Journal of Clinical & Translational Endocrinology 8 (2017) 15–21

Contents lists available at ScienceDirect

Journal of Clinical & Translational Endocrinology

journal homepage: www.elsevier.com/locate/jcte

Original research

Temporal trend of autonomic nerve function and HSP27, MIF and PAI-1 in type 1 diabetes

Malin Zimmerman [a,b,*], Sara Rolandsson Enes [c], Hanna Skärstrand [d], Kaveh Pourhamidi [e], Anders Gottsäter [f], Per Wollmer [g], Olov Rolandsson [e], Gunilla Westergren-Thorsson [c], Lars B. Dahlin [a,b]

[a] Department of Translational Medicine – Hand Surgery, Lund University, Skåne University Hospital, Jan Waldenströms gata 5, 205 02 Malmö, Sweden
[b] Department of Hand Surgery, Skåne University Hospital, Jan Waldenströms gata 5, 205 02 Malmö, Sweden
[c] Department of Experimental Medical Science Lund, Lung Biology Unit, Lund University, Sölvegatan 19, Klinikgatan 32, BMC D12, Lund, Sweden
[d] Department of Clinical Sciences Malmö, Unit of Diabetes and Celiac Diseases, Skåne University Hospital, 205 02 Malmö, Sweden
[e] Department of Public Health and Clinical Medicine, Family Medicine, Umeå University, 901 85 Umeå, Sweden
[f] Department of Vascular Diseases, Skåne University Hospital, 205 02 Malmö, Sweden
[g] Department of Translational Medicine – Clinical Physiology and Nuclear Medicine, Lund University, 205 02 Malmö, Sweden

ARTICLE INFO

Article history:
Received 17 October 2016
Received in revised form 26 February 2017
Accepted 11 March 2017

Keywords:
Type 1 diabetes mellitus
Diabetic autonomic neuropathy
Heat-Shock Protein 27
Macrophage Migration Inhibition Factors
PAI-1

ABSTRACT

Aim: Diabetes mellitus type 1 (T1D) has numerous complications including autonomic neuropathy, *i.e.* dysfunction of the autonomous nervous system. This study focuses on Heat Shock Protein 27 (HSP27), Macrophage Migration Inhibitory Factor (MIF), Plasminogen Activator Inhibitor-1 (PAI-1) and HbA1c and their possible roles in effects of diabetes on the autonomic nervous system.
Methods: Patients with T1D (n = 32, 41% women) were recruited in 1985 and followed up on four occasions (1989, 1993, 1998, and 2005). Autonomic function was tested using expiration/inspiration (E/I-ratio). Blood samples, *i.e.* HSP27 (last three occasions), MIF, PAI-1 (last two occasions) and HbA1c (five occasions), were analyzed.
Results: Autonomic nerve function deteriorated over time during the 20-year-period, but levels of HSP27, MIF, and PAI-1 were not associated with cardiovascular autonomic neuropathy. MIF and PAI-1 were lower in T1D than in healthy controls in 2005. Increased HbA1c correlated with a decrease in E/I-ratio.
Conclusions: Neither the neuroprotective substance HSP27 nor the inflammatory substances, MIF and PAI-1 were associated with measures of cardiovascular autonomic nerve function, but a deterioration of such function was observed in relation to increasing HbA1c in T1D during a 20-year follow-up period. Improved glucose control might be associated with protection against autonomic neuropathy in T1D.

Introduction

Diabetes mellitus (DM) causes numerous complications [10,22] and increases mortality [37], partly due to an increasing risk for atherosclerosis and thus cardiovascular disease [15]. Another factor contributing to cardiovascular mortality among patients with diabetes is autonomic neuropathy [8,21]. Cardiovascular autonomic neuropathy (CAN), *i.e.* the impairment of autonomic nerve function in the cardiovascular system in patients with DM [30], leads to inability to compensate for changes in posture or activity level [8]. CAN ranges from being subclinical and barely detectable on continuous electrocardiography (ECG) early in the disease process to resting tachycardia and orthostatic hypotension as the disease progresses [8]. Other complications due to CAN include exercise intolerance, silent myocardial ischemia, diastolic dysfunction and sudden cardiac death [36,38]. Even though the knowledge of CAN is improving, additional research is needed to explore the natural history of CAN and its impact on cardiovascular disease [8].

The pathophysiology of autonomic nerve dysfunction is believed to differ between type 1 diabetes (T1D) and type 2 diabetes (T2D). Improved glucose control prevents the development of diabetic polyneuropathy and CAN to a larger extent in T1D than in T2D [5,30]. Both parts of the autonomic nervous system, the sympathetic and the parasympathetic, are affected in individuals with diabetes [12]. Diminished parasympathetic function can be an early feature of CAN since longer nerve fibers, such as the vagal nerve, are affected first [9].

The small Heat Shock Protein 27 (HSP27) might be an important factor in protecting peripheral neurons from diabetes [26] and

* Corresponding author at: Department of Translational Medicine – Hand Surgery, Lund University, Jan Waldenströms gata 5, SE-205 02 Malmö, Sweden.
E-mail address: malin.zimmerman@med.lu.se (M. Zimmerman).

http://dx.doi.org/10.1016/j.jcte.2017.03.001
2214-6237/

possibly also Schwann cells from undergoing apoptosis [1]. Higher concentrations of HSP27 are associated with fewer signs of peripheral sensorimotor neuropathy, as well as with better peripheral nerve function [26]. HSP27 has to the best of our knowledge not previously been studied in relation to autonomic nerve function in humans with T1D. Macrophage Migration Inhibitory Factor (MIF) is a key part in the development and pathogenesis of T1D [6,31]. MIF stimulates pancreatic beta-cell apoptosis, cytokine inflammatory responses and inflammation in pancreatic islets [28]. MIF is also associated with an increased risk of cardiovascular events in patients with coronary artery disease and T2D [18]. Plasminogen Activator Inhibitor-1 (PAI-1), an important component of the fibrinolytic system, forms complexes together with Tissue Plasminogen Activator (tPA). These complexes may be a predictor of diabetic complications in insulin-dependent DM and circulating levels of such complexes are higher in men with than without diabetic neuropathy, contributing to insulin resistance [20]. Current data also suggest a role of low-grade inflammation as a potential therapeutic target for diabetic neuropathy [25] making the inflammatory markers MIF and PAI-1 interesting subjects for research concerning their role in the development of neuropathy in DM.

The aims of our study were to 1) investigate the temporal trend of cardiovascular autonomic neuropathy in patients with T1D, and 2) evaluate whether the progression of such autonomic nerve dysfunction was associated with serum concentrations of HSP27, the inflammatory proteins MIF and PAI-1 as well as with HbA1c.

Methods

Study population

In 1984–1985, 110 patients with T1D at the Diabetes Clinic at Malmö University Hospital, Sweden, were invited to a neuropathy study. The patients had developed T1D between the ages of 15 and 25 (median 19) years and had a diabetes duration of between 2 months and 30 years. Insulin treatment had been initiated directly after diagnosis and C-peptide values were negative or low in all patients when the study started. Fifty-eight (53%) patients agreed to participate. Thirty-two (55%) of the original 58 patients were included in this follow-up study; three had died, seven had moved, nine declined to participate, one got pregnant, and six only participated in the clinical examination. Out of these 32 participants, 24 participated in all five follow-ups in 1985, 1989, 1992, 1998, and 2005 (Fig. 1). The study population and some neuropathy data, not related to HSP27 and inflammatory parameters, have been described previously [2,7,11,12,23,29].

Ethics

Informed consent was obtained from the participants and the study was approved by the Regional Ethical Review Board in Lund, Sweden (663/2005). The study was conducted in accordance with the Helsinki Declaration and followed Good Clinical Practice.

Measurements

On every follow-up visit, patients underwent autonomic testing, measurement of blood pressure (BP), collection of blood samples, and nerve conduction studies to assess peripheral nerve function [data presented elsewhere [27]].

Blood sample analyses included glycosylated hemoglobin (HbA1c) from all follow-up visits. At the last two follow-ups (*i.e.* 1998 and 2005) low-density lipoprotein (LDL) and high-density lipoprotein (HDL) were also analyzed. In addition, serum (s-) concentrations of HSP27 were analyzed on the last three follow-up visits [*i.e.* 1992, 1998, and 2005; data from1992 and 2005 already published [27]], whereas s-concentrations of MIF and PAI-1 were measured at the last two follow-ups (*i.e.* 1998 and 2005).

Autonomic testing consisted of measurements of expiration/inspiration (E/I)-ratio, acceleration (AI), and brake (BI) indices [3]; E/I ratio reflecting cardiac parasympathetic function, AI reflecting partly withdrawal of vagal tone and partly sympathetic function, and BI reflecting reinstitution of vagal tone (see below).

Expiration/inspiration ratio (E/I-ratio)

Cardiac parasympathetic activity, mediated by the vagal nerve, was assessed by measuring the E/I ratio, while deep breathing [33]. During continuous ECG, six maximal inspirations and expirations were performed in a supine position. Using the mean value of the shortest R-R intervals (the time between the R-waves in two adjacent ECG-complexes) during inspiration and the mean value of the longest R-R intervals during expiration, the E/I-ratio was calculated and expressed as standard deviation (SD) from age-corrected normal values. Abnormal E/I was defined as less than −1.64 SD below the age-related reference value [11]. Patients were instructed to take their medication as usual, not eat any bigger meal two hours before testing and to refrain from physical exercise two hours before testing. Relations of some of these data and autoantibodies have been presented earlier [29]. The coefficient of variation for repeated measurements of the E/I ratio in the laboratory was 8.7% and 3.5% for healthy subjects and type I diabetes patients, respectively. Corresponding values for AI were 17.8% and 21.5%, respectively, and for BI 12.0% and 38.1%, respectively [35].

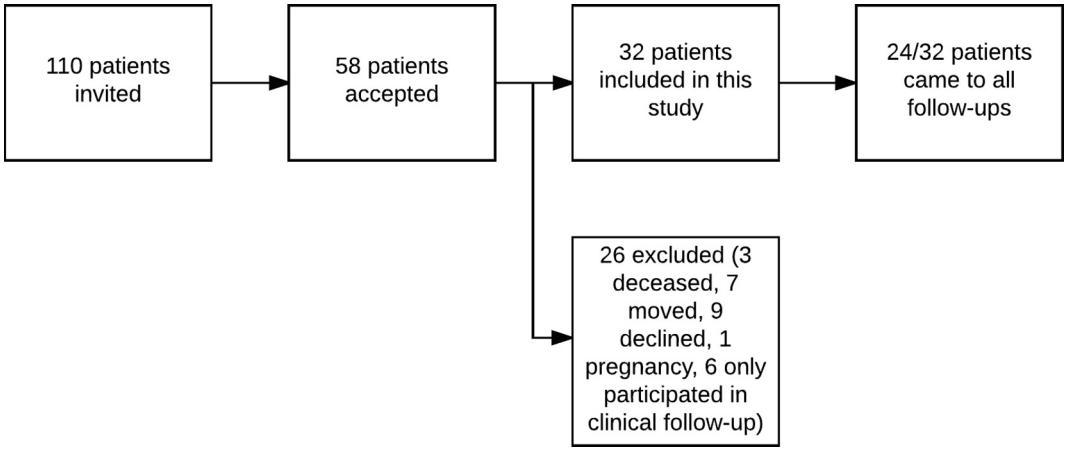

Fig. 1. Flowchart of inclusion process.

All measurements were made by a small group of three experienced technicians.

Acceleration index (AI)

To calculate the acceleration index (AI), which reflects partly withdrawal of the vagal tone and partly sympathetic activity [34], the patient was tilted rapidly (2 s) from a supine position to an upright position (90°) during continuous ECG registration. The ten last R-R intervals during the first minute before the tilt were measured and their mean was calculated (A). Directly after the tilt, the shortest R-R interval before the transient deceleration in heart rate was measured (B), as well as the longest R-R interval (C). The AI was then calculated using the following formula: ([A − B]/A) * 100 and expressed as SD from age-corrected normal values [3]. A value of <1.64 SD of the mean of an age matched control group was considered abnormal [2].

Brake index (BI)

Brake index (BI) in the orthostatic testing is induced by reinstitution of vagal tone due to vasoconstriction [32]. Using the same variables as above, the BI is calculated using ([C − B]/A) * 100 and expresses as SD from age-corrected normal values. A value of <1.64 SD of the mean of an age matched control group was considered abnormal [2].

HSP27 – analysis and control subjects

S-concentrations of HSP27 in the T1D patients from the three last follow-ups (stored in −80 °C freezer) were compared to HSP27 concentrations in gender- and age-matched healthy controls previously evaluated in relation to peripheral sensorimotor neuropathy [27]. The controls were obtained from healthy blood donors without any known cases of diabetes mellitus or cardiovascular disease in 2004. Serum concentrations of HSP27 were analyzed using an ELISA kit (Calbiochem, San Diego, CA) with a lowest detection limit of 312 pg/ml. The analyses of HSP27 were performed at the same time (2010) at the same laboratory. This was also true for MIF and PAI-1 (analysis performed in 2016).

MIF and PAI-1 – analyses and control subjects

S-concentrations of MIF and PAI-1 in T1D patients were determined in the last two follow-ups (*i.e.* years 1998 and 2005) and compared to MIF and PAI-1 concentrations of matched controls (gender- and age-matched). The control subjects were found in the group of healthy blood donors (see above; [27,29]. S-concentrations of MIF and PAI-1were analyzed using ELISA (R&D Systems, cat. No. DMF00B and cat. No. DTSE100, respectively) according to the manufacturer's instructions.

Statistics

Statistical calculations were performed using IBM SPSS Statistics, version 22 (SPSS Inc., Chicago, IL, USA) (IBM SPSS Statistics). Since most of the variables were not normally distributed, median and interquartile range (IQR) were used consistently as measure of central tendency.

Differences over time were analyzed using the related-samples with Friedman's two-way analysis of variance by ranks, and post hoc analysis was conducted, where appropriate, between adjacent monitoring times (1985–1989, 1989–1993, 1993–1998, and 1998–2005). Multivariate linear regression analysis was used to study the impact of age at baseline (1985), gender, baseline HbA1c (1985) and HSP27 (1993) on change in E/I-ratio (delta-E/I: E/I-values in 2005 – E/I-values in 1985) over time. The same model was also built for MIF (values from 1998) and PAI-1 (values from 1998). Another model was built to analyze the impact of change in HbA1c levels (1993–2005) on the change in E/I (1993–2005), adjusted for age and gender. To compare the frequency of normal values between follow-up visits related samples Cochrane's Q test was used. Wilcoxon signed-ranks test was used to compare MIF and PAI-1 concentrations over time, as well as differences between MIF and PAI-1 in cases and controls.

A Spearman's rank order correlation was run to determine the correlation between concentrations of HSP27, MIF, PAI-1 and E/I-ratio, AI and BI, as well as between HbA1c and E/I-ratio. A Mann-Whitney *U* test was used to compare concentrations of HSP27 and the inflammatory variables in patients with abnormal E/I-ratio to concentrations of HSP27 and the inflammatory variables in patients with a normal E/I-ratio. A P-value of <0.05 was considered statistically significant.

Results

Clinical characteristics

Most of the T1D patients were in their early thirties at their first visit (median 31, interquartile range; IQR 11 years) (Table 1). Nineteen (59%) patients were men and 13 (41%) were women. Systolic blood pressure (BP) tended to increase over time (p = 0.054) whereas diastolic BP tended to increase overall during the 20-year follow-up, with a significant increase between 1985 and

Table 1
Clinical characteristics of the 32 T1D patients at five visits during 20 years of follow-up between 1985–2005.

	1985 n = 32	1989 n = 28	1993 n = 27	1998 n = 30	2005 n = 31	p-value
Age (years)	31 [11]	35 [11]	39 [11]	44 [11]	51 [11]	
Duration of diabetes (years)	14 [11]	18 [11]	22 [11]	27 [11]	34 [11]	
BMI (kg/m^2)				23 [4]	24 [4]	
Systolic BP (mmHg)	125 [18]	125 [10]	125 [15]	128 [25]	130 [20]	ns
Diastolic BP (mmHg)	**75 [10]**	**80 [15]**	80 [15]	77 [20]	75 [15]	**<0.05**
HbA1c (%)	6.7 [1.8]	**6.2 [1.3]**	**7.1 [1.0]**	7.4 [1.3]	7.5 [1.3]	**<0.0001**
HbA1c (mmol/mol)	59 [19]	**54 [13]**	**64 [11]**	67 [14]	68 [13]	**<0.0001**
HDL (mmol/l)				1.4 [0.5][f]	1.4 [0.6]	
LDL (mmol/l)				2.8 [1.0][f]	2.7 [0.9]	
LDLHDL-ratio				2.1 [0.7]	1.9 [1.0]	
Smoking, n (%)			6 (26)	8 (33)	8 (25)	
Statin treatment, n (%)				3 (9)	9 (29)	
Immunosuppressive therapy, n (%)				2 (6)	2 (6)	
Betablocker treatment, n (%)				3 (9)	2 (6)	

Data presented as median [interquartile range, IQR] if not otherwise stated. The two values that differ statistically significant for each value are marked in bold. Friedman's test with post hoc analysis used for calculations with multiple time points. Wilcoxon was used in the calculations with only two time points. Abbreviations: BMI = Body Mass Index, BP = Blood pressure, HDL = High Density Lipoprotein, LDL = Low Density Lipoprotein, ns = non-significant, T1D = type 1 diabetes.

1989 ($p < 0.05$, Table 1). Also, the HbA1c values increased over time ($p < 0.001$, Fig. 5), the difference occurring between 1989 and 1993 ($p = 0.001$, Table 1).

HSP27, MIF and PAI-1 concentrations in patients and controls

There were no differences in s-HSP27 concentrations between follow-up visits (Table 2).

In 1998, no difference in MIF concentrations was found between T1D patients and control subjects ($p > 0.05$, Table 2, Fig. 3). In 2005, the T1D patients had significantly lower MIF concentrations compared to control subjects ($p < 0.05$, Table 2, Fig. 3).

There were no significant differences in PAI-1 concentrations between T1D patients and healthy controls in 1998 (Table 2, Fig. 4). In samples from 2005, PAI-1 levels were significantly lower in T1D patients compared to controls ($p < 0.05$, Table 2, Fig. 4).

Both MIF ($p < 0.001$) and PAI-1 ($p < 0.05$) concentrations were lower in the patients with T1D 2005 compared to 1998, which was not observed in the healthy controls (Table 2).

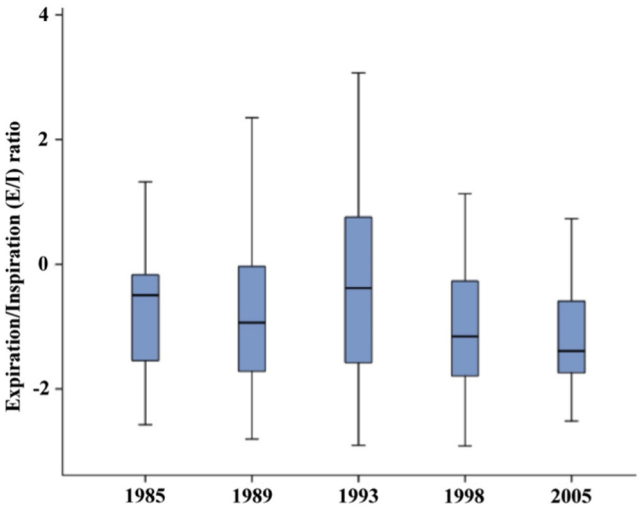

Fig. 2. E/I-ratio during follow-up (1985–2005) in 32 patients with T1D. E/I-ratio = Expiration/Inspiration-ratio, T1D = Diabetes Mellitus type 1.

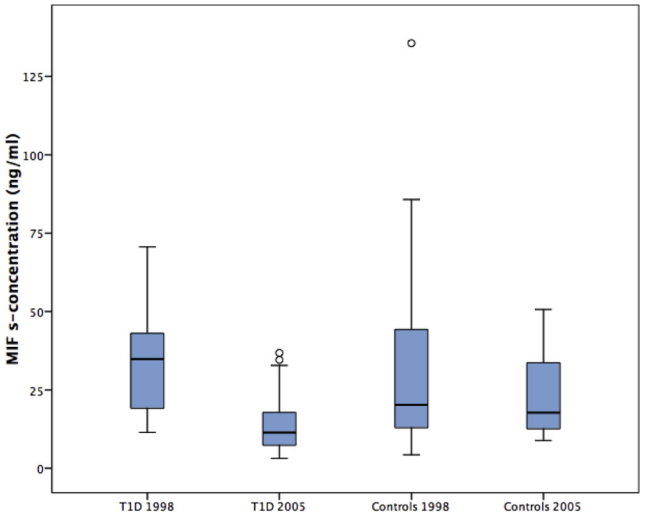

Fig. 3. S-concentration of MIF (ng/ml) in T1D patients compared to healthy controls in 1998 and 2005.

Correlation between levels of HSP27, MIF and PAI-1

HSP27 levels correlated with MIF (r_s 0.46; $p < 0.05$), but not with PAI-1 levels in 2005, whereas there were no correlations between HSP27 and MIF or PAI-1 in 1998.

Cardiovascular autonomic nerve function

Expiration/inspiration ratio (E/I-ratio)

E/I-ratio was higher in the T1D patients in 1993 compared to 1998 ($p < 0.05$; Table 2, Fig. 2), although the number of patients with an abnormal E/I-ratio was consistent over time ($p > 0.05$; Table 2). In the multivariate linear regression analysis, none of the variables had a significant impact on the change in E/I over time.

Acceleration index (AI)

Regarding AI, we observed a significant difference over time ($p < 0.05$; Table 2), with a deterioration occurring between 1998 and 2005 ($p < 0.05$). The proportion of patients with normal AI was consistent over time ($p > 0.05$; Table 2).

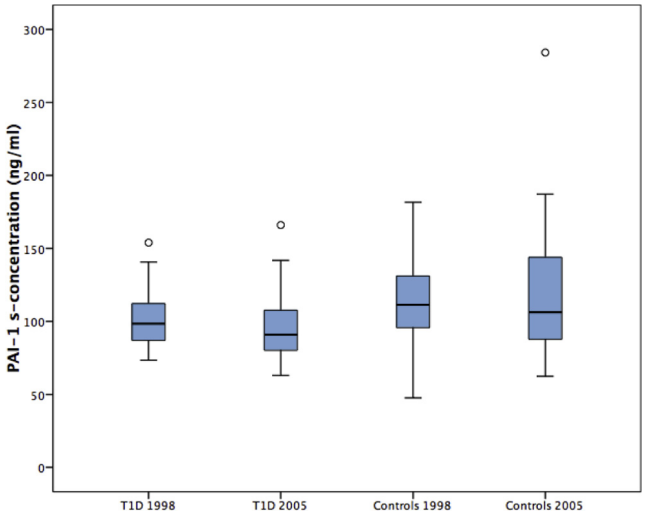

Fig. 4. S-concentration of PAI-1 (ng/ml) in T1D patients compared to healthy controls in 1998 and 2005.

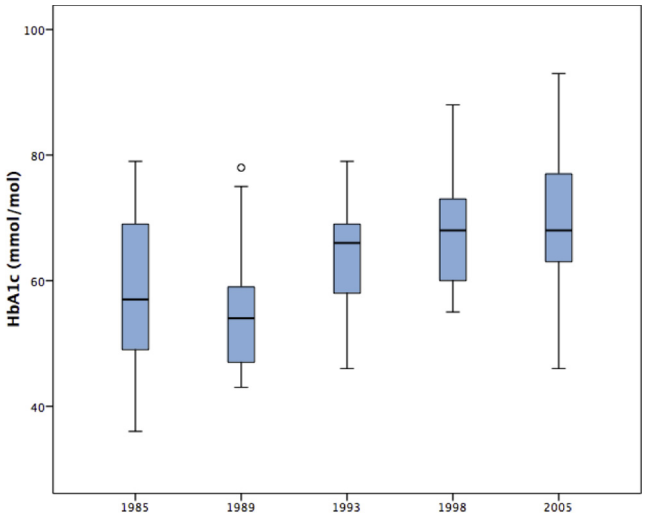

Fig. 5. HbA1c (mmol/mol) in 32 T1D patients over time.

Table 2
Autonomic nerve function, HSP27, MIF, and PAI-1 concentrations in the 32 patients with T1D during 20-years of follow-up.

	1985 n = 32	1989 n = 28	1993 n = 27	1998 n = 30	2005 n = 31	p-value
HSP27 pg/ml			388 [578]	433 [705]	452 [561]	ns
HSP27 matched controls pg/ml*				**1103 [500]**	**496 [864]**	**<0.05**
MIF ng/ml				**34 [27]**	**11 [10]**	**<0.0001**
MIF matched controls ng/ml				20 [30]	18 [21]	ns
PAI-1 ng/ml				**100 [35]**	**93 [29]**	**<0.05**
PAI-1 matched controls ng/ml				111 [37]	107 [56]	ns
E/I-ratio	−0.68 [2.03]	−0.94 [1.99]	**−0.52 [2.35]**	**−1.32 [1.39]**	−1.43 [1.16]	**<0.05**
Abnormal E/I-ratio, n (%)	9 (28)	10 (31)	7 (22)	11 (34)	13 (41)	ns
Normal E/I-ratio, n (%)	23 (72)	18 (56)	20 (63)	19 (59)	18 (56)	ns
Acceleration index (AI)	−0.85 [1.15]	−1.21 [1.36]	−1.16 [1.65]	**−1.48 [1.56]**	**−0.62 [1.25]**	**<0.05**
Abnormal AI, n (%)	7 (22)	10 (31)	9 (28)	13 (40)	7 (22)	ns
Normal AI, n (%)	19 (59)	18 (56)	18 (56)	17 (53)	23 (72)	ns
Brake index (BI)	−0.89 [2.13]	−1.15 [1.30]	**−1.1 [1.32]**	**−0.73 [1.80]**	−0.26 [1.00]	**<0.0001**
Abnormal BI, n (%)	9 (28)	8 (25)	9 (28)	**4 (13)**	**1 (3)**	**<0.05**
Normal BI, n (%)	17 (53)	20 (63)	18 (56)	**26 (87)**	**29 (91)**	**<0.05**

Data presented as median [interquartile range, IQR] if not otherwise stated. The two values that differ statistically significant for each value are marked in bold. Friedman's test with post hoc analysis used for calculations with multiple time points. Wilcoxon was used in the calculations with only two time points.
Abbreviations: E/I-ratio = expiration/inspiration-ratio, AI = acceleration index, BI = brake index, ns = non-significant, T1D = type 1 diabetes.
* Data already published [27] and [26].

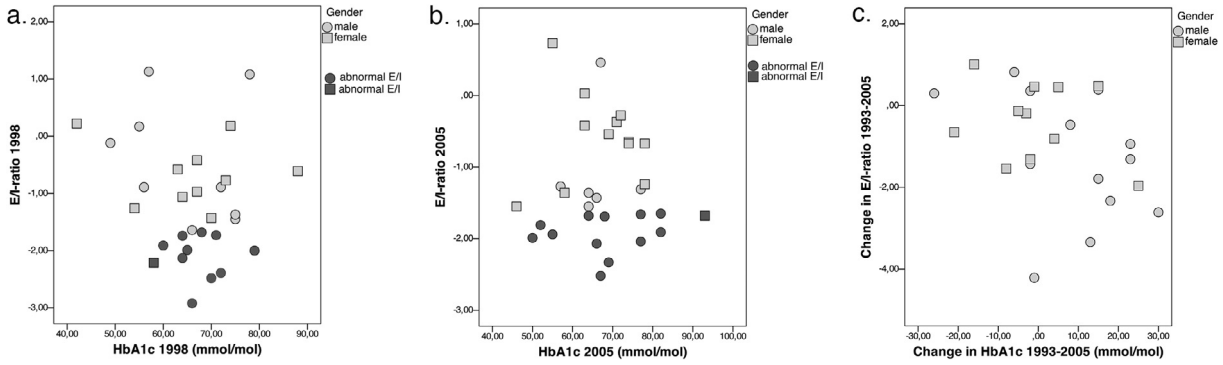

Fig. 6. E/I-ratio and HbA1c in a) 1998 and b) 2005 and c) the change in E/I-ratio over time versus the change in HbA1c over time in T1D patients.

Brake index (BI)

BI changed over time ($p < 0.001$), deteriorating between 1993 and 1998 ($p = 0.001$; Table 2). There were differences in the number of patients with abnormal BI between the follow-up visits ($p < 0.05$), explained by the difference between 1998 and 2005 ($p < 0.05$; Table 2).

HSP27, MIF, PAI-1 concentrations in correlation with E/I-ratio

There were no significant correlations between HSP27 concentrations and E/I-ratio among T1D patients in 1993, 1998 or 2005.

We found no difference when comparing concentrations of HSP27 in patients with abnormal and normal E/I-ratios neither in 1993 nor in 2005 ($p > 0.05$). In 1998, however, we found significantly higher concentrations of HSP27 in patients with abnormal E/I-ratio compared to patients with normal E/I-ratio ($p < 0.05$). We could not demonstrate any correlation when plotting delta-HSP27 between 1993 and 2005 against delta-E/I-ratio over the same period. There was no correlation between concentrations of neither MIF nor PAI-1 with E/I-ratio. Delta-MIF (the change in MIF concentration between 1998 and 2006) did not correlate with delta-E/I (adjusted R^2 0.029). Delta-PAI-1 did not correlate with delta-E/I (adjusted R^2 −0.019).

HSP27, MIF, PAI-1, acceleration and brake indices

In 1993, HSP27 and AI were correlated ($r_s = -0.43$, $p < 0.05$), whereas in 1998 or 2005 no significant correlations were observed.

There were no correlations between HSP27 and BI in 1993, 1998, or 2005. Neither did we find any correlation between MIF and AI or BI, or between PAI-1 and AI or BI.

HbA1c

There were no correlations between HbA1c and E/I-ratio in 1993, 1998, or 2005, using a Spearman rank order correlation test. However, delta-HbA1c and delta-E/I-ratio correlated (1993–2005, Spearman: $r_s = -0.42$; $p < 0.05$).

HbA1c and MIF correlated in 2005 (r_s 0.43; $p < 0.05$), but not in 1998. There were no correlations between HbA1c and PAI-1 or HSP27. In the regression model when studying the impact of change in HbA1c on the change in E/I-ratio, the model did not turn out statistically significant. The distribution of E/I-ratio in relation to HbA1c levels are presented in Fig. 6.

Discussion

Cardiovascular autonomic dysfunction, expressed as an abnormal E/I ratio, deteriorated over time in 32 patients with T1D during a 20-year period. Despite this, we found no correlations between cardiovascular autonomic nerve function and HSP27 or the inflammatory markers MIF and PAI-1 concentrations in our study. However, deterioration of autonomic nerve function was related to increasing HbA1c in T1D during the 20-year follow-up.

To the best of our knowledge, no previous research has studied HSP27 or the presently used markers of inflammation, MIF and

PAI-1, in relation to cardiovascular autonomic neuropathy in human diabetes. However, an importance of HSP27 in the cellular response in peripheral neuropathy [14,24] is suggested by HSP27 being associated with distal symmetric polyneuropathy in T1D patients. In addition, patients with polyneuropathy have higher concentrations of HSP27 [13], and sensory neurons could be protected from diabetic neuropathy by up-regulation of HSP27 [16,26]. Therefore, we hypothesized that HSP27 might be associated with cardiovascular autonomic nerve function in T1D, yet our results did not support this hypothesis. The earliest available blood samples for HSP27 were collected in 1993, when patients already had their diabetes for an average of 22 years; HSP27 levels were already then lower than those in the healthy control group. The corresponding levels of the inflammatory variables were only available from the last two follow-ups. Unfortunately, we do not have data of HSP27, MIF, or PAI-1 at study baseline 1985. One might therefore speculate that at onset, neuropathy could cause a rise in HSP27 as an initial response in an attempt to rescue neurons, and with time the response fades, leaving the patient with subnormal HSP27, indicating that neuropathy has to be detected early with an attempt to optimize treatment. Furthermore, development of autonomic or peripheral neuropathy might also induce an inflammatory response as a consequence of a degeneration process. Hyperglycemia is known to cause oxidative stress on a cellular level, and this stress causes HSP27 to be released into the circulation, eliciting an autoimmune response [4]. It is also possible that the diabetic state in itself lowers circulating concentrations of HSP27. Other possible hypotheses are a genetic predisposition related to the genes predisposing for T1D may cause the low concentrations of HSP27, or some environmental factor not addressed in our study may influence HSP27. HSP27 levels dropped in the control subjects. We speculate that this might be an effect of aging.

We did not find any relationship between autonomic neuropathy in T1D and circulating PAI-1 levels, and even though PAI-1 is known to be involved in diabetic complications, as part of a hypercoaguable state. Our results are in accordance with a previous study by Madan et al. where high PAI-1 levels were associated with microvascular complications, but not with symptoms of peripheral and autonomic neuropathy complications [17].

In our last year of follow-up, 2005, patients with diabetes had lower levels of both MIF and PAI-1 compared to healthy controls. With a small study and significant results from only one of the measuring occasion, this calls for careful interpretation. One can speculate if the well-regulated diabetes found in our study group (with a mean HbA1c of 68 mmol/mol after a mean duration of 34 years) can contribute to a diminished inflammatory state.

We found a weak, but significant, correlation between MIF and HbA1c at the last follow-up. In T2D, MIF is associated with insulin resistance and obesity [28], two factors therapeutically targeted in T2D management to lower HbA1c. In T1D, where instead lack of insulin is the main problem, higher levels of MIF might perhaps be associated with higher levels of inflammatory activity leading to dysregulation of the disease and higher HbA1c.

We found deteriorating cardiovascular autonomic nerve function measured as E/I-ratio and BI. These findings are consistent with the notion that diabetic neuropathy affects the parasympathetic system before the sympathetic system [9,32,34], and confirms that cardiovascular autonomic nerve function deteriorates over time in patients with T1D. In addition, our data indicate a fluctuation over time, not a continuous deterioration, where cardiovascular autonomic function seems to improve some years and deteriorate some.

Our data did not reveal whether subclinical neuropathy progresses into overt neuropathy over time. One would have expected the number of patients with abnormal autonomic function to increase if it progressed over the years. There was a negative correlation between E/I-ratio and HbA1c. This would fit well with current knowledge of the pathophysiology of peripheral neuropathy in T1D; good glucose control is believed to be important for the prevention of development of neuropathy [5]. However, recent results from two major trials, the Diabetes Control and Complications Trial (DCCT) and Epidemiology of Diabetes Interventions and Complications (EDIC) studies, show that glucose control is not enough to protect patients with diabetes from development of peripheral neuropathy and CAN [19]. In the light of this fact, low-grade inflammation has come into focus as a potentially new area of interest for research and development of new treatment strategies [25].

Strengths and limitations

The strengths of our study was the long follow-up time of 20 years and well matched control subjects (age- and gender-matched) concerning levels of HSP27, MIF and PAI-1. Our study population is small comprising only 32 patients, and not all patients came to all follow-ups, which is a limitation. Another limitation is that we did not evaluate development of cardiomyopathy, which could be associated with autonomic dysfunction.

Conclusion

The present study demonstrated a deterioration of cardiovascular autonomic nerve function in relation to increasing HbA1c in T1D patients followed for a period of 20 years. We observed no association between HSP27, MIF or PAI-1 and measures of cardiovascular autonomic neuropathy. Improved glucose control might be associated with protection against autonomic neuropathy in T1D.

Funding

The study was supported by grants from the Swedish Research Council (Medicine), Svenska Diabetesförbundet, Diabetesföreningen i Malmö, Sigurd och Elsa Goljes Foundation, the County of Skåne, and Funds from Malmö University Hospital, Sweden.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

We are grateful to the late professor Göran Sundqvist who initiated and followed the cohort study and for the analyses of HSP27 concentrations by Kristina Eriksson at Umeå University Hospital, Sweden. Furthermore, thanks to Ann Radelius and Victoria Granberg, who organized the follow up in 2005, and to Professor Jonas Björk and Tommy Schyman for statistical advice.

References

[1] Benn SC, Perrelet D, Kato AC, et al. Hsp27 upregulation and phosphorylation is required for injured sensory and motor neuron survival. Neuron 2002;36:45–56.
[2] Bergstrom B, Lilja B, Osterlin S, Sundkvist G. Autonomic neuropathy in type I diabetes: influence of duration and other diabetic complications. Acta Med Scand 1987;222:147–54.
[3] Bergstrom B, Lilja B, Rosberg K, Sundkvist G. Autonomic nerve function tests. Reference values in healthy subjects. Clin Physiol 1986;6:523–8.
[4] Burut DF, Borai A, Livingstone C, Ferns G. Serum heat shock protein 27 antigen and antibody levels appear to be related to the macrovascular complications associated with insulin resistance: a pilot study. Cell Stress Chaperones 2010;15:379–86.

[5] Callaghan BC, Hur J, Feldman EL. Diabetic neuropathy: one disease or two? Curr Opin Neurol 2012;25:536–41.
[6] Cvetkovic I, Al-Abed Y, Miljkovic D, et al. Critical role of macrophage migration inhibitory factor activity in experimental autoimmune diabetes. Endocrinology 2005;146:2942–51.
[7] Dahlin LB, Granberg V, Rolandsson O, Rosen I, Dahlin E, Sundkvist G. Disturbed vibrotactile sense in finger pulps in patients with Type 1 diabetes–correlations with glycaemic level, clinical examination and electrophysiology. Diabet Med 2011;28:1045–52.
[8] Dimitropoulos G, Tahrani AA, Stevens MJ. Cardiac autonomic neuropathy in patients with diabetes mellitus. World J Diab 2014;5:17–39.
[9] Edwards JL, Vincent AM, Cheng HT, Feldman EL. Diabetic neuropathy: mechanisms to management. Pharmacol Ther 2008;120:1–34.
[10] Federation ID. Diabetes Atlas, 6th edn.. Brussels, Belgium2013. http://www.idf.org/diabetesatlas: International Diabets Federation.
[11] Forsen A, Kangro M, Sterner G, et al. A 14-year prospective study of autonomic nerve function in Type 1 diabetic patients: association with nephropathy. Diabet Med 2004;21:852–8.
[12] Freccero C, Svensson H, Bornmyr S, Wollmer P, Sundkvist G. Sympathetic and parasympathetic neuropathy are frequent in both type 1 and type 2 diabetic patients. Diabetes Care 2004;27:2936–41.
[13] Gruden G, Bruno G, Chaturvedi N, et al. Serum heat shock protein 27 and diabetes complications in the EURODIAB prospective complications study: a novel circulating marker for diabetic neuropathy. Diabetes 2008;57:1966–70.
[14] Hunt D, Raivich G, Anderson PN. Activating transcription factor 3 and the nervous system. Front Mol Neurosci 2012;5:7.
[15] Kannel WB, McGee DL. Diabetes and cardiovascular disease. The Framingham study. JAMA 1979;241:2035–8.
[16] Korngut L, Ma CH, Martinez JA, et al. Overexpression of human HSP27 protects sensory neurons from diabetes. Neurobiol Dis 2012;47:436–43.
[17] Madan R, Gupt B, Saluja S, Kansra UC, Tripathi BK, Guliani BP. Coagulation profile in diabetes and its association with diabetic microvascular complications. J Assoc Phys India 2010;58:481–4.
[18] Makino A, Nakamura T, Hirano M, et al. High plasma levels of macrophage migration inhibitory factor are associated with adverse long-term outcome in patients with stable coronary artery disease and impaired glucose tolerance or type 2 diabetes mellitus. Atherosclerosis 2010;213:573–8.
[19] Martin CL, Albers JW, Pop-Busui R. Neuropathy and related findings in the diabetes control and complications trial/epidemiology of diabetes interventions and complications study. Diabetes Care 2014;37:31–8.
[20] Maser RE, Ellis D, Erbey JR, Orchard TJ. Do tissue plasminogen activator-plasminogen activator inhibitor-1 complexes relate to the complications of insulin-dependent diabetes mellitus? Pittsburgh epidemiology of diabetes complications study. J Diabetes Complications 1997;11:243–9.
[21] Maser RE, Pfeifer MA, Dorman JS, Kuller LH, Becker DJ, Orchard TJ. Diabetic autonomic neuropathy and cardiovascular risk. Pittsburgh epidemiology of diabetes complications study III. Arch Intern Med 1990;150:1218–22.
[22] Nathan DM. Long-term complications of diabetes mellitus. N Engl J Med 1993;328:1676–85.
[23] Nilsson H, Bergstrom B, Lilja B, Juul-Moller S, Carlsson J, Sundkvist G. Prospective study of autonomic nerve function in type 1 and type 2 diabetic patients: 24 hour heart rate variation and plasma motilin levels disturbed in parasympathetic neuropathy. Diabet Med 1995;12:1015–21.
[24] Patodia S, Raivich G. Role of transcription factors in peripheral nerve regeneration. Front Mol Neurosci 2012;5:8.
[25] Pop-Busui R, Ang L, Holmes C, Gallagher K, Feldman EL. Inflammation as a therapeutic target for diabetic neuropathies. Curr DiabRep 2016;16:29.
[26] Pourhamidi K, Dahlin LB, Boman K, Rolandsson O. Heat shock protein 27 is associated with better nerve function and fewer signs of neuropathy. Diabetologia 2011;54:3143–9.
[27] Pourhamidi K, Skarstrand H, Dahlin LB, Rolandsson O. HSP27 concentrations are lower in patients with Type 1 diabetes and correlate with large nerve fiber dysfunction. Diabetes Care 2014;37:e49–50.
[28] Sanchez-Zamora YI, Rodriguez-Sosa M. The role of MIF in type 1 and type 2 diabetes mellitus. J Diab Res 2014;2014:804519.
[29] Skarstrand H, Dahlin LB, Lernmark A, Vaziri-Sani F. Neuropeptide Y autoantibodies in patients with long-term type 1 and type 2 diabetes and neuropathy. J Diabetes Complications 2013;27:609–17.
[30] Spallone V, Ziegler D, Freeman R, et al. Cardiovascular autonomic neuropathy in diabetes: clinical impact, assessment, diagnosis, and management. Diab Metab Res Rev 2011.
[31] Stosic-Grujicic S, Stojanovic I, Maksimovic-Ivanic D, et al. Macrophage migration inhibitory factor (MIF) is necessary for progression of autoimmune diabetes mellitus. J Cell Physiol 2008;215:665–75.
[32] Sundkvist G. Autonomic nervous function in asymptomatic diabetic patients with signs of peripheral neuropathy. Diabetes Care 1981;4:529–34.
[33] Sundkvist G, Almer L, Lilja B. Respiratory influence on heart rate in diabetes mellitus. Br Med J 1979;1:924–5.
[34] Sundkvist G, Lilja B, Manhem P, Almer LO. Responses of plasma catecholamines to tilt in patients with diabetes mellitus. Acta Med Scand 1984;216:223–7.
[35] Sundkvist G, Lilja B. Autonomic neuropathy in diabetes mellitus: a follow-up study. Diabetes Care 1985;8:129–33.
[36] Vinik AI, Maser RE, Mitchell BD, Freeman R. Diabetic autonomic neuropathy. Diabetes Care 2003;26:1553–79.
[37] WHO. Global status report on noncommunicable diseases 2010. Geneva, World Health Organization, 2011.
[38] Willenheimer RB, Erhardt LR, Nilsson H, Lilja B, Juul-Moller S, Sundkvist G. Parasympathetic neuropathy associated with left ventricular diastolic dysfunction in patients with insulin-dependent diabetes mellitus. Scand Cardiovasc J 1998;32:17–22.