Hepatitis C Virus Infection in Haemodialysis Patients: "Wolf in Sheep's Clothing"

Col GS Chopra, ^{SM*}, Lt Col RM Gupta⁺, Col SR Gedela[#], Col PP Varma, ^{vSM**},
Lt Gen R Rai, ^{AVSM, VSM, PHS⁺⁺}, Col SK Nema^{##}

Abstract

Background: 170 million people are infected with the Hepatitis C virus (HCV) around the world. Approximately 50%-70% patients infected with HCV develop chronic liver disease. Haemodialysis patients constitute an especially important group with high HCV prevalence. Outbreaks of HCV infection in dialysis units have been documented. Detection of anti-HCV antibodies is a convenient and conventional mode of documentation. However, in this group, it has its own caveats.

Methods: 48 patients who had undergone or were on haemodialysis (HD) and had undergone a minimum of 15 dialysis sittings were studied. HCV infection was documented both by anti-HCV antibody detection and HCV RNA testing. A comparative evaluation of results by both tests was done.

Results: Out of a total of 48 patients, HCV RNA was detected in 38 (79.16%) and anti-HCV antibodies in 13 (27.07%). Out of 48 patients 10 (20.83%) were negative for both parameters. 22.91% (11/48) of patients were positive for both HCV RNA and anti-HCV antibody. 56.25% (27/48) were HCV RNA positive but anti-HCV antibodies were not detectable in their sera. 2 patients (04.16%) had a positive anti-HCV antibody status despite HCV RNA being negative. In 20.83% (10/48) both parameters were undetectable.

Conclusion: Chronic liver disease (CLD), particularly due to HCV infection, is a major complication amongst haemodialysis (HD) patients. Without reliable assays for antigenemia and the inability of antibody tests to define viremia in all cases, the detection of viral nucleic acid is necessary for diagnosis of active HCV infection.

MJAFI 2005; 61 : 241-244

Key Words : Hepatitis C virus; Haemodialysis

Introduction

170 million people are infected with the hepatitis C virus (HCV) around the world and the number is increasing at a rapid pace [1]. Approximately 50%-70% patients infected with HCV develop chronic liver disease (CLD). Broadly, HCV transmission patterns have been divided into parenteral and non-parenteral. Parenteral transmission occurs in transfusion recipients, plasma product recipients, organ transplant recipients, health care professionals and intravenous drug abusers, and haemodialysis patients. Haemodialysis patients constitute an especially important group with high HCV prevalence [2,3]. Among patients on maintenance haemodialysis, the prevalence averages 20%, although there are wide geographical variations. The main risk factors for HCV infection in haemodialysis units include blood transfusions, renal transplantation and treatment within a dialysis unit. Outbreaks of HCV infection in dialysis units have been attributed to poor infection control practices [4]. Nosocomial infection has been documented by phylogenetic analyses [5].

The detection of anti-HCV antibodies using third generation ELISA is a convenient and conventional mode of documenting past or present HCV infection. The most widely used supplemental assay for confirmation of a positive result on ELISA is the recombinant immunoblot assay (RIBA, Chiron Corporation, Emeryville, CA). Immunoassays have also been used for detection of HCV nucleocapsid protein in serum [6]. Without reliable tests for antigenemia and inability of antibody tests to define viremia in all cases, the detection of viral genome is necessary for diagnosis of active HCV infection. HCV probably circulates in the serum at a concentration of 10^2 to 5×10^7 particles per milliliter [7], making it possible to detect HCV-RNA in plasma or tissues using polymerase chain reaction (PCR) [8]. HCV RNA has been found in the blood long before other markers and often within days of infection [9].

This study was carried out in a tertiary care hospital to document HCV infection in a dialysis setting and to correlate the putative roles of anti-HCV antibody

*Senior Advisor (Pathology and Immunology), **Senior Advisor (Medicine and Nephrology), Army Hospital (R&R), +Reader, Department of Microbiology, ##Professor and Head, Dept of Pathology, Armed Forces Medical College, Pune-40, #Senior Adviser (Medicine and Nephrology), Military Hospital, Bareilly, ++Director General Medical Services (Army), New Delhi.

detection and measurement of HCV RNA in this subset of high-risk patients.

Material and Methods

A total of 48 patients [38 of chronic renal failure and 10 post renal transplant (RT) who had undergone or were presently on haemodialysis (HD) at the nephrology center] were included. All patients included had undergone a minimum of 15 sittings of HD. Informed consent was taken prior to collection of samples from the patients. The blood for EIA and RT-PCR was collected by venipuncture in a sterile vacutainer under all biosafety precautions. The sera for EIA were stored at -20°C and those for RT-PCR at -70°C till the tests were carried out.

EIA for anti-HCV antibody detection: The EIA was carried out using third generation ELISA (Murex anti-HCV, version III). In the test, diluted sample was incubated in micro wells coated with highly purified HCV antigens. The intensity of the color measured in optical density (OD) was proportionate to the level of anti-HCV antibodies in the sample. Positive and negative control samples provided with the kit were used to validate the runs and calculate the cut-off values (COVs) as per the manufacturer's guidelines.

RT-PCR for detection of HCV RNA: HCV RNA was detected in clinical samples using *Clonit* (Italy) HCV RNA RT PCR kit. The reaction protocol consisted of three steps: RT, Outer PCR, followed by Nested PCR. The primers were designed to amplify the 5' untranslated region of the HCV RNA Genome, the most conserved region within the various HCV strains. During RNA extraction, extreme caution was observed to prevent Rnase mediated loss of the HCV RNA in the sample. All material including the glassware and plastic ware, the biosafety cabinets were rendered Rnase free. Also DEPC (Diethyl polycarbazide) water was used for preparation of glassware and plastic ware.

Estimation of ALT,AST and GGT : In all cases the three biochemical parameters were estimated at the time of sample collection. The blood chemistry (AST,ALT and GGT estimation) was done on OPERA-2000 Chemistry System (BAYER).

Results

Out of total of 48 patients, 38 patients had CRF and were on HD, whereas 10 patients were post transplant and had undergone HD prior to the transplant. Females constituted 58.33% of the study population whereas 41.67% were males. The number of HD varied from 15 to 196 with a mean of 38 sittings. In all patients HCV RNA, anti-HCV antibody, AST, ALT and GGT were done.

Table 1
HCV RNA and anti-HCV antibody status (n=48)

HCV RNA	Anti-HCV antibody	Total	Percentage (%)
Positive	Positive	11/48	22.91%
Positive	Negative	27/48	56.25%
Negative	Positive	2/48	04.16%
Negative	Negative	8/48	16.66%

Out of a total of 48 patients HCV RNA was detected in 38 (79.16%) and anti-HCV antibodies in 13(27.07%). Anti-HCV antibodies were present prior to dialysis in only two of the patients included in the study population. 11 of them acquired the antibodies during the course of the study. Out of 48 patients 8 (16.66%) were negative for both parameters.

The relationships of HCV RNA and anti-HCV antibody status in case of all 48 patients are shown in Table 1. 22.91%(11/48) of patients were positive for both HCV RNA and anti HCV antibody. 56.25%(27/48) had HCV RNA positive but anti-HCV antibodies were not detectable in their sera. 02 patients (04.16%) had a positive anti-HCV antibody status in spite of HCV RNA being negative. In 16.66% (8/48) both parameters were undetectable.

Results of biochemical parameters estimated in the study population who was HCV RNA positive (n=38) are shown in Table 2. Among patients with positive HCV RNA, majority in our study (52.63%) showed normal hepatic enzymes (ALT,AST and GGT). The AST and ALT were raised in 47.36%, only ALT in 31.57% and only AST in 10.52% of these patients. In all our subjects except one, the GGT levels were within normal limits.

Discussion

Chronic liver disease (CLD), particularly due to HCV infection, is a major complication amongst haemodialysis (HD) patients [10]. A high prevalence of HCV infection among patients being treated with maintenance HD has been attributed to transfusion requirements in this risk group [3]. In the present study, HCV RNA was used as the gold standard to document HCV infection. A correlation with anti-HCV antibody detection was included. A prevalence of 79.16% and 27.07% was observed based on nucleic acid (NA) detection using PCR and anti-HCV antibody detection respectively. Among patients on maintenance HD, the documented prevalence of HCV infection averages 20%, although there are wide geographic variations, ranging from less than 5% for Europe to 30-50% in Japan and Egypt. Intermediate prevalences from 5% to 30% have been reported from India [11,12].

Longitudinal studies have demonstrated the persistence of anti-HCV in patients with chronic HCV infection, in contrast to the transient sero-positivity in those with resolved infection. Also, anti-HCV may

Table 2
Biochemical profile of hepatic function in HCV RNA positive patients (n=38)

ALT	AST	GGT	Total (n=38)	Percentage (%)
Normal	Normal	Normal	20/38	52.63%
Raised	Raised	Normal	18/38	47.36%
Raised	Normal	Normal	12/38	31.57%
Normal	Raised	Normal	04/38	10.52%
Normal	Normal	Raised	01/38	02.63%

persist for many years in patients who have recovered completely from acute infection [13]. One of the most important issues related to anti-HCV antibody detection is that in spite of good immune response, majority of infected individuals fail to clear the infection. Also, anti-HCV antibodies are isolate restricted and are not able to protect super-infection with variant strains of HCV. In view of above, there are significant discrepancies between the prevalence data generated by use of serology and that by NA detection. In the present study only a 28.94% positive concordance was observed between the results of the two techniques.

Without reliable assays for antigenemia and the inability of antibody tests to define viremia in all cases, the detection of viral NA is necessary for diagnosis of active HCV infection. HCV infection leads to a chronic carrier state in many individuals, marked by the presence of HCV RNA but with a negative serology [14], as was seen in 56.25% cases. Negative serology in spite of HCV viremia has been documented in haemodialysis patients [15]. HCV viremia in the absence of anti-HCV may occur in the viremic stage early in acute infection, and is of great importance as such individuals are more likely to transmit the infection in a high risk setting of a HD unit. Contrary to this, it is likely that in some individuals serology is positive without detectable viremia. This occurs both in the immunosuppressed and immunocompetent. In our study, 04.16% of the patients had a positive serology but HCV RNA was undetectable. In two of our patients, the anti-HCV antibodies were detected at the initial screening. In these two subjects the HCV RNA was however negative. These two individuals remained negative for viremia despite a positive serology throughout the course of study. Such a situation has been explained on the basis of three factors: non-establishment of a carrier state, low-level undetectable viremia and intermittent viremia. The latter two are more likely in a dialysis setting.

Among patients with positive HCV RNA, majority in our study (52.63%) showed normal hepatic enzymes (ALT, AST and GGT). The AST and ALT were raised in 47.36%, only ALT in 31.57% and only AST in 10.52% of these patients. In all our subjects except one, the GGT levels were within normal limits. Contrary to our observations, in a 12-year follow-up study, GGT activity was a pertinent marker for assessing the severity of the chronic HCV infection in HD patients [16]. Presence of HCV markers in only 50% of patients of HD with recurrent ALT elevations, so also normal ALT levels in 66.7% of patients who were HCV positive has been documented [17]. These two studies [16,17] and our observations indicate that ALT, AST and possibly GGT may not be useful as surrogate markers of HCV infection

in dialysis patients.

To conclude, our observations and those from others underline the difficulties and caveats in establishing a reliable diagnosis of primary and chronic HCV infection in dialysis patients. The identification and interpretation of atypical seroconversion profiles such as slight, fluctuating or non-existent changes in ALT, AST and GGT activities, delayed detection or absence of anti-HCV antibodies and/or long term persistence of positive or negative serology pose great challenges. A direct test for HCV viremia is thus, probably the only answer to more accurately determine the epidemiology of HCV infection, to identify infectious subjects and to assess the impact of chronic HCV infection on morbidity and mortality in HD patients. Most important of all, it would give us a cutting edge in preventing nosocomial transmission of this agent in a high risk-group setting like haemodialysis.

References

1. World Health Organization : Hepatitis C – global prevalence (update). *Wkly Epidemiol Rec* 2000;75:17-28.
2. Zeldis JB, Depner TA, Kuramoto IK et al. The prevalence of HCV antibodies among haemodialysis patients. *Ann Intern Med* 1990;112:958-60.
3. Chan TM, Lok ASF and Cheng IKP. Hepatitis C infection among dialysis patients: a comparison between patients on maintenance haemodialysis and continuous ambulatory peritoneal dialysis. *Nephrol Dial Transpl* 1991; 6:944-7.
4. Niu MT, Coleman PJ, Alter MJ. Multicenter study of Hepatitis C infection in chronic haemodialysis patients and haemodialysis center staff members. *Am J Kidney Dis* 1993;22:568-73.
5. Sampeiro M, Badalamenti S, Salvadori S, et al. High prevalence of a rare hepatitis C virus in patients treated in the same haemodialysis unit: evidence for nosocomial transmission of HCV. *Kidney Int* 1995;47:911-17.
6. Tanaka E, Kiyosawa K, Matsumoto A, et al. Serum levels of HCV core protein in patients with chronic hepatitis C treated with interferon alfa. *Hepatology* 1996;23:1330-33.
7. Simmonds P, Zhang LQ, Watson HG, et al. Hepatitis C quantification and sequencing in blood products, haemophiliacs and drug users. *Lancet* 1990;339: 1469-72.
8. Kato N, Yokosuka O, Omata M, et al. Detection of HCV RNA in the serum by amplification with PCR. *J Clin Invest* 1990; 86:1764-64.
9. Farei P, Alter HJ, Wong D, et al. A long-term study of hepatitis C virus replication in non-A non-B Hepatitis. *N Engl J Med* 1991;325:98-104.
10. Zeldis JB, Depner TA, Kuramoto IK et al. The prevalence of HCV antibodies among haemodialysis patients. *Ann Intern Med* 1990;112:958-60.
11. Chadha MS, Arankalle VA, Jha J and Banerjee K. Prevalence of hepatitis B and C virus infections among haemodialysis patients in Pune (western India). *Vox Sang* 1993; 64:127-38.
12. Arankalle VA, Chasa MS, Jha J, et al. Prevalence of anti-HCV antibodies in western India. *Ind J Med Res* 1995; 101:91-3.

13. Dittman S, Roggendorf M, Durkop J, et al. Long-term persistence of hepatitis C virus antibodies in a single source outbreak. *J Hepatology* 1991; 13:323-7.
14. Curthbertson B, Reid KJ, Foster PR. Viral contamination of human plasma and procedures for preventing virus transmission by plasma products. Pp.385-435. In Harris JR (ed): *Blood Separation and Plasma Fractionation*. Wiley-Liss, New York, 1991.
15. Goldblum SE, Reed WP. Host defenses and Immunologic alterations associated with chronic haemodialysis. *Ann Intern Med* 1980;93:597-613.
16. Simon N, Courouze AM, Lemarrec N, et al. A twelve-year natural history of hepatitis C virus infection in haemodialized patients. *Kidney International* 1994; 46:504-11.
17. Chan TM, Lok ASF, Cheng IKP and Chan RT. Prevalence of HCV infection in dialysis patients: A longitudinal study comparing the results of RNA and Antibody tests. *Hepatology* 1993; 17:5-8.

Circular from the Office of DGAFMS/DG-3B

Scientific Articles for the Awards

1. Scientific articles are invited from the serving Medical/Dental officers of the Army, Navy and Air Force for the following awards :
 - (a) Chief of Army Staff Award for best published article in the field of Pathology, Microbiology, Hematology and Biochemistry.
 - (b) Chief of Naval Staff Award for best published article in the field of Medicine and allied specialities including Marine Medicine and Aviation Medicine.
 - (c) Chief of Air Staff Award for best published article in the field of Surgery and allied specialities including Dental Surgery.
 - (d) Senior Colonel Commandant & DGAFMS Award for best published article in the field of Preventive and Social Medicine including Epidemiology, Bio-statistics, Health and Hospital administration.
 - (e) Late Lt Gen RS Hoon, PVSM, AVSM (Retd) Award (Cardiac Sciences) for best article in Cardiology carried out by Specialists in the field of Medicine and Surgery and allied specialities.
 - (f) Late Maj Gen Latey Memorial Prize : Essay Competition : 2005 - for best article pertaining to Cardiology, Cardio-thoracic diseases, Heart and Lung diseases and allied specialities like Anaesthesia, Paediatrics, Radiology, Oncology published during the preceding calendar year in any Medical/Scientific Journal including MJAFI.
2. Articles should be submitted through proper channels as per the instructions to the authors printed in Jan 2005 issue of MJAFI. Ten copies each of the article should reach this office by 31st August 2005. Articles received after due date and not through proper channel will not be considered for award.
3. The awards will be presented in the 54th Armed Forces Medical Conference at AFMC, Pune in Feb 2006.
4. Officers will be entitled to submit only one article for consideration of each award. A Board of officers at the office of the DGAFMS will evaluate the articles for selection of the best paper(s). The recipient(s). The recipient(s) of the award will be given an opportunity, subject to exigencies of service to present his/her work at the time of presentation of the award during AFMRC.
5. Officers working in the office of DGAFMS are not eligible to compete for the awards. Articles submitted by the officers other than those working in the office of DGAFMS but having co-authors working in the office of DGAFMS will, however, be eligible for consideration of the award. However, the officers will not be eligible for any award certificate or shared prize money.