A Prospective Study of Hepatitis 'C' Virus Infection in Hemodialisis Patients in Jeddah, Saudi Arabia

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HCV infection is significantly higher in hemodialysis patients in most countries than in the general population[1]. The rate of infection in some countries may be relatively low, such as in the Netherlands (3%) and Belgium (9.4 %), Intermediate, such as in Italy (22.5%) and Turkey (31.4%), or high such as in Saudi Arabia (57%), Bulgaria (65.8%), and Egypt (80%). This is contrasted with a much lower prevalence in the general population amounting only to 0.1 % in the Netherlands, 0.9% in Belgium, 0.5% in Italy, 1.5% in Turkey, and 1.8% in Saudi Arabia[1]. Notably, such figures only give the average prevalence, as large variations in the rate of infection can exist between different hemodialysis centers in the same country.

The importance of studying the problem of hepatitis C in hemodialysis centers in Saudi Arabia is indicated by the following reasons. First, HCV infection is very common in some of the major centers in this country [2-8]. Second, HCV infection has grave consequences for the dialysis patients, resulting in reduced survival[9,10]. It may result in cirrhosis in about 10% of patients. It can adversely affect the survival of renal transplants in previously infected recipients. HCV infection is also a major factor in mortality after renal transplantation[9,11]. Third, cross infection is recognized as the main source of transmission of the virus[1]. Finally, it is possible to reduce this

problem to a large extent by adherence to strict infection control measures and vigilant monitoring of patient status.

The two types of tests most widely used for detection of HCV infection are the 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 [1,11-14]. 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[15]. 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[12]. On the other hand, HCV RNA may often be found in the patient's serum within the first week after exposure[11]. 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.* reported that 2.6% of dialysis patients in Norway who were sero-negative by second generation ELISA were positive for viral RNA by PCR[7]. 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[1].

Another reason why HCV RNA detection may be of particular importance in the hemodialysis setting is the partial immuno-suppression in these patients, resulting in an inadequate anti HCV response [11,16-18].

Methods of Study

Specimen Collection and Processing: 347 specimens were collected from 67 patients regularly attending the hemodialysis center at the King Abdulaziz University Hospital (average 5.2 samples/patient) over a period of 18 months from February 2001 till August 2002, and on 39 specimens, one time in July 2002, from 39 patients at King Fahd General Hospital, Jeddah. Specimens constituted of 5–10 ml whole blood collected from the hemodialysis ward and transferred to plain glass. Serum was separated and divided into two aliquots which were stored at – 80C.

Specimen Numbering and patient data was carefully programmed into the computer with particular emphasis on ID numbers, date of collection, sex, age, duration of therapy, nationality and other serological test results.

Instruments: For manual PCR procedures a thermalcycler (Techne Progene) and a horizontal gel electrophoresis unit (Midicell EC 350) were used. However some automated analyzers from Molecular Biology department at the K.A.A.U.hospital were also utilized such as Cobas Amplicor (Roche diagnostics).

RNA extraction: high quality contamination free RNA extraction was performed using QIAmp extraction kit that is based upon mini-columns to

provide RT-PCR-able RNA.

Nested PCR and detection: Furthermore nested PCR was performed using two pairs of sequence specific primers. The product was then subjected to 2% agarose gel electrophoresis prior to staining with ethedium bromide and detection on UV light.

COBAS – AMPLICOR: The fully automated Cobas-Amplicor was used to analyze 211 specimens from KAAUH and 39 specimens from KFGH for HCV-RNA. Serum samples were pooled together in groups of five for initial testing to reduce costs. Sample pooling was made possible as indicated by the manufacturer and is commonly followed procedure in blood banks where sample volume for screening is much greater. Up to 24 samples of serum can be pooled together before RNA extraction. All samples in a 'positive pool' were repeated individually to identify the exact sample.

Results and Discussion

I. KAAUH patient samples: The number of patients enrolled in the unit was 75, while 8 of them died during the study, seven were not tested. This left us with 60 patients. Initially 20 specimens were tested by manual PCR. Subsequently we decided to switch to the Cobas-Amplicor automated system where we validated the 20 initially tested and continued with the rest for the sake of uniformity. A total of 211 samples were tested from 60 patients (an average of 3.5 sample/ patient)

Manual PCR

Out of 20 patients at KAAU that were tested individually with manual PCR, 19 were negative while 1 patient was positive. The positive patient had been tested positive even by the serological tests.

Cobas- Amplicor

Four of the sixty samples tested on Cobas-Amplicor gave positive PCR results where three of these were also sero-positive where fourth was sero-negative. Three more gave equivocal results. This negative to positive conversion detected by PCR during our study period indicated recent infection. Three other patients were positive for anti HCV but negative by PCR, indicating that they had past infections and were viremic. Thus, our findings indicate that there was only one recent infection among the 51 patients which were negative for anti HCV. This represents a ratio of 2% (1/51). However these result indicated a low rate of HCV infection at KAAUH dialysis center. None of the patients with both positive PCR and anti HCV had significantly elevated liver profile.

II. 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 six months as indicated by international recommendations [18]. We concentrated our efforts on patients considered to be anti HCV negative (39 patients).

Table 3 gives 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 also positive for anti HCV and 7 were negative. Of the 39 patients, 17 were positive for anti HCV. Of these, 15 patients were also positive with PCR and 2 patients were negative

Discussion

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 were retrospective studies that measured anti HCV in patients using first, or second generation ELISA[1,2,7,10,11,13]. These studies provided an indication of the seriousness of the problem but did not follow infection prospectively however some international studies did prospectively measure the rate of sero-conversion and gave results varying between 7-50% over several months to several years.

In our study we 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. Currently we are not aware of any hemodialysis center in Saudi Arabia, which employs periodic

PCR testing of patients.

Initially manual PCR was tested as a cost effective method, however once established and validated on 23 samples we switched to fully automated Cobas-Amplicore for the sake of sensitivity, speed, and additional reliability which was based on the presence of internal controls. The main part of the study was thus performed using the Cobas-Amplicor. This system has the advantage of automation and, more importantly, a modified base to eliminate cross contamination with the amplified product, and internal and external controls to ensure validity of the results. It is the main system widely used for diagnostic testing of HCV in hospitals and health care centers.

The present study indicates that the guidelines of testing dialysis patients for anti HCV semiannually will be grossly inadequate in centers with a high transmission rate. Thus of 39 patients at KFGH not known to be infected when the test was performed, 22 (56%) were shown to be viremic. The anti HCV test could identify only 15 of these patients, while 7 (18%) were negative for antibody. 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 institution 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 immuno-suppression, thereby making HCV RNA detection imperative. Our results are in agreement with those of Schneeberger[14] 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 [16-19].

HCV Transmission in hemodialysis centers at the present time is largely thought to occur by cross infection [9,11]. 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 our 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. Speedier detection of new infection will make these units have a clearer picture of 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.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. We are planning to perform genotyping of HCV RNA-positive samples obtained at KFGH to have a clearer picture of the relationship of these isolates to each other. This would confirm the cross infection as the

main source of transmission.

Conclusion

The research study had come to a conclusion that 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 particles.

The study also indicate that the guideline of testing dialysis 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 recommendation is essential, but may not be adequate in centers with high transmission.

It is recommended that these units conduct tests much more frequently, preferably every 2-3 months. Such units will greatly benefit from using PCR testing for viral RNA. The use of fully automated Cobas-Amplicore is very effective for the sake of sensitivity, speed and additional reliability which was based on the presence of internal controls, and its automation. Most importantly, the system has a modified base to eliminate cross contamination with amplified product, and the internal and external controls to ensure validity of the results.

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دراسة تطلعية عن عدوى التهاب الكبد الفيروسي من النوع «ج» (Hepatitis 'C') لدى مرضى الغسيل الكلوي بجدة

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المستخلص : متابعة حالات الإصابة بمرض التهاب الكبد الوبائي من النوع (ج) لدى المرضى المنتظمين على الغسيل الكلوي في مدينة جدة.

على مدى ١٨ شهرا تم جمع ٢١١ عينة دم من ٦٠ مريضا يراجعون مركز غسيل الكلى في مستشفى جامعة الملك عبد العزيز (٥١ مريضا لم تثبت إصابتهم بالمرض سابقا) بطريقة تطلعية لملاحظة الإصابات الحديثة بالمرض فور وقوعها ، وكذلك تم اختبار ٣٩ عينة دم من ٣٩ مريضا لم تعرف إصابتهم بالمرض سابقا في مستشفى الملك فهد العام بجدة ، و قد فحصت هذه العينات باستخدام طريقة تفاعل البلمرة التسلسلي (PCR) للتعرف على وجود الحامض النووي الريبوزي (رنا RNA) لفيروس التهاب الكبد الوبائي نوع (ج).

تم تشخيص حالة إصابة حديثة واحدة لدى المرضى المراجعين لمركز غسيل الكلى بمستشفى جامعة الملك عبد العزيز كانت غير مشخصة لسلبيتها بالفحص المصلي، ومن بين ٣٩ مريضا يراجعون مركز غسيل الكلى في مستشفى الملك فهد العام تم تشخيص ٢٢ حالة إصابة بواسطة (PCR) تبين أن ١٥ منهم كانت إصابتهم سابقة إذ كانت نتائجهم إيجابية بالفحص المصلي.

هذه النتائج تؤكد فائدة استخدام تفاعل البلمرة التسلسلي (PCR) بالإضافة إلى الاختبارات المصلية لسرعة التعرف على الإصابة بعدوى التهاب الكبد الوبائي (ج) بين المرضى المراجعين لمراكز غسيل الكلى، مما يساعد على تحديد طريقة حدوث هذه العدوى والعمل على تفادي انتشارها بين المرضى .