

SPECIFICITY OF CLINICAL COURSE AND RISK FACTORS ASSOCIATED WITH THE PROGRESSION OF HEPATITIS C IN PATIENTS OF HAEMODIALYSIS

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Summary

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Aim of the study: To determine the clinical course of chronic hepatitis C and risk factors associated with the progression of the liver disease in patients on maintenance hemodialysis with retrospective-prospective clinical research for a period of 4 years. In this study we included 80 hemodialysis patients (51 male and 29 female, mean age of 55.8 years) with chronic hepatitis C, divided in two groups according to the clinical course of the HCV infection: Group 1 consisted of 34 patients with chronic hepatitis C with aminotransferase levels within the reference values and Group 2 consisted of 46 patients with chronic hepatitis C with variable aminotransferase levels. **Results:** Group 1 of patients with aminotransferase levels within the reference values had significantly longer duration on hemodialysis ($P=0.048$), significantly longer duration of HCV infection ($A=0.005$), and significantly lower serum levels of aminotransferases ($A=0.000$) compared to Group 2 of patients with variable aminotransferases. The results from the Doppler ultrasound examination of the abdominal organs and portal vein revealed 37 (46.3%) patients with compensated cirrhosis and 43 (53.7%) patients with chronic liver disease. **Conclusions:** The clinical course of chronic hepatitis C in hemodialysis patients was not associated with serum aminotransferase activity. None of the analyzed risk factors was associated with the progression of liver disease. Doppler ultrasound measurement of the liver morphology, liver echogenicity, and portal vein flow velocity could accurately predict the progression of liver disease in hemodialysis patients with chronic hepatitis C.

Key words: clinical course, haemodialysis patients, hepatitis C, risk factors

INTRODUCTION

Hepatitis C virus (HCV) infection is a serious health problem worldwide. More than 240 million people worldwide have chronic hepatitis C (Thursz *et al.*, 1999). The prevalence of HCV in the world is determined by seroprevalence as regards the prevalence of antibodies to HCV in serum of infected patients. Prevalence of HCV infection in the general population show variations depending on the socio-

economic development of the geographic region. In North America prevalence of antibodies to HCV from 0.5% to 1.4% in the serum of voluntary blood donors, 0% to 1.3% in Europe, 0.3% to 1.3% in Asia, and 2.3% in Australia were registered. In Thailand, Vietnam and Egypt high prevalence to HCV was registered – from 4% to 22% in voluntary blood donors, in soldiers and preschool population (Keeffe,

2002; Polenakovic *et al.*, 2003). In R. Macedonia, established prevalence of HCV infection was 1.15% in voluntary blood donors, or from 1654 tested volunteers, 19 (1.15%) were positive for HCV infection antibodies (Polenakovic *et al.*, 2003). Epidemiological studies show that only 20% of infected people with HCV spontaneously eliminate the virus, and in the remaining 80% chronic infection that can be converted in liver cirrhosis after 20 years, and after 28 to 30 years liver failure or adenocarcinoma have occurred (Alberti *et al.*, 1999; Keffe, 2002; Renou *et al.*, 2002; Yoshizawa *et al.*, 2003). Chronic HCV infection is a leading indication for liver transplantation in USA. HCV virus belongs to the Flaviviridae family. HCV is a virus with a core containing a single strain RNA made of 9600 nucleotides. The core is wrapped in shell containing structural proteins E1 and E2. The virus includes seven nonstructural proteins: p7, HC2, HC3, HC4A, HC5B, HC5A, HC5B (Fig. 1).

Appearance of extra hepatic manifestations (mixed cryoglobulinaemia and membranoproliferative glomerulopathy). Chronic infection is associated with autoimmune and lymphoproliferative conditions for viral replication and cytopathic effect of B lymphocytes with subsequent clonal expansion of specific B lymphocyte clones. Cryoglobulins occur as a result of association of IgM –Fc on HCV IgG antibody (IgM-Fc IgG) and precipitate when body temperature will drop. Cryoglobulins can be detected in half of infected with HCV, but only in 10–15% are with

clear symptoms (Keeffe, 2002). Cryoglobulins are deposited in the glomerular mesangium and induce development of membranoproliferative glomerulopathy. By electron microscopy mesangial and subendothelial deposits as finger prints can be detected (Fig 2, A3), and with indirect immunofluorescence – as subendothelial IgM deposits (Fig. 2, A2) Cryoglobulins can be deposited in glomerular capillaries forming eosinophilic thromb (Fig. 2, A1), and subsequent development of vasculitis with fibroid necrosis (Jaudol *et al.*, 2004).

Progression of chronic HCV infection described by retrospective and prospective studies.

- Retrospective studies: monitoring duration 9–29 years; development of cirrhosis 17–55% (average 42%); HCC development 1–23% and liver associated mortality 4–15%.
- Prospective studies: monitoring duration 8–16 years; development of cirrhosis 1–16% (average 11%); HCC development 0.7–1.3%; liver associated mortality 1.3–3.7%.

The results of described retrospective and prospective studies highlight that the natural course or progression of HCV infection is highly variable, or is under the influence of risk factors associated with the virus and host (Jaudol *et al.*, 2004). Risk factors that affect the progression of chronic HCV infection are divided into 3 groups:

- Host risk factors: sex, age, duration of infection and immunological response;



Fig. 1. Structure of HCV.

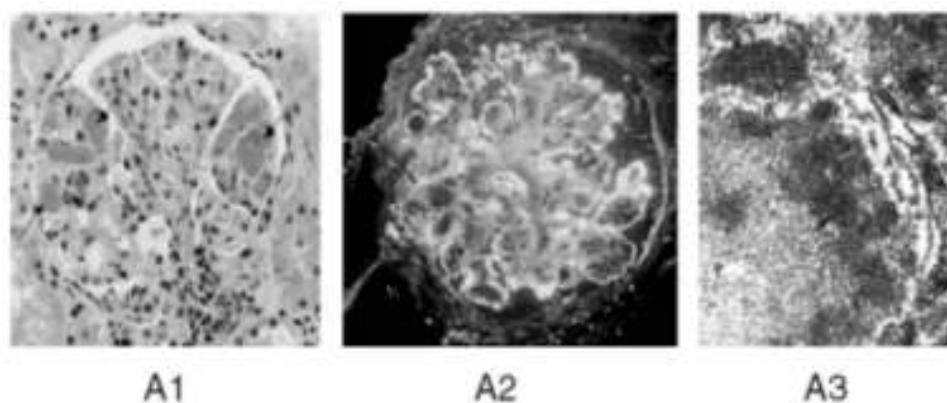


Fig. 2. Cryoglobulin glomerular deposits detected with immunofluorescence and electron microscopy.

- Virus risk factors: virus genotype and virus concentration in initial inoculation;
- External risk factors: HBV and/or HIV coinfection, alcohol consumption.

Male sex is a risk factor for rapid progression of inflammation and fibrosis, that females have protective role, possibly because female hormones that are part of liver metabolism (Poynard *et al.*, 1997; Degos *et al.*, 2000; Serafimovski, 2004). In terms of age it is shown that infection with HCV over the age of 40 years carries a higher risk of morbidity and mortality from liver changes (Poynard *et al.*, 2000; Thomas *et al.*, 2000). One of the possible explanation for the influence of age and the infection progression is inability of immune system that ages along with the body to fight HCV infection (Zavaglia *et al.*, 1998). Regarding the duration of HCV infection, it is difficult to determine it with accuracy, because the diagnosis of HCV infection is commonly set with detection of HCV antibodies in serum in infected organism. T cell response towards the antigen of HCV differs quantitatively and qualitatively between patients who come to spontaneous elimination of the virus

and recovery, and these who are developing chronic infection. Patients with spontaneous elimination of HCV virus have more significant T cell response against viral antigen (Roudot-Thraval *et al.*, 1997; Bellentani *et al.*, 1999).

Virus factor may influence on infection clinical outcome by viral inoculation concentration and by viral genotype. Feature of transfusion transmission of HCV is an inoculation of a high concentration of virus that was associated with high viral cytotoxicity (Hussein *et al.*, 2007). Studies report that infection with genotypes 1 and 4 leads to more liver damage than genotypes 2 and 3. In a prospective study, the team of Bellentani covered 162 HCV PHA positive patients where genotype 1 was present in 70% of patients with cirrhosis (Gallego *et al.*, 2006).

By external factors, coinfection with HBV (hepatitis B virus) and/or HIV (human immunodeficiency virus) was associated with rapid progression of chronic HCV infection (Alberti *et al.*, 1999; Degos *et al.*, 2000; Keefe, 2002). Also chronic excessive alcohol consumption (>50 g per day) is associated with four time higher risk for cirrhosis development

and higher incidence of hepatocellular carcinoma in patients with HCV infection (Bellentani *et al.*, 1999; Hussein *et al.*, 2007).

The high prevalence of HCV infection in patients on chemodialysis and expectation to increase the number of patients on chemodialysis with serious liver damage by increasing the length of their lives as a result of improving the technology of chemodialysis treatment was the motive of the study.

One of objectives of the study was to determine the specificity of the clinical course of chronic HCV infection in patients on chemodialysis: the prevalence of patients with chronic HCV infection, aminotransferase level in a reference or fluctuating values and the serum aminotransferase serum level association with progression of chronic hepatitis C in patients on chemodialysis. The second objective was to define risk factors which are associated with the progression of chronic HCV infection in patients on chemodialysis.

MATERIAL AND METHODS

Study design: retrospective-prospective, cohort clinical research. Duration of clinical research: January 2004 to January 2008. Total of 185 patients with chronic renal insufficiency in the terminal phase of chronic dialysis programme at the Clinic of Nephrology, 86 were chemodialysis patients with chronic infection. Six patients were excluded from the survey due to the presence of co-infection with HBV, so the survey subjects were 80 patients on chemodialysis programme with chronic hepatitis C. In patients included in survey there were not specific HIV antibodies and none of them was a user of narcotics. Demographic characteristics and basic renal disease in HCV positive

patients on chemodialysis included in study are presented in Table 1.

The patients included in study had a haemodialysis programme in the Department for chronic dialysis programme on Clinic of Nephrology. In all patients standard bicarbonate haemodialysis treatment with three times a week frequency and haemodialysis duration of one treatment for 4 to 5 hours was applied. Dialysis programme in Department for chronic hemodialysis programme is carried out in two shifts, two patients per day on one haemodialysis unit. All haemodialysis units are sterilised and disinfected after each treatment. For haemodialysis, 1.3 to 1.7 m² polysulfone dialysis membranes were used. For each new chemodialysis programme new sterile dialysis membranes were used. Blood flow through chemodialysis membrane was 250 to 300 mL/min and the flow of the hemodialysis fluid was 500 mL/min.

According the clinical course in chronic HCV infection, patients were divided into two groups:

- First group: patients with chronic HCV infection and reference aminotransferase levels;
- Second group: patients with chronic HCV infection and fluctuating aminotransferase levels.

The first group of patients with chronic HCV infection and reference aminotransferase levels consisted of 34 patients with the following criteria:

- Persistent positive HCV antibodies detection in blood serum;
- Positive on HCV RNA in blood serum, proven at least once during the study after a minimum of 6 months after HCV antibodies detection;
- Reference aminotransferase serum levels (ALT: 10–45 U/L, AST: 10–34 U/L) during the study.

Table 1. Demographic characteristics and basic renal disease in HCV positive patients on dialysis.

	Number	%	Mean±SD	Range
Male	51	63.75		
Female	29	36.25		
Age (years)			55.88±11.95	29–79
< 30	2	2.50		
31–45	18	22.50		
46–60	39	48.75		
>61	21	26.25		
Dialysis service (months)			152.70±68.04	60–336
Basic renal disease				
Glomerulopathy	22	27.50		
Nephro arteriosclerosis	17	21.25		
Diabetic nephropathy	4	5.00		
Interstitial pyelonephritis	9	11.25		
Adult polycystic kidney disease	8	10.00		
Obstructive nephropathy	4	5.00		
Undifferentiated nephropathy	16	20.00		

The second group of patients with chronic HCV infection and fluctuating aminotransferase level, consisted of 46 patients with the following criteria:

- Persistent positive HCV antibodies detection in blood serum;
- Positive on HCV RNA in blood serum, proven at least once during the study after a minimum of 6 months after HCV antibodies detection;
- Fluctuating aminotransferase serum level (times with referent aminotransferase serum level and times with aminotransferase serum level above the referent level).

In the study were used standard diagnostic assays for HCV: serology assay for HCV antibodies detection in serum and molecular assay for virus (HCV RNA) detection in serum.

In patients on haemodialysis on Clinic of Nephrology, HCV antibodies were determined every 6 months as part of regular virology and serological tests by standards for haemodialysis treatment in R. Macedonia (Treatment haemodialysis standards

in R. Macedonia, Nephrologist association in R. Macedonia, Macedonian Medical Association, Skopje 2005). Virology and serological tests were conducted in the Institute of Clinical Biochemistry – Skopje; and Clinic for infectious disease – Skopje.

HCV antibodies were detected by MELA (*Microparticle Enzyme Immunoassay*) which is ELISA (*Enzyme Linked Immunoassay*) assay modification. When performing of MELA assay, patient serum was mixed with microparticles tagged with antibodies directed to HCV antibodies and the HCV antibodies and anti-antibodies complex was formed. The complex binds 4-methylumbelliferyl phosphate (4-MUP) and conjugate that contains alkaline phosphatase. Alkaline phosphatase hydrolyses 4-MUP and emits fluorescent methylumbelliferyl (MU). MU concentration is proportional to the concentration of tested HCV antibodies. For virus detection in patients positive on HCV antibodies was used HCV RT-PCR (AMPLICOR® HCV Test, version 2.0, Roche Molecular Systems Inc., Branch-

burg NJ 08876 USA) assay (Fabrizi *et al.*, 1996). It is qualitative *in vitro* diagnostic assay for HCV RNA detection in patient serum. The HCV RT-PCR is very sensitive assay that can detect from a few to 100 viral copies/mL. For HCV genotype determination in HCV RNA positive patients, can be use oligonucleotide hybridization analysis. With this type of analysis can be determinant 4 main genotypes and subtypes 1a, 1b, 2a, 2b, 3a, 3b and 4a. This analysis are conducted in Research center for Genetic Engineering and Biotechnology at the Macedonian Academy of Sciences and Arts (MANU).

RESULTS AND DISCUSSION

In this study, 80 out of 86 positive patients had chronic HCV infection and absence in co-infection with HBV. Patients with chronic hepatitis C were positive on HCV serum antibodies and serum HCV RNA was detected at least 6 months after detection of HCV antibodies. The dominant HCV genotype in patients on haemodialysis programme with chronic hepatitis C was 1a, detected in 97.5% of patients examined, and the rest 2.5% were infected with 4a genotype (Fig. 3).

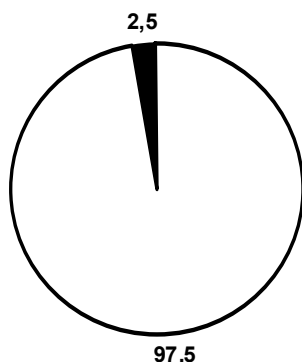


Fig. 3. HCV genotype distribution in patients with chronic hepatitis C on dialysis.

The study included HCV/HD starting date calculation in HCV positive patients on haemodialysis programme. HCV/HD starting date is the time period passed from the date of initiation of haemodialysis till the date of HCV antibodies detection. HCV/HD starting date was expressed in months. The mean HCV/HD starting date was 66.98 months (range: 6–276 months) (Fig. 4).

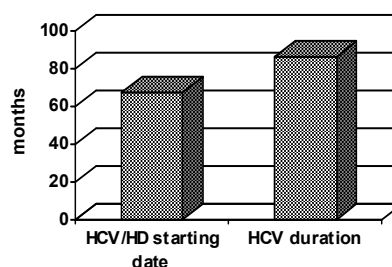


Fig. 4. Mean HCV/HD starting date and mean HCV duration in positive patients in the dialysis programme.

Duration of HCV infection in months (HCV duration) was calculated as a difference between closing date of study and the date of first HCV antibodies detection in patients on chemodialysis program. The mean HCV duration was 85.50 months (range: 54–120 months) (Fig. 4).

Included HCV positive patients in the dialysis programme received an average of 6.26 (range: 0–30) blood and blood products transfusions during the dialysis service.

Table 2 presents the average values of the tested parameters in all 80 HCV positive hemodialysis patients included in the study.

Mean serum AST, total and direct bilirubin were in reference range in patients included in the study, but mean serum ALT was at the upper reference

Table 2. Average values of the tested parameters in HCV positive hemodialysis patients included in the study

Parameters	X	SD	Reference range
AST (U/L)	32.71	15.99	10–34
ALT (U/L)	48.40	26.96	10–45
Total bilirubin ($\mu\text{mol/L}$)	7.34	1.19	6.8–20.5
Direct bilirubin ($\mu\text{mol/L}$)	3.48	0.65	1.5–6.8
Size of liver (mm)	168.96	23.05	160–240
Liver morphology	0.52	0.10	< 0.60
Spleen surface (cm^2)	65.38	19.42	< 45
D – portal vein (mm)	9.51	1.87	< 12
V – portal vein flow (cm/s)	15.47	4.16	> 15
Cumulative point	63.00	29.71	0–200

D – diameter; V – velocity.

Table 3. Comparison of the examined parameters between both study groups of patients with referent aminotransferase level and fluctuating aminotransferase level

Parameters	I group		II group		p
	Mean	SD	Mean	SD	
Age (years)	54.88	12.06	56.61	11.94	0.526
Hemodialysis duration (months)	170.12	67.33	139.83	66.37	0.048
HCV/HD starting date (months)	73.94	48.74	61.83	51.53	0.291
HCV duration (months)	97.05	29.74	77.48	30.00	0.005
Transfusion number	7.26	7.38	5.52	6.31	0.256
AST (U/L)	22.21	5.92	40.48	16.69	0.000
ALT (U/L)	28.83	7.33	62.86	27.09	0.000
Total bilirubin ($\mu\text{mol/L}$)	7.05	0.72	7.55	1.41	0.065
Direct bilirubin ($\mu\text{mol/L}$)	3.31	0.26	3.61	0.81	0.044
Size of liver (mm)	171.03	23.13	167.43	23.13	0.493
Liver morphology	0.51	0.10	0.53	0.10	0.228
Liver surface (cm^2)	64.80	20.10	65.81	18.76	0.821
D – portal vein (mm)	9.81	1.71	9.29	2.06	0.221
V – portal vein flow (cm/s)	15.25	4.36	15.64	3.92	0.681
Cumulative point	62.57	27.89	63.32	32.44	0.912

value. Mean size and the morphology of the liver and the portal vein diameter were within the reference range but the mean spleen surface was above the reference range. Mean velocity of portal vein flow was equivalent to the reference velocity. Mean cumulative point in all 80 HCV positive hemodialysis patients was 63.

In the study, HCV positive haemodialysis patients were divided in two groups: patients with chronic HCV infection and

reference aminotransferase levels and patients with chronic HCV infection and fluctuating aminotransferase levels. The comparison of the examined parameters between both study groups of patients with referent aminotransferase level and fluctuating aminotransferase level are presented in Table 3.

The first patients group with chronic hepatitis C and reference aminotransferase levels was characterised with significantly

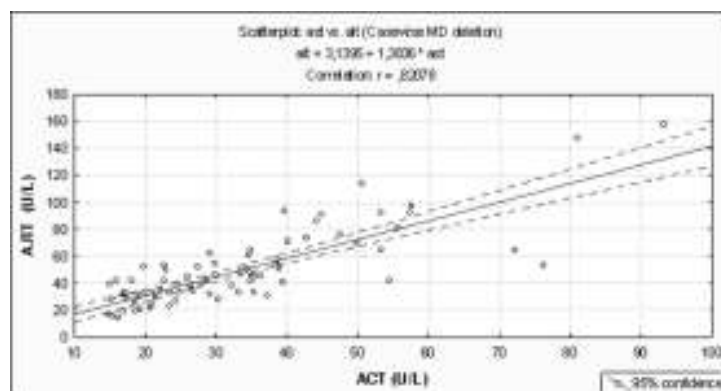


Fig. 5. Correlation between serum ALT and AST level in patients with chronic C hepatitis.

longer haemodialysis (HD) duration time ($P=0.048$) and HCV duration ($P=0.005$) but significantly lower direct bilirubin serum level ($P=0.044$) compared with the second group of patients with chronic hepatitis C and fluctuating aminotransferases. Patients with fluctuating aminotransferase had significantly higher serum aminotransferase levels compared with the patients group with reference aminotransferase levels. The number of blood and blood products transfusions was higher but without significance compared with the number of transfusions in the second group. Between both patient groups there was no significant difference in examined morphological and haemodynamic ultrasonographic parameters.

Among male patients, probability for fluctuating aminotransferase levels was 1.16 times (95% CI, 0.43–2.68) higher compared with female patients. With increasing age in HCV positive patients on haemodialysis programme increased the probability for fluctuating aminotransferase levels, in patients older than 50 years the probability for fluctuating aminotransferase levels was 1.42 times higher (95% CI, 0.48–2.84) compared to those younger than 50 years. With the increas-

ing the HD duration time and HCV duration decreased probability for fluctuating aminotransferase level in patients with chronic HCV infection on haemodialysis. In patients with HD duration time less than 120 months, the probability for fluctuating aminotransferase level was higher 2.6 times (95% CI, 0.61–3.79) compared to those with HD duration time longer than 120 months. In patients with HCV duration less than 60 months the probability for fluctuating aminotransferase level was higher 3.25 times (95% CI, 0.66–4.23) compared to those with duration HCV longer than 60 months. Dominant HCV genotype in both study groups of patients with chronic hepatitis C was 1a. In first group all patients were infected with 1a genotype (100%), in second group, 95.6% were infected with genotype 4a. In patients with <5 transfusions, the probability for fluctuating aminotransferase levels was 1.44 times higher (95% CI, 0.47–2.95) compared with patients that with > 5 blood and blood products transfusions.

Serum ALT level in all patients with chronic hepatitis C correlated positively with serum AST level ($r=0.82$, $P=0.00$) as shown in Fig. 5.

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