

The prevalence of HCV RNA positivity in anti-HCV antibodies-negative hemodialysis patients in Thrace Region. Multicentral study

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Abstract

Introduction HCV infection in patients under hemodialysis for end stage chronic kidney disease (ESCKD) may exist despite the absence of anti-HCV antibodies. Molecular methods are widely accepted as “gold standard” techniques for the detection of viral RNA. However, the molecular methods are more expensive in comparison to conventional methods and their replacement is not cost-effective. The aim of this study was to estimate the prevalence of HCV RNA positivity in anti-HCV negative hemodialysis patients and evaluate new diagnostic methods for the detection and the monitoring of hepatitis C in ESCKD patients.

Methods The study was performed in four hospitals of Thrace region of Greece and 233 patients with no history of hepatitis C were enrolled. Measurement of anti-HCV antibodies and HCV core antigen was performed by microparticle chemiluminescence immunoassay. Molecular detection of viral RNA was performed by the real-time RT PCR.

Results The mean age of the patients was 64.9 ± 23.3 years. HCV-Ag was positive in 2/233 patients (0.86%). Nevertheless, viral RNA was negative in those patients.

Conclusions The results of the present study showed that the incidence of HCV-RNA in patients with negative anti-HCV Abs, in hemodialysis patients in Thrace region of Greece was negligible (0/233).

Keywords HCV, hemodialysis, HCV RNA

Introduction

The prevalence of hepatitis C virus (HCV) according to the World Health Organization is about 3% worldwide.¹ However, the estimates published in medical literature widely vary. The prevalence of hepatitis C in Greece ranges from 0.5% to 2%.² In developed countries, most HCV infections occur among drug abusers. However, in lower to middle-income settings HCV infection is mostly associated with inadequate

infection control and viral transmission from one person to other.³

Patients on hemodialysis (HD) belong to the high-risk group for HCV infection, with recorded transmission of HCV during dialysis. Akhtar S. et al., in a metanalysis study reported that the HCV prevalence in hemodialysis patients ranged from 1.4-2.3% in developed countries to 4.7-41.9% in developing countries.^{4,5} The dialysis units must implement effective infection prevention and

Received: 06 August 2020; revised: 05 October 2020 and 07 January 2021; accepted: 12 January 2021.

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control programs to prevent HCV transmission. However, small-scale HCV outbreaks were reported and HCV transmission in the dialysis unit (DU) represents a public health problem, especially in developing countries.⁶ HCV infection has been proven as an independent risk factor for fatal liver complications. The diagnosis of HCV infection is currently based on the detection of anti-HCV antibodies by EIA and is confirmed by the presence of HCV ribonucleic acid (HCV RNA). According to Kidney Disease Improving Global Outcomes (KDIGO) guidelines of 2018, the diagnosis of HCV infection in end stage chronic kidney disease (ESCKD) is based on the detection of anti-HCV antibodies.⁷ Measurement of anti-HCV antibodies is recommended every 6 to 12 months for all HCV negative patients. In contrast, previous studies concluded that anti-HCV antibody testing has less reliability for detecting HCV infection in hemodialysis patients.^{8,9} The new clinical guidelines have suggested to search for HCV-RNA after anti-HCV antibodies have been detected, but molecular methods carry several limitations such as cost and high technical skill requirement. The availability of new automated assays such as hepatitis C core antigen (HCVAg) enabled us to propose this kit for the diagnosis of HCV.¹⁰ Furthermore, Moini M. et al., reported their experiences for prognostic value of HCV core antigen assay for detection and its special application for hemodialysis patients.¹¹

The aim of the present study was to evaluate

the prevalence of HCV infection using HCV core antigen and HCV RNA tests among anti-HCV antibodies-negative HD patients from four DU in Thrace, Greece, and assess the efficacy of these two methods.

Methods

The study was approved by the Bioethics Committee of the University General Hospital of Alexandroupolis, Greece (Ethics Committee 13619).

Study population

All dialysis centers of National Health System (NHS) in the Thrace region of Greece were invited and accepted to engage in this study. The study population consisted of >18 years old patients on dialysis program (three times a week), without history of HCV infection. The total number of patients that agreed to participate in the study and signed an informed consent form was 233. More specifically, 41 from General Hospital of Xanthi (Hospital 1), 75 from General Hospital of Komotini (Hospital 2), 74 from General University Hospital of Alexandroupolis (Hospital 3) and finally, 43 patients from General Hospital of Didymoteicho (Hospital 4). The exclusion criteria for this study were as following:

1. Patients who did not give a written consent for participation in this study or were unable to do so.
2. Age under 18 years.
3. Patients with a history of chronic hepatitis C.
4. Patients treated for chronic hepatitis C.

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Article downloaded from www.germs.ro

Published March 2021

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ISSN 2248 – 2997

ISSN – L = 2248 – 2997

5. Patients on peritoneal dialysis.

A questionnaire with clinical and epidemiological data was completed from an interview with the patients, considering the following information: age, gender, duration of hemodialysis (in months), etiology of chronic renal failure, frequency of blood transfusion and jaundice in the past six months, previous renal transplant. All clinical and epidemiological data were registered. The causes of ESKD were recorded according to the personal folder of each patient. Blood samples were obtained from the patients before the initiation of the hemodialysis session. The amount of blood that was drawn was 10 mL. Consequently, it was collected in two tubes, and finally centrifuged. Thereafter, one tube was used for routine laboratory investigations, completed by automated methods. The serum from the second tube was collected and stored at -80°C for HCV core antigen testing and RNA extraction.

HCV core antigen test

The HCV core Ag assay was performed using the Microparticle Immunochemical Luminescence (CMIA) method (Architect, Abbott Diagnostics Division, USA). The cut-off point for positive result was ≥ 3.00 fmol/L. The method has a specificity of $\geq 99.5\%$ and a sensitivity of 97.8%.¹²

Molecular detection of HCV RNA

Viral RNA from the serum samples was extracted by using the Viral RNA/DNA Nucleospin Virus kit, (Macherey-Nagel, Germany), 200 μ L of serum sample were used for RNA extraction. One step real time PCR kit for the qualitative detection of HCV in human plasma (Sacace Biotechnologies, Como, Italy) was used. This qualitative assay was run by SaCycler-96 Real Time PCR System (Sacace Biotechnologies, Italy) with detection limit of 50 IU/mL. Moreover, positive samples were examined by quantitative RT real-time PCR assays with detection limit: 6.00 ± 0.9 IU/mL with Cobas AmpliPrep/Cobas TaqMan HCV v2.0 (Roche Molecular Systems, USA). For each run, positive and negative controls as well as internal control for extraction were included.

Dialysis process

Patients were dialyzed for 4 hours thrice weekly. All patients, except one, were dialyzed in one center only. The anti-HCV positive patients were given dialysis in isolated rooms. A monitoring of the hepatic enzymes such as aminotransferase and anti-HCV testing, on a regular basis, were performed for early detection of HCV acquisition in HD patients. All participants gave their written informed consent to be included into the study.

Statistical analysis

For quantitative variables like age, duration of hemodialysis and number of blood transfusions we calculated mean $\pm$ standard deviation (SD). The data for qualitative variables like gender, cause of kidney failure, prior renal transplant is presented as percentages. Nonparametric tests (Mann-Whitney and Kruskal-Wallis) and the Chi-square (χ^2) test were performed. A value of $p < 0.05$ was considered as statistically significant.

Results

In the present study 233 patients who met the inclusion criteria were enrolled. The mean age of the patients was 64.9 ± 23.3 years (range 18 to 89); men were 59.7% ($n = 139$). The most common causes of ESKD were hypertension and diabetes mellitus. The main demographic and epidemiological characteristics of the study groups are presented in Table 1 and laboratory findings of the cohort in Table 2.

HCV core antigen

The measurement of HCV core antigen was positive in two patients. The first patient was from Hospital 1 (2.4%) and the second one from Hospital 3. There were no positive patients from Hospitals 2 and 4.

The first case was a 49-year-old male with ESKD, secondary to diabetic renal disease and polycystic nephropathy. From his past medical history, he suffered from multiple sclerosis and coronary heart disease treated with coronary artery bypass grafting and angioplasty. In addition, he also suffered from myelodysplastic syndrome, for which he had required blood transfusions the last year. The HCV-Ag value was

Table 1. Demographic characteristics of patients

	Hospital 1 n=41	Hospital 2 n=75	Hospital 3 n=74	Hospital 4 n=43	Total n=233
Age (years), mean $\pm$ SD	58.9 $\pm$ 12.3	68.9 $\pm$ 21.5	66.7 $\pm$ 25.7	68.1 $\pm$ 16.7	65.9 $\pm$ 4.9
Gender (M/F)	25/16	39/36	46/28	29/14	139/94
Dialysis duration (years), mean $\pm$ SD	6.2 $\pm$ 5.3	5.2 $\pm$ 3.1	6.2 $\pm$ 4.1	5.3 $\pm$ 4.1	5.7 $\pm$ 2.4
Cause of ESCKD, n (%)					
Hypertension	7 (17.1%)	13 (17.3%)	20 (27%)	17 (39.5%)	54 (24.5%)
Glomerulonephritis	7 (17.1%)	13 (17.3%)	15 (20.3%)	3 (7%)	38 (16.3%)
Diabetes mellitus	10 (24.4%)	10 (13.3%)	9 (12.2%)	3 (7%)	32 (13.7%)
Polycystic kidney disease	6 (14.6%)	6 (8%)	1 (1.4%)	3 (7%)	16 (6.9%)
Obstructive nephropathy	3 (7.3%)	7 (9.3%)	1 (1.4%)	2 (4.7%)	13 (5.6%)
Bladder reflux	-	2 (2.7%)	1 (1.4%)	-	3 (1.3%)
Cardiorenal syndrome	-	2 (2.7%)	-	1 (2.3%)	3 (1.3%)
Other	8 (19.5%)	22 (29.3%)	47 (63.5%)	14 (32.6%)	91 (39.1%)
History of blood transfusions	6 (14.6%)	12 (16%)	10 (13.5%)	8 (18.6%)	36 (15.4%)
History of transplantation	-	3 (4%)	6 (8.1%)	2 (4.7%)	11 (4.7%)

ESCKD – end stage chronic kidney disease; SD – standard deviation.

Table 2. Laboratory characteristics of the cohort

	Hospital 1 n=41	Hospital 2 n=75	Hospital 3 n=74	Hospital 4 n=43	p value
Hemoglobin (g/dL)	10.3 $\pm$ 2.6	11.3 $\pm$ 2.58	11.3 $\pm$ 2.1	10.3 $\pm$ 1.9	0.801
WBC $\times 10^3/\mu\text{L}$	5.1 $\pm$ 3.1	6.3 $\pm$ 3.2	6.01 $\pm$ 0.9	5.9 $\pm$ 1.1	0.31
Biochemical tests					
SGOT (U/L)	13.5 $\pm$ 1.2	14.5 $\pm$ 1.8	13.8 $\pm$ 2.8	14.4 $\pm$ 2.9	0.909
SGPT (U/L)	15.1 $\pm$ 1.9	13.04 $\pm$ 2.7	15.6 $\pm$ 3.4	18.6 $\pm$ 12.4	0.073
GGT (U/L)	20.8 $\pm$ 3.7	21.8 $\pm$ 9.1	20.4 $\pm$ 6.1	32.1 $\pm$ 19.8	0.088
Creatinine (mg/dL)	6.9 $\pm$ 1.7	7.3 $\pm$ 0.9	7.4 $\pm$ 1.1	6.3 $\pm$ 1.1	0.095
CRP (mg/dL)	1.9 $\pm$ 0.4	2.3 $\pm$ 0.9	1.5 $\pm$ 0.9	1.8 $\pm$ 1.7	0.761
Total proteins (g/dL)	6.1 $\pm$ 1.4	7.04 $\pm$ 1.2	5.9 $\pm$ 0.7	6.4 $\pm$ 0.8	0.401
Albumin (g/dL)	3.5 $\pm$ 0.8	4.1 $\pm$ 0.6	3.4 $\pm$ 0.4	3.4 $\pm$ 0.4	0.612

Data in the table is presented as mean $\pm$ SD.

CRP – C-reactive protein; GGT – gamma-glutamyl transpeptidase; NSS – non-statistically significant; SD – standard deviation; SGOT – serum glutamic oxaloacetic transaminase; SGPT – serum glutamic pyruvic transaminase.

14.49 fmol/L the first time and 15.11 fmol/L the second time. Unexpectedly, the RT PCR was twice negative.

The second case was a 62-year-old female, with ESCKD secondary to diabetic renal disease. The HCV-Ag value was 4.49 fmol/L, and 3.69 fmol/L, in two measurements. In this patient as well, the RT PCR was twice negative.

HCV RNA test

All patients were negative for HCV RNA with qualitative real-time RT PCR. Even for the two patients who were found positive for HCV

core antigen the test could not be confirmed either with highly sensitive quantitative real-time RT PCR, or qualitative RT PCR.

Discussion

Previous studies have demonstrated that patients on HD had an over 10-fold higher risk of HCV infection compared to other chronic patients.¹³⁻¹⁵ Several factors such as longer duration on HD and transfusion of blood components are incriminated as predisposing factors for HCV transmission.¹⁵ In addition,

older age, dialysis treatment in more than one different center, history of transplantation, co-infections with hepatitis B and/or HIV, and diabetes mellitus as comorbidity are all an independent risk factors.^{13,16} Furthermore, HCV infection is associated with the progression of chronic kidney disease and higher mortality in HD patients. Nosocomial transmission of HCV infection can occur through hemodialysis procedures, surgical manipulations, dental treatment, and even by environmental contamination.¹⁷ Previous studies have reported that viremia in anti-HCV negative patients is 0-16%.^{9,18} Dalekos GN et al. recently concluded that routine HCV RNA testing is not necessary, because all patients were negative.¹⁸ However, in a subsequent study the same authors reported that HCV RNA assay increased their diagnostic accuracy by more than 33%.¹⁹ Sakamoto N et al. postulated that HD patients may have prolonged period of HCV seronegativity, secondary to immunosuppression.²⁰ Furthermore, anti-HCV antibody negative patients usually have normal liver function tests and they are not routinely further investigated for the possibility of HCV infection.²¹ It was reported in previous studies that HD patients have lower viremia levels, through membrane-dependent absorption of HCV RNA. According to Etik OD et al., blood specimens for HCV RNA testing should be drawn prior to a dialysis procedure for two reasons: (1) the presence of heparin in the sample can induce a false-positive PCR result; (2) a patient's HCV viral load can decrease during the HD session and return to baseline within 48 h. A patient's HCV RNA could be falsely low due to several reasons. Firstly, the restraint of HCV particles to the dialysis membrane, since HCV virions are larger (30-40 nm) than the membrane's pores (10-20 nm). Finally, with the destruction of HCV particles during the HD sessions these particles rarely escape into the dialysate.²² Studies failed to detect HCV-RNA in the dialysis ultrafiltrate. The previously reported results by R Hubmann et al. indicate that no HCV RNA passed through the dialysis membranes.²³ Nevertheless, the impact of the HD procedure, during hemofiltration and hemodiafiltration, on HCV viral kinetics requires

further investigation. In any case, to avoid false negative results, all samples were obtained prior to the HD sessions.

Previous studies suggest that HCV Ag testing is a sensitive and specific method that can be used for HCV diagnosis in immunodeficient patients.¹⁰ HCV core Ag is a trustworthy marker of viral replication which correlates with HCV RNA. Moreover, Mederacke I et al. reported that HCV core Ag correlates with HCV RNA in HCV-infected patients undergoing hemodialysis.²⁴ Our results are in accordance with previously published studies. A multicentric HD study in France found that only 2 patients out of 4357 anti-HCV negative (0.05%) were positive for HCV RNA.²⁵ Papadopoulos N et al. reported that none out of 41 anti-HCV-negative hemodialysis patients were proven to be viremic.⁹

The presence of two cases with positive HCV antigen and negative HCV RNA tests could be due to the low detection limit of qualitative PCR analysis. We hypothesize that these two patients had low viremic load that could not be detected by our used test. Another explanation is that these are false positive results.

The above mentioned is the main limitation of the present study, and more specifically the detection limit of 50 IU/mL in qualitative RT real-time PCR for detection of HCV RNA.

Conclusions

In conclusion, in this study, in HD patients in Thrace region, we found an exceptionally low level of occult HCV infection. In patients with negative anti-HCV Ab, HCV core Ag as well as HCV RNA was not detected. Therefore, HCV core Ag testing of dialysis patients with negative anti-HCV antibodies is adequate for early detection of HCV infection and subsequently elimination of HCV transmission in DU.

Molecular detection of HCV RNA could be limited only to HCV core Ag positive patients with quantitative RT PCR.

Authors' contributions statement: EIK, EGK and AK contributed to conceptualization, methodology and validation. EF, VR, EK, MK and SZ contributed to laboratory investigation. GR, ET, KK and ET contributed to clinical data curation. TK contributed to writing – original

draft preparation. GM, DC and KM contributed to writing – review and editing. MP and KM contributed to supervision. All authors read and approved the final version of the manuscript.

Conflicts of interest: All authors – none to declare.

Funding: None to declare.

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Please cite this article as:

Konstantinidou EI, Kontekaki EG, Kefas A, Konstantinidis T, Romanidou G, Fotiadou E, Rekari V, Triantafyllidou E, Zisaki S, Kasmeridou E, Andreadou M, Kantartzi K, Mavromatidis K, Martinis G, Cassimos D, Thodis E, Panopoulou M, Mimidis K. The prevalence of HCV RNA positivity in anti-HCV antibodies-negative hemodialysis patients in Thrace Region. Multicentral study. *GERMS.* 2021;11(1):52-58. doi: 10.18683/germs.2021.1240