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EXTRAHEPATIC MANIFESTATIONS IN PATIENTS WITH CHRONIC HEPATITIS C

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Abstract. The aim of the study was to determine extrahepatic manifestations (EHM) of chronic hepatitis C (CHC) in patients with genotype 1 and 3a infections.

Materials and methods. We examined 83 patients with CHC: 42 (50.6%) with genotype 1, and 41 (49.4%) with genotype 3a; 49 (59.0%) were males, 34 (41.0%) were females; the mean ± SD age of patients was 39.3±12.3 (range: 17 – 72) years. Chronic hepatitis was diagnosed in 75 (90.4%) patients, and cirrhosis – in 8 (9.6%). Clinical, laboratory, histological, biochemical and virological data (AbHCV, viral RNA) were obtained.

Results. EHM were observed in 27 (32.5%) patients: in 18 (42.9%) with genotype 1 infection, and in 9 (21.9%) with genotype 3a infection. The following manifestations were diagnosed: arthralgia (14.5%), purpura (8.4%), glomerulonephritis (6.0%), thyroiditis (6.0%), type 2 diabetes mellitus (3.6%), non-Hodgkin lymphoma (1.2%), Raynoud's phenomenon (1.2%), psoriasis (1.2%), vitiligo (1.2%). Cryoglobulinemia was detected in 9 (10.8%) patients. On average, EHM were revealed 2 times more frequently in females (25.3%) than in males (7.2%).

Conclusion. Extrahepatic manifestations in CHC were observed in 32.5% of patients, more frequently in patients infected with genotype 1 virus and in females, than in patients with genotype 3a infection and males.

Keywords: extrahepatic manifestations, chronic hepatitis C, genotype 1, genotype 3a.

Background

Chronic hepatitis C (CHC) is often accompanied by involvement of other organs and systems in pathological process with development of cutaneous vasculitis, arthritis, glomerulonephritis, thyroiditis, Sjogren's syndrome, cutaneous porphyria, neuropathy, lymphoproliferative and other disorders (Ali, 2005; Galossi, 2007; Krel, 2009; Baĭkova, 2013). Multisystem affection in chronic HCV (hepatitis C virus) infection is caused by many reasons: the ability of hepatitis C virus to replicate not only in the liver, but in other organs as well; the circulation of immune complexes in the blood; polyclonal activation of B-cells infected by the virus; mixed cryoglobulinemia; HCV mutations and emergence of crisscross immune reactions; peculiarities of immune reactivity of the host. Sometimes extrahepatic manifestations (EHM) of chronic HCV-infection are more severe and unfavorable than the liver lesion. The frequency, type, and clinical relevance of EHM in CHC can vary depending on the geographic region, prevalence of CHC in this region, duration of chronic HCV-infection, adequacy of patient examination and other conditions.

The aim of our study was to determine forms and prevalence of extrahepatic manifestations in chronic hepatitis C patients carrying HCV of different subtypes.

Materials and methods

CHC patients admitted to the Petrozavodsk Railway Hospital during 2005-2013 were involved in research. HCV-infection was confirmed by the presence of anti-HCV antibodies using ELISA test-system ("Vector-Best", Novosibirsk, Russia) and by positive test for HCV-RNA using PCR test-system ("AmpliSens HCV", Central Research Institute of Epidemiology, Moscow, Russia). The HCV genotypes were identified by "AmpliSens HCV genotype" test-system (Central Research Institute of Epidemiology, Moscow, Russia).

Liver biopsy was performed in 70 (84.3%) patients; the grade of histological activity was estimated according to the Knodell system and the stage of fibrosis – according to the METAVIR.

Cryoglobulins were detected as described in (Kallemuchikkal, 1999), specifically: 10 ml of venous blood were obtained from a patient in a warmed up to 37^{o} C test-tube, after that serum was separated by centrifugation, the supernatant was incubated in a capillary in vertical position at 4°C for 7 days and then examined for cryoprecipitate.

Laboratory analyses, abdominal and thyroid gland ultrasonography, esophagogastroscopy were carried out routinely in all patients. All patients engaged in research were informed and gave voluntary written consent to medical manipulations. The design of research was approved by the ethic committee of the Faculty of Medicine, Petrozavodsk State University. Statistical analysis was conducted using Statistics 6 software. The p-value = 0.05 was considered statistically significant according to Student t-test, Mann-Whitney U-test, χ^2-test.

Results

We have examined 83 patients with CHC: 49 (59.0%) were males, 34 (41.0%) were females; the mean age was 39.3±12.3 (range: 17 – 72) years. Chronic hepatitis was diagnosed in 74 (89.2%) patients, and cirrhosis – in 9 (10.8%) patients. Genotype 1 HCV was determined in 42 (50.6%) patients, among them subgenotype 1b was revealed in 36 (43.4%) patients and subgenotype 1a – in 6 (7.2%) patients. Patients with subgenotype 1b and 1a were combined into one group. Genotype 1 group included 24 males (57.1%) and 18 (42.9%) females (the average age 37.1±14.6 years). Genotype 3a HCV was determined in 41 (49.4%) patients: males – 25 (61.0%), females – 16 (39.0%) (the average age 41.1±10.7 years).

Extrahepatic manifestations were observed twice as often in patients with genotype 1 HCV than in patients bearing HCV 3a genotype : 18 (42.9%) vs. 9 (21.9%) patients, respectively) (Table 1). Arthralgia and cryoglobulinemic vasculitis were the most frequent systemic manifestations

found in patients carrying genotype 1 HCV. Arthralgia was observed in 5 (25.3%) patients, and a complex of polyorganic lesions (cutaneous vasculitis, glomerulonephritis and arthralgia) was detected with the same frequency – in 5 (25.3%) patients with genotype 1 HCV. Arthralgia was localized mainly in large joints of upper and lower extremities and was not accompanied by clinical or roentgenologic signs of inflammation or structural alterations. Our data are in accordance with the results of other authors reporting on arthralgia as the most frequent extrahepatic manifestation in CHC (Ferri, 2004; Ali, 2005; Galossi, 2007; Krel, 2009; Baĭkova, 2013).

Simultaneous symptoms of cutaneous vasculitis, glomerulonephritis and arthralgia were observed in 5 patients. Cutaneous vasculitis developed as purpura in lower legs in 4 patients, and in legs and buttocks in 1 patient. Glomerulonephritis manifested itself in the form of proteinuria and hematuria in 4 patients , and in the form of proteinuria, hematuria and hyperkreatininemia (130-250 mkmol/l) – in 1 patient. Arterial hypertension and nephrotic syndrome were not observed. Mixed cryoglobulinemia was detected in all 5 patients. According to many authors, membranoproliferative glomerulonephritis is a typical renal manifestation in CHC and is characterized by mild course in more than 50% of cases despite that glomerulonephritis *per se* is a negative prognostic factor (Daghestani, 1999; Ferri, 2004).

Isolated purpura without glomerulonephritis symptoms was observed in 2 (10.5%) females displayed positive result in a mixed cryoglobulinemic test.

Raynaud's phenomenon was observed in 1 (2.4%) 35-year man with cryoglobulinemia, and was characterized by progressive course and development of necrosis of distal phalanges of a few fingers. For this patient, CHC had subclinical course with minimal alterations in laboratory tests – doubly elevated ALT and AST (alanine / aspartate aminotransferase) levels. As it follows from literature data, Raynaud's phenomenon is detected in 30% of cases in cryoglobulinemia and develops more frequently in females than in males (Ferri, 2004), although its occurrence in CHC is not sufficiently studied.

Type 2 diabetes mellitus was detected in 1 female with genotype 1 infection. According to literature data, diabetes is a CHC-associated disease, but its pathogenesis is not yet well defined. Knobler et al. have shown that insulinoresistance and deterioration of B-cell functions occur in CHC patients due to TNFα effect (Knobler, 2005). Several researchers observed diabetes mellitus more frequently in patients with genotype 2a HCV (Mason, 1999) and advanced liver fibrosis (Papatheodoridis, 2006). At the same time, some authors have found that patients with diabetes mellitus are more susceptible to infection by hepatitis C virus (Guo, 2013). Other researchers suppose that HCV serves as a trigger in the development of type 1 diabetes in susceptible persons. In our case, type 1 diabetes was not detected among the examined CHC patients.

Autoimmune thyroiditis was diagnosed in 3 (15.8%) CHC patients carrying HCV subtype 1. Its occult course was proved by thyroid ultrasonography and laboratory tests including thyroid hormones and anti-thyroglobulin autoantibodies. According to epidemiological data, autoimmune thyroiditis is a CHC-associated disease, however, additional evidence of its close etiological and pathogenic connection with chronic HCV-infection is still required (Khattab, 2010).

Table 1. The frequency of extrahepatic manifestations observed in CHC patients with different HCV genotypes

Signs	Genotype 1 HCV	Genotype 3a HCV	Total
Total number of patients, n (%)	42 (50.6%)	41 (49.4%)	83
Patients with extrahepatic manifestations, n (%)	18 (42.9%)	9 (22,0%)*	27 (32.5%)
Arthralgia, n (%)	5 (16.7%)	2 (4.9%)*	7 (8.4%)

Purpura, glomerulonephritis, arthralgia, n (%)	5 (9.5%)	0%*	5 (6.0%)
Purpura, n (%)	2 (4.8)	0%*	2 (2.4%)
Thyroiditis, n (%)	3 (7.1%)	3 (7.5%)	6 (7.2%)
Type 2 diabetes mellitus, n (%)	1 (2.4%)	2 (5.0%)	3 (3.6%)
Non-Hodgkin lymphoma, n (%)	1 (2.4%)		1 (1.2%)
Raynoud's phenomenon, n (%)	1 (2.4%)	-	1 (1.2%)
Vitiligo, n (%)	0%	1 (2.4%)	1 (1.2%)
Psoriasis, n (%)	0%	1 (2.4%)	1 (1.2%)

*- $p<0.05$

Non-Hodgkin lymphoma was diagnosed in a 56-year male by means of biopsy of enlarged neck lymph nodes and bone marrow. CHC revealed by ELISA and PCR was characterized by mild activity according to clinical and laboratory data. Non-Hodgkin lymphoma as well as diabetes belongs to CHC-associated diseases. The risk of its occurrence increases by 20-30% in CHC patients as compared with common population (Giordano, 2007), but pathogenesis of neoplasm development is still unclear. The ability of HCV to infect B-cells could be explained by tropism of viral envelop protein E2 to CD81 receptors on the surface of B-cells. As a result of infection, B-cells become activated and start to produce polyclonal and monoclonal cryoglobulins. Furthermore, in infected B-lymphocytes apoptotic pathway is destroyed and expression of oncogenic proteins is increased leading to lymphoma development. In addition to other infections, various environmental and genetic factors can induce neoplastic transformation of B-cells (Nicolau, 2011).

Five patients with purpura plus glomerulonephritis, who displayed the highest level of hepatocellular inflammation and fibrosis, were the oldest in the group and had the largest disease duration. Four of them (80.0%) had liver cirrhosis (Table 2).

Table 2. Clinical, laboratory and histological parameters for patients with HCV type 1 infection and various extrahepatic manifestations (M±m)

Signs	Arthral-gia	Purpura, glomerulo-nephritis, arthralgia	Purpura	Thyro-iditis	Type 2 Diabe-tes	Non-Hodgkin Lym-phoma	Ray-noud's pheno-menon	Total
Number of patients, n (%)	5 (25.3%)	5 (26.3%)	2 (10.5%)	3 (15.8%)	1 (5.3%)	1 (5.3%)	1 (5.3%)	18
Males, n (%)	2 (40.0%)	0	0	1	0	1	1	5 (27.8%)
Females, n (%)	3 (60.0%)	5	2	2	1	0	0	13 (72.2%)
Age, years	57.02± 4.8	55.5± 5.5	37.6± 14.8	39.0± 10.9	39.0	51.0	35.0	48.7± 11.0
Disease duration, years	19.5± 7.2	19.7± 9.6	10.0± 7.2	5.7±3.0	10	9	7	11.8± 8.6
Hepatitis, n (%)	2 (40.0%)	1	2	2	1	1	1	10 (55.6%)
Cirrhosis, n (%)	3 (60.0%)	4	0	1	0	0	0	8 (44.4%)

ALT, U/l	109.1± 35.4	146.3± 41.3	64.9± 29.5	74.9± 29.6	70.8	100.3	76.7	92.6± 41.3
AST, U/l	79.7± 23.6	92.6± 17.7	53.1± 19.3	60.77± 20.4	53.6	95.5	60.3	71.02± 21.0
Bilirubin, mkmol/l	16.3± 2.7	16.8± 1.9	15.2± 1.3	20.9± 4.1	14.5	13.6	16.8	17.1± 6.2
HAI, score	8.6±4.1	14.1± 2.3	12.5± 3.5	11.0± 6.5	5	-	-	12.6± 4.7
F, score	2.4±1.5	3.5±0.4	2.0±1.4	2.3±1.5	1	-	-	3.0±1.2

Liver cirrhosis was also diagnosed in 3 (60.0%) of 5 patients with arthralgia. These patients did not show high level of serum ALT and histological activity compared to patients with combined purpura and glomerulonephritis. Cryoglobulinemia was detected in 8 (19.0%) patients bearing genotype 1 HCV and developing purpura, glomerulonephritis and Raynaud's phenomenon.

Within the group of genotype 1 HCV infected patients, extrahepatic manifestations were found more often in females (13 – 72.2%) than in males (5 – 27.8%, $p<0.05$). Some authors also report on gender dependence of certain systemic manifestations of CHC, with more frequent occurrence in females, than in males (Stefanova-Petrova, 2007). At the same time, it is accepted that the risk of CHC is higher in men than in women.

The frequency of EHM in patients with genotype 3a virus was 22.2%, which is 2 times less than for patients with genotype 1 virus. Thyroiditis was revealed in 3 (33.3%) patients (Table 3). It had subclinical course as diagnosed by ultrasonography of thyroid gland and elevated levels of thyroid hormones. Arthralgia and type 2 diabetes were observed in equal number of patients (22.2%). Vitiligo was defined in 1 (11.1%) patient as well as psoriasis.

Liver cirrhosis was not diagnosed in patients with HCV of 3a genotype. Clinical and laboratory activity of CHC did not differ from that observed in patients with HCV genotype 1, however, histological activity index and fibrosis in patients with HCV 3a were significantly lower than those in patients with HCV type 1 ($p<0.05$).

Cryoglobulines were identified only in 1 (11.1%) HCV3a-positive CHC-patient with arthralgia versus 8 (19.0%) patients with HCV type 1 infection. Other authors report on the higher frequency of cryoglobulinemia development in HCV2a/c-positive patients (Franguel, 1996).

Within the group of HCV3a-infected patients, systemic manifestations of HCV-infection were observed more often in females (7 – 77.7%) than in males (2 – 22.2%) which is similar to the group of HCV type 1 positive patients.

Table 3. Clinical, laboratory and histological parameters for patients with HCV type 1 infection and various extrahepatic manifestations (M±m)

Signs	Arthralgia	Thyroi-ditis	Type 2 Diabetes mellitus	Vitiligo	Psoriasis	Total
Number of patients, n (%)	2 (22.2%)	3 (33.3%)	2 (22.2%)	1 (11.1%)	1 (11.1%)	9
Males, n (%)	0	0	1 (11.1%)	0	1 (11.1%)	2 (22.2%)
Females, n (%)	2 (22.2%)	3 (33.3%)	1 (11.1%)	1 (11.1%)	0	7 (77.8%)
Age, years	49.5±4.8	49.0±5.6	47.5±19.2	42	61	54.7±13.9
Disease duration, years	11.5±4.6	6.0±5.6	6.0±1.4	10	4	6.7±7.2
Hepatitis, n (%)	2 (22.2%)	3 (33.3%)	2 (22.2%)	1 (11.1%)	1 (11.1%)	9 (100%)
Liver cirrhosis, n (%)	0	0	0	0	0	0

ALT, U/l	97.9±53.4	56.1±39.5	186.5±52.3	221.7	142.4	132.6±56.3
AST, U/l	73.1±26.1	24.4±8.1	71.9±9.4	80.3	74.1	49.1±14.3
Bilirubin, mkmol/l	17.2±5.3	23.1±21.0	13.5±0.9	15.6	16.3	17.5±10.2
HAI, score	7.2±0.8	7.5±0.7	11.5±2.1	6	5	8.0±3.0
F, score	1.0±0.0	1.0±0.0	1.0±0.0	1	1	1.1±0.0

Conclusions

1. Extrahepatic manifestations were detected in 32.5% of patients with chronic hepatitis C and characterized by high frequency of arthralgia, purpura and glomerulonephritis caused by cryoglobulinemia.
2. Extrahepatic manifestations occurrence depends both on viral and host factors.
3. HCV genotype, comparing to other viral factors, significantly affected the frequency of extrahepatic manifestations in CHC-patients: EHM occurred twice as often in group of HCV type 1 positive persons than in group of HCV 3a positive.
4. As for the host factors, it was gender that significantly influenced the frequency of extrahepatic manifestations in CHC: EHM occurred 3 times more frequently in females than in males.

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