THE PREVALENCE AND RISK FACTORS OF AUTONOMIC AND SENSORY NERVE DYSFUNCTION IN PATIENTS WITH NEWLY DIAGNOSED TYPE 1 DIABETES MELLITUS AND PRIMARY BILIARY CIRRHOSIS

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SUMMARY

Autonomic and sensory nerve dysfunction is a serious complication of both diabetes mellitus and chronic liver diseases. This is the first study that evaluates the frequency and predisposing factors of autonomic and peripheral sensory neuropathy in patients with newly diagnosed Type-1 diabetes mellitus and with primary biliary cirrhosis (PBC).

Our data confirm that autonomic and sensory nerve dysfunctions are frequent complications in newly diagnosed Type-1 diabetes mellitus and seem to be closely related to each other. Autonomic and sensory neuropathy may be significant even when the disease is diagnosed, involving both the parasympathetic and the sympathetic systems, as well as small and large fibres. Traditional cardiovascular risk factors (smoking, hypertension and serum cholesterol) should be considered as potential risk factors for the development of neuropathy, even in newly diagnosed Type-1 diabetic patients. These observations may confirm the role of vascular factors in the pathogenesis of neuropathy. There is a relationship between autonomic nerve dysfunction and QT-interval prolongation even in newly diagnosed Type-1 diabetes, which justifies that the standard autonomic reflex-tests should be performed on patients with a prolonged QT-interval.

Autonomic and sensory nerve dysfunction are frequent complications in patients with PBC and seem to be mutually related. As a novel finding a significant reduction of both time domain and frequency domain parameters of heart rate variability (HRV) were proven in PBC. The HRV analysis was found to be more sensitive for detecting autonomic impairment than standard reflex-tests and revealed the synchronic impairment of parasympathetic and sympathetic systems. Autonomic neuropathy is closely related to the severity and duration of liver disease as well as to the markers of hepatocellular dysfunction. We provided the first evidence that hyperesthesia is a characteristic feature of sensory neuropathy, and might contribute to the itching, a typical symptom of PBC. The sympathovagal imbalance is significantly related to the reduction of casual, and 24-hour mean blood pressure, as well as to lower blood pressure variability. These observations indicate that autonomic neuropathy may play a role in the development of systemic vasodilatation characteristic of hyperdynamic circulation.
INTRODUCTION

In the majority of cases, neuropathy is not a separate clinical entity, but a component or complication of other diseases. Therefore it is evident that neuropathy is a par excellence interdisciplinary subject, comprising related fields from neurology and internal medicine in particular, as well as from surgery, vascular surgery, rheumatology, orthopedics, pediatrics, urology and rehabilitation. Practical clinical issues connected with the above related fields of neuropathy made me decide to examine the clinical and possible common features of neuropathy in diabetic and chronic liver disease patients.

Autonomic and sensory neuropathy are considered as essentially progressive forms of the disease, whose clinical and prognostic importance has been recognised mainly in the last 5 to 10 years. Follow-up studies have proven that the survival patterns of patients with autonomic neuropathy are similar in diabetes mellitus and in chronic liver diseases, as the presence of autonomic neuropathy carries a 5-fold risk of mortality irrespective of the etiology. The poor prognosis of peripheral sensory neuropathy was confirmed in a recent 6-year long prospective study where peripheral nerve dysfunction was the best predictor of new foot ulcer and mortality. Considering the poor prognosis, we designed the overall examination of autonomic and sensory nerve function in newly diagnosed Type-1 diabetes mellitus and in primary biliary cirrhosis, as related examinations had not been done before.

Despite considerable research recently, the exact cause of increased mortality associated with autonomic neuropathy has not been identified. Several mechanisms have been considered including resting tachycardia, the increased incidence of silent myocardial ischaemia and infarction, cardiorespiratory arrests during or right after anesthesia and cardiac dysfunction associated with autonomic neuropathy even in the absence of ischaemic heart disease. The imbalance of sympathetic-parasympathetic activity plays a pivotal role in the flattening of the nocturnal reduction of heart rate and blood pressure, leading to an increased left ventricular mass, and has also been shown to increase mortality. Recently, attention has been focused on the investigation of heart rate variability (HRV), as its attenuation due to autonomic imbalance has a major prognostic importance. The poor prognosis can be related to QT-interval prolongation associated with autonomic neuropathy leading to an increased risk of major arrhythmias and sudden cardiac death. Considering the prognostic significance of QT-interval prolongation, we found clarifying the relation between autonomic neuropathy and QT-interval important even in newly diagnosed Type 1 diabetic patients.

Nowadays, it has been proven that autonomic and sensory neuropathy is associated with cardiovascular risk factors. In the EURODIAB IDDM Complications Study significant correlations were observed between the prevalence of neuropathy and age, smoking, the presence of hypertension, lower HDL-cholesterol, and higher serum triglyceride levels. It is evident from what has been said so far that surveying and controlling the risk factors of autonomic and sensory nerve dysfunction may be
one of the most important means of preventing complications and improving prognosis. Based on these findings I examined the risk factors of autonomic and sensory neuropathy in cases of newly diagnosed Type-1 diabetes and primary biliary cirrhosis (PBC).

Autonomic and peripheral sensory neuropathy are frequent complications of chronic liver diseases. Our study group was the first to demonstrate that in chronic alcoholics, the severity of neuropathy correlates with that of alcoholic liver disease. Moreover, as neuropathy had been found also in non-alcoholic liver disease, chronic liver damage was considered an independent etiologic factor in the pathogenesis of neuropathy. Our previous data provided evidence of a relation between the presence and severity of autonomic neuropathy and QT-interval prolongation in chronic alcoholic and non-alcoholic liver diseases. Prolongation of the QT-interval is also involved in the poor prognosis of autonomic neuropathy accompanying chronic liver disease. Recently, some data suggest that autonomic neuropathy may play a role in the development of hyperdynamic circulation and portal hypertension in chronic liver diseases. Reduced HRV has been found not only in cardiovascular diseases but in chronic liver diseases too. As in PBC the complete examination of the autonomic function, including traditional reflex tests and HRV analysis had not been done before, one of my aims has been the complete evaluation of the autonomic function in PBC. In order to further clarify the supposed connection between the systemic hypotension characterising hyperdynamic circulation and the autonomic function we compared the parameters of the autonomic function to blood pressure values found during 24-hour monitoring.
AIMS OF THE STUDY

Based on the aforementioned, the objectives of the study were:

1. To assess the frequency and severity of autonomic neuropathy in newly diagnosed Type-1 diabetes mellitus.
2. To evaluate the frequency and to determine the characteristics of sensory neuropathy in newly diagnosed Type-1 diabetes mellitus.
3. To examine the possible relationship between autonomic and sensory nerve dysfunction in patients with newly diagnosed Type-1 diabetes mellitus.
4. To determine the potential risk factors of autonomic and sensory nerve dysfunction in patients with newly diagnosed Type-1 diabetes mellitus.
5. To establish whether there is a relationship between autonomic neuropathy and QT-interval prolongation in newly diagnosed Type-1 diabetes mellitus.
6. To assess the frequency of autonomic neuropathy and to determine whether patients with primary biliary cirrhosis have decreased heart rate variability.
7. To observe the frequency and characteristics of sensory neuropathy in patients with primary biliary cirrhosis.
8. To reveal whether the autonomic function and the clinical and biochemical characteristics of primary biliary cirrhosis are connected.
9. To identify whether there is a relation between sensory nerve function and clinical and biochemical features of primary biliary cirrhosis.
10. To investigate the connection between autonomic and sensory nerve function in primary biliary cirrhosis.
11. To evaluate the relationship between autonomic function and 24-hour blood pressure profile in patients with primary biliary cirrhosis.
SUBJECTS AND METHODS

Forty patients with newly diagnosed Type-1 diabetes mellitus with a mean age of 34.7 years and 25 healthy control subjects (mean age: 38.3 years) were included in the study for evaluating the risk factors of neuropathy in newly diagnosed Type 1 diabetes. Type 1 diabetes was defined by classical clinical symptoms and by blood glucose values according to 1999 WHO criteria. The relationship between autonomic function and QT-interval prolongation was analysed in 27 patients with newly diagnosed Type-1 diabetes (mean age: 23 years). None was taking any medication, apart from insulin.

Twenty-four female patients with PBC (mean age: 60.4 years) and twenty age-matched healthy female controls (mean age: 59.3 years) were recruited for the study evaluating the risk factors of PBC. The diagnosis of PBC was based on characteristic clinical and laboratory data, AMA M2 positivity and liver biopsy. The severity of liver disease was assessed by the histologic stage classification. None was taking any drugs apart from ursodeoxycholic acid, vitamin D and calcium supplementation, and none had ascites. Every participant was asked to refrain from consuming caffeine and alcoholic beverages, and tobacco products 12 hrs before autonomic testing.

The autonomic function was explored by the five standard cardiovascular reflex-tests. All reflex-tests were performed by using a computerized ECG-recording-analysing system developed by Innomed Inc, Hungary. Heart rate variations during deep breathing, standing and Valsalva manoeuvre mainly reflect parasympathetic function, while blood pressure responses to sustained handgrip and standing primarily allow the assessment of sympathetic integrity. The results of each of the five tests were scored as 0 (normal), 1 (borderline) or 2 (abnormal), and a final autonomic score was calculated (range 0-10) to express the severity of the overall autonomic disorder. Patients with at least one abnormal or two borderline cardiovascular tests were considered to have autonomic neuropathy. The corrected QT (QTc) interval was determined by Bazett’s formula.

Evaluating 24-hour heart rate variability and ambulatory 24-hour-long blood pressure monitoring were done by CardioTens-01 combined ABPM and ECG monitoring system (Meditech, Budapest, Hungary). Analysis of stored data was done by Medibase software. The device was programmed to measure blood pressure at 20 minute intervals throughout daytime and at 30 minute intervals during night-time.

To characterize 24-hour heart rate variability, both time domain and frequency domain methods were used. A simple geometric time domain parameter, HRV triangular index (HRVTI) was also computed, characterising the overall variability. HRVTI is the integral of the density distribution (the number of all NN intervals divided by the maximum of the density distribution). In the frequency domain analysis (power spectral density analysis), total power (TP), low- (0.04-0.15 Hz) and high- (0.15-0.4 Hz) frequency bands of the power spectrum (power distribution as function of frequency) were analysed.
Peripheral sensory function was characterized by the evaluation of the CPT (current perception threshold) with the Neurometer(r) diagnostic stimulator (Neurotron, Baltimore, MD), which permits transcutaneous testing at three sinusoidal frequencies (2000 Hz, 250 Hz and 5 Hz). Median and peroneal nerves (digital branches) were studied. As demonstrated by the results of comparative trials conducted earlier, CPT values measured during high frequency stimulation correlate best with tests of large fibre function and low frequency CPT values correlate with tests of small fibre function.

Statistical analysis was performed using Statistica Software. Data are expressed as mean±SD and were compared between groups by Student’s t-test. In cases of categorical variables the differences were tested by χ² test. Within the patient groups, relationships were also assessed by analysis of regression and calculation of the correlation coefficient. Evaluating the association between neuropathy and potential risk factors, correlations between variables were also analysed by partial correlation coefficient calculation adjusted for age. A P-value of <0.05 was regarded as statistically significant.

RESULTS

1. The frequency and severity of autonomic neuropathy in newly diagnosed Type-1 diabetes mellitus

Autonomic neuropathy was present in 12 (30%) newly diagnosed Type-1 diabetic patients. Among these patients 9 had mild and 3 had moderate autonomic impairment. Parasympathetic neuropathy was found in six patients, sympathetic nerve dysfunction was observed in three patients, and three subjects had both parasympathetic and sympathetic damage. A significant decrease of heart rate variation after standing was found in diabetic patients compared to control subjects (P=0.003).

2. The frequency and characteristics of sensory neuropathy in newly diagnosed Type-1 diabetes mellitus

Sensory neuropathy was observed in 10 patients (25%) with newly diagnosed Type-1 diabetes mellitus. All these patients had hypesthetic type sensory nerve dysfunction, in 6 cases mild, in the other 4 cases moderate somatic nerve impairment was proven. Higher CPT values indicating hypesthesia were found in the diabetic group compared with control subjects at peroneal nerve testing at 250 Hz (P=0.03) and 5 Hz (P=0.007), just as at median nerve testing at 5 Hz (P=0.048).

3. Relationship between autonomic and sensory nerve dysfunction in patients with newly diagnosed Type-1 diabetes mellitus

There was a significant negative correlation between the deep breathing test and the CPT values testing the median nerve at 2000 Hz (P=0.005) as well as at 250 Hz (P=0.01). The response of systolic blood pressure to standing correlated negatively with CPT values at the peroneal nerve
at 2000 Hz (P=0.032), 250 Hz (P=0.014), and 5 Hz (P=0.042). The severity of autonomic nerve dysfunction was closely related to those of sensory neuropathy (P=0.01).

4. **Risk factors of autonomic and sensory nerve dysfunction in patients with newly diagnosed Type-1 diabetes mellitus**

A significant negative correlation was found between the duration of smoking and the deep breathing test (P=0.006). The duration of smoking was associated with the severity of both autonomic (P=0.006) and parasympathetic (P=0.002) neuropathy. Serum cholesterol was positively related to the severity of parasympathetic damage (P=0.047). The diastolic blood pressure values correlated negatively with the heart rate changes after standing (P=0.044). There was a positive relationship between systolic blood pressure and the CPT values stimulating median nerve at 5 Hz (P=0.012).

5. **Relationship between autonomic neuropathy and QT-interval prolongation in newly diagnosed Type-1 diabetes mellitus**

In this study we examined 27 patients with newly diagnosed Type-1 diabetes, of whom autonomic neuropathy was proven in 7 (26%) patients. Prolonged QTc interval (>440 ms) was found in three patients among those 7 with autonomic neuropathy and none of those 20 patients without autonomic neuropathy (P<0.01). The QTc interval was longer in patients with autonomic neuropathy than in patients with normal reflex-test results (P<0.001). Analyzing the relationship between QTc-interval and the five reflex parameters separately, there was a significant negative correlation between QTc lengthening and the sustained handgrip test (P<0.05). This is in accordance with data suggesting that sympathetic dysfunction has a role in the development of QTc prolongation.

6. **The autonomic function in primary biliary cirrhosis**

Using standard cardiovascular reflex-tests, 14 patients with PBC (58%) had autonomic neuropathy. Among these patients parasympathetic neuropathy was found in 8 patients, sympathetic nerve dysfunction was observed in 2 patients, and 4 subjects had both parasympathetic and sympathetic damage. The heart rate response to deep breathing (P=0.001), as well as to standing (P=0.03) and Valsalva manoeuvre (P=0.01) was significantly lower in PBC patients than in age-matched control subjects. A more profound decrease of systolic blood pressure after standing (P=0.03) was found in patients with PBC compared to healthy controls. Each patient had at least one abnormal parameter of HRV. As a novel finding we proved that both time domain and frequency domain parameters of HRV were significantly reduced in PBC patients compared to controls. PBC patients showed significantly lower HRV triangular index (P=0.004), total power (P=0.0001), power of low frequency band (P=0.0007) and of high frequency band (P=0.004) than healthy control subjects.

7. **The sensory function in primary biliary cirrhosis**
At least one abnormal sensory parameter was detected in 13 patients (54%), of whom 12 had hyperesthetic type, and only one had hypesthetic type sensory nerve dysfunction. Among patients with sensory neuropathy the lower extremities were affected in all 13 patients, while 3 patients had abnormal CPT values at upper extremities testing. Lower CPT values - indicating hyperesthesia - were found in PBC patients compared with age-matched controls at peroneal nerve testing at all three frequencies (all P(0.01) as well as at median nerve testing at 250 Hz (P=0.03). Patients with hyperesthesia stimulating median and peroneal nerve at 5 Hz had itching.

8. **Associations of autonomic function with the clinical and biochemical characteristics of PBC**

The longer duration of PBC was associated with less prominent increase of diastolic blood pressure during sustained handgrip test (P=0.01). Duration of the disease also correlated with reduced time domain HRV parameters. The severity of PBC (stage) was found to negatively correlate with HRVTI (P=0.01). Partial correlation analysis revealed that lower prothrombin activity was associated with lower heart rate response to standing (P=0.006) as well as to deep breathing (P=0.04). The serum albumin was positively related to HRVTI (P=0.02). Serum AST and ALT levels negatively correlated with HRV (both P=0.01). These relationships remained significant after age adjustment.

9. **Relations of sensory nerve function to clinical and biochemical features of PBC**

Negative correlations of serum ALT with CPT values at median nerve testing at 250 Hz (P=0.005) as well as with CPT at peroneal (P=0.03) and median nerve (P=0.02) at 5 Hz were revealed. AST and ALP levels were inversely related to CPT values at peroneal nerve testing at all three frequencies, as well as to those at median nerve testing at 250 Hz and 5 Hz. An inverse relationship was also found between serum bilirubin levels and CPT values at median nerve testing at 5 Hz (P=0.04). None of these relationships were altered by adjustment for age. Interestingly, no correlation was found between peripheral sensory nerve function and the duration and severity of PBC.

10. **Connections between autonomic and sensory nerve function in PBC**

Reduced total power was associated with lower CPT values testing median nerve at 250 Hz (P=0.0001), and at 5 Hz (P=0.002), as well as with those testing peroneal nerve at 2000 Hz (P=0.01). HF-power was positively related to CPT values at peroneal nerve testing at 2000 Hz (P=0.01); LF-power correlated positively with CPT values testing median nerve at 250 (P=0.01), as well as at 5 Hz (P=0.03). A significant positive correlation was also observed between the time domain parameters of HRV and the CPT values testing both median and peroneal nerve at all three frequencies, even after adjustment for age.

11. **Relationships between autonomic function and 24-hour blood pressure profile in PBC**
Although casual blood pressure values of PBC patients were similar to those of healthy controls, in patients with PBC significantly lower 24-hour mean systolic and diastolic blood pressure values (both $P=0.01$) were found than in control subjects. Decreased heart-rate variability was associated with lower casual blood pressure values in PBC patients. Patients with lower HRV triangular index had lower 24-hour mean systolic ($P=0.002$) and diastolic ($P=0.036$) blood pressure values. The severity both of autonomic and parasympathetic dysfunction was inversely related to 24-hour mean systolic and diastolic blood pressure values. Time domain parameters of HRV positively correlated with 24-hour blood pressure variability, characterised by 24-hour systolic and diastolic blood pressure standard deviations. Attenuation of 24-hour blood pressure variability was closely related to diminished nocturnal blood pressure changes.
CONCLUSIONS

1. Autonomic and sensory nerve dysfunction are quite frequent complications in newly diagnosed Type-1 diabetic patients and seem to be closely related to each other.
2. Autonomic nerve dysfunction may be significant even in newly diagnosed Type-1 diabetic patients, involving both the parasympathetic and the sympathetic systems.
3. Traditional cardiovascular reflex-tests are suitable for evaluating the autonomic function even in newly diagnosed Type-1 diabetic patients.
4. Significant sensory impairment, involving both large and small fibres, may already have developed in newly diagnosed Type-1 diabetic patients.
5. Our data suggest that among traditional cardiovascular risk factors smoking, hypertension and increased serum cholesterol should be considered as potential risk factors for the development of neuropathy as well, even in newly diagnosed Type-1 diabetic patients. These observations may confirm the role of vascular factors in the pathogenesis of neuropathy.
6. There is a close relationship between autonomic nerve dysfunction and QT-interval prolongation even in newly diagnosed Type-1 diabetes.
7. The analysis of the QT-interval may be a useful additional method for the screening of autonomic neuropathy even in newly diagnosed Type-1 diabetic patients, and in patients with a prolonged QT-interval cardiovascular reflex-tests are worth performing.
8. Our results suggest that a treatment strategy aiming at reducing all the risk factors may even prevent the development of neuropathy.
9. Autonomic and sensory nerve dysfunction are frequent complications in patients with PBC and seem to be mutually related.
10. HRV analysis is more sensitive than standard cardiovascular reflex-tests for detecting parasympathetic and sympathetic neuropathy even in PBC.
11. Both time domain and frequency domain parameters of HRV were significantly reduced in PBC, according to the parallel parasympathetic and sympathetic damage.
12. Our data provide the first evidence that hyperesthesia is characteristic of sensory neuropathy in PBC, which might partly be responsible for itching, a typical symptom in PBC.
13. Autonomic neuropathy is related to the severity and duration of liver disease as well as to the markers of hepatocellular dysfunction. Sensory neuropathy was associated with the markers of hepatocellular damage and cholestasis indicators, independent of the severity and duration of the disease.
14. Impaired parasympathetic and sympathetic integrity, leading to autonomic imbalance, seems to be associated with lower 24-hour mean blood pressure values just as with decreased blood pressure variability in PBC.

15. Our data support that autonomic dysfunction may be responsible for systemic hypotension, a typical symptom of hyperdinamic circulation.