Diabetic Peripheral Neuropathy as a Predictor of Asymptomatic Myocardial Ischemia in Type 2 Diabetes Mellitus: A Cross-Sectional Study

Adv Ther (2016) 33:1840–1847
DOI 10.1007/s12325-016-0399-1

BRIEF REPORT

Diabetic Peripheral Neuropathy as a Predictor of Asymptomatic Myocardial Ischemia in Type 2 Diabetes Mellitus: A Cross-Sectional Study

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Received: July 18, 2016 / Published online: August 23, 2016

ABSTRACT

Introduction: Diabetic peripheral neuropathy (DPN) is one of the most common complications of diabetes and has been associated with cardiovascular disease, the leading cause of mortality in diabetes. As asymptomatic myocardial ischemia (MI) is frequent in diabetes, we hypothesized that DPN may be associated with MI in patients with type 2 diabetes mellitus and no history of cardiovascular events.

Methods: Eighty-two patients with DPN ($n = 41$) or without DPN ($n = 41$) were included. Among the DPN group, 15 had active foot ulcers. All subjects underwent Technetium-99 m sestamibi single-photon emission computed tomographic imaging for the estimation of myocardial ischemia, expressed as Summed Stress Score (SSS). The Neuropathy Disability Score (NDS) was used to quantify DPN and abnormal ratio of the longest electrocardiographic RR interval between the 28th and 32nd beats, after standing to the shortest interval between the 13th and 17th

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Electronic supplementary material The online version of this article (doi:10.1007/s12325-016-0399-1) contains supplementary material, which is available to authorized users.

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beats (RR ratio) was used as an index of cardiovascular autonomic neuropathy (CAN).

Results: Abnormal SSS was observed in 9.8% of patients without DPN and in 46.3% of patients with DPN ($p < 0.001$). In the multivariate analysis, NDS was the strongest predictor for SSS ($\beta = 0.32$, $p = 0.003$). When excluding patients with abnormal RR ratio ($\beta = 0.32$, $p = 0.003$) or with foot ulcers ($\beta = 0.24$, $p = 0.04$), this association remained significant. The RR ratio was also significantly associated with SSS in univariate ($\rho = -0.30$, $p = 0.005$) and multiple regressions ($\beta = 0.24$, $p = 0.02$).

Conclusions: MI was strongly associated with DPN, and this association remained significant in patients with normal RR ratio. These results suggest that DPN assessment could help in identifying patients at risk of cardiovascular disease (CVD).

Keywords: Cardiovascular autonomic neuropathy; Diabetes; Diabetic foot ulcer; Diabetic peripheral neuropathy; Myocardial ischemia; Type 2 diabetes mellitus

INTRODUCTION

Diabetic peripheral neuropathy (DPN) is the most prevalent neuropathy in diabetes and a common cause of morbidity. It leads to foot ulceration and predisposes to limb loss, as almost 28% of all foot ulcers ultimately require some form of lower extremity amputation [1]. Among patients with diabetes mellitus (DM) cardiovascular mortality accounts 43.6% of all deaths [2].

Diabetes is a pivotal cause of asymptomatic myocardial ischemia (MI) even in the absence of coronary artery disease [3]. Therefore, early identification of patients with DM at high risk for asymptomatic MI consists of a significant challenge. Evidence suggests an association between cardiovascular autonomic neuropathy (CAN) and an increase in overall mortality or silent myocardial ischemia [4, 5]. Although DPN has recently been shown to be associated with incident cardiovascular events [6, 7], the relationship between DPN and MI has never been established.

We hypothesized that DPN may be associated with MI in patients with type 2 DM (T2DM) and no history of cardiovascular events. This association could provide additional information on the link between the risk of cardiovascular disease (CVD) and T2DM, and be useful to better identify patients at risk of MI.

METHODS

Recruitment and Imaging

Eighty-two adult subjects with diagnosed T2DM were recruited from the Diabetes Mellitus Outpatient Clinic of the 3rd Department of Internal Medicine, situated at Papageorgiou General Hospital, Thessaloniki, Greece, and divided into two groups according to the presence or absence of DPN. The DPN group was additionally subdivided according to the presence of active ulcers that were located below the ankle join. The inclusion criteria were: type T2DM, according to ADA and IDF criteria [8], and age >18 years. The exclusion criteria were: history of myocardial infraction, stroke, coronary revascularization or cardiac bypass, active liver disease, any chronic renal disease, any autoimmune disease, HIV infection, malignancy, primary neurologic disorders (previous spinal injury, a history of lumbar or cervical discopathy, carpal tunnel syndrome, alcoholism, inherited neuropathy),

vitamin B9 or B12 deficiency, concomitant use of glucocorticoid, isoniazid or metronidazole. The Ethics Committee of the Papageorgiou General Hospital approved the study's protocol and patients gave their informed consent prior to participation in accordance with the principles of the Declaration of Helsinki as revised in 2008.

MI was diagnosed via electrocardiographically gated Technetium-99 m (Tc-99 m) sestamibi single-photon emission computed tomographic (SPECT) images, a method attributing added value to non-gated SPECT perfusion images. The Summed Stress Score (SSS) which represents the extent and severity of a perfusion abnormalities was obtained and considered abnormal when ≥ 4 [9]. Additionally, the percentage of ischemia was estimated for each patient, as described in the European Association of Nuclear Medicine guidelines [10]. Patients were instructed to fast for a minimum of 4 h and withhold beta-blockers, calcium channel blockers, nitrates, methyl-xanthene containing drugs and caffeinated foods and beverages for at least 24–48 h prior to the examination [10]. DPN was evaluated with the neuropathy disability score (NDS) by testing the sensations of pain, touch, cold, and vibration in both legs of each patient and assigning a score according to the level of impaired sensation. An NDS score ≥ 5 was indicative of DPN [11]. Additionally, the group without DPN was determined based on a negative 10-g monofilament test [12]. The ratio of the longest electrocardiographic RR interval between the 28th and 32nd beats after standing to the shortest interval between the 13th and 17th beats (RR ratio) and orthostatic hypotension were used as markers of CAN. One observer calculated the RR ratio. Abnormal autonomic function was defined as a loss of heart rate variability with an RR ratio of less than 1.04, postural hypotension with a fall in systolic blood pressure of 20 mmHg or more, or both [7]. Peripheral arterial disease was diagnosed with the ankle-brachial index (ABI), with a technique that has been previously described, and the results were considered pathological when the values were <9 or >1.3 [13].

Statistical Analysis

Continuous data were expressed as mean ± standard deviation or as the median (interquartile range) when lacking normality in distribution. Between-group comparisons were analyzed by one-way ANOVA. Post hoc comparisons were performed using the Tukey test. When application conditions were not met, nonparametric tests (Kruskal–Wallis and Mann–Whitney) were used. Categorical data were expressed as frequencies and percentages and then compared with the Chi-squared test. Spearman's correlations were used to assess the relationship between SSS and other continuous variables. We subsequently included all variables that correlated with a p value <0.15 in a multiple linear regression model to identify predictors of SSS. In order to assess the performance of using the NDS to classify patients with ischemia, receiver-operating characteristic (ROC) curve was plotted and the area under the ROC curve (AUC) with its confidence interval was computed. p values <0.05 were considered as significant. Statistical analysis was performed using SPSS software (v.22.0; IBM, Armonk, NY, USA).

RESULTS

Patient characteristics and between-groups comparisons are presented in Table 1. Patients with DPN had a higher risk of abnormal SSS (OR = 7.99, 95% CI 2.4–26.5), ischemia (OR = 4.25, 95% CI 1.4–12.9), and abnormal

Table 1 Participant characteristics and post hoc analysis among groups

	No DPN ($n = 41$)	DPN ($n = 41$)		p value
		No ulcer ($n = 26$)	Ulcer ($n = 15$)	
Sex (male), n (%)	17 (41)	16 (61)	8 (53)	0.27
Age (years)	62.5 ± 9.8	68 ± 9	65.5 ± 10	0.08
Diabetes duration (years)	10 (6–12)	20 (11–28)*	16 (12–23)*	<0.001
HbA1c (%)	6.9 (6.2–7.5)	7.1 (6.6–7.6)	7.2 (6.9–7.9)	0.13
HbA1c (mmol/mol)	51.9 (44.3–57.4)	54.1 (48.6–59.6)	55.2 (51.9–62.8)	0.13
BMI (kg/m^2)	31.2 (28.0–34.0)	31.0 (27.6–34.1)	34.9 (28.8–38.9)	0.32
Waist circumference (cm)	107 ± 12	108 ± 12	112 ± 9	0.31
TC (mg/dL)	177 (154–210)	179 (154–218)	169 (152–202)	0.76
TG (mg/dL)	133 (100–163)	125 (102–168)	113 (87–178)	0.72
HDL (mg/dL)	45 (41–52)	48 (42–51)	41 (36–50)	0.27
LDL (mg/dL)	103 (83–126)	104 (86–122)	101 (73–132)	0.94
Smoking, n (%)	7 (14)	5 (19)	4 (27)	0.72
GFR (mL/min/1.73 m^2)	96 ± 32	96 ± 29	106 ± 30	0.56
ABI	1.00 (1.00–1.00)	1.00 (0.93–1.00)	1.00 (0.97–1.18)	0.15
NDS	0 (0–2)	8 (6–10)*	12 (10–14)*§	<0.001
Abnormal RR ratio, n (%)	5 (12.1)	5 (19.2)	10 (66.7)*§	<0.001
Ischemia, n (%)	3 (7.3)	7 (26.9)*	9 (60)*§	<0.001
Abnormal SSS, n (%)	4 (9.8)	10 (38.5)*	9 (60)*	<0.001

Continuous variables are expressed as mean ± standard deviation or as the median (interquartile range) when the distribution was not normal. Categorical variable are expressed as frequency (%)

NDS Neuropathy Disability Score, *BMI* body mass index, *ABI* ankle-brachial index, *SSS* Summed Stress Score, *TC* total cholesterol, *TG* triglycerides, *HDL* high-density lipoprotein, *LDL* low-density lipoprotein, *GFR* glomerular filtration rate

* $p < 0.05$ compared to no DPN, § $p < 0.05$ compared to DPN without ulcers

RR ratio (OR = 4.15, 95% CI 1.34–12.87). Moreover, abnormal RR ratio and ischemia were significantly higher in patients with DPN and ulcers than in patients with DPN without ulcers or in patients without DPN (Table 1).

Univariate and multivariate regressions identified NDS, waist circumference and gender as significant predictors of SSS (adjusted $R^2 = 0.22$), with NDS being the most strongly related ($\beta = 0.32$, $p = 0.003$) (Table 2). Sensitivity analyses removing patients with abnormal RR ratio or without ulcers yielded to similar results, with NDS significantly associated with SSS ($\beta = 0.32$, $p = 0.003$ and $\beta = 0.24$, $p = 0.04$, respectively) (supplementary Table S1). The AUC of the ROC curve was 0.76 (95% CI, 0.65–0.86; $p < 0.001$). The cutoff point of the NDS for which sensitivity and specificity are optimal was 5, i.e. the threshold value that is commonly accepted as indicative of DPN.

Table 2 Regression analyses predicting the Summed Stress Score (SSS)

	Univariate analyses		Multivariate analysis	
	Spearman's correlation coefficient (ρ)	p value	Standardized coefficients (β)	p value
NDS	0.38	<0.001	0.32	0.003
Waist circumference	0.19	0.089	0.22	0.03
Age	0.31	0.004	0.09	0.13
Sex (male)	−0.42	<0.001	−0.24	0.02
LDL	−0.17	0.12	−0.04	0.67
Duration of diabetes	0.12	0.28	–	–
HbA1c	0.14	0.21	–	–
ABI	−0.08	0.46	–	–
GFR	−0.10	0.36	–	–

$R^2 = 0.22$; remove: $p \geq 0.100$

NDS Neuropathy Disability Score, *SSS* Summed Stress Score, *TC* total cholesterol, *LDL* low-density lipoprotein, *GFR* glomerular filtration rate

The RR ratio was also significantly associated with SSS in univariate ($\rho = -0.30$, $p = 0.005$) and multiple regressions (adjusted $R^2 = 0.18$, $\beta = 0.24$, $p = 0.02$) (supplementary Table S2). Orthostatic hypotension was diagnosed only in 3 (3.4%) patients with DPN.

DISCUSSION

Large cohort studies have established the relationship between DPN and cardiovascular risk factors [7] or events [6]. However, the mechanisms behind this link are still not clear, as recently published endothelial dysfunction occurs early in the pathophysiology of diabetes and could be a potential link between cardiovascular risk factors and DPN [14]. In the present study, we further show that DPN is strongly associated with asymptomatic MI assessed with Technetium-99 m sestamibi SPECT imaging in T2DM patients who were free of cardiovascular events, independently from other risk factors as was shown in the regression analysis. Moreover, AUC of the ROC curve showed a fair to good performance of the NDS to discriminate patients with ischemia. We would like to point out that, interestingly, the Detection of Ischemia in Asymptomatic Diabetics (DIAD) study did not find any association between peripheral neuropathy and MI using the same technique. This discrepancy might be explained by the use of differing methods of testing and evaluating DPN in our study. The DIAD study used the presence of two or more signs and symptoms of diabetic neuropathy. Although a trend towards higher frequency of DPN was observed among patients with moderate-to-large stress perfusion abnormality, it was not significant [4]. Using continuous scales (i.e. DPN and SSS) might have provided us with more sensitivity to detect this relationship. Another explanation could be the duration of diabetes as the average duration of diabetes was shorter

in the DIAD study (8.1 vs. 14.8 years in our study) [4], therefore patients were less likely to have developed complications. Considering the importance of diabetes duration as a determinant for DPN and ischemia, we ran a sensitivity analysis including diabetes duration in the model. However, this did not significantly affect the findings.

On the other hand, we found similar results to the DIAD study regarding markers of CAN. Abnormal valsalva ratio was identified as the strongest predictor of ischemia in the DIAD study. In our study, the estimation of autonomic neuropathy was performed with the RR ratio and orthostatic hypotension, as suggested by the European Diabetes (EURODIAB) Prospective Complications Study due to the risk of retinal hemorrhage [7]. A significant correlation between the RR ratio and MI was observed in the univariate and multivariate analyses. This finding is in compliance with numerous studies stressing an association between CAN in early stages and increased overall mortality or silent myocardial ischemia [4, 5]. The Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial suggested the role of CAN as a possible predictor for CVD mortality [5].

Interestingly, sensitivity analysis after removing patients with abnormal RR ratio did not affect the main finding of our study. This suggests that the association between diabetic neuropathy and MI exceeds autonomic neuropathy. DPN had previously been associated with increased incidence of CVD [6]. Although the link between these two conditions has not been fully elucidated, they probably share similar pathophysiological pathways. Indeed, DPN, which is traditionally counted among microvascular complications, might be caused by neuronal cells abnormalities, such as oxidative or inflammation stress [15] that also affect the endothelium.

One limitation of the present study is that we only included 15 patients with ulcers and we did not reach statistical significance when assessing the effect of both DPN (OR = 3.88, 0.97–15.4; $p = 0.054$) and ulcers (OR = 3.73, 0.87–161; $p = 0.077$) on abnormal SSS, probably because of a lack of power. Although proper sample size calculation could not be done a priori considering that the variables to be included in the multivariate analysis (primary endpoint) were not known, our final sample provided satisfactory power if we consider the rule of thumb of a minimum of 10 participants required per variable included in the model. Additionally, the trend we observed is consistent with the 2.2 increase in relative risk of fatal MI in patients with ulcers [2] confirming other studies [16]. This could be partly due to increased chronic inflammation, which is involved in the development of atherosclerosis in these patients [2]. Yet, removing patients with active ulcers from analysis did not significantly change the main conclusion. Another limitation is that this study is cross-sectional and the absence of follow-up does not allow us to draw definitive conclusions on any causal relationship between factors. Finally, the diagnosis of DPN is observer-dependent and could be prone to subjectivity. In order to decrease the risk of objective bias, the same experienced physician, prior to SPECT imaging, performed the DPN assessments.

CONCLUSIONS

In conclusion, the present study indicates that, besides autonomic neuropathy, DPN is strongly associated with asymptomatic MI. This suggests

that NDS could be a useful tool to appraise the risk of early cardiovascular complications among diabetic patients, and needs to be further evaluated in larger-scale prospective studies.

ACKNOWLEDGMENTS

This study was funded by Papageorgiou General Hospital, Thessaloniki. D. Baltzis received a scholarship from the North Greece Association of Diabetes. M. Roustit received funding from the Fondation AGIR pour les Maladies Chroniques. D. Baltzis collected the data and contributed to writing the manuscript, M. Roustit and M. G. Grammatikopoulou performed the statistical analyses and contributed to writing the manuscript, D. Baltzis, M-C Trakatelli and C. Manes were responsible for the conception and design of the study. I. Iakovou, D. Katsaboukas and V. Athanasiou performed the Technetium-99m Sestamibi SPECT imaging and analyzed the data. A. Veves contributed in data interpretation and manuscript drafting. All authors critically revised the manuscript for important intellectual content and approved the final version of the manuscript. All named authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship for this manuscript, take responsibility for the integrity of the work as a whole, and have given final approval for the version to be published.

Disclosures. D. Baltzis, M. Roustit, M. G. Grammatikopoulou, D. Katsaboukas, V. Athanasiou, I. Iakovou, A. Veves, C. Manes and M.-C. Trakatelli have nothing to disclose.

Compliance with Ethics Guidelines. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964, as revised in 2008. Informed consent was obtained from all patients for being included in the study.

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