Research Paper

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Study of Seroprevalence of Hepatitis C Viral Infection in Haemodialysis Patients in Tirunelveli

Medical Science

KEYWORDS : Prevalence, Hepatitis C Virus, Haemodialysis, ELISA

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ABSTRACT

Hepatitis C is an emerging infection in India. This study was aimed to determine the prevalence of HCV infection among patients who were on haemodialysis for more than six months and to assess the risk factors associated with it and find means to reduce the transmission of HCV infection. This study was conducted at Tirunelveli medical college hospital from October 2010 to November 2011. A total of 121 patients were included in the study. The third generation commercial ELISA kit was used for detection of antibodies to Hepatitis C Virus in the patient's serum. In the present study, Out of the 121 patients tested, 7 (5.8 %) patients were seropositive for HCV infection. The mean age of the positives was 36.9±12.9 years. All of them were male. The results revealed that there was a very strong association between the number of blood transfusions and HCV positivity (P<0.001). The positivity increased according to the number of haemodialysis centres visited, which was statistically very highly significant. (P<0.001) The mean duration of haemodialysis among the HCV positive patients was 28.7 ± 18.3 months with statistical significance. There was an association between HCV positivity and blood transfusion. The possibility of acquiring HCV infection was more, when the patient visited more than one haemodialysis centre and with increased duration of haemodialysis. Strict adherence to universal precautions, well screened blood transfusion, early screening and isolation of patients, proper disinfection measures in dialysis units will definitely reduce the risk of HCV infection transmission.

Introduction

Hepatitis C is an emerging infection in India. Hepatitis C infection is defined as the inflammation of liver caused by Hepatitis C virus (HCV), an envelope, single stranded, positive sense RNA virus.[WHO- Hepatitis 2002] The main transmission route of HCV is parenteral, like[1,2,3]. blood (0.5% to 13%)and blood products transfusion(66%) , haemodialysis (20%),organ transplant (50 to100%), nosocomial (2to20%), intravenous drug users (70 to 90 %),tattooing(12.6%),mother to child (4 to7%) ,sexual (0.23%) and household contacts(4%) [1].However, approximately 10% of the cases are sporadic, without well-defined transmission routes [4,5]. Hepatitis C Virus has a causative role in chronic hepatitis, cirrhosis of liver and hepatocellular carcinoma.

The term chronic renal failure applies to the process of continuing significant irreversible reduction in nephron number, and typically corresponds to Chronic Kidney Disease (CKD stages 3-5).The End Stage Renal Disease (ESRD) represents stage 5 of CKD.[6] In these patients with renal challenge, haemodialysis is the mainstay. Haemodialysis is an artificial means of removing nitrogenous and other toxic products of metabolism from the blood. Infection due to Hepatitis C virus is a significant problem in haemodialysis patients [7]

Patients on haemodialysis are at high risk of infection by HCV because these patients are often anaemic and require multiple blood transfusions. Moreover, they have prolonged vascular access and frequent parenteral interventions. In addition, a state of partial immunosuppression prevailing in this population aggravates the risk of HCV infection.[8,9]. Hepatitis C virus infection also affects the post kidney transplant outcome and has been associated with greater morbidity and mortality[8].

The estimated global prevalence of HCV infection is around 3% of the world population, resulting in about 170 million HCV infected persons.[10]This is nearly five times more than Human Immunodeficiency Virus (HIV) infected individuals. The WHO has estimated that 3 to 4 million people worldwide are newly infected with HCV infection every year.The prevalence is consistently higher in haemodialysis patients (12-15%) than in the general population (1.8-2.5%) [11-13].

The treatment is often ineffective and expensive. Particularly in Indian patients with socioeconomic constraint, main emphasis is on preventive strategies.[14]Hence, HCV screening and careful attention to infection control precautions are mandatory for dialysis units to prevent spread of HCV. An early diagnosis in the course of the disease can increase the chances of successful treatment and can limit cross-infection.

This study attempts to find the prevalence of HCV infection in haemodialysis patients and its association with risk factors and the means to reduce HCV infection in haemodialysis units.

The present study was aimed at finding the sero prevalence of HCV infection in haemodialysis patients and to analyse the risk factors for acquiring HCV infection. It was conducted at Tirunelveli Medical College Hospital, Palayamkottai, Tirunelveli district, TamilNadu from October 2010 to November 2011. The third generation commercial ELISA kit was used for detection of antibodies to Hepatitis C Virus in human serum.

Materials and Methods

Study group

A total of 121 samples were collected from chronic renal failure patients who underwent haemodialysis for more than 6 months.

Inclusion criteria:

- Adult chronic renal failure patients who underwent haemodialysis for more than 6 months were included.

Exclusion criteria:

- Adult chronic renal failure patients who underwent haemodialysis for less than 6 months were excluded.
- Paediatric population were excluded.

Ethical clearance:

As this study involved in collection of blood from human beings, ethical committee clearance for conducting the study was obtained from the college ethical committee before the commencement of the study.

Informed consent:

All the patients and relatives were explained about the study in their mother tongue and informed consent was obtained from them before collecting the sample.

Questionnaire:
A printed questionnaire was filled regarding the details of the patients name, age, sex,previous blood transfusion , surgery, history of jaundice, history of IV drug abuse, sharing of razors and tooth brushes, haemodialysis details-first haemodialysis ,number, mode of haemodialysis, number of centres visited, number of times A-V fistula made, filter reuse , general condition ,pre-existing disease.

Sample collection and serum separation:
About 2-5 ml of venous blood was collected from all subjects under aseptic precautions in disposable centrifuge tubes and labelled. The collected blood samples were allowed to stand for 30 mts and subjected to centrifugation for separation of serum at 2000 rpm for 3 minutes. The separated serums were transferred to disposable cuvettes with the help of micropipette and were properly labelled. The haemolysed samples were discarded and fresh samples were collected in those patients.Samples were stored at -20° in a deep freezer, until testing.

Enzyme Immuno Assays (EIA):
Principle:
The ERBALISA KIT is a solid phase immunoassay, utilising a mixture of synthetic peptides and recombinant proteins of HCV, CORE, NS3, NS4 and NS5 for detection of HCV antibodies present in the serum and plasma. When human serum or plasma is added to the well, the bound antigen present in well form a stable complex with the anti HCV antibodies present in the test or positive control specimen .After washing anti human IgG-HRPO is ae to the wells Only the bound antigen –antibody complex present in the well ,will react with the conjugate molecule. The second washig step will remove the unbound conjugate molecule Addition of colour reagent, will develop colour only in positive control wells and wells containing anti- HCV antibodies in the serum(test sample). The intensity of colour development is directly proportional to the presence of bound HCV antibodies in the respective wells.

ELISA kit:
The third generation ELISA kit for detection antibodies to Hepatitis C Virus in human serum and plasma was purchased from ERBA diagnostics, Germany. Serum samples were screened for anti-HCV antibodies according to the manufacturers' instructions.

Interpretation of results:
Non – Reactive: If the absorbency of the test serum is less than the cut –off value, then the sample was considered as non – reactive.

Reactive: If the absorbency of the test serum is equal or greater than the cut – off value, then it was considered as initial reactive. This sample was retested as duplicate. If the absorbency of duplicate retest result was less than cut – off value, then the specimen was considered as non - reactive .If both of the duplicate and retest results were found reactive, then the specimen was considered as repeatedly reactive. Manufacturers' instructions were strictly followed.

Results
The prevalence of HCV infection among the patients is shown inTable-1.A total of 121 samples were collected from chronic renal failure patients who underwent haemodialysis for more than six months. Out of the 121 patients tested, 7 (5.8 %) patients were seropositive for HCV infection and 114 patients were anti- HCV negative. The prevalence was 5.8% and the same among the population was within the range of 1.6% to 10% at 95% confidence intervals. The mean age of the positives were 36.9±12.9 years and the same of the negatives were 45.9±13.7 years.

Blood transfusion and HCV positivity
The table given below [Table -2] explains the relationship between the number of blood transfusions given and the HCV positivity. Out of the HCV positive group, one patient had a history of blood transfusion less than 5 times and one had a history of 5 to 10 times. Five of the HCV positive patients had a history of blood transfusion more than 10 times .The results revealed that there was a very strong association between the number of blood transfusions and HCV positivity ($P<0.001$).

Duration of haemodialysis and HCV positivity
The mean duration of haemodialysis among the HCV positive patients was 28.7 ± 18.3 months. The mean duration in the HCV negative group was 18.4 ± 11.8 months. The difference of means between the two groups was 10.3 months and the same was statistically significant ($P< 0.05$).

Kidney transplantation and HCV positivity
The association between the kidney transplantation and occurrence of HCV among the haemodialysis patients is shown in the Table -3.Two patients with HCV positivity had previous history of kidney transplantation. The association between kidney transplantation and HCV positivity was statistically significant. ($P < 0.001$)

Association of Risk Factors with HCV Positivity
Among the study group 18 persons had undergone surgery earlier. Two persons had acupuncture and tattooing. When the duration of haemodialysis was more than one year, the patient had a risk of HCV positivity of 11.33 times more. Patients who had blood transfusion more than five times had 6 times the risk for acquiring HCV infection. Similarly when a patient had undergone haemodialysis in more than two centres there was nearly a risk of 1.3 times. Those with kidney transplantation had an equal risk as others.

Discussion
Issues like HCV prevalence with respect to age and sex and the risk factors associated with HCV positivity like blood transfusion, number of haemodialysis centres visited, duration of haemodialysis and kidney transplantation are discussed here.

In the present study, the prevalence of HCV infection among the study group was observed to be 5.8% In comparison to other Indian studies the prevalence rate observed in this study was low.[13,15,16].The low prevalence rate may be attributable to the proper adherence to universal precautions, early screening of patients undergoing haemodialysis, and use of dedicated machines (separate haemodialysis machines for HCV positive patients).

The mode of spread of HCV infection among haemodialysis patients was mainly blood transfusion before 1990, but nowadays the scenario has changed since highly sensitive screening tests have been employed in blood banks [17]. However, despite screening of blood for HCV and the wide use of erythropoietin, some patients still become infected by HCV during haemodialysis. The reason for this may be, the donor was in the window period masking the infection or improper screening in blood banks. The other contributing factors supporting transmission of HCV infection are nosocomial factors, longer duration of haemodialysis, and visiting more than one centre.

In the study interestingly there was a correlation between kidney transplantation and HCV positivity.The blood transfusions given during surgery and the state of immunosupression prevailing in these patients, favour occurrence of HCV infection in them. The prevalence of HCV infection in haemodialysis patients varies worldwide from country to country, region to region and also from one unit to another (1%-62[18,19]. There are only a few studies done on prevalence of HCV in haemodialysis patients in India. The prevalence of HCV on haemodialysis patients in India was in the range of 3 to 45%.[20]

Reddy et al studied the prevalence of HCV infection on haemodialysis patients in Nizam Institute of Medical Sciences, Hyderabad .They have reported a prevalence of 5.9% of HCV positivity.[21]Another study by EghlimNemati etal from Iran had a similar prevalence of 5.3% HCV positivity among patients on haemodialysis. [22]. A HCV prevalence of 4.3% was reported by Agarwal et al among the patients who underwent haemodialy-

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sis at the All India Institute of Medical Sciences, New Delhi[23]. Harmankaya et al study showed a prevalence of 4.7% of HCV positivity in patients of a haemodialysis unit in Turkey.[68] The above studies were within the range of the HCV prevalence of the present study.

Murthy et al's study also reported a prevalence of 7.5% of HCV positivity, in patients on haemodialysis from Vellore [14]. A study from Jammu and Hyderabad, by Reddy et al and Sudan etal had an anti- HCV prevalence of 8% and 9.9% respectively. [24].Jasuja et al, evaluated the prevalence of HCV viremia in haemodialysis patients at a tertiary care hospital in Delhi .A positivity of 27.7% was reported.[16] In Jain et al's study on maintenance haemodialysis patients in Jaipur, 30% had HCV infection. [25] Another study by Chandra et al reported 46% HCV positivity in patients on maintenance haemodialysis from Hyderabad. [13]This was a higher HCV prevalence report from India The above studies postulate that the risk factors are haemodialysis environment, blood transfusion ,duration of dialysis,

However some studies showed a very low prevalence. A national survey of hepatitis C virus (HCV) infections among dialysis patients in Netherlands performed by Schneeberger et al reported a HCV prevalence of 3.5% in patients on haemodialysis suggesting the existence of local risk factor in dialysis centres for acquiring HCV infection.[26]

Alavian et al report a prevalence of HCV positivity as 13.2%, Rais jalali et al-5.5%, and Ansar et al -55.5%, among haemodialysis patients in Iran. There was a wide variation in the HCV prevalence in Iran like India. Blood transfusion and nosocomial transmission were risk factors for HCV transmission. [26]

The present study shows that the patients who had blood transfusion more than five times had six times the risk for acquiring HCV infection. Blood transfusion plays a role in HCV transmission in haemodialysis patients, despite screening of blood products for HCV and the wide use of erythropoietin, which reduces blood transfusion requirements, some patients still become infected by HCV during haemodialysis.[27,28].

In the present study, one patient who had undergone haemodialysis over a period of 6 to 12 months (1 year) was HCV positive .Two patients were positive with a duration of 12-24 months (2 years), three patients who showed HCV positivity, had a history of haemodialysis for 24-36 months (3 years) and one patient was positive with a duration of dialysis of 60 -72 months (5 years).The mean duration of haemodialysis of the HCV positive patients was 28.7 ± 18.3 months and of the negative patients was 18.4 ± 11.8 months. The difference of means between the two groups was 10.3 months and the same was statistically significant ($p < 0.05$).When the duration of haemodialysis was more than one year the risk of HCV positivity was 11.33 times more.

In present study, four patients had undergone kidney transplantation among them, two were HCV positive (50%).This was statistically significant (P< 0.001).In a report from Vellore, out of a group of 68 renal transplant patients 60.5% were anti HCV positive [29].Another study by Sampathkumar et al from Madurai, report a seropositivity of 54% in post transplant patients.[30] The prevalence of anti-HCV-positivity among renal transplant recipients ranges from 6%to 46% with wide variation among different centres and countries with lower incidence (5% to 15%) in developed countries, and much higher frequency in developing countries[31].

The prevalence of Hepatitis C infection has been found to be significantly high in renal transplant patients as well. The state of immunosuppression prevailing in these patients and blood transfusions given during kidney transplantation may favour the spread of HCV infection.

Hepatitis C virus (HCV) infection is an important consideration in kidney transplantation candidates since mortality rates are higher among HCV-infected dialysis patients than HCV-negative patients. Similarly, kidney transplant recipients who are positive for HCV antibodies (anti-HCV) have higher rates of liver complications and lower survival rates after transplantation than anti-HCV–negative recipients. On the basis of United Network for Organ Sharing data from January 2004, anti-HCV is present in 5.2% of patients who receive renal transplants.[31]

In this study, data supporting the well recognised risk factors for transmission of HCV infection among haemodialysis patients such as intravenous drug abuse, needle stick injury, sharing of razor blades, IM/IV injection administration, previous surgery, acupuncture or tattooing, history of sexually transmitted diseases could not be obtained.

In conclusion, the present findings showed that attention should be given to strict adherence to infection control measures in dialysis setting.and proper disinfection of dialysis units. Health education for patients undergoing haemodialysis, on infectious diseases and the modes by which they can be acquired may be given. Training and orientation programmes for health care workers about HCV infection hazards.HCV screening should be done monthly for all the patients who are undergoing haemodialysis.

Table 1: Prevalence of HCV among the haemodialysis Patients

Screened	Frequency	%	Estimated percentages in the population at 95% confidence interval
Positive	7	5.8	1.6% to 10.0 %
Negative	114	94.2	90.0% to 98.4%
Total	121	100.0	

Table 2: Comparison of blood transfusion and HCV positive and negative

Blood transfusion	Haemodialysis Patients			c^2	degree of freedom (Df)	p value
	Positive	Negative	Total			
<5 times	1	78	79			
5-10 times	1	23	24	19.049	2	P<0.001
>10 times	5	13	18			
Total	7	114	121			

Table-3: Comparison Positive and negative of HCV in Kidney transplantation and among patients who have not undergone kidney transplantation

Kidney transplantation	HCV			c^2	d.f	P-value
	Positive	Negative	Total			
Yes	2	2	4			
No	5	112	117	14.838	1	P<0.001
Total	7	114	121			

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