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PATTERNS OF VIRAEMIA IN HAEMODIALYSIS PATIENTS WITH HEPATITIS C

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A b s t r a c t: Clinical features, aminotransferases levels, and antibody to HCV have only limited correlation with the activity of liver disease and cannot accurately predict persistence versus eradication of the virus in haemodialysis patients. Although permanent loss of serum HCV RNA appears to correlate with resolution of the disease, little is known about the predictive value of a single HCV RNA value. The aim of the study was to evaluate the viraemia in the serum of HCV antibody positive haemodialysis patients during a period of 3 years.

The study group consisted of 65 HCV antibody positive patients from our dialysis unit. HCV antibodies were measured every 6 months by ELISA third-generation assay. The presence of serum HCV RNA was assessed by reverse-transcriptase polymerase chain reaction (RT-PCR) once a year during the period of 3 years. Serum levels of aminotransferases were measured monthly with standard automated analyzers.

There were three different patterns of viraemia after the third assesment of the serum HCV RNA in HCV antibody positive patients: 47% (30/65) were persistently HCV RNA positive, 38% (25/65) were intermittently HCV RNA positive, and 15% (10/65) were persistently HCV RNA negative. The dominant genotype was 1a, detected in 97% of the patients positive for HCV RNA. The HCV RNA persistently positive patients had significantly higher levels of ALT compared to HCV RNA persistently negative patients (50.07 ± 30.0 *vs.* 28.5 ± 10.0 U/L, $p < 0.027$). There was no significant difference between the three groups of patients according to age, haemodialysis duration, and serum levels of AST.

This pattern of intermittent viraemia clearly showed that a single negative result of the presence of serum HCV RNA in an HCV antibody positive patient should not be taken as a proof of a persistent resolution of HCV. Thus, repeated testing for HCV RNA is necessary to assess viraemia accurately in HCV antibody positive patients. HCV antibody positive patients who were persistently serum HCV RNA negative could be potentially infectious because of the possibility of the persistence of occult hepatitis C.

Key words: hepatitis C, haemodialysis patients, intermittent viraemia, occult hepatitis C.

Introduction

A growing literature has described a very high prevalence of hepatitis C virus (HCV) infection in haemodialysis patients. Diagnosis of HCV infection is based on serological detection of HCV antibodies in the serum of haemodialysis patients. The investigators reported a prevalence of 1% to 80% in haemodialysis patients in contrast to a prevalence of 0.3% to 1.5% in the general population [1–5].

Retrospective and prospective studies of the natural history of HCV infection in the general population demonstrated that acute infection progressed to chronic infection in 85% of the infected persons and spontaneous clearance of the virus was assessed in 15% of the patients [6]. The assessment of the natural history of HCV infection in haemodialysis patients was difficult to obtain for several reasons. The clinical course of HCV infection usually extends over decades rather then years, whereas haemodialysis patients generally have higher morbidity and mortality rates than the general population due to their age and co-morbid conditions, making the long-term consequences of HCV infection difficult to establish. Another reason was limited performance of liver biopsy in haemodialysis patients due to possible hemorrhagic complications [7, 8].

Clinical features, aminotransferases levels, and antibody to HCV have only a limited correlation with the activity of liver disease and cannot accurately predict persistence versus eradication of the virus. Reversed transcriptase-polymerase chain reaction (RT-PCR) has been introduced as a sensitive test to detect HCV RNA in the serum of infected persons [9, 10]. Although permanent loss of serum HCV RNA appears to correlate with resolution of the disease, little is known about the predictive value of a single HCV RNA value.

The aim of this study was to evaluate the viraemia in the serum of HCV antibody positive haemodialysis patients during a period of 3 years.

Patients and methods

The total number of HCV antibody positive patients was 108 (60%) in our dialysis unit. The study group included 65 HCV antibody positive patients. The rest of the patients were not enrolled into the study for reasons of failure to provide informed consent or an inadequate observation period. None of the patients from the study group underwent antiviral treatment during the study and there were no HBs Ag positive patients. All patients received bicarbonate dialysis, and dialysis treatments were performed 3 times per week with a duration of 4 hours.

HCV antibodies were measured every 6 months by ELISA third-generation assay. The presence of HCV RNA in the serum of the study group was assessed by reverse-transcriptase polymerase chain reaction *(AMPLICOR Hepatitis C Virus Test, version 2.0, Roche*) once a year during the period of 3 years. HCV genotype was analyzed by reverse transcriptase polymerase chain reaction followed by hybridization of amplified products.

Serum levels of alanine and aspartat aminotransferases (ALT and AST) were measured monthly with standard automated analyzers. The normal range for our laboratory was 4 to 34 U/L for AST and 3 to 45 U/L for ALT.

Statistical analysis

Numerical data were expressed as means. One way ANOVA was used for group mean comparison between the different groups of patients according to serum HCV RNA status and p less than 0.05 was considered significant. Statistical analysis was performed using the Statistica version 7 program for Microsoft Windows.

Results

The study group included 65 HCV antibody positive haemodialysis patients. The mean age of the patients was 54.1 years (range, 27 to 87 years). The mean haemodialysis duration was 148.1 months (range, 7 to 312 months). The demographic characteristics of patients enrolled in the study are given in Table 1.

There were three different patterns of viraemia after the third assesment of the presence of HCV RNA in the serum of HCV antibody positive haemodialysis patients. Most of the patients, 47% (30/65), were persistently HCV RNA positive, 38% of the patients (25/65) were intermittently HCV RNA positive, and 15% of the patients (10/65) were persistently HCV RNA negative, Figure 1. The dominant genotype was 1a, detected in 97% of the patients positive for HCV RNA.

Table 1 – Табела 1

Demographic characteristics of patients from the study group
Демографски карактеристики на испитуваните пациенти

	No
Male/female	38/27
	X ± SD
Age (years)	54.12 ± 12.38
HD duration (months)	148.17 ± 72.84
Renal disease	**No**
Glomerulonephritis	21
Nephrosclerosis	8
Diabetic nephropathy	3
Interstitial nephritis	13
Adult polycystic renal disease	4
Unknown cause	16

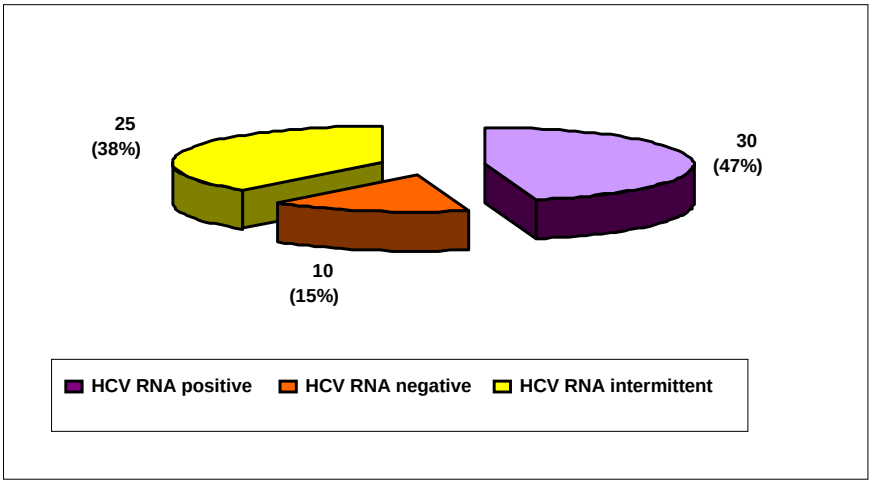

Figure 1 – Distribution of HCV RNA status in the HCV antibody positive patients after the third assesment of viraemia

Слика 1 – Дистрибуција на ХЦВ РНА статусот кај пациентите со позитивен наод на протителата кон ХЦВ, по третото одредување на виремијата

Group mean comparison between the patients with different HCV RNA status according to age, haemodialysis duration, and serum levels of amino-transferases is presented in Table 2.

Table 2 – Табела 2

Group mean comparison between patients with different HCV RNA status
Споредба помеѓу пациентите со различен ХЦВ РНА статус

HCV RNA status	***Age (yr)***	***HD duration (mo)***	***AST (U/L)***	***ALT (U/L)***
HCV RNA positive	53.50 ± 13.77	142.63 ± 78.95	37.00 ± 16.03	50.77 ± 20.52¥
HCV RNA negative	59.40 ± 13.50	176.40 ± 65.74	27.66 ± 13.97	28.49 ± 11.20¥
HCV RNA intermittent	52.76 ± 12.38	143.52 ± 67.84	29.54 ± 13.08	42.23 ± 28.62

¥ $p < 0.027$ HCV RNA positive vs. HCV RNA negative patients

As is shown in Table 2, HCV RNA persistently positive patients had significantly higher levels of ALT compared to HCV RNA persistently negative patients (50.07 ± 30.0 *vs.* 28.5 ± 10.0 U/L, $p < 0.027$). There was no significant difference in serum levels of ALT between HCV RNA persistently positive patients and those with intermittent viraemia, the same as in the comparison between HCV RNA persistently negative patients and those with intermittent viraemia. In addition, there was no significant difference between the three groups of patients according to age, haemodialysis duration, and serum levels of AST.

Disscusion

The natural history of chronic hepatitis C in haemodialysis patients has not been studied extensively. To date, several prior reports have appeared in the literature that considered fluctuations of hepatitis C viraemia over time. Fabrizi F. *et al*. prospectively studied fluctuations of HCV RNA in serum from 52 HCV antibody positive haemodialysis patients, measuring viraemia monthly during a period of 13 months [11]. They observed three different patterns of viraemia: persistent viraemia in 44% of the patients, intermittent viraemia in 33%, and an absence of viraemia in 23% of the patients. Patients with persistent viraemia had a significantly higher viral load and significantly higher serum levels of

aminotransferases compared to patients with intermittent viraemia. Umlauft F. *et al*. documented that 65% (20/31) of HCV antibody positive haemodialysis patients were persistently HCV RNA positive and 35% (11/31) of the patients had a fluctuating course of viraemia with virus-free intervals of up to 4 weeks [12]. With the exception of a lower initial viral load in patients with fluctuating viraemia, there was no statistical difference in age, sex, duration of haemodialysis or serum ALT levels compared to patients with persistent viraemia. In our study, 38% of HCV antibody positive patients had a fluctuating course of viraemia. There was no significant difference in age, haemodialysis duration and serum levels of aminotransferases between persistently HCV RNA positive patients and those with a fluctuating course of viraemia. Several possible causes of the fluctuating course of viraemia among haemodialysis patients were described: the passage of HCV particles into the dialysis compartment [13, 14], the entrapment of HCV particles at the surface of the dialysis membrane [15, 16], destruction of HCV particles by the pressure applied for haemodialysis [17], and an increase in native interferon activity by the dialysis membrane [18, 19].

We found that 15% of HCV antibody positive patients were persistently serum HCV RNA negative over the 3-year study. Does repeatedly negative HCV RNA in serum necessarily reflect spontaneous clearance of the virus in HCV antibody positive patients? Carreno V. *et al*. analyzed 12 HCV antibody positive patients with undetectable serum viral RNA and normal ALT levels in order to determine if there was a clearance of HCV [20]. These patients underwent a programmed interventional laparoscopy for performing the liver biopsy. The HCV RNA was detected in the liver in 10/12 (85%) of the patients, and it was also found that HCV was replicating in the hepatocytes of those patients. Viral RNA was also found in the peripheral blood mononuclear cells (PBMCs) in 6/12 (50%) of the patients.

Occult hepatitis C is a new, recently-characterized entity, which can be presented in two different clinical forms [21]:

– HCV antibody positive patients with normal levels of liver enzymes and without serum HCV RNA and
– HCV antibody and HCV RNA negative patients with abnormal liver function

Castillo *et al*. first described the role of occult hepatitis C in chronic liver disease of unknown etiology in January 2004 [22]. They performed liver biopsies on 100 patients with persistently abnormal liver function, but repeatedly HCV antibody and serum HCV RNA negative. A reverse-transcription polymerase chain reaction found that 57% of them had HCV RNA in their liver. Moreover, 70% of the patients with intra-hepatic HCV RNA also had viral RNA in their PBMCs. Once occult hepatitis C infection was identified, the important question was whether the clinical characteristics of this infection

differ from the clinical course found in chronic hepatitis C. Trying to answer this question, the biochemical, virological and histological features of a group of 68 patients with occult hepatitis C were compared with those of a group of 69 patients with chronic hepatitis C [23]. Serum levels of alanine aminotransferases, necroinflamatory activity and fibrosis, and the percentage of HCV-infected hepatocytes were significantly higher in the group of patients with chronic hepatitis C than in the group with occult hepatitis C. It was concluded that occult hepatitis C is a milder disease, with less liver damage than chronic hepatitis C. To study the issue whether the virus could replicate in PBMCs, 18 patients with occult hepatitis C (intra-hepatic HCV RNA positive) were tested for HCV RNA in their PBMCs [24]. It was found that 61% of the patients with occult hepatitis C had HCV RNA in their PBMCs. Testing for viral RNA in PBMCs should be much more reliable in identifying patients with occult infection when liver biopsy is not available.

To date, there are only two studies which have identified the occult hepatitis C in haemodialysis patients. Thongsawat S. *et al*. enrolled 231 haemodialysis patients from three dialysis centres in Thailand [25]. The presence of HCV antibodies and serum HCV RNA was tested every 6 months for 3 years. Isolation of peripheral blood mononuclear cells (PBMCs) and their additional testing for the presence of viral RNA was also done during the follow-up. Their results showed that 35 patients were HCV antibody and serum HCV RNA positive at the time of enrolment, 51 patients seroconverted during the follow-up, and 2 out of 11 HCV antibody positive patients who transiently lost the detectable serum HCV RNA during the follow up tested positive for viral RNA in their PBMCs. Barril G. *et al*. tested 109 haemodialysis patients who were repeatedly HCV antibody and serum HCV RNA negative, and also had abnormal liver function of unknown cause [26]. Occult hepatitis C was found in 45% (49/109) of the patients, determined by the presence of HCV RNA in their PBMCs. Patients with occult hepatitis C had spent a significantly longer time on haemodialysis and had significantly higher mean alanine aminotransferase levels during the 6 months before the study entry.

In conclusion, three different patterns of viraemia in the serum of HCV antibody positive haemodialysis patients were observed: persistent viraemia, interminent viraemia, and an absence of viraemia. Patients with persistent viraemia had greater aminotransferase levels than those observed in haemodialysis patients with intermittent or with absence of viraemia. This pattern of intermittent viraemia clearly showed that a single negative result of the presence of HCV RNA in the serum of HCV antibody positive patient should not be taken as proof of a persistent resolution of HCV infection. Thus, repeated testing for HCV RNA is necessary to assess viraemia accurately in HCV antibody positive patients. HCV antibody positive patients who were persistently serum HCV RNA negative could be potentially infectious because of the possibility of

the persistence of occult hepatitis C. Thus serum HCV RNA testing should be supplemented with PBMCs testing to maximize the diagnostic sensitivity of HCV infection in the haemodialysis population. Further studies involving a large number of patients with a much longer observation period are required to elucidate the natural history and clinical implications of occult hepatitis C in haemodialysis patients.

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Р е з и м е

ВИДОВИ ВИРЕМИЈА КАЈ ХЕМОДИЈАЛИЗНИТЕ ПАЦИЕНТИ СО ХЕПАТИТ Ц

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Клиничките карактеристики, аминотрансферазната активност и противтелата кон хепатит Ц вирусот (ХЦВ) слабо корелираат со степенот на црнодробното оштетување и не можат да го предвидат присуството или елиминацијата на вирусот кај хемодијализните пациенти. Исчезнувањето на ХЦВ РНА во серумот се поврзува со елиминацијата на вирусот, но сепак малку се знае за предикативната вредност на еден единствен негативен резултат при тестирањето на ХЦВ РНА. Целта на студијата беше да се одредат видовите на серумската виремија (ХЦВ РНА) кај хемодијализните пациенти, со позитивен наод на противтелата кон ХЦВ, во текот на тригодишното иследување.

Во студијата беа вклучени 65 пациенти на хемодијализа со позитивен наод на противтелата кон ХЦВ. Присуството на противтелата кон ХЦВ беше одредувано според методот на ЕЛИСА од третата генерација, на секои 6 месеци. Присуството на ХЦВ РНА во серумот на испитаниците беше одредувано според методот на верижна реакција на полимеразата со реверзна транскриптаза, еднаш годишно во период од три години. Серумските вредности на аминотрансферазите беа одредувани еднаш месечно.

Испитаниците беа поделени во три групи според видот на серумската виремија: постојано ХЦВ РНА позитивни беа 47%, 38% имаа интермитентна виремија и 15% беа постојано ХЦВ РНА негативни. Доминантен генотип беше 1а, присутен кај 97% од испитуваните пациенти со позитивен наод на ХЦВ РНА. Пациентите кои беа постојано ХЦВ РНА позитивни се одликуваа со сигнификантно повисоки вредности за АЛТ споредено со пациентите со постојано негативен наод на ХЦВ РНА (50.07 ± 30.0 *vs.* 28.5 ± 10.0 U/L, $p < 0.027$). Не постоеше сигнификантна разлика помеѓу трите групи на пациенти во однос на нивната возраст, хемодијализниот стаж и серумската вредност на АСТ.

Присуството на интермитентната виремија покажува дека еден единствен негативен резултат при тестирањето на ХЦВ РНА во серумот не смее да се земе како доказ за елиминација на ХЦВ од организмот. Потребно е повеќекратно тестирање на ХЦВ РНА во серумот кај пациентите со противтела кон ХЦВ со цел да се одреди виремичниот статус. Кај пациенти кои

се позитивни за противтелата кон ХЦВ, но се постојано ХЦВ РНА негативни, постои опасност потенцијално да се инфективни заради постоењето на окултна инфекција со ХЦВ.

Клучни зборови: хепатит Ц, хемодијализни пациенти, интермитентна виремија, окултен хепатит Ц.

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