Autonomic nervous system function in patients with chronic hepatitis C virus infection

Ph.D. thesis

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Introduction

Hepatitis C virus (HCV), that was first isolated in 1989, is classified as the only member of the Hepacivirus genus, in Flaviviridae family. The 9.6 kb long single positive-stranded RNA genome encodes a single polyprotein of about 3,000 amino acids in length, which is processed by both host and viral proteases into structural (at least three) and nonstructural (six) proteins.

HCV is transmitted parenteral, thus, blood transfusion was a major risk factor for HCV transmission before the introduction of sensitive and specific anti-HCV testing in 1992. This route of transmission has been virtually eliminated in countries where screening of blood donors is implemented, e.g in Hungary. Transfusion-related hepatitis has almost disappeared in these countries, leaving injection drug use as the most common mode of transmission. Overall, the WHO estimated global prevalence of HCV infection was 3% or 170 million individuals in 2000, and it has been risen to 200 million individuals, according to estimation in 2008. The prevalence in Hungary is 1.5%, parallel to the European average.

Clinical significance of HCV infection is shown by the fact, that it persists in about 80% of infected persons over 6 months and became chronic, and 20% of patients with chronic HCV go on to develop cirrhosis. HCV is responsible for a significant proportion of primary liver cancer cases globally and is a leading cause of HCC in Western countries. Thus, HCC represents one of the major HCV complications associated with mortality. However, chronic HCV infection may remain asymptomatic for many years. Patients may present with the physical signs are associated with either decompensated cirrhosis, or HCC, or the extrahepatic manifestations of the disease.

According to the numerous studies and case reports, 40–74% of patients may develop at least one extrahepatic manifestation during the course of the disease. At least 36 extrahepatic disease manifestations, mainly autoimmune disorders, have been reported to be associated with HCV infection, including

cryoglobulinemia, HCV-associated glomerulo- and nephropathies, different skin complications, arthropathies, endocrine diseases and neurologic manifestations.

Among extrahepatic manifestations, neurologic complications are known to involve the peripheral or the central nervous system, leading to mild to severe symptoms and complaints. Manifestations of HCV-associated central nervous system impairment include fatigue, depression, as well as cognitive impairment. However, the commonest and best established neurologic complication of HCV infection is peripheral neuropathy. Clinically, the neuropathy presents as a distal symmetric sensorimotor polyneuropathy, mononeuropathy multiplex, or mononeuropathy.

Autonomic system function in patients with chronic hepatitis C virus infection has not been studied before. However, presence and clinical importance of autonomic dysfunction in chronic liver diseases has been examined and proved by several studies. The significant relationship between cardiovascular morbidity and mortality, and the cardiovascular autonomic nervous system function, assessed by heart rate variability and baroreflex sensitivity, is well established. In chronic liver diseases, autonomic neuropathy carries a 5-fold risk of mortality within 4 years, in patients with chronic liver diseases independent from the severity of the liver disease. Autonomic neuropathy may also be regarded as a potential etiologic factor of hyperdynamic circulation and portal hypertension.

Aims

I aimed to answer the following questions:

1. Is there a difference in cardiovagal autonomic function between treatment-naïve patients with chronic HCV infection and healthy control people?

2. Is there a correlation between autonomic indices and markers of liver cell damage (serum aminotransferases), liver synthetic capacity (serum albumin), glucose metabolism, cryoglobulins and serum HCV RNA level in patients with chronic HCV infection?

3. How does the current standard of antiviral therapy affect the cardiovagal autonomic function during the course of the treatment?

4. Is there an association between with autonomic function indices and the anthropometric and laboratory variables (age, BMI, cryoglobulinemia, HCV RNA level, ALT, albumin, glucose) and the response to therapy during antiviral treatment?

Methods

For the cross-sectional study, forty-five treatment-naïve patients with chronic HCV infection (range 26–62 years of age, mean 48.2) were recruited from three outpatient liver clinics in Budapest, Hungary. Forty healthy subjects (range 28–67 years of age, mean 44.6) were recruited from the medical and assistant staff of different medical departments of Semmelweis University and served as controls. Both patients and controls underwent laboratory examinations and cardiovagal autonomic function (heart rate variability and baroreflex sensitivity) assessments once. For the follow-up study, twenty-two patients with chronic HCV infection were recruited from the same three outpatient liver clinics in Budapest. Autonomic function and laboratory examinations were performed one day before therapy, then on week 12, week 24 and week 48, depending on the individualized duration of antiviral therapy. All individuals gave written informed consent to participate

in the studies that were approved by the Ethics Committee of the Semmelweis University, Budapest, Hungary.

One day before autonomic function tests the following routine laboratory analyses were performed: aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin and blood glucose. In patients with HCV infection, HCV RNA was quantified by real-time PCR (COBAS TaqMan, Roche Diagnostics, Meylan, France). Genotyping was performed by reverse hybridization (INNO-LiPA HCV II, Innogenetics, Ghent, Belgium). For the detection of mixed cryoglobulins, blood samples were kept at 37 °C until complete coagulation and were analyzed by standard methods.

Cardiovagal autonomic function was determined by assessing the time- and frequency domain indices of heart rate variability (HRV) and baroreflex sensitivity (BRS). During the 10 minutes long recording, patients and controls were in a resting supine position. R-R intervals were measured from R-wave threshold crossings on continuously recorded ECGs. Radial artery pressure was monitored continuously with an automated tonometric device (Colin CBM-7000; Colin Corp., Komaki City, Japan) for determination of BRS indices.

Time and frequency domain measurements of HRV from 10 min recordings of RRIs were calculated using the WinCPRS program (Absolute Aliens Oy, Turku, Finland). The following parameters were determined: the standard deviation of the RRI (termed NNSD), the root-mean-square of successive differences (termed RMSSD), the percentage of successive RRIs which differed by more than 50 ms (termed pNN50), as well as low frequency (0.05–0.15 Hz) and high-frequency (0.15–0.4 Hz) power of RRI variability (termed LF and HF, respectively).

To assess baroreflex sensitivity, coupling between spontaneous fluctuations in heart rate and SBP was determined by the sequence method and by spectral analysis. The software used (WinCPRS) detected the ECG R wave, computed R-R intervals and radial artery SBP time series and

identified spontaneously occurring sequences in which SBP and RRI concurrently increased and decreased over three or more consecutive beats (BRS_{seq}). To determine spectral indices, the signals were interpolated, resampled and their power spectra were determined using Fast Fourier Transformation-based methods. The LF_{gain} (LF [low-frequency] transfer function gain) was determined, which expresses RRI and SBP cross-spectral magnitude in the frequency range of 0.05–0.15 Hz, where coherence is greater than 0.5.

For the follow-up examinations, patients were treated with pegylated interferon (PEG-IFN) alfa-2a (40kd, 180μg) or PEG-IFN alfa-2b (12kd, 1.5 μg/kg) subcutaneously once per week and ribavirin (1000 mg if < 75 kg and 1200 mg if >75 kg) per os daily, according to the guidelines. Treatment responses were characterized by the results of HCV RNA testing. Early virologic response was defined as a decrease in serum HCV RNA concentration to <50 IU/mL or a decrease of at least 2 log units from baseline viral load at week 12 of therapy. SVR was defined as negativity for HCV RNA in serum by a PCR test at the end of treatment and 6 months later.

Between controls and patients, differences in variables were analyzed using unpaired Student's *t* tests. To assess the independent effect of different parameters (age, body mass index [BMI], SBP, DBP, serum levels of AST, ALT, albumin, glucose, cryoglobulinemia, HCV RNA) on autonomic indices (HRV and BRS), we performed a multivariate analysis using full information maximum likelihood regression. To assess the change over time in variables of interest, repeated variance analysis was used with post hoc Tukey tests. To evaluate the effect of potential determinants (age, body mass index [BMI], serum levels of ALT, albumin, glucose, cryoglobulinemia, HCV RNA, and response to therapy) on autonomic (HRV and BRS) indices during therapy, multivariate analysis using full information maximum likelihood regression was performed including all selected participants and correcting for attrition. Statistical analyses were performed using SPSS program package version 14

(SPSS Inc., Chicago, IL, USA) and Mplus version 5.2 (Muthen and Muthen, Los Angeles, CA, USA).Significance was accepted at $p<0.05$.

Results

In the cross-sectional study, there was no difference in BMI, blood pressure, heart rate, serum albumin and glucose levels between patients and controls, while AST and ALT levels were significantly higher in patients with chronic HCV infection ($p<0.001$). As shown in **Table 1.**, heart rate variability sequence indices (NNSD, RMSSD, pNN50), frequency-domain indices (LF and HF), baroreflex sensitivity sequence index (BRS_{seq}) and frequency-domain index (LF_{gain}) were lower in patients with HCV infection compared to controls ($p<0.01$).

	Patients	**Controls**
NNSD (ms)	30.5 ± 10.7 *****	42.7 ± 19.2
RMSSD (ms)	21.7 ± 12.2 *****	32.9 ± 22.7
pNN50 (%)	4.4 ± 6.8 *****	13.4 ± 18.0
LF (ms^2)	168.5 ± 160.9 *****	370.7 ± 349.4
HF (ms^2)	182.6 ± 198.1 *****	388.9 ± 361.3
BRS_{seq} (ms/mmHg)	7.1 ± 3.4 *****	11.5 ± 6.5
LF_{gain} (ms/mmHg)	5.2 ± 3.3 *****	7.9 ± 5.3

Table 1. Cardiovagal autonomic function indices of cross-sectional study cohort

Values are given as means ± SD. NNSD indicates SD of RR intervals; RMSSD, root mean square of successive RR-interval differences; pNN50, percentage of RR intervals that differ >50 ms; LF, low-frequency (0.05–0.15 Hz) power of RR-interval variability; HF, high-frequency (0.15–0.4 Hz) power of RR-interval variability. BRS_{seq} indicates baroreflex sensitivity sequence index; LF_{gain}, cross-spectral transfer gain in the low-frequency range. ***** $p< 0.01$, compared with controls.

	Before treatment	**Treatment week 12**	**Treatment week 24**	**Treatment week 48**
n (male)	22 (11)	22 (11)	21 (11)	19 (10)
AST (IU/l)	92.1 ± 56.2	45.7 ± 27.7 *	39.5 ± 26.7 *	42.16 ± 23.8*
ALT (IU/l)	113.3 ± 71.5	37.8 ± 26.1 *	33.2 ± 27.8 *	33.7 ± 21.8*
Albumin (g/l)	45.8 ± 7.1	43.8 ± 4.3	45.6 ± 3.7	43.9 ± 4.3
Glucose (mmol/l)	5.1 ± 0.5	5.1 ± 0.7	5.1 ± 0.6	5.0 ± 0.5
cryoglobulinemia (%)	8 (35%)	5 (22%)	2 (8%)	1 (5%)
Heart rate (beats/min)	73.1 ± 6.0	76.2 ± 7.5	73.4 ± 8.1	75.2 ± 9.2
NNSD (ms)	36.8 ± 11.9	27.5 ± 10.8 †	31.3 ± 16.1	34.1 ± 11.3
RMSSD (ms)	28.0 ± 14.7	19.9 ± 13.5	29.9 ± 23.8	30.2 ± 17.3
pNN50 (%)	5.5 ± 6.2	1.4 ± 2.3 †	3.4 ± 4.4 ‡	4.4 ± 4.9
LF (ms^2)	253.0 ± 156.1	111.6 ± 81.9 †	183.4 ± 169.6 ‡	211.6 ± 149.1
HF (ms^2)	230.1 ± 165.1	105.0 ± 97.9 †	254.5 ± 333.4 ‡	251.8 ± 290.4
$\mathbf{BRS_{seq}}$ (ms/mmHg)	8.1 ± 3.9	5.6 ± 2.0 †	8.3 ± 4.1 ‡	8.8 ± 2.9
$\mathbf{LF_{gain}}$ (ms/mmHg)	6.8 ± 3.6	5.0 ± 2.2 †	5.9 ± 2.6 ‡	6.7 ± 2.4

Table 2. Laboratory data and autonomic function indices in patients with chronic hepatitis C during antiviral treatment

Values are given as means ± SD. *****Significantly different, $p< 0.001$ from "Before treatment" value; †significantly different, $p< 0.01$ from "Before treatment" value; ‡ significantly different, $p< 0.05$ from "Treatment week 12" value.

Analyzing the possible associations between autonomic function and clinical characteristics with multivariate analysis, neither anthropometric (age, BMI), nor hemodynamic (SBP, DBP), nor specific serum variables (serum HCV RNA, glucose, albumin level, cryoglobulinemia) were independently associated with any of the examined autonomic function indices. ALT was the only parameter independently associated with RMSSD, pNN50 and HF [standardized coefficient (β) = -0.58; -0.649; -0.642, respectively, $p<0.05$].

In our follow-up study, genotype analyses proved HCV subtype 1b in 21/22 patients (95%), and subtype 1a in 1/22 patients (5%). Study and treatment protocol was discontinued in one patient in week 14 because of severe fatigue, one patient died in traffic accident in week 31, and one patient refused study examinations in week 48. Among the 22 patients 15 (68%) showed an early virological response at week 4 or 12, and 11/19 (58%) patients achieved a sustained virological response (SVR).

The patients' laboratory data and cardiovagal autonomic indices before and during antiviral treatment are given in **Table 2.** The initially elevated AST/ALT levels were nearly normalized by week 12 of therapy and remained normal throughout weeks 24 and 48 ($p< 0.001$). Glucose or albumin levels remained unchanged before and during therapy. Both heart rate variability sequence indices (NNSD, pNN50), frequency-domain indices (LF and HF), and baroreflex sensitivity indices (BRS_{seq}, LF_{gain}) significantly decreased by week 12 ($p< 0.01$), and then increased by week 24 ($p< 0.05$) to reach pre-treatment levels by week 48 of antiviral therapy.

Multivariate analyses did not identify a significant correlation between any of the anthropometric or laboratory variables (age, BMI, cryoglobulinemia, HCV RNA level, ALT, albumin, glucose) and changes of autonomic function indices during antiviral treatment. Response to therapy was also not associated with the changes of autonomic function.

Conclusions

1. Patients with chronic HCV infection have impaired cardiovagal autonomic function, comparing to healthy controls.

2. Among the examined clinical variables, serum ALT level is the only marker independently associates with autonomic dysfunction; neither liver synthetic capacity, nor cryoglobulinemia, nor serum HCV RNA level correlates with impaired autonomic function, suggesting common pathophysiology underlying HCV-induced liver disease and neurological manifestations, through cryoglobulin-independent ways.

3. We found a significant decrease in autonomic function occurred by week 12 of treatment to be followed by a significant improvement by week 24 and a return to pre-treatment values by week 48 of antiviral therapy.

4. Multivariate analyses does not identify a significant correlation between any of the anthropometric or laboratory variables (age, BMI, cryoglobulinemia, HCV RNA level, ALT, albumin, glucose) and changes of autonomic function indices during antiviral treatment. Response to therapy is also not associated with the changes of autonomic function.

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List of publications

List of publications regarding to dissertation

- **Osztovits J,** Horváth E, Tax J, Csihi L, Horváth T, Littvay L, Tóth T, Abonyi M, Lakatos PL, Kollai M, Fehér J, Szalay F, Blum HE:
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