Seroprevalence of Hepatitis C virus (HCV) in patients on maintenance haemodialysis at tertiary care hospitals of Pune, Maharashtra, India

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Abstract

Introduction: High prevalence of Hepatitis C virus (HCV) has been reported among the dialysis patients throughout the world. Aim of the study was to evaluate prevalence of HCV in haemodialysis patients at tertiary care hospitals of Pune, Maharashtra, India.

Materials and Methods: A cross sectional study design was conducted among 250 patients from five dialysis centres in pune city including both government and corporate hospitals who reported to the nephrology department for haemodialysis in a period of one year. Cases were studied in detail about complete history, clinical and demographical profile as well as several biochemical parameters. A predesigned, pretested questionnaire was used for data collection. SPSS 21.0 version software was used to analyze the data.

Result: Prevalence of HCV Infection in patients on hemodialysis was found to be (18.8%). Out of total anti-HCV antibody positive patients (59.6%) were found to be male and (40.4%) were female. Out of total anti-HCV antibody positive cases (72.3%) had been on dialysis for more than 5 years. About (68.1%) of total positive cases received blood transfusion once or more in life time.

Conclusion: In developing countries with a high endemic background of HCV infection surveillance programs and efforts to increase awareness, improve diagnosis and facilitate treatment of acute HCV will have far reaching implications for the management of chronic HCV. Thus preventive measures and the adherence to universal precautions for HCV control remain a priority.

Keywords: Seroprevalence, Hepatitis C, Haemodialysis, Tertiary care hospitals.

Introduction

High prevalence of Hepatitis C virus (HCV) has been reported among the dialysis patients throughout the world.[1] Chronic infection with hepatitis C virus (HCV) is one of the major causes of liver cirrhosis and hepatocellular carcinoma. The high rate of chronicity combined with the lack of a successful vaccine makes HCV infection a serious public health challenge.[2]

Hepatitis C virus is a single stranded enveloped positive stranded RNA virus which belongs to genus hepacivirus, family flaviviridae initially recognized as non A non B hepatitis virus in 1974.[3, 4] In 1989 agent responsible for non A non B hepatitis was cloned & identified as HCV.[6] HCV Infection is major public health problem with an estimated Global Prevalence of approx 3% i.e. 180 million people.[1] In India 12.5 million people are infected with HCV infection.[2] An estimated 5-20% of HCV infected patients have or will develop cirrhosis, 1-4% of whom will annually develop hepatocellular carcinoma. Well known routes for HCV transmission include intravenous drug use, blood transfusion, organ transplantation, chronic haemodialysis, occupational exposure among health care workers, unprotected sexual contact and vertical transmission.[5]

Prevalence of HCV Infection among dialysis patient is much higher than healthy blood donors.[3] Studies held in dialysis centre from different countries revealed that prevalence ranges from 1-84.6% & there is particular concern because HCV chronic infection causes significant morbidity and mortality among patient undergoing haemodialysis. Studies in developed countries shows prevalence of 5% to 35% in patients undergoing maintenance haemodialysis.[1]

Increased incidence of HCV infection is noted universally in patients undergoing haemodialysis & study of prevalence of HCV is important to prevent device induced infection in a nephrology setting. This helps in profiling the incidence, prevalence and gravity of the problem & so also the viral load. Cirrhosis and other liver related deaths are reported more frequently in HCV infected dialysis patients than in those without the virus. Evaluation of patients with chronic HCV infection is warranted to determine the stage of disease and need for HCV therapy.

In this background this study was conducted to evaluate prevalence of HCV in haemodialysis patients at tertiary care hospitals of Pune, Maharashtra, India.

Materials and Methods

This was a prospective study carried out in tertiary care hospitals of Pune from July 2014 to June 2015. The study comprised of 250 patients from five dialysis centres in pune (2 government hospitals and 3 corporate (private) hospitals who reported to the nephrology department for haemodialysis. Their clinical and

demographical profile as well as several biochemical parameters was recorded**.** Informed consent was obtained from concerned departments & patients.

Blood was collected in a 10 ml vacutainer aseptically and serum was separated using centrifuge at 2000 rpm for 5 min. The serum so collected was stored at -70^0 C for Anti- HCV antibody detection.

Data collected was complied in Microsoft excel software and checked for its completeness and correctness before data was analyzed. Descriptive statistical analysis has been carried out in the present study. Results on categorical measurements are presented in numbers (%). Chi-square test been used to find the significance of study parameters on categorical scale between two or more groups. P-value of <0.05 was considered to be statistically significant. SPSS 21.0 version software was used to analyze the data.

Anti-HCV Antibody Detection

HCV antibody was detected using kit manufactured by SD HCV ELISA. This is a 3rd generation enzyme immunoassay in which the micro plate wells is coated with HCV recombinant antigens (Core, NS3, NS4, and NS5).

Principle

The HCV antibody kit is based on "indirect sandwich" ELISA principle. The antigens are derived from "core" and "NS" conserved region encoding for immunodominant antigenic determinants.

Procedure

Either fresh sera or plasma (EDTA, Heparin and Citrate) can be used for the assay. The sample can also be stored at 2-8 degree Celsius for 1 week or in case of longer storage freezed at- 20 degree Celsius. The sample was dispensed into the well, reacted with the solid phase and the antibodies to HCV, if present were captured by antigens. After washing in ELISA washer all the other components of the sample, in the second incubation, bound antibodies to HCV were detected by the addition of goat anti- human IgG antibody, labelled with horse radish peroxidase (HRP). The enzyme captured on the solid phase, acts on the chromogen/substrate solution (TMB), generates an optical signal that is proportional to the amount of antibodies to HCV present in the sample. The procedure was carried out as per manufacturer's protocol. The assay was considered valid if the optical density (OD) of the Al blank well was < 0.100. Higher values were index of chromogen/substrate contamination; after blanking on Al, the OD 450 nm mean value of the negative control (NC) was < 0.200. Abnormal values may be observed when the washing instrument does not work correctly or the washing Procedure has not been adapted to the assay. The OD 450nm value of the positive control (PC) was > 1.000. Lower values could result when the storage temperature was not optimal or with an incorrect operative procedure. After the validity of the assay was confirmed, cut-off value was calculated through the formula;

Cut-off = NC mean + 0.400

Grey – zone = +/- 10%

Interpretation

Samples with OD value (measured in ELISA reader) below the lower limit of the grey-zone were reported as negatives. Samples with an OD value within or exceeding the upper limit of the grey-zone were reported as initially reactive. The sample was re-tested in duplicate. As per manufacturer the sensitivity of the kit was 99.5% and had 99.6 % specificity.

Result

The study involved 250 patients on maintenance haemodialysis. The largest number of cases (36%) were in the age group of 51-60 years & lowest number of cases (2.4%) were in age group of 11-20 years. Mean age of cases in the study was 47.3 years with the range 11 to 70 years. (82.8%) cases were males and (17.2%) were females. (Table 1)

The largest number of cases (78.4%) were of chronic kidney disease which includes chronic renal failure, end stage renal disease & chronic glomerulonephritis. Least number of cases were found to be malignant (5.2%). (Table 2)

Prevalence of HCV Infection in patients on hemodialysis was found to be (18.8%). Out of total 207 males about (13.5%) were found to be positive for anti-HCV antibody and out of total 43 females (44.1%) were found to be positive for anti-HCV Antibody. This association was found to be highly statistically significant with p value < 0.01.This shows that sex is significantly associated with anti-HCV positivity. (Table 3)

Out of total anti-HCV antibody positive cases about (72.3%) had been on dialysis for more than 5 years. Out of total 250 patients 141 patients were on dialysis for moré than five years & out of these about (24.1%) were positive for anti-HCV antibody. Whereas out of total 250 patients 109 patients were on dialysis for less than five years & out of these only (11.9%) were positive for anti-HCV antibody. This association was found to be statistically significant with p value < 0.05. This shows that >5 years of dialysis is an important risk factor for anti-HCV antibody positivity. (Table 4)

About (68.1%) of total positive cases received blood transfusion once or more in life time. Out of total 250 patients 114 gave history of blood transfusion & out of these about (28.1%) were found to be anti-HCV antibody positive. Whereas out of total 250 patients 136 gave negative history of blood transfusion & out of these only (11%) were found to be positive for anti-HCV antibody. This association was found to be

statistically highly significant with p value < 0.01. Positive history of blood transfusion was found to be significant for anti-HCV antibody positivity and blood transfusion was found to be important risk factor for HCV infection. (Table 5)

Table 1: Age and sex distribution of hemodialysis patients (n=250)

Age Groups (Years)	Male	Female	Total
11-20	4 (66.7%)	2 (33.3%)	6 (2.4%)
21-30	31 (86.1%)	5 (13.9%)	36 (14.4%)
31-40	18 (75%)	6 (25%)	24 (9.6%)
41-50	51 (85%)	9 (15%)	60 (24%)
51-60	77 (85.6%)	13 (14.4%)	90 (36%)
61-70	26 (76.5%)	8 (23.5%)	34 (13.6%)
Total	207 (82.8%)	43 (17.2%)	250 (100%)

Table 2: Clinical Profile of Study Population (n=250)

S.No.	Clinical Profile	No. of Patients	Percentage
1	Chronic Kidney Disease	196	78.4%
2	Diabetic Nephropathy	24	9.6%
3	Reno vascular Hypertension	17	6.8%
4	Malignancy	13	5.2%
Total		250	100%

Table 3: Prevalence of anti-HCV antibody among all Haemodialysis patients (n=250)

Gender	Anti HCV antibody			Total
	Positive	Negative	% Positivity	
Male	28	179	13.5	207
Female	19	24	44.1	43
Total	47	203	18.8	250

N = 250, X^2 = 21.924, DF = 1, p < 0.01 (0.000003)

Table 4: Distribution of anti HCV antibody positive patients according to duration of Dialysis

Year Range	Anti HCV antibody			Total
	Positive	Negative	% Positivity	
< 5	13	96	11.9	109
> 5	34	107	24.1	141
Total	47	203	18.8	250

N = 250, X^2 = 5.981, DF = 1, p < 0.05 (0.0145)

Table 5: Distribution of anti HCV antibody positive patients according to history of blood transfusion

Blood Transfusion	Anti HCV antibody			Total
	Positive	Negative	% Positivity	
Yes	32	82	28.1	114
No	15	121	11	136
Total	47	203	18.8	250

N = 250, X^2 = 11.79, DF = 1, P < 0.01 (0.0006)

Discussion

The relation between HCV infection and kidney disorders is well recognized. Patients with renal disease have been at increased risk of acquiring HCV because of prolonged vascular access as well as the potential for exposure to infected patients and contaminated equipment. Liver disease is a significant cause of morbidity and mortality in patients with chronic kidney disease treated by dialysis in renal dialysis patients.

In current study demographic data of the patients was collected from five dialysis centres in Pune including both government and private hospitals. Mean age of cases in the study was 47.3 years and most of them were in age group of 51-60 years. Out of total 250 samples collected (82.8%) were males and (17.2%) were females.

In this study third generation anti-HCV ELISA was performed. Third generation anti-HCV ELISA is in use since the mid 1990s, has a sensitivity of 95%-99% and can detect HCV antibodies 6-8 weeks after exposure. The prevalence of HCV in haemodialysis patients was found to be (18.8%). Initial studies by Arankalle et al in 1995 and Gosavi et al in 1997 reported very high anti-HCV positivity rates in such patients accounting for nearly 24- 28% of cases.[6, 7] In a study from Hyderabad by Chandra et al that comprised of both renal transplant and renal failure patients on haemodialysis, the HCV prevalence was as high as 46%.[8] More recently, a study from Delhi by Agarwal et al in 1999 noted that the prevalence of HCV in 208 patients undergoing haemodialysis was 4.3%.[9] There is wide geographical variation ranging from 5% in northern Europe to 30-50% in Japan and Poland, 30.25% in Saudi Arabia, 39% in brazil prevalence between 5% and 30% has been reported from united states, 23.3% in newyork.[10]

The prevalence of HCV infection was higher in females as compared to males. About (44.1%) of females were anti-HCV antibody positive and only (13.5%) of males were positive. The prevalence of acute HCV in both genders is controversial. While some studies showed higher HCV incidence among men. Other population based surveys showed slightly higher rates in women than in men. However additional epidemiological studies are needed to confirm the risk of HCV transmission according to gender.

In this study assessment of various risk factors responsible for high HCV infection in haemodialysis patients was done. Significant association was found between the duration of dialysis, blood transfusion and HCV infection indicating towards the nosocomial transmission of infection. Mean duration of dialysis of positive HCV patients was found to be 6.03 yrs. Out of total anti-HCV antibody positive cases about (72.3%) had been on dialysis for more than 5 years. Out of total 250 patients 141 patients were on dialysis for moré than five years & out of these about (24.1%) were positive for anti-HCV antibody. Whereas out of total 250 patients 109 patients were on dialysis for less than five

years & out of these only (11.9%) were positive for anti-HCV antibody. This association was found to be statistically significant with p value < 0.05. This shows that >5 years of dialysis is an important risk factor for anti-HCV antibody positivity. Various other studies confirmed that duration of haemodialysis was considered one of the risk factors for acquiring HCV infection.[11, 12] Almost all recent surveys on the subject have congruently suggested the length of time on haemodialysis as a risk factor for HCV seropositivity.

Blood transfusion constitute an important part of treatment in many haemodialysis patients and thus exposed them greatly to HCV infection.[1] In current study about (68.1%) of total positive cases received blood transfusion once or more in life time. Out of total 250 patients 114 gave history of blood transfusion & out of these about (28.1%) were found to be anti-HCV antibody positive. Whereas out of total 250 patients 136 gave negative history of blood transfusion & out of these only (11%) were found to be positive for anti-HCV antibody. This association was found to be statistically highly significant with p value < 0.01. Positive history of blood transfusion was found to be significant for anti-HCV antibody positivity and blood transfusion was found to be important risk factor for HCV infection.

Historically, the numbers of blood transfusions received were consistently reported in the literature to be associated with increased prevalence of HCV positive dialysis patients. However several recent reports could not recognize blood transfusion as independent risk factor in HCV spread among dialysis patients. History of organ transplantation, older age, dialysis in multiple centres. The haemodialysis unit hepatitis B infection human immunodeficiency virus infection and diabetes mellitus were other factors suggested by some studies to be associated with HCV positivity.[13]

Conclusion

In developing countries with a high endemic background of HCV infection surveillance programs and efforts to increase awareness, improve diagnosis and facilitate treatment of acute HCV will have far reaching implications for the management of chronic HCV, where current disease management and health outcome strategies are less effective. Thus preventive measures and the adherence to universal precautions for HCV control remain a priority. Hence it is recommended to use dedicated dialysis equipment, trained nursing staff, separate washing areas and the screening of the patients once in 3 months for preventing cross-infection. Furthermore the present study demonstrated that along with the duration of hemodialysis, blood transfusion was also an important risk factor for acquiring the HCV infection.

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