ORIGINAL ARTICLE

# HCV-RNA PCR POSITIVITY IN HCV ANTIBODY NEGATIVE PATIENTS UNDERGOING HAEMODIALYSIS

**Ismaa Ghazanfar Kiani, Adil Naseer Khan*, Batool Butt, Sohail Sabir**, Sundas Ejaz, Adeeba Perveen, Eijaz Ghani**

**Military Hospital, Rawalpindi, *Department of Gastroenterology, Ayub Medical College Abbottabad, **Department of Nephrology, CMH Lahore-Pakistan**

**Background:** It's estimated that almost 2.2% of the world's inhabitants suffer from hepatitis C virus (HCV). The most common cause of chronic liver disease in haemodialysis centres is due to HCV. In 1993, it was first described by Bukh and colleagues that HCV viremia can occur without any detectable antibodies to the HCV. Keeping this in mind the purpose of this cross-sectional study was to assess the frequency of HCV in haemodialysis patients by PCR who are serologically negative for HCV. **Methods:** This cross-sectional study was conducted from 1st June to 31st December 2016 on all haemodialysis patients at MH Rawalpindi. Epidemiological data for gender, age, duration on haemodialysis, cause of chronic renal failure and any associated risk factor for acquiring hepatitis C infection was asked. Patients undergoing haemodialysis were investigated by fourth generation ELISA for Anti HCV antibodies, HCV DNA polymerase chain reaction, HCV genotype (where required) and liver function test were also done. **Results:** A total of 201 patients were undergoing haemodialysis. Among these patients 73 were hepatitis "C" negative and 128 were hepatitis "C" positive. Among the 73 patients who were hepatitis C negative by ELISA method 17 (23%) were PCR positive. Of the 17 patients 13 (76.5%) were men and 4 (23.5%) were women. The mean age of the patients was 49.7±18.0 years and mean duration of haemodialysis was 4.4±4.1 months. The most common cause of CKD requiring haemodialysis was hypertension (64.7%). The most common genotype was type 1 (58.8%) followed by genotype 3 (41.2%). The mean viral load was 23583615.70 IU. **Conclusion:** HCV-RNA detection by PCR should be used as standard of care to detect HCV infection in patients undergoing haemodialysis.

**Keywords:** End stage renal disease; Hepatitis C virus; Renal replacement therapy

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## INTRODUCTION

It's estimated that almost 2.2% of the world's inhabitants suffer from hepatitis C virus (HCV).[1] Ali SA and colleagues recently conducted a nationwide survey in Pakistan to look for the prevalence and its associated risk factors for transmission of HCV and HBV.[2] Pakistan has the second highest rate of chronic HCV (4.8%) following Egypt(22%).[3] The most common cause of chronic liver disease in haemodialysis centres is due to HCV.[4] To assess the prevalence of HVC infection in haemodialysis centres most epidemiological studies have employed hepatitis C antibody testing but in recent years polymerase chain reaction (PCR) is used to diagnose HCV viremia by detecting HCV-RNA copies and at some and at some centres it has become the protocol test for detecting HCV viremia.[5] In 1993, Bukh and colleagues were one of the first investigators to identify that HCV viremia can be present without the presence of HCV antibodies.[6] This has been evinced through numerous small patient populations studies, therefore emphasizing the fact that checking out for hepatitis C antibodies alone is indecisive for screening of HCV.[7]

Routine hepatitis C virus screening is recommended for high risk group patients especially in chronic renal failure patients requiring regular maintenance haemodialysis at least twice or thrice weekly. Anti-HCV antibody testing has less reliability for detecting HVC viremia as haemodialysis patients are immunocompromised and the antibody response is blunted due to depressed immune state of such patients. It has also been proposed that the window period between acquiring the infection and production of antibody is prolonged as a result these patients have a negative test antibody test. The objective of the present cross-sectional study was to assess the frequency of HCV in haemodialysis patients by PCR who are serologically negative for HCV reporting to military hospital Rawalpindi.

## MATERIAL AND METHOD

This study was a cross-sectional study conducted at Military Hospital Rawalpindi from 1st June to 31st December 2016. The sampling technique applied in this study was non-probability convenient sampling technique. All male and female patients presenting to the haemodialysis unit irrespective of age who gave consent were included in the study. Prior to initiation of this study informed consent was taken from all the participants and

full disclosure was explained and privacy was maintained for the participants wellbeing. A questionnaire comprising of the epidemiological data for the participants age, gender, duration of haemodialysis (in months), gender, cause of chronic renal failure, frequency of blood transfusion in the past six months, previous renal transplant and history of jaundice in the past was filled by the investigator. The participants were also asked about the risk factor for acquiring HCV from other sources such as intravenous drug abuse, sharing of needles, haematological conditions (requiring blood transfusion), and if they already had chronic liver disease. Laboratory investigations included HCV antibody screening using fourth generation ELISA by Adaltis(Italy) kit, HCV DNA polymerase chain reaction (PCR) by Sacace Biotechnology (Italy), HCV genotype test by real-time hybridization-fluorescence detection technique on Sa Cycler™ (Sacace Biotechnology), liver function test, i.e., aspartate aminotransferase (AST), alanine aminotransferase (ALT), bilirubin and alkaline phosphatase were also carried out. Patients who were HCV positive and didn't give consent were excluded from the study. Data analysis was done using SPSS version 14. Mean and standard deviation (SD) for quantitative variables like age, duration of haemodialysis and number of blood transfusion were calculated. Frequencies and percentages were calculated for qualitative variables like gender, cause of kidney failure, prior renal transplant, history of jaundice and risk factors for acquiring HCV infection.

## RESULTS

A total of 201 patients were getting regular four-hour sessions of haemodialysis done at least twice or thrice a week. The department caters for both hepatitis C positive and hepatitis C negative patients. Haemodialysis machines are separate for positive and negative patients. Fifteen machines are dedicated to hepatitis C positive patients and 12 haemodialysis machines for hepatitis C negative patients. Of the 201 patients 73 were hepatitis C negative and 128 were hepatitis C positive. The 73 patients were hepatitis C negative by ELISA method. Real time PCR was done on blood sample of all the 73 patients who were having negative serology for HCV antibodies, 17 of the 73 patients were PCR positive (23%). Of the 17 patients 13 (76.5%) were males and 4 (23.5%) were female participants.

The mean age of those 17 patients was 49.76±18.00 years. The mean duration of haemodialysis was 4.47±4.14 months. The most common cause of end stage renal disease requiring haemodialysis was hypertension (64.7%), followed by diabetes mellitus (17.6%) and chronic glomerulonephritis (17.6%), autosomal dominant polycystic kidney disease (5.9%), multiple myeloma (5.9%) and obstructive nephropathy (5.9%). History of previous blood transfusion was also asked from the patients, 82.4% gave a history of blood transfusion and 17.6% gave no such history. 11.8% of the participants gave a history of jaundice and 88.2% denied history of jaundice in the past. None of the patients had had a renal transplant in the past (Table-1). All the patients denied history of drug abuse, immunosuppressive therapy was used by 3 patients before they developed chronic renal failure and none of the patients had haematological conditions requiring blood transfusion. The most common genotype was type 1(58.8%) followed by genotype 3 (41.2%). The mean viral load was 23583615.70 IU. The mean serum bilirubin was 8.00±5.89 umol/l, mean serum alanine transferase was 61.82±5.89U/l, mean serum aspartate transferase was 55.88±6.75 U/l and mean serum alkaline phosphatase was 408.64±288.76 IU/l (Table-2).

**Table-1: Demographic data of anti-HCV antibody negative haemodialysis patients**

| Characteristics | | Results |
|---|---|---|
| Gender (n=73) | Male | 50 (68.4%) |
| | Female | 23 (31.5%) |
| HCV DNA PCR | Positive | 17 (23.2%) |
| | Negative | 56 (76.7%) |
| Gender of HCV DNA PCR positive patients (n=17) | Male | 13 (76.5%) |
| | Female | 4 (23.5%) |
| Mean age of HCV DNA PCR positive patients (n=17) | | 49.76±18.00 years |
| Mean duration of HD of HCV DNA PCR positive patients (n=17) | | 4.47±4.14 months |
| Cause of chronic renal failure (n=17) | Hypertension | 11 (64.7%) |
| | Diabetes Mellitus | 3 (17.6%) |
| | Glomerulonephritis | 3 (17.6%) |
| | Autosomal dominant polycystic kidney disease | 1 (5.9%) |
| | Multiple myeloma | 1 (5.9%) |
| | Obstructive nephropathy | 1 (5.9%) |
| History of blood transfusion (n=17) | Yes | 14 (82.4%) |
| | No | 3 (17.6%) |
| History of jaundice (n=17) | Yes | 2 (11.8%) |
| | No | 15 (88.2%) |
| History of previous renal transplant(n=17) | Yes | 0(0%) |
| | No | 17(100%) |

**Table-2: Laboratory investigations of Hepatitis C PCR positive patients and negative antibodies by ELISA**

| Characteristics | | Results |
|---|---|---|
| HCV Genotype | Genotype 1 | 10 (58.8%) |
| | Genotype 3 | 7 (41.2%) |
| Mean quantitative HCV DNA PCR | | 23583615.70 IU |
| Liver function tests | Serum Alanine transferase | 61.82±5.89U/l |
| | Serum Aspartate transferase | 55.88±6.75U/l |
| | Serum bilirubin | 8.00±5.89umol/l |
| | Serum alkaline transferase | 408.64±288.76 IU/l |

## DISCUSSION

The most common cause of chronic liver failure of viral aetiology is due to hepatitis C virus. Even in haemodialysis patients it is the most common reported cause of liver injury and failure. Various factors are involved in the spread of this virus in haemodialysis centres nation as well as worldwide which include the need for repeated and prolonged access to the bloodstream, multiple patients being treated in the same area, the need for blood transfusion and also the duration of haemodialysis increased the risk of exposure to blood borne pathogens such as HCV and Hepatitis B in patients who are on maintenance haemodialysis. The introduction of recombinant erythropoietin had reduced the occurrence of acquiring hepatitis C over the years thus decreasing the requirement for blood products; however nevertheless it remains high in comparison to the general populace.[7]

The survival in haemodialysis patients affected by HCV has a poor quality of life due to the complications of the disease as well as due to the side effects for the antiviral therapy used to treat HCV. Life span of such patients is further reduced. Renal transplant recipients who acquire HCV infection are at an increased risk of acute and chronic allograft dysfunction and also hepatocellular carcinoma leading to a reduction in long term survival. Treatment of HCV in haemodialysis patients before kidney transplantation has become the standard of care thus reducing the complication rate and also preventing the potential risk of transplant rejection.[8] Therefore, kidney transplant patients should be thoroughly evaluated for the presence of HCV infection in the pre-transplant period. The antibody detection with the help of sensitive assays has not culminated in complete elision of the risk of HCV transmission from blood products. A diagnostic test which is authenticated and cost effective is required for detection and management of HCV infection in ESRD sufferers because of increased risk of infection due to prolonged window period.[8]

It is estimated that the prevalence of HCV infection in patients undergoing haemodialysis in the US is approximately 8–10%. However, its prevalence varies the world over ranging from as low as 1–70% in patients on maintenance haemodialysis.[9] The data is scarce regarding HCV infection is patients with chronic renal failure in the developing countries and even less information from a country like ours. A literature review in countries from South America revealed that the rate of hepatitis C infectivity among patients on maintenance haemodialysis varies between 4.2% and 83.9%. This data is mostly obtained from countries such as Argentina, Mexico, Chile, Brazil, Venezuela, Peru and Cuba.[10]

The frequency of HCV infection in our study was 23.2% by HCV PCR quantitative assay. The prevalence of HCV antibody negative viremia The rate of HCV antibody negative viremia identified by PCR method is estimated to be between 0–12% in haemodialysis dependent patients worldwide.[10] In the study done by Bukh and *et al* in 340 Danish haemodialysis patients 8 patients were found to be HCV-PCR positive but were negative for antibodies.[6] Similar results were seen in a study conducted by Kuhn and *et al*[11] and also in a study done by Seelig and *et al* from Germany in which 4.1% of the participants were HCV-PCR positive and HCV-antibody negative[12].

Studies have shown the liver function tests to be normal or near normal in patients who are HCV-PCR positive and antibody negative as a result these patients are not routinely further investigated for the possibility of HCV infection. In our study, the mean serum bilirubin was 8.00±5.89 U/l, mean serum alanine transferase was 61.82±5.89 U/l, mean serum aspartate transferase was 55.88±6.75 U/l and mean serum alkaline phosphatase was 408.64±288.76 U/l which is slightly on the higher side thus arousing suspicion and prompting to investigate the patient further.

In a study done in Iran by Maryam Moini and *et al* 6% (11) of the 181 patients who were anti-HCV antibody negative had detectable HCV RNA with a viral load of 40-336543 IU/ml by PCR.[13] The mean viral load was 23583615.70 IU/ml in our study. All of this data suggests that HCV RNA detection by polymerase chain reaction is essential for the diagnosis of HCV infection in haemodialysis patients.[13]

The shortcoming in our study was that it only included one haemodialysis centre with a scarce number of patients. A larger number of participants

from various centres should be included but none the less, HCV-RNA PCR should be used for hepatitis C infection detection.

## CONCLUSION

From our study, certain recommendations can be made in regards to detection of HCV in our own country. 1. HCV-RNA detection by real time PCR can be used to identify HCV infectivity in haemodialysis patients who are antibody negative. 2. If HCV viremia is detected by real time PCR decision regarding selection of treatment strategy is based upon quantitative PCR and HCV genotype. Additional tests such as liver function tests including serum albumin, alpha fetoprotein levels and diagnostic imaging should be done regularly. Our haemodialysis protocol is to do these tests quarterly in a year.3. Dedicated haemodialysis machines for HCV infected patients in a specified room in order to reduce the spread of HCV transmission. 4. Astringent adherence to universal precautionary protocols, especially strict compliance to all exigent biosafety procedures while in the haemodialysis centre are considered to be the cornerstone to minimize HCV transmission.[14,15] These measures include: (a) using disposable haemodialyzer and proper disposal to prevent dialyser reuse; (b) institute based protocol for proper decontamination of all disposable and non-disposable equipment after each patients haemodialysis session; (c) prohibit the use of the same medication by several patients in the haemodialysis centre such as intravenous fluids and multiuse vials of heparin; (d) prohibit the sharing of instruments such as blood pressure apparatus and tourniquets; (e) separate medication preparing room; (f) disinfecting haemodialysis station surfaces timely; (g) hand sanitization and changing disposable gloves before attending a new patient and (h) scheduled training of haemodialysis staff in haemodialysis unit.

## AUTHORS' CONTRIBUTION

IGK: Data collection, data analysis, discussion. writing. ANK: Data analysis, discussion writing. HS: Data analysis, data collection. BB, SS, SE, AP & EG: Data collection. SS: Data collection.

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## Address for Correspondence:

**Dr. Adil Naseer Khan,** MBBS, FCPS, Department of Gastroenterology, Ayub Medical College Abbottabad-Pakistan
**Cell:** +92 333 509 1321
**Email:** adil_alizai@hotmail.com