

## A Prospective Study of Hepatitis C Virus Infection in Hemodialysis Patients in Jeddah, Saudi Arabia

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**Abstract:** Hepatitis C Virus (HCV) infection is one of the major health problems worldwide. Hemodialysis patients are at an increased risk of acquiring HCV and the prevalence of HCV in hemodialysis patients is generally higher than the general population. The present research is a prospective followup study of the HCV infection in hemodialysis patients attending two dialysis centers in Jeddah, Saudi Arabia. About 75 and 39 patients were enrolled from King Abdulaziz University Hospital and King Fahd General Hospital in Jeddah, respectively. Blood samples were tested for HCV-Ab and HCV RNA at enrollment and negative samples were followed up for a period of 18 months. At KAUH center, researchers found an HCV RNA prevalence of 4/60 (6.7%) one of them had negative HCV-Ab result indicating a case of recent infection. Three other cases were HCV-Ab positive but negative for RNA. Of the 39 cases enrolled at KFGH, we found 22 samples to be positive for HCV RNA 15 of them were HCV-Ab positive while 7 were HCV-Ab negative (recent infection). The results showed that the recommended screening of the hemodialysis patients every 6 months is inadequate in centers with high transmission rate. Strict screening of such transmission is essential preferably every 2-3 months. Researchers recommend to use the HCV-RNA screening by the most sensitive PCR assays, in addition to HCV-Ab, to screen the patients attending hemodialysis centers to decrease the window period, help to detect recent infections and better control transmission.

**Key words:** Hepatitis C infection, hemodialysis, dialysis, RNA, Saudi Arabia

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### INTRODUCTION

Hepatitis C Virus (HCV) infection is one of the major health problems worldwide (Al-Ahdal and Kessie, 1997; Al-Meshari *et al.*, 1995; Al-Mugeiren *et al.*, 1992). Hemodialysis patients are at an increased risk of acquiring HCV and the prevalence of HCV in hemodialysis patients is generally higher than the general population this is due to prolonged vascular access and the potential for exposure to infected patients and contaminated equipment. The rate of infection in developed countries is generally low; 3% in the Netherlands (0.1% in the general population) and 9.4% in Belgium (0.9% in the general population). While the rate of infection in the developing countries much higher; 57% in Saudi Arabia (1.8% in the general population) and 80% in Egypt (15% in the general population) (Al-Ahdal and Kessie, 1997).

An estimated 5-20% of HCV-infected patients have or will develop cirrhosis, 1-4% of whom will annually develop hepatocellular carcinoma (Ayoola *et al.*, 1991). Hepatitis C is the most common cause of liver disease in

patients on HD while liver disease itself is a significant cause of morbidity and mortality in patients with End-Stage Renal Disease (ESRD) treated by dialysis or transplantation (Al-Nasser *et al.*, 1992).

Screening HCV infection is achieved either by serological tests to detect HCV antibodies (Enzyme Linked Immunosorbent Assay, ELISA) and Recombinant Immunoblot Assay (RIBA) to detect current or past infection and by molecular tests to detect HCV-RNA (Babakim *et al.*, 1991). Negative results for HCV-Ab are most common very early after infection (it takes 6-8 weeks for third generation ELISA to yield positive results) or in patients who have an impaired immune system e.g., patients with human Immunodeficiency Virus (HIV) infection, those on Hemodialysis (HD) and patients on chemotherapy drugs (Huraib *et al.*, 1995; Mitwalli *et al.*, 1993; Fabrizi *et al.*, 2002).

A 5 years follow-up study in Aljouf, Saudi Arabia on 36 HCV seronegative HD patients who were under strict infection control regulations and were screened for

HCV-Ab, HBsAg and HIV every 3 months showed that the incidence of HCV was zero (Mohamed, 2010). The two types of tests most widely used for detection of HCV infection are the Enzyme-Linked Immunosorbent Assay (ELISA) anti HCV antibody test which reflects the immune response and the viral RNA test as determined by the Polymerase Chain Reaction (PCR) which measures viremia. These tests differ in their sensitivity and kinetics making it important to give proper interpretation to the results of each test depending on the particular setting in which it is used. The two tests have been compared by several investigators (Al-Ahdal and Kessie, 1997; Bukh *et al.*, 1993; De Medina and Schiff, 1995; Gretch, 1997; Schneeberger *et al.*, 1998).

In general, a significant delay is observed between the detection of HCV RNA to the appearance of anti HCV. In one study this delay has been reported to be  $6.9 \pm 4.1$  months (Furusyo *et al.*, 2001). In another study it was concluded that anti HCV antibodies are not detectable for at least 6 weeks and may not appear for several months (De Medina and Schiff, 1995). On the other hand, HCV RNA may often be found in the patient's serum within the 1st week after exposure. This early detection of infection by testing for HCV RNA may have special implications in the hemodialysis setting. Thus, serologic tests for anti HCV and abnormalities in liver function assays can be negative despite the presence of viremia in these patients.

Bukh *et al.* (1993) reported that 2.6% of dialysis patients in Norway who were seronegative by second generation ELISA were positive for viral RNA by PCR (Huraib *et al.*, 1995). Lower figures were subsequently reported with third generation ELISA. Such figures are expected to increase in proportion to a higher rate of HCV transmission. This makes PCR testing a valuable addition to serology for the monitoring of HCV infections in hemodialysis units (Al-Ahdal and Kessie, 1997). Another reason why HCV RNA detection may be of particular importance in the hemodialysis setting is the partial immunosuppression in these patients, resulting in an inadequate anti HCV response (Bukh *et al.*, 1993; Goldbloom and Reed, 1980; Jadoul *et al.*, 1993; Kuhns *et al.*, 1994).

## MATERIALS AND METHODS

**Specimen collection and processing:** About 75 samples were enrolled and followed up in the study who regularly attended the hemodialysis center at King Abdulaziz University Hospital (KAUH), eight of them died and 7 patients withdrew from the study because they attended another dialysis center. A total of 211 specimens

were collected from these patients (average 3.5 samples/patient) over a period of 18 months from February 2001 till August 2002. Another 39 patients were enrolled from King Fahd General Hospital (KFGH), Jeddah with unknown HCV status. Serum was separated from 5-10 mL whole blood separated, aliquoted into two aliquots and stored at  $-80^{\circ}\text{C}$  till testing.

Data collected from patients included date of collection, gender, age, duration of therapy, nationality and other serological test results.

**Instruments:** For manual PCR procedures a thermal cycler (Techne Progene, UK) and a horizontal gel electrophoresis unit (BioRad, USA) were used. Cobas Amplicor (Roche diagnostics, UK) automated analyzer was used for HCV RNA extraction, amplification and detection.

**RNA extraction:** RNA extraction was performed using QIAamp viral RNA mini kit (Qiagen, Germany) according to manufacturer recommendations.

**Nested PCR and detection:** It was performed using two pairs of sequence specific primers amplifying a 235 bp fragment of the 5'-UTR of the HCV viral genome according to Stuyver *et al.* (1993), the products were run on a 2% agarose gel then visualized by ethidium bromide staining and detection under UV light.

**Cobas-Amplicor:** The fully automated Cobas-Amplicor kits and instruments were used to analyze 211 specimens from KAUH for HCV-RNA following manufacturer instructions. Serum samples were pooled together in groups of five for initial testing to reduce cost, all samples in a positive pool were retested individually to identify the positive sample.

## RESULTS AND DISCUSSION

**KAUH patient samples:** Researchers started the PCR testing with the in house PCR assay for the sake of cost effectiveness. Initially 20 specimens were tested by in house PCR, subsequently we decided to shift to the Cobas-Amplicor automated system where we validated first the 20 in house tested samples where results were concordant then, we continued with this technique for the rest of the study for simplicity, sensitivity and minimizing contamination.

**Manual PCR:** Out of 20 patients at KAUH that were tested individually with manual PCR, 19 were negative while 1 patient was positive (found positive for HCV-Ab).

Table 1: HCV RNA and anti HCV test results for 39 patients at KFGH

Test results	No. of samples
HCV RNA positive and HCV-Ab positive	15
HCV RNA positive only	7
HCV-Ab positive only	2

**Cobas-Amplicor:** Four of the sixty samples tested on Cobas-Amplicor gave positive PCR results where three of them were seropositive and the fourth was seronegative. This negative to positive conversion detected by PCR during the study period indicated a recent infection. Three other patients were positive for anti-HCV but negative by PCR indicating that they had past infections and cleared the viremia. Thus, the findings indicate that there was only one recent infection among the 53 patients which were negative for anti HCV. This represents a ratio of about 1.9% (1/53). However, these results indicated a low rate of HCV infection at KAUH dialysis center. None of the patients with both positive PCR and anti HCV had significantly elevated liver enzymes.

**KFGH patient samples:** The number of enrolled patients in the dialysis center at KFSH exceeds 350. Previous information indicated that HCV infection rate (as measured by anti HCV) exceeded 60%. Patients were tested for anti HCV every 6 months as indicated by international recommendations. Researchers concentrated the efforts on patients considered to be anti HCV negative based on their last screening results (39 patients).

Table 1 shows a summary of HCV RNA and anti HCV testing at KFGH. Of the 39 patients, 22 gave PCR positive results (57%). Of these, 15 were positive for anti HCV and 7 were negative. Of the 39 patients, 17 were positive for anti HCV, 15 of whom were RNA positive and 2 patients were RNA negative.

The findings of this study indicate that HCV infection is a serious and continuing problem in hemodialysis centers in this country and the extent varies between different centers depending on patient population, patient load and the level of adherence to infection control practices. However, with patient movement from center to center, it is expected that the effect of the problem will be widespread.

Most previous studies on hepatitis C infection in hemodialysis units in Saudi Arabia provided an indication of the seriousness of the problem. In the study researchers used PCR to detect HCV RNA and periodically retested patients so as to reduce the window period of infection. This did not lead to dramatic results in the center with a low rate of transmission and a small patient load but still showed the usefulness of PCR in detecting infection before sero-conversion occurred. The results were more dramatic at the center where the rate of transmission was very high.

**PCR testing of patients:** Initially manual PCR was used for testing as a cost effective method, however once established and validated on 23 samples we switched to fully automated Cobas-Amplicore as it was the system used for diagnostic HCV detection in hospitals and health care centers at the time. So, the main part of the study was performed using the Cobas-amplicor. This system has the advantage of automation, a modified base to eliminate cross contamination with the amplified product and internal and external controls to ensure validity of the results.

The present study indicates that the guidelines of testing dialysis patients for anti HCV semi-annually will be grossly inadequate in centers with a high transmission rate. Thus of 39 patients at KFGH who were not known to be infected at the start of the study, 22 (56%) were found viremic. The anti HCV test could identify only 15 of these patients while 7 (18%) were negative for antibody indicating a recent infection. This shows the value of PCR especially in centers with high transmission rate as it will provide a useful lead time of several weeks to several months over serologic testing. Such a lead time would greatly help in the faster application of control measures. These results also indicate the need to perform anti HCV testing more frequently, perhaps every 2-3 months. In a few patients it should be expected that antibody response may remain inadequate due to immune-suppression, thereby making HCV RNA detection imperative. The results are in agreement with those of Schneeberger *et al.* (1998) who concluded that the gold standard for detecting HCV infection in hemodialysis patients should include testing for viral RNA as well as testing for anti HCV antibody. This is supported by other studies which have indicated that serologic assays alone are not sufficient for the diagnosis of HCV infection in dialysis patients.

HCV Transmission in hemodialysis centers at the present time is largely thought to occur by cross infection. Efficient screening of donated blood made transfusion a less likely route of HCV transmission. Strict application of such recommendation is essential but may not be adequate in centers with high transmission. More frequent anti HCV testing e.g., every 2-3 months and HCV RNA testing are recommended in the view. Although, use of separate dialyzers is not indicated by the CDC recommendations for HCV patients, we feel that it should be considered in high transmission settings.

It is recommended that these units conduct testing much more frequently, preferably every 2-3 months. Such units will greatly benefit from using PCR testing for viral RNA. Prompt detection of new infection will make these units have a clear picture of the patient HCV status and the sources of transmission and an additional handle on applying control measures. Pooling is very cost effective at centers with a low prevalence of HCV infection.

However, at centers with a high rate of HCV the cost effectiveness of pooling is diminished as most samples in the pools have to be repeated individually but on applying HCV RNA screening in these centers the rate of transmission is expected to be lower and the testing will be cost effective. Several recent studies in Saudi Arabia determined the genotype of the prevalent strains of the virus in order to see whether particular genotypes are more frequently associated with infection in different settings and may also benefit patients receiving IFN therapy. Genotyping of HCV RNA-positive samples obtained at KFGH will give a clear picture of the relationship of these isolates to each other. This would confirm the cross infection as the main source of transmission.

### CONCLUSION

HCV is a serious and continuing problem in hemodialysis centers in Saudi Arabia and the extent varies between different centers depending on patient population, patient load and the level of adherence to infection control practices.

The study indicates that the guideline of testing dialysis patients for anti HCV semiannually is grossly inadequate in centers with high transmission rate. HCV transmission in hemodialysis centers at present time is largely thought to have occurred through cross infection. Efficient screening of donated blood, made transfusion a less likely route of HCV transmission. Strict screening of such transmission is essential but may not be adequate in centers with high transmission.

### RECOMMENDATION

Researchers recommend that these units conduct tests preferably every 2-3 months. Such units will greatly benefit from using PCR testing for HCV RNA. The use of very sensitive real time PCR system is very effective for the sake of sensitivity and speed. Additional reliability can be achieved by the presence of internal and external controls. Systems which have a modified base to eliminate cross contamination with amplified product further increase the validity of the results.

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