), insulin requirement ($P = 0.04$) and neuropathy disability score ($P = 0.047$).

Corneal nerve fibre length was significantly lower in participants with cardiac autonomic neuropathy than in those without (mean ± SD 5.5 ± 2.4 vs 9.2 ± 3.8; $P = 0.005$). This difference remained significant after adjustment for disease duration ($P = 0.02$), insulin requirement ($P = 0.027$) and neuropathy disability score ($P = 0.012$), but not after adjustment for age ($P = 0.097$) and gender (0.197).

No significant differences in the branching grade (mean ± SD 1.4 ± 0.8 vs 1.9 ± 0.7; $P = 0.06$), nerve tortuosity (grade 2–3, 36.4 vs 64%; $P = 0.159$) or in the number of nerve beadings (mean ± SD 14.8 ± 4.2 vs 15.3 ± 3.2, $P = 0.719$) were found between participants with and participants without cardiac autonomic neuropathy.

Discussion

Cardiac autonomic neuropathy is one of the most overlooked but life-threatening complications of diabetes mellitus [7]. Although small nerve function correlates with corneal nerve structure, and corneal confocal microscopy has been proposed as a useful marker of small fibre dysfunction, corneal nerve changes have never been specifically investigated in cardiac autonomic neuropathy [20–22]. To the best of our knowledge, the present study shows, for the first time, that corneal confocal microscopy can be effectively used to specifically investigate cardiac autonomic neuropathy in a population with Type 1 diabetes. As subtle corneal nerve changes anticipate diabetic retinopathy [23], it could be argued that all molecular cascades eventually damaging the sub-basal corneal plexus could also negatively affect autonomic nervous fibres.

According to previous data [7], people with cardiac autonomic neuropathy are older and have a longer disease duration; however, the difference in corneal nerve density remained in the present study after adjusting for both age and disease duration, showing that the ability of *in vivo* corneal confocal microscopy to screen people with Type 1 diabetes for cardiac autonomic neuropathy was not affected by these confounding factors. In addition, because symptoms of cardiac autonomic neuropathy are usually found late in the diagnostic process [7], corneal confocal microscopy could also provide an early diagnosis of asymptomatic cardiac autonomic neuropathy. Indeed, in the present study, no patient had symptoms of cardiac autonomic neuropathy. Nevertheless, the present study was not designed to evaluate differences in corneal innervation among different stages of cardiac autonomic neuropathy and further studies would be required to investigate this.

A limitation of the present study is its small sample size. In addition, we used only three of the available cardiovascular provocative tests to detect cardiac autonomic neuropathy and therefore there is the possibility that we missed some diagnoses of cardiac autonomic neuropathy in our population; for example, we did not perform the Valsalva manoeuvre test. Nevertheless, Stranieri *et al.* [24] have recently shown that using a selection from the Ewing battery of tests has good accuracy. They also showed that the deep-breathing heart variation test, one of the tests used in the present study, was the best single Ewing test for the diagnosis of cardiac autonomic neuropathy. Another limitation of the present study is that corneal confocal microscopy evaluation is subject to inter- and intra- examiner variability.

In conclusion, to our knowledge the present study is the first to show that corneal confocal microscopy can represent a new and non-invasive tool to specifically and easily investigate cardiac autonomic neuropathy in people with Type 1 diabetes. It opens the way for larger studies to further define the role of corneal confocal microscopy in the diagnosis of cardiac autonomic neuropathy.

Funding sources

None.

Competing interests

None declared.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Extensive description of methods used in this paper for cardiac autonomic neuropathy assessment and for corneal confocal microscopy.