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• BRIEF REPORTS •

Autonomic and sensory nerve dysfunction in primary biliary cirrhosis

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Abstract

AIM: Cardiovascular autonomic and peripheral sensory neuropathy is a known complication of chronic alcoholic and non-alcoholic liver diseases. We aimed to assess the prevalence and risk factors for peripheral sensory nerve and autonomic dysfunction using sensitive methods in patients with primary biliary cirrhosis (PBC).

METHODS: Twenty-four AMA M2 positive female patients with clinical, biochemical and histological evidence of PBC and 20 age matched healthy female subjects were studied. Five standard cardiovascular reflex tests and 24-h heart rate variability (HRV) analysis were performed to define autonomic function. Peripheral sensory nerve function on median and peroneal nerves was characterized by current perception threshold (CPT), measured by a neuroselective diagnostic stimulator (Neurotron, Baltimore, MD).

RESULTS: Fourteen of 24 patients (58%) had at least one abnormal cardiovascular reflex test and thirteen (54%) had peripheral sensory neuropathy. Lower heart rate response to deep breathing ($P = 0.001$), standing ($P = 0.03$) and Valsalva manoeuvre ($P = 0.01$), and more profound decrease of blood pressure after standing ($P = 0.03$) was found in PBC patients than in controls. As a novel finding we proved that both time domain and frequency domain parameters of 24-h HRV were significantly reduced in PBC patients compared to controls. Each patient had at least one abnormal parameter of HRV. Lower CPT values indicated hyperaesthesia as a characteristic feature at peroneal nerve testing at three frequencies (2000 Hz: $P = 0.005$; 250 Hz: $P = 0.002$; 5 Hz: $P = 0.004$) in PBC compared to controls. Correlation of autonomic dysfunction with the severity and duration of the disease was observed. Lower total power of HRV correlated with lower CPT values at median nerve testing at 250 Hz ($P = 0.0001$) and at 5 Hz ($P = 0.002$), as well as with those at peroneal nerve testing at 2000 Hz ($P = 0.01$).

CONCLUSION: Autonomic and sensory nerve dysfunctions are frequent in PBC. Twenty-four-hour HRV analysis is more sensitive than standard cardiovascular tests for detecting of both parasympathetic and sympathetic impairments. Our novel data suggest that hyperaesthesia is a characteristic feature of peripheral sensory neuropathy and might contribute to itching in PBC. Autonomic dysfunction is related to the duration and severity of PBC.

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INTRODUCTION

Autonomic neuropathy (AN) is frequent complication of both alcoholic and non-alcoholic chronic liver diseases[1]. Cardiovascular AN represents a serious complication as it carries a 5-fold risk of mortality in patients with chronic liver diseases[2]. In a 10-mo long follow-up study in patients awaiting for liver transplantation the mortality was significantly higher in patients with AN (27%) compared to those without AN (0%), suggesting that AN should be taken into consideration for early liver transplantation in patients with advanced liver disease[3]. Up to now the precise explanation of increased mortality associated with AN has not been identified. Beside the most severe complications of AN-silent myocardial ischaemia and infarction, cardiorespiratory arrest, major arrhythmias[4] -the attenuation of circadian variation of blood pressure and heart rate may contribute to the higher death rate[5,6]. Prolongation of the QT-interval is also involved in the poor prognosis of AN accompanying chronic liver disease [7,8]. Autonomic neuropathy may also be regarded as a potential etiologic factor of hyperdynamic circulation and portal hypertension[9].

Recently, attention has been focused on the importance of 24-h heart rate variability (HRV). It has been confirmed that HRV is a strong and independent predictor of mortality after an acute myocardial infarction[10]. Time and frequency domain analysis of HRV proved to be a reliable, noninvasive tool to provide quantitative information on cardiovascular autonomic function differentiated into vagal and sympathetic components[11]. Additionally, assessment of HRV is a sensitive method for early detection of autonomic neuropathy even if the standard cardiovascular reflex tests are normal[12]. Depressed HRV has been described not only in cardiovascular disorders, but also in chronic liver diseases[5,13-15]. Although autonomic and sensory neuropathy is known as a common extrahepatic manifestation in chronic liver diseases[1,3,7,13], there are only few data on risk factors of neuropathy in PBC[16,17].

The aim of our study was to assess the frequency and predisposing factors of autonomic and peripheral sensory neuropathy in PBC.

MATERIALS AND METHODS

Patients

Twenty-four female patients with PBC (mean age: 60.4±7.1 years; range: 45-73 years) from the Hepatological Outpatient Unit of Semmelweis University, Budapest and 20 age-matched healthy female controls (mean age: 59.3±6.8 years; range: 44-72 years) were recruited for this cross sectional study. 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 histologic classification. Stage I: 2, stage II: 5, stage III: 12 and stage IV: 5 patients. Full medical history was taken, followed by thorough physical and neurologic

examination in each patient and control. Patients were only included if they were normotensive, ie. no history of hypertension, and at the time of inclusion visiting office blood pressure <140/90 mmHg calculated by the mean of three measurements using Korotkov's technique and no evidence of disease known to affect autonomic function (e.g. other hepatic disease, cardiovascular, kidney, endocrinologic, neurologic and psychiatric disorders including alcoholism). None was taking any antihypertensive drugs or other medications, apart from ursodeoxycholic acid, vitamin D and calcium supplementation[18], and none had ascites. The healthy controls were recruited from the staff of our institution and their family members. Every participant was asked to refrain from consuming caffeine and alcoholic beverages, and tobacco products 12 h before autonomic testing.

Methods

The autonomic function was explored by the *five standard cardiovascular reflex tests*[19]. Heart rate tests (heart rate responses to deep breathing, the 30/15 ratio and the Valsalva ratio) mainly reflect parasympathetic function while blood pressure responses to sustained handgrip and standing primarily allow the assessment of sympathetic integrity. Patients with at least one abnormal or two borderline cardiovascular tests were considered to have autonomic neuropathy. The same research assistant using a computerized ECG-recording-analyzing system developed by Innomed Inc, Budapest, Hungary, performed all reflex tests.

Two channel 24-h ECG recordings were done by CardioTens equipment (Meditech, Budapest, Hungary). This device complies with the requirements of the British Hypertension Society and the Association for the Advancement of Medical Instrumentation protocols. Automatic filters were used to continuously restore baseline and filter background and muscle noise. Analysis of stored data was done by Medibase software. The recording was also edited using visual control and manual corrections were made to omit ectopic beats, arrhythmic events and noise effects and only normal-to-normal beats (NN intervals) were used for further analysis. Ratio of normal beats to total number of beats was >95% in both groups.

To characterize 24-h *HRV*, time domain and frequency domain methods were used. Since there were several parameters cited in the literature to assess HRV, we selected a limited number of parameters according to the recommendations of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology[11]. Statistical time domain parameters could be calculated from either the direct measurements of NN intervals or from the differences between NN intervals. The following parameters were calculated from direct measurements of NN intervals: standard deviation of all NN (SDNN) intervals reflecting all the cyclic components responsible for variability and standard deviation of the averages of NN (SDANN) intervals in all 5-min segments of the entire recording, an estimate of the changes in heart rate due to cycles longer than 5 min.

Statistical time domain parameters deriving from NN interval differences are RMSSD and pNN50. RMSSD (the square root of the mean of the sum of the squares of differences between adjacent NN intervals) is an estimate of short-term components of HRV. PNN50 (the proportion of adjacent NN intervals differ by more than 50 ms) was considered to reflect the vagal tone of the heart.

A simple geometric time domain parameter, HRV triangular index (HRVTI) was also computed. HRVTI is the integral of the density distribution (the number of all NN intervals) divided by the maximum of the density distribution. HRVTI represents overall HRV.

In the frequency domain analysis (power spectral density analysis) of heart period oscillations low- (0.04-0.15 Hz) and high- (0.15-0.4 Hz) frequency bands of the power (i.e. variance) spectrum (power distribution as function of frequency) was performed. The following frequency domain measures were computed: TP (total power: variance of all NN intervals), LF (power in the low frequency range) which is under both sympathetic and parasympathetic influences, HF (power in the high frequency range) which is an acknowledged measure of the parasympathetic (i.e. vagal) modulations.

Peripheral sensory function was characterized by the evaluation of the current perception threshold (CPT) with a neuroselective diagnostic stimulator (Neurotron, Baltimore, MD, USA), which permits transcutaneous testing at three sinusoidal frequencies (2000 Hz, 250 Hz and 5 Hz). The intensity of the stimulating current was changed within the range from 0.01 to 9.99 mA. The neurometer is the first instrument designed for the overall assessment of all types of sensory fibres. As demonstrated by the results of comparative trials conducted earlier[20], CPT values measured during high frequency stimulation correlated best with tests of large fibre function and low frequency CPT values correlated with tests of small fibre function. Median and peroneal nerves (digital branches) were studied.

The Local Regional Committee of Science and Research Ethics approved the study. Written informed consent was obtained.

Statistical analysis

All analyses were performed using Statistica Software. Data are expressed as mean±SD and were compared between groups by Student's *t*-test. Correlations between variables were analysed by partial correlation coefficient calculation adjusted for age. $P<0.05$ was regarded as statistically significant.

RESULTS

Autonomic function

Using standard cardiovascular tests[18], 14 PBC patients (58%) had at least one abnormal autonomic function test. Among these patients parasympathetic neuropathy was found in 8 (57.1%) patients, sympathetic nerve dysfunction was observed in 2 patients, and 4 subjects had both parasympathetic and sympathetic damage.

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. Each patient had at least one abnormal parameter of HRV.

Results of cardiovascular reflex tests and HRV parameters are presented in Table 1. 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 compared to healthy controls. By HRV analysis most of the time-domain indices were significantly lower in patients than in controls (PNN50: $P = 0.0008$; HRVTI: $P = 0.004$; RMSSD: $P = 0.006$ and SDNN: $P = 0.015$). PBC patients also showed significantly lower total power ($P = 0.0001$), power of LF band ($P = 0.00007$) and of HF band ($P = 0.004$).

Peripheral sensory nerve function

At least one abnormal sensory parameter was detected in 13 patients (54%), of whom 12 had hyperaesthetic type, and only one had hypoaesthetic 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 hyperaesthesia, were found in PBC patients compared with age matched controls at peroneal nerve testing at all three frequencies ($P<0.01$) as well as at median nerve testing at 250 Hz ($P = 0.03$). The CPT values of patients and controls are shown in Table 2.

Table 1 Results of cardiovascular reflex tests and 24-h heart rate variability (HRV) parameters in patients with PBC and age matched healthy controls

	Patients with PBC (n = 24)	Age-matched controls (n = 20)	P value
Cardiovascular reflex tests			
Deep breathing test (beats / min)	11.3 (4.4)	17.5 (6.6)	0.001
30 / 15 ratio	1.18 (0.1)	1.29 (0.2)	0.03
Valsalva ratio	1.32 (0.1)	1.48 (0.2)	0.01
Orthostatic test (mmHg)	-7.1 (8.6)	-1.5 (3.7)	0.03
Handgrip test (mmHg)	20.4 (8.1)	22.3 (4.4)	NS
Time domain parameters of HRV			
SDNN (ms)	119 (42)	151 (37)	0.015
SDANN (ms)	142 (102)	165 (66)	NS
RMSSD (ms)	23 (10)	38 (22)	0.006
PNN50 (%)	2.4 (4)	11 (10)	0.0008
HRVTI	28 (8)	38 (13)	0.004
Frequency domain parameters of HRV			
TP (ms^2)	1506 (701)	4032 (2787)	0.0001
LF (ms^2)	299 (176)	1213 (977)	0.00007
HF (ms^2)	150 (148)	525 (565)	0.004

Table 2 Current perception threshold (CPT) values in PBC patients and controls at median and peroneal nerve testing at three different frequencies

	Patients with PBC (n = 24)	Age-matched controls (n = 20)	P value
CPT (mA)-Median nerve			
2000 Hz	2.45 (0.75)	2.94 (0.87)	0.076
250 Hz	0.81 (0.33)	1.10 (0.46)	0.030
5 Hz	0.42 (0.23)	0.50 (0.17)	0.242
CPT (mA)-Peroneal nerve			
2000 Hz	2.91 (0.71)	3.61 (0.66)	0.005
250 Hz	1.01 (0.39)	1.40 (0.26)	0.002
5 Hz	0.68 (0.40)	1.06 (0.28)	0.004

Associations of autonomic function with clinical and biochemical characteristics

After adjustment for age, the longer duration of the disease was associated with less prominent increase of diastolic blood pressure during sustained handgrip test (r = -0.52, P = 0.01). Duration of the disease also correlated with reduced SDNN and SDANN (r = 0.47 and r = -0.45, P<0.05, for both). The severity of PBC (stage) was found to negatively correlate with lower HRVTI (r = -0.6, P = 0.01) and lower SDANN (r = -0.49, P = 0.04) as well.

Partial correlation analysis revealed that lower prothrombin activity was associated with lower heart rate response to standing (r = 0.79, P = 0.006) as well as to deep breathing (r = 0.63, P = 0.04). The serum albumin positively correlated with SDNN and HRVTI (r = 0.47 and 0.57, P<0.05, for both). Serum AST and ALT levels negatively correlated with SDNN (r = -0.54, P = 0.01, for both). Positive correlations of SDNN (r = 0.62, P = 0.004) and Valsalva ratio (r = 0.51, P = 0.02) with serum trigliceride levels were found. These relationships remained significant after age adjustment.

Correlations of peripheral sensory nerve function with clinical and chemical characteristics

Negative correlations of serum ALT with CPT values at median nerve testing at 250 HZ (r = -0.56, P = 0.005) as well as with CPT at peroneal (r = -0.48, P = 0.03) and median nerve (r = -0.45, 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 (r = -0.43, P = 0.04). None of these relationships was altered by adjustment for age.

Interestingly no correlation was found between peripheral sensory nerve function and duration and severity of PBC. Furthermore, no association was found between sensory nerve function and serum lipid levels, prothrombin activity and serum albumin levels.

Associations between autonomic and peripheral sensory nerve function

Reduced total power was associated with lower CPT values testing median nerve at 250 Hz (r = 0.69, P = 0.0001), and at 5 Hz (r = 0.62, P = 0.002), as well as with those testing peroneal nerve at 2000 Hz (r = 0.53, P = 0.01). HF-power was positively related to CPT values at peroneal nerve testing at 2000 Hz (r = 0.51, P = 0.01), LF-power correlated positively with CPT values testing median nerve at 250 (r = 0.53, P = 0.01), as well as at 5 Hz (r = 0.47, P = 0.03). A significant positive correlation was observed between the SDNN and the CPT values testing the median nerve at 2000 Hz (r = 0.60, P = 0.003), as well as at 250 Hz (r = 0.53, P = 0.01) and at 5 Hz (r = 0.50, P = 0.02). Lower SDANN values were associated with lower CPT values at the median nerve at 2000 Hz (r = 0.52, P = 0.01). The PNN50 values correlated positively with CPT values testing the peroneal nerve at 2000 Hz (r = 0.50, P = 0.02) and the beat-to-beat variation was also positively related to CPT values at the peroneal nerve at 250 Hz (r = 0.47, P = 0.02), even after adjustment for age.

DISCUSSION

Somatic neuropathy accompanying advanced stage primary biliary cirrhosis, was described as early as 1964 by Walker and Thomas[21]. In the last two decades, autonomic neuropathy has been found as a common complication of this type of liver disease[1,16,17,22]. The poor prognosis of neuropathy has been widely known even in chronic liver diseases, primarily as regards the impairment of autonomic functions[2,3]. During the 4-year follow-up study of Hendrickse *et al*., mortality was 30% among patients with autonomic neuropathy and 6% in those without AN[2]. As suggested by the description of clinical features, the prognosis of sensory neuropathy was rather poor. In their 14-year follow-up study conducted on diabetic patients, Coppini *et al.* showed that sensory neuropathy was an independent predictor for mortality[23].

There are only few studies on the prevalence of autonomic and sensory neuropathy in PBC, and the characteristics of the study population have a strong impact on prevalence data. Nevertheless, our data are consistent with previous findings showing that autonomic and sensory neuropathy were frequent complications in patients with PBC[1,16,17].

Sensory neuropathy has also been found as a common complication in chronic liver diseases, yet there are no data on its prognostic importance.

Although many studies have been published on autonomic and sensory neuropathy in chronic liver diseases, some of the results were conflicting. To our knowledge this is the first study in PBC conducted on the evaluation of autonomic function assessed both by the standard cardiovascular reflex tests and by 24-h HRV analysis. HRV analysis could indicate the synchronic impairment of the parasympathetic and sympathetic systems in PBC. To date only one systematic study has assessed the factors that predispose to autonomic and sensory nerve dysfunction in PBC[17], but in their study the autonomic function was only evaluated by the standard tests. We confirmed previous data showing that autonomic dysfunction was related to the severity of liver damage[1,14,15,24], contrary to Oliver[25] who did not show similar results. As a novel finding not only the severity, but also the duration of PBC was related to autonomic dysregulation. It would be worthy to investigate neuropathy in asymptomatic PBC population[26]. We have also found a close correlation between decreased serum albumin and prothrombin activity with the autonomic dysfunction, which differed from the data of Lazzeri *et al*.[5], but these results were consistent with two other studies[3,15]. Moreover, in our study the serum AST and ALT levels were inversely related to the autonomic function. The markers of cholestasis did not correlate with the autonomic function, in keeping with the data of Coelho[15]. An interesting finding in our study was that lower serum trigliceride level was associated with impaired autonomic function. This is of interest, since PBC related somatic neuropathy was originally attributed to lipid deposition[21]. Later, however, no significant association of hyperlipidaemia with autonomic and sensory neuropathy was demonstrated[17], which is consistent with our data regarding the serum cholesterol level.

Sensory impairment might involve both large myelinated fibres and small sensory fibres as demonstrated by Kempler[16]. These data are consistent with the present results showing abnormal CPT values at all types of sensory fibres, testing both median and peroneal nerves. Abnormal CPT values were more frequent on the lower extremities, which were in accordance with previous observations that longer fibres were damaged earlier[27]. The present data extended previous results by analysing which type of sensory nerve dysfunction was specific for PBC. Our data provide the first evidence that hyperesthaesia is a feature of peripheral sensory neuropathy in PBC. In this phase of neuropathy the degeneration and regeneration of non-myelinated small fibres occur concomitantly. These processes were inaccesible to earlier methods for sensory testing, but the neurometer could permit the detection of this early phase of sensory nerve impairment[28]. Evaluating the current perception threshold by neurometer seems a simple and comprehensive way of assessing even early abnormalities of peripheral sensory nerve function in patients with PBC. Recent studies have demonstrated that itching, a characteristic symptom in PBC, could be evoked by activation of peripheral unmyelinated C-fibers[29,30]. In our study, patients with hyperaesthaesia at 5 Hz had itching. Considering that 5 Hz CPT values demonstrate the unmyelinated C-fibre function, our results support the possible role for unmyelinated C-fibre damage in hyperaesthaesia in the pathogenesis of pruritus in PBC.

Serum bilirubin and albumin were found to be associated with peripheral nerve function in the only one study published on correlation of sensory nerve dysfunction in PBC[17]. Our results confirmed these data regarding serum bilirubin, but not serum albumin. Moreover, in our study not only elevated serum bilirubin, but higher serum AST, ALT and ALP were also related to lower CPT values.

Our results were consistent with those of Hendrickse[17], showing that peripheral sensory nerve function correlates with cardiovascular autonomic function in patients with PBC. Prospective studies are required to evaluate the prognostic importance of sensory neuropathy in PBC.

In summary, autonomic and sensory nerve dysfunctions are frequent complications in patients with PBC and seem to be mutually related. The novel findings of reduced time and frequency domain parameters of 24-h HRV analysis indicate the synchronic impairment of parasympathetic and sympathetic systems. HRV analysis is more sensitive than standard cardiovascular tests for detecting autonomic neuropathy. This study provides the first evidence that hyperaesthesia involving all types of fibres is characteristic for sensory neuropathy in PBC. Hyperaesthaesia of unmyelinated fibres might partly be responsible for itching, a characteristic symptom in PBC. Our data suggest that autonomic neuropathy is related to the severity and duration of liver disease as well as to the markers of hepatocellular dysfunction.

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