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A 14-year prospective study of autonomic nerve function in Type 1 diabetic patients: association with nephropathy

A. Forsén*, M. Kangro†, G. Sterner‡, K. Norrgren§, O. Thorsson¶, P. Wollmer¶ and G. Sundkvist*

Abstract

**Aims** Prospective studies of autonomic nerve function are rare. We have followed the progression of autonomic dysfunction in relation to nephropathy over 14 years in Type 1 diabetic patients.

**Methods** Autonomic nerve function was assessed by heart-rate responses to deep breathing (E/I ratio) and tilting (acceleration and brake indices) and by the postural blood pressure reaction in 58 patients, 43 of whom were reassessed after 14 years. Nephropathy was evaluated by the degree of albuminuria (albuminuria > 20 µg/min or > 0.03 g/24 h) and glomerular filtration rate ($^{51}$Cr-EDTA plasma clearance). The acceleration index had deteriorated after 7 years ($P = 0.0155$), whereas the E/I ratio ($P = 0.0070$) and the diastolic postural blood pressure reaction ($P = 0.0054$) had deteriorated 14 years after the baseline examination (age-corrected values). All those with albuminuria at the third examination showed signs of autonomic neuropathy at baseline (10 of 10) compared with only nine of 22 without ($P = 0.0016$). Multiple regression analysis showed that the association between autonomic dysfunction and future albuminuria was due to the E/I ratio. In addition, individuals with an abnormal postural diastolic blood pressure fall ($n = 7$) at baseline showed a greater fall in glomerular filtration rate more than others 7–14 years later [29 (16.5) ml/min/1.72 m$^2$ vs. 11 (9) ml/min/1.72 m$^2$; $P = 0.0074$].

**Conclusion** Autonomic nerve function had deteriorated after 14 years. Autonomic neuropathy and abnormal postural diastolic blood pressure falls at baseline were associated with future renal complications.

**Keywords** autonomic neuropathy, nephropathy, neuropathy, proliferative retinopathy, retinopathy, symptoms of neuropathy

**Abbreviations** DCCT, Diabetes Control and Complication trial; E/I ratio, expiration/inspiration ratio; GFR, glomerular filtration rate
Table 1  Clinical features at baseline and at the final examination 14 years later amongst Type 1 diabetic patients

<table>
<thead>
<tr>
<th></th>
<th>All patients</th>
<th>Patients followed up on four occasions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>14 year later</td>
</tr>
<tr>
<td>Number of patients</td>
<td>58</td>
<td>48</td>
</tr>
<tr>
<td>Age (years)</td>
<td>33±10</td>
<td>46±9</td>
</tr>
<tr>
<td>Duration of diabetes (years)</td>
<td>13±9</td>
<td>26±8</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>7.15±1.48</td>
<td>7.41±1.20</td>
</tr>
<tr>
<td>Supine blood pressure (mmHg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>130±19</td>
<td>135±22</td>
</tr>
<tr>
<td>Diastolic</td>
<td>77±10</td>
<td>82±11</td>
</tr>
<tr>
<td>Hypertension (= 140/90 mmHg)</td>
<td>14/58 (24%)</td>
<td>15/48 (31%)</td>
</tr>
<tr>
<td>Anti-hypertensive therapy</td>
<td>1 (2%)</td>
<td>14/48 (29%)</td>
</tr>
<tr>
<td>Albuminuria</td>
<td>18/36 (50%)</td>
<td>11/45 (24%)</td>
</tr>
<tr>
<td>Retinopathy</td>
<td>24/58 (41%)</td>
<td>42/46 (91%)*</td>
</tr>
</tbody>
</table>

Results are given as mean ± SD or number (%).
*Retinopathy not assessed in two patients.

renal complications were secondary to autonomic nerve dysfunction.

**Patients and methods**

In 1984–85, 58 patients (22 women), all diagnosed with Type 1 diabetes between the ages of 15 and 25 years (age 17–56 years, median 33; duration of diabetes 2 months—30 years, median 12) were evaluated with regard to autonomic nerve function [8]. In 1989 (i.e. 4 years after the first examination), all were invited to a second examination and 44 of 58 accepted. In 1992 (7 years after the first examination), subjects were invited to a third examination and 41 of 58 agreed [9]. In 1998 (13–14 years after the original examination), all patients were invited to a fourth examination and 43 of 58 (16 women), median age 46 years (range 30–67) and median duration of diabetes 25 years (13–42), agreed to undergo autonomic nerve function tests. Fifteen did not participate in the fourth examination. Among them, three had died (one of probable myocardial infarction, one of aortic valvular disease, and one of multi-infarction dementia), two had moved and five completed a clinical examination only. A total of 34 individuals underwent autonomic nerve function in all four studies. Prospective data are based on these 34. Table 1 records their clinical features which were representative for the whole group. Informed consent was obtained from all subjects. The Ethics Committee, Lund University, Sweden, approved the study.

**Cardiac autonomic nerve function tests**

**Deep breathing test (R-R interval variation)**

Six maximal expirations and inspirations were performed during 1 min in the supine position during the recording of a continuous ECG and the R-R intervals were recorded. The E/I ratio, a test of parasympathetic vagal nerve function, was calculated as the mean of the longest R-R interval during expiration (E) divided by the mean of the shortest R-R interval during inspiration (I) [10].
A urine sample was available for 36 of 58 subjects at baseline, 32 of 44 at the second examination, 27 of 41 subjects at the third examination and 45 of 48 at the final examination. In 36 of the individuals, albuminuria was assessed in at least three of the four examinations. Amongst the 45 patients tested for albuminuria at the final examination, 31 had been tested at baseline. Among the 36 evaluated at baseline, four of five not followed up showed albuminuria at baseline. Three of the four with albuminuria at baseline had died during the observation period. Urine albumin was measured by immunonephelometry on a Beckman Array Protein system instrument (Beckman Instruments, Fullerton, CA, USA). During the study period, the method for collecting urine samples was changed, i.e. from collection during 24 h (the first three examinations) to overnight collection (final examination). Microalbuminuria was therefore defined as an albumin excretion of 0.03–0.30 g/24 h in the first three examinations (24-h collection) and 20–200 µg/min (over-night collection) at the final examination; macroalbuminuria was defined as an albumin excretion above those levels [15]. In this report, patients with micro or macroalbuminuria are considered together as patients with albuminuria.

Glomerular filtration rate (GFR)

Glomerular filtration rate (GFR) was assessed at the third and fourth examination. GFR was evaluated by the $^{51}$Cr-EDTA plasma clearance method [16]. Reference values were taken from [17] and age-corrected values expressed as Z scores were calculated. A Z score value less than $-1.64$ SD (95% confidence interval, one-sided test) was considered abnormally low.

Statistical analysis

Repeated measures ANOVA followed by paired $t$-tests were used in within group comparisons. As the number of individuals was low ($n < 20$) in the different subgroups, non parametric Mann–Whitney $U$-test was used in comparisons between two groups. Chi-square, Fisher’s test, and Mac Nemar’s test were used for evaluating differences in frequencies. Spearman’s test ($r_s$), Pearson’s correlation coefficient and multiple regression were used to test associations. A $P$-value $< 0.05$ was considered significant. Data are presented as mean ± sd, if not otherwise stated.

Results

Autonomic nerve function

Cardiac autonomic nerve function

Figure 1(a) shows that the E/I ratio and the acceleration index deteriorated ($P < 0.0001$) during the 14 years of observation. However, there was a difference between the two tests. The acceleration index had deteriorated after 7 years, whereas the E/I ratio had decreased 14 years after baseline, as was the case from [17] and age-corrected values expressed as Z scores were calculated. A Z score value less than $-1.64$ SD (95% confidence interval, one-sided test) was considered abnormally low.

Figure 1  (a) The progression of autonomic nerve function during 14 years amongst 34 Type 1 diabetic subjects examined at all four examinations. Results expressed in absolute values and presented as mean ± sem. The E/I ratio and the acceleration index deteriorated ($P < 0.0001$) during the 14 years of observation. (b) The progression of autonomic nerve function during 14 years amongst 34 Type 1 diabetic patients examined at all four examinations. Results expressed in age-corrected Z-scores and presented as mean ± sem. The acceleration index deteriorated after 7 years ($P = 0.0155$; first vs. third examination) and the E/I ratio after 14 years ($P = 0.007$; third vs. fourth examination). The brake index decreased after 7 years ($P = 0.006$; baseline vs. third examination) to normalize after 14 years ($P < 0.001$; third vs. fourth examination).
for age-corrected values (Fig. 1b) ($P = 0.0155$ for the acceleration index, first vs. third examination; $P = 0.0070$ for the E/I ratio, third vs. fourth examination). The brake index also changed during the study ($P < 0.0001$), albeit differently. In agreement with the acceleration index, the brake had decreased after 7 years ($P = 0.0066$ in age-corrected values) to then, different from the acceleration index, be normalized after 14 years ($P < 0.001$ age corrected values; third vs. fourth examination). There was a significant correlation ($r_s = 0.58; P = 0.006$) between the age-corrected brake index at the third examination vs. the age-corrected acceleration index at the final examination.

**Postural blood pressure reaction**

Using absolute values, there was no significant difference \(\text{(ANOVA; } P = 0.0979)\) in the development of the postural diastolic blood pressure reaction (Fig. 2). However, using age-corrected values, the postural diastolic blood pressure reaction deteriorated \(\text{(ANOVA; } P = 0.0101)\) with a significant decrement in the postural diastolic blood pressure in the final examination \(P = 0.0054\); fourth vs. the third examination).

**Individual autonomic tests results**

Table 2 shows the prevalence of abnormal cardiac autonomic nerve function at baseline and after 14 years in all subjects as well as in the 34 patients examined on all four occasions. Half had signs of autonomic neuropathy (i.e. one or more abnormal test) at baseline and the prevalence was not significantly increased

<table>
<thead>
<tr>
<th>Any abnormal test</th>
<th>Baseline $n = 58$</th>
<th>14 year later $n = 43$</th>
<th>Baseline $n = 34$</th>
<th>14 year later $n = 34$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any</td>
<td>34 (53%)</td>
<td>27 (65%)</td>
<td>18 (52%)</td>
<td>17 (50%)</td>
</tr>
<tr>
<td>E/I</td>
<td>20 (34%)</td>
<td>18 (42%)</td>
<td>9 (26%)</td>
<td>13 (38%)</td>
</tr>
<tr>
<td>Acceleration index</td>
<td>12 (21%)</td>
<td>16 (37%)</td>
<td>6 (18%)</td>
<td>12 (35%)</td>
</tr>
<tr>
<td>Brake index</td>
<td>15 (31%)</td>
<td>3 (7%)</td>
<td>10 (29%)</td>
<td>3 (9%)</td>
</tr>
<tr>
<td>One abnormal test</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E/I</td>
<td>9 (16%)</td>
<td>8 (19%)</td>
<td>4 (12%)</td>
<td>7 (21%)</td>
</tr>
<tr>
<td>Acceleration index</td>
<td>4 (7%)</td>
<td>6 (14%)</td>
<td>3 (9%)</td>
<td>6 (18%)</td>
</tr>
<tr>
<td>Brake index</td>
<td>9 (16%)</td>
<td>3 (7%)</td>
<td>6 (18%)</td>
<td>0*</td>
</tr>
<tr>
<td>E/I or acceleration index or brake index</td>
<td>23 (40%)</td>
<td>19 (44%)</td>
<td>13 (38%)</td>
<td>11 (32%)</td>
</tr>
<tr>
<td>Two abnormal tests</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E/I and acceleration index</td>
<td>5 (9%)</td>
<td>7 (16%)</td>
<td>3 (9%)</td>
<td>3 (9%)</td>
</tr>
<tr>
<td>E/I and brake index</td>
<td>3 (5%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Acceleration index and brake index</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total two abnormal tests</td>
<td>8 (14%)</td>
<td>7 (16%)</td>
<td>3 (9%)</td>
<td>3 (9%)</td>
</tr>
<tr>
<td>Three abnormal tests</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E/I, acceleration index and brake index</td>
<td>3 (5%)</td>
<td>3 (7%)</td>
<td>2 (6%)</td>
<td>3 (9%)</td>
</tr>
<tr>
<td>Two or three abnormal tests</td>
<td>11 (19%)</td>
<td>10 (23%)</td>
<td>5 (15%)</td>
<td>6 (18%)</td>
</tr>
</tbody>
</table>

Results are given as number (%).

E/I, expiration/inspiration ratio.

* $P = 0.0246$ vs. first study.
14 years later. Amongst those tested both at baseline and after 14 years, 10 of 12 with an abnormal E/I and five of six with an abnormal acceleration index at baseline had abnormalities in the corresponding test 14 years later. The prevalence of an abnormal postural diastolic blood pressure response was 11/58 (19%) at baseline and 12/42 (29%) after 14 years.

**Autonomic nerve function and glycaemic control**

There was no significant relationship between HbA_{1c} at baseline and autonomic nerve function. However, HbA_{1c} at baseline and at the second examination was related to the brake index at the third examination ($r = -0.40; P = 0.0215$ and $r = -0.433; P = 0.0116$, respectively). Further, HbA_{1c} at the second examination showed a significant negative correlation with the E/I ratio at the third examination ($r = -0.35; P = 0.0394$). There were also significant negative correlations between HbA_{1c} at the third examination vs. the E/I ratio ($r = -0.40; P = 0.0186$) and the acceleration index ($r = -0.35; P = 0.0405$) at the fourth examination. Patients with an abnormal E/I ratio as well as those with an abnormal brake index at the third examination showed significantly higher HbA_{1c} values at the second examination (7.84 ± 1.39 vs. 6.84 ± 1.15%; $P = 0.0194$) and 7.86 ± 1.59 vs. 6.74 ± 0.93%; $P = 0.0447$, respectively). The number of patients with definite abnormalities (Table 2) did not increase.

**Albuminuria**

Overall, half [18 of 36 (50%); 5/18 macroalbuminuria] of patients had albuminuria at baseline as compared with eight of 39 (21%; 3/8 macroalbuminuria) after 4, 10 of 33 (30%; 2/10 macroalbuminuria) after 7, and 11 of 45 (24%; 4/11 macroalbuminuria) after 14 years. All patients with albuminuria at third examination had autonomic neuropathy at baseline, of 10 as compared with nine of 22 without ($P = 0.0016$). Correlation analysis between autonomic nerve function tests, HbA_{1c}, supine blood pressures and albumin excretion at baseline vs. the albumin excretion 14 years later showed the age-corrected correlation coefficient of 0.403. When albumin excretion, 14 years after the baseline examination (dependent variable) was included with the E/I ratio, HbA_{1c}, albumin excretion, and supine diastolic pressure variables at baseline (independent variables) in a multiple regression analysis, only the adjusted partial coefficient for the E/I ratio remained significant ($r = -0.524; P = 0.0054$). Those with albuminuria at the final examination (Fig. 3) showed significantly lower E/I ratios than those without at all examinations (baseline, $P = 0.0162$; second examination, $P = 0.0149$; third examination, $P = 0.0004$; fourth, $P = 0.0002$, respectively).

There was a significant negative correlation between the brake index at baseline vs. the decrease in GFR (age-corrected) between the third and fourth examination ($r = -0.442; P = 0.0124$). The decrease in GFR between the third and fourth examination was also significantly higher in those with an abnormal postural diastolic blood pressure fall at baseline ($n = 7$) vs. those without ($n = 32$) (Fig. 4; 29.16.5 ml/min/1.72 m² vs. 11.9 ml/min/1.72 m²; $P = 0.0074$; age corrected 2.09 (0.80) vs. 0.60 (0.74); $P = 0.0080$).

**Discussion**

This prospective study showed that autonomic nerve function deteriorated during the 14 years of observation. Initially, acceleration index decreased and this was later followed by deteriorations in the E/I ratio and the postural diastolic blood pressure. Low E/I ratios at baseline, not supine blood pressures, HbA_{1c} or albumin excretion, were associated with increased albuminuria 14 years later. Moreover, those with abnormal postural diastolic blood pressure falls or low brake indices at baseline deteriorated in GFR more than expected 7–14 years after the baseline examination.

**Signs of autonomic neuropathy**

In our prospective study, three different indices of autonomic neuropathy were used. The deep breathing test evaluates the heart-rate variation during 1 min during deep breathing and the results are expressed as the E/I ratio, a marker of parasympathetic nerve function [10]. This test is reproducible [18] and has recently
Autonomic neuropathy is associated with an increased risk for death [1], but in one prospective study, mortality was less than expected; 27% in those with autonomic neuropathy vs. 11% in those without [7]. Those who died in the current study had signs of autonomic neuropathy at the examination prior to their death, but the number of fatalities was low [three of 58 (5%) patients during 14 years].

We have previously suggested a causal connection between autonomic neuropathy and diabetic nephropathy [3,4], confirmed by other investigators [5,6]. In diabetic individuals with autonomic neuropathy, there is loss of the normal nocturnal decrease in blood pressure [22–24], suggesting that intraglomerular pressure is increased at night in autonomic neuropathy. This and the fall in intraglomerular pressure during the day due to postural falls in blood pressure may be a consequence of autonomic nephropathy [4]. The current study, supports the hypothesis that autonomic neuropathy precedes development of albuminuria. Disturbed parasympathetic nerve function (low E/I ratios) at baseline, not supine blood pressures, HbA1c values, or albumin excretion, was associated with increased albumin excretion 14 years later. As parasympathetic neuropathy (disturbed E/I ratio) was predominant in our patients with albuminuria, it is possible that parasympathetic nerve damage disturbs the balance between the parasympathetic and sympathetic nervous system with a nocturnal predominance of sympathetic nerve activity explaining the loss of normal nocturnal drop in blood pressure [25]. This increases the intraglomerular pressure, causing an increased leakage of albumin [26,27]. Indeed, it was recently shown that autonomic neuropathy precedes microalbuminuria in Type 1 diabetic patients [28]. Autonomic neuropathy is also associated with albuminuria in subjects with impaired glucose tolerance [29] and non-diabetic relatives of Type 2 diabetic patients [30]. The recent observation that microalbuminuria is reversible in half of Type 1 diabetic patients [31] suggests that autonomic neuropathy may be a permissive factor for the development of constant and persistent microalbuminuria.

Because of intensified anti-hypertensive treatment during recent years, the prognosis of nephropathy has improved. The decline in GFR is nowadays not steeply progressive but is more variable and albuminuria is not the only progression promoter [32]. As in other work [3–6], our study suggests that autonomic neuropathy may be one such factor. As we have observed previously [3,4], a low brake index at baseline was associated with higher than expected decreases in GFR between the third and fourth examination, as was an abnormal postural diastolic blood pressure fall at baseline. This underlines the idea of sympathetic denervation as another promoter of nephropathy progression [4].

In conclusion, autonomic neuropathy is a frequent complication in Type 1 diabetic patients. Autonomic dysfunction is a progressive disorder associated with future albuminuria and deterioration in GFR.

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