Genotyping and prevalence of hepatitis c virus in dialysis patients in Gandhi general hospital - A tertiary care centre

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Abstract

Context: The present study was undertaken to determine the genotyping and prevalence of hepatitis c virus in dialysis patients as HCV genotyping has become a part of the pre-treatment evaluation. Studies correlating risk factors and genotypes of HCV infection prevalent in Haemodialysis patients are scanty. Therefore our study is conducted to study the prevalence, genotypes and risk factors in order to detect and try to curb HCV infection.

Aims: To study the genotypes of hepatitis C infection and its prevalence in hemodialysis patients.

Settings and Design and Period: Study was conducted in Gandhi hospital Secunderabad in 1 year period as Cross-sectional study.

Materials and Methods: A total of 225 serum samples were collected from chronic renal failure patients who were undergoing Haemodialysis over a period of 1 year. The samples were screened for anti HCV antibodies by third generation ELISA test. 25 anti-HCV positive samples and 40 anti-HCV negative samples were randomly selected for HCV RNA detection using Trizol-Chloroform-Isopropyl alcohol method, HCV RNA was reverse transcribed with 200 U of maloney murine leukemia virus reverse transcriptase and random hexamers. Nested PCR was done. HCV RNA positive samples were genotyped with primers specific for core region of different HCV types by type specific PCR.

Statistical Analysis Used: Statistical analysis of the data was done by chi-square (χ^2) test using EPIINFO 2000 software. The values of chi square test are interpreted as not significant >0.05, highly significant < 0.001.

Results: Out of 225 hemodialysis patients 38 (16.8%) patients were anti HCV positive. Duration of dialysis was significantly longer in anti-HCV antibodies positive group with dialysis duration more than 2 years. Seropositivity is more in HD patients having dialysis at more than one centre. HCV RNA was detected in randomly selected 13/25 (52%) anti HCV positive patients and in 5/40 (12.5%) anti HCV negative patients. The genotype distribution was as 3a (61.1%), 2a (11.11%), 2b (5.5%), mixed genotypes (16.6%), untypable (5.5%).

Conclusions: Duration of dialysis, number of centres used for dialysis and number of times dialysis was done are important associations for anti-HCV antibodies positivity**.** Genotype 3 was predominant (61.11%). Detection of genotypes helps in early initiation of specific therapy and helps in early prediction of prognosis in patients of chronic renal failure on hemodialysis.

Keywords: Chronic renal failure, Seropositivity.

Introduction

Hepatitis C virus (HCV) infection is a major public health problem, with an estimated global prevalence of 3% occurring in about 180 million carriers and approximately 4 million people have been newly infected annually.[1] Hepatitis C virus (HCV) infection is a major health problem among dialysis patients in developing countries. Dialysis patients remain a high-risk group for hepatitis C virus (HCV) infection.[2] Six major hepatitis C virus genotypes have been identified to date, genotypes 1, 2, 3, 4, 5 and 6. HCV genotype influences response to therapy with both mono (alpha interferon alone) and combination therapy (alpha interferon and ribavirin) HCV genotype has emerged as part of the pretreatment evaluation in chronically infected patients.[3]

Genotypes of HCV is useful for understanding the molecular epidemiological status, is useful in identifying source of infection, designing control program, evaluating the response to treatment and development of diagnostic methods and vaccine production. Therefore evaluation of HCV genotype has become an integral part of investigation.

Thus the present study conducted at our hospital aimed to determine the prevalence of HCV antibodies, associated risk factors and genotypes of hepatitis C virus isolated from hemodialysis patients.

Methodology

Sample Collection: Prior approval was obtained from Institution Ethical committee to carry out the study. Informed consent was taken from all patients. All patients were interviewed for demographic data and risk factors to HCV infections including history of number of blood transfusions, surgical interventions, and number of years on dialysis and change of the centers.

About 5ml of blood was collected under aseptic conditions from HD patients and sera were separated, fresh samples were used for detection of anti HCV antibodies with ELISA and stored in two aliquots and frozen at -70°C. These frozen samples were used for HCV RNA detection and genotyping.

Antibodies Screening: All the subjects were screened for anti HCV antibodies by third generation ELISA test according to the manufacture's Instructions.

HCV Detection and RNA Isolation: HCV RNA Detection was done in randomly selected 25 anti-HCV positive samples and 40 anti-HCV negative samples. RNA was isolated from 250 µL serum, using Trizol-Chloroform-Isopropyl Alcohol Method

Reverse Transcription PCR: HCV RNA was reverse transcribed with 200 U of Maloney murine leukemia virus reverse transcriptase (Fermentas USA) and Random hexamers.

PCR Reaction of cDNA: Nested PCR[4] was done for the detection of hepatitis C virus in serum samples. The amplification of cDNA was carried out with nested primers specific for 5' un translated region of HCV as per manufacturers instructions.

The following primers were used Table 1

Amplified product was subjected to 1.5% agarose gel for electrophoresis.

HCV Genotyping: HCV RNA positive samples were genotyped with primers specific for core region of different HCV types by type specific PCR.

Type Specific PCR: First PCR product was used as a template for genotyping. PCR was performed for each sample, amplified product was subjected to 1.5% agarose gel for electrophoresis and to confirm amplification of genotype specific PCR product. Eight micro liters of the PCR product was electrophoresed on a 2% agarose gel, stained with ethidium bromide, and evaluated under UV light. The HCV genotype for each sample was determined by identifying the genotype specific cDNA bands.

Oligonucleotide primers used for PCR and genotyping Table 2 and Table 3.

For contamination control RNA extraction, cdna amplification and electrophoresis were carried in separate areas. Both negative and positive controls were run parallel to the patient samples in each batch.

Control Group: Total 118 sera of normal healthy controls were screened for anti-HCV antibodies using the same ELISA kit which was used for screening of anti-HCV antibodies in HD patients.

Statistical Analysis: Statistical analysis of the data was done by chi-square (X2) test using EPIINFO 2000software. The values of chi square test are interpreted as $P<0.05$ significant.

Results

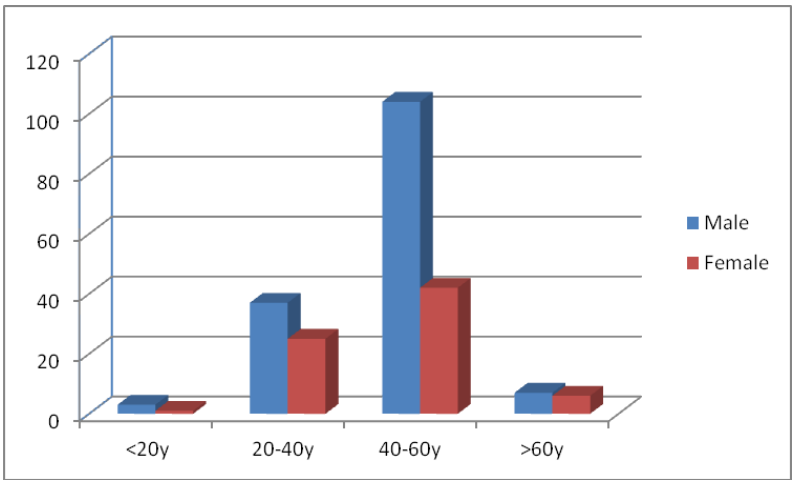

Fig. 1: Age and sex wise distribution in HD patients included in the study

Most of the cases belonged to the age group 40 – 60years.

Table 1: Males and Females distribution included in the study

Males	151 (67.11%)
Females	74 (32.8%)
Total	225

Male to Female ratio of total HD patients 2.04:1

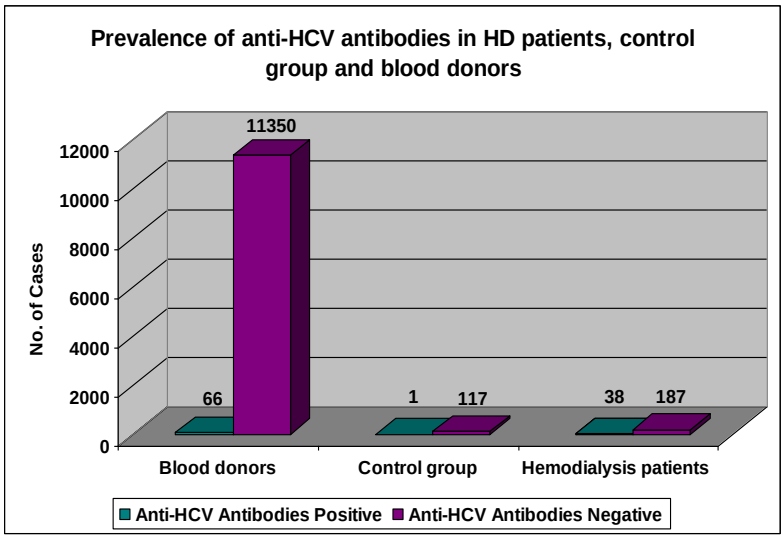

Fig. 2:

During one year period of study Prevalence of Anti-HCV antibodies tested by 3rd generation ELISA in blood donors was 66/11350 (0.57%), control group 1/117 (0.85%) and in hemodialysis patients was 38/187 (16.8%) seropositive. Highest prevalence was observed in HD Patients in our study and it was insignificant in blood donors

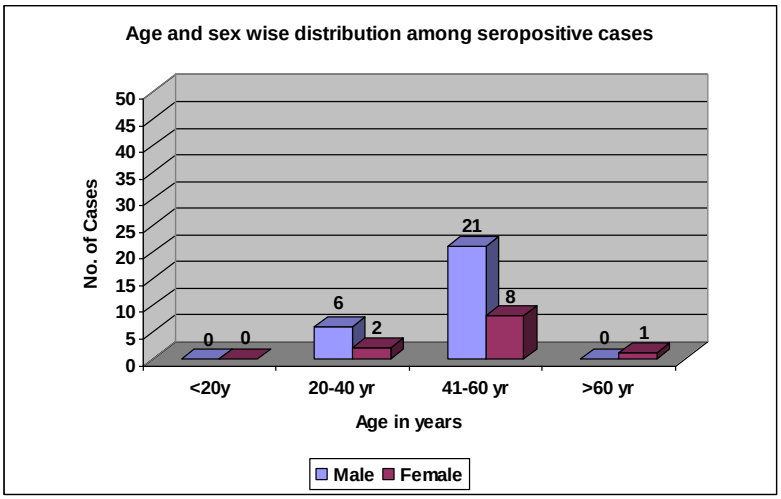

Fig. 3:

Most of the seropositive cases belonged to the age group: 40 – 60years
Male to Female ratio among seropositive cases of HD patients in our study: 2.45:1

Table 2: Comparison of HCV positive and negative patients on dialysis with regard to gender

	Anti-HCV Positive	Anti-HCV Negative	Total	P value
Male	27	124	151	0.705
Female	11	63	74	
Total	38	187	225	

Out of 38 seropositive, 27 were Males and 11 were Females.

P value = 0.705 is considered to be not statistically significant. As p values above 0.05 is taken as statistically not significant as per statistical analysis of data done by chi square test.

Risk Factors Associated with HCV Infection

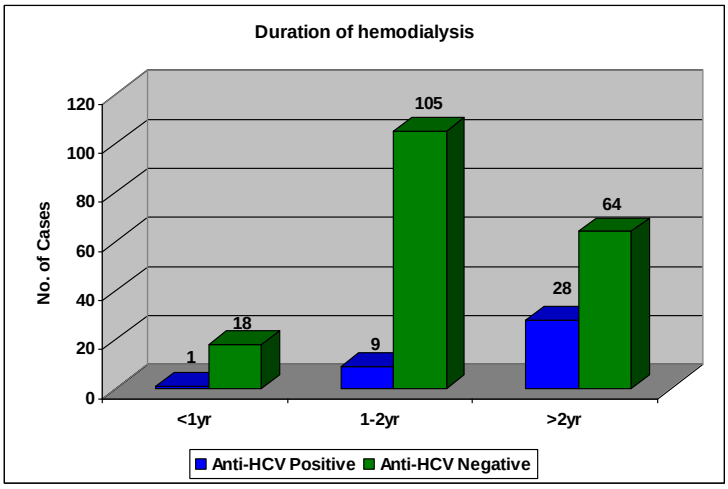

Fig. 4:

Out of 225 HD patients, 114 patients were with 1-2yrs of hemodialysis, but seropositivity was more in patients having hemodialysis more than 2yr (30.43%)

Table 3: No. of centers patients underwent hemodialysis

No. of Centers	Anti-HCV Positive	Anti-HCV Negative	Total
1	9(7.9%)	104	113
2	20(20.4%)	78	98
>2	9(64.2%)	5	14
Total	38	187	225

Regarding no. of centers where they have undergone dialysis, 113 patients in 1center, 98 in 2 centers and 14 in >2 centers. 25.8% had seropositivity who had hemodialysis in more than 1 center.

Table 4: Number of blood transfusions among the seropositive and seronegative HD patients

Units	Seropositive	Seronegative	Total
No Transfusions	17(16.3%)	87	104
1-3	27(25.9%)	77	104
4 & Above	4(23.5%)	13	17
Total	38	187	225

Table 5: Risk factors of HCV infection in hemodialysis patients

		Anti-HCV Positive	Anti-HCV Negative	Total	P Value
Duration of Dialysis	Upto2	10	123	133	<0.0001
	>2	28	64	92	
No. of Centers	1	9	104	113	0.0003
	>1	29	83	112	
Blood transfusion	yes	20	84	104	0.4758
	No	18	103	121	

Among the risk factors for HCV infection in HD patients duration of dialysis, No. of centers and Blood transfusion, duration of dialysis and different dialysis centers had p value <0,05 which are extremely significant whereas for blood transfusion p value is 0.4758 which is not very significant.

Nested PCR for detection of HCV RNA

Table 6: PCR Results

	HCV RNA Positive	HCV RNA Negative	Total
Anti-HCV Positive	13(52%)	12	25
Anti-HCV Negative	5(12.5%.)	35	40
Total	18(100%)	47	65

Total number of samples tested for HCV RNA by PCR were 65 samples (25+40)

Nested PCR was done for 25 seropositive cases which showed 52 % positive for HCV RNA indicating replication stage and out of 40 seronegative cases 12.5% showed positivity indicating increased sensitivity of HCV detection by PCR as compared to ELISA.

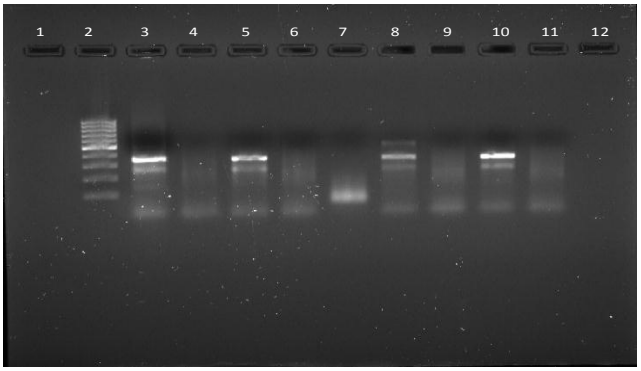

Fig. 5:

Nested PCR analysis of samples of HD patients showing Lane 2 – 100bp DNA ladder, Lane 3 – positive control, Lane 4- negative control, Lane 5,8,10 – serum samples showing the presence of HCV specific 352bp amplicon, Lane 6,7,9,11- serum samples negative for 355bp amplicon.

Table 7: HCV genotypes in HCV RNA positive samples

Genotypes	No (%)
3a	11(61.11%)
2a	2(11.11%)
2b	1(5.55%)
1b+3a	2(11.11%)
1b+1a	1(5.55%)
Untypable	1(5.55%)
Total	18(100%)

Genotyping was done was done USING genotype –specific primers, in our study the genotype distribution was 3a 11(61.1%), 2a 2(11.1%), 2b1 (5.5%), 1b+3a – 2(11.1%), 1b+1a -1(5.5%), untypable 1(5.5%).

Table 8: Distribution of HCV genotypes by age and sex

		3a	2a	2b	1b+3a	1b+1a	Untypable	Total
Age	<20yr	-	-	-	-	-	-	-
	20-40yr	3	-	-	-	-	-	3
	41-60y	8	2	1	2	1	-	14
	>60yr	-	-	-	-	-	1	1
	Total	11	2	1	2	1	1	18
Sex	Male	7	2	1	2	-	-	12
	Female	4	-	-	-	1	1	6
	Total	11	2	1	2	1	1	18

Maximum No. of genotypes were in age group 41-60yr, and more in males than females

Table 9: Correlation of genotypes with risk factors

		3a	2a	2b	1b+3a	1b+1a	Indeterminate	
Blood transfusion	Yes	7	2	1	1	1	1	13
	No	4	-	-	1	-	-	5
Total		11	2	1	2	1	1	18
Duration of HD	<2yr	2	-	-	-	-	1	3
	>2yr	9	2	1	2	1	-	15
Total		11	2	1	2	1	1	18
No. of Centers	1	3	-	-	-	-	-	3
	>1	8	2	1	2	1	1	15
Total		11	2	1	2	1	1	18

H/O blood transfusion is present in 13/18 genotypes, and duration of dialysis >2yr and dialysis more than 1 center was seen in 15/18 genotypes. Indicating again that duration of dialysis and number of centers are important risk factors.

Discussion

Hepatitis C virus (HCV) ia a 50-60 nm virus with a linear, single standard RNA genome enclosed within a core and surrounded by an envelope, carrying glycoprotein spikes. It belongs to genus: Hepacivirus, family: Flaviviridae and exists as six major genotypes and more than 60 defined subtypes. Patients with renal disease are at an increased risk of acquiring HCV Infection as they undergo repeated dialysis HCV is the most common liver disease in HD patients, and liver disease which invariably leads to morbidity and mortality in patients with end stage renal disease treated by dialysis or transplantation[5] The seroprevalence of HCV ranges between 0.2 -2% globally[6] in India among blood donors it varies from 0.48% in Vellore[7] to 1.85% in New Delhi.[8] In a study by R.N. Makroo et al New Delhi reported seroprevalence of HCV 0.66% in blood donors[9] which is on par with our study which reports 0.57% control group reported 0.85%. The hemodialysis patients are at high risk for development of hepatitis C infection. However the data on the prevalence of anti HCV among Indian hemodialysis patients is scanty.

In our study the prevalence of anti-HCV positive cases among study group (hemodialysis patients) was 16.8%, correlating with a study of Medhi S et al[10] from New Delhi which reported 17.2%.

Out of 225 hemodialysis patients, male to female ratio 2.04:1 which is correlating with the study of Jasuja et al[11] who reported 1.85:1 and Khan et al[12] and Arwa Mujahid et al[13] who reported 1.7:1.

Third-generation anti-HCV ELISA is used as the screening test for the diagnosis of HCV infection.

It has shown better performance than the previous two generations of anti-HCV tests with a mean window period of 70 days.[14] Detection of HCV RNA by reverse transcriptase PCR is the 'gold standard' to identify HCV infection.[15]

Risk Factors in Hemodialysis Patients: Duration on HD is considered one of the risk factors for acquiring HCV infection, the number of years on dialysis is the major risk factor independently associated with higher rates of HCV Infection.[16]

Our study showed a statistically significant difference in the prevalence of HCV infection between patients who were on dialysis for more than two years, and patients who were on dialysis for less than two years (P = <0.001) this is in accordance with the study by Bdour et al[17] which reported >48 months of duration of hemodialysis is a significant risk factor for HCV infection. Risk factors associated with HCV infection among hemodialysis patients include history of blood transfusions, the volume of blood transfused, and years on dialysis. However our study did not show any increase in seroprevalence of HCV in blood transfused patients correlating with the study of Agarwal SK et al[18] which reported blood transfusion is not an important source of HCV infection in HD patients. The risk of acquiring post –transfusion HCV infection has significantly declined primarily because of availability of better screening test for HCV, use of advanced machines and observing strict universal precautions. In our study there is increased prevalence of HCV infection in HD patients who had dialysis in more than one center.

Previous studies have shown that de novo infections in haemodialysis units may still occur in the absence of other Parenteral risk factors.[19] Some reports state that the duration of haemodialysis is an independent predictor of HCV infection in chronic haemodialysis patients. Thus hospital acquired infectionsof HCV is a probability in haemodialysis units.

HCV RNA Detection: By RT-PCR which detects acute infections in early stages while the virus is replicating i.e., within weeks of contacting infection as against ELISA which detects the infection only after the appearance of Anti-HCV antibodies. Thus, proving PCR is advantageous over ELISA.

In Our study HCV RNA was detected in 52% (13/25) of anti-HCV seropositive patients which is higher than study of de Jesus et al[20] which reported as 42%.

This deviation between PCR and ELISA results may be due to intermittent viraemia, or the level of viraemia is below the lower limit of PCR detection.

HCV RNA Extracted in HCV Antibody Negative Samples: Several studies have shown that serological assays alone are not sufficient for diagnosis of HCV infection and the detection of HCV RNA is required to identify all infected patients.[21] So detection of HCV RNA is more reliable than serology in detecting ongoing HCV infection in HD patients who may not mount an adequate antibody response.

In our study, HCV RNA was detected in serum of 12.4% patients of the anti-HCV negative (5/40).

Genotyping of HCV: The distribution and prevalence of the HCV genotypes prevail in certain geographic areas. In India various studies showed the predominant HCV genotype is 3 followed by genotype 1. Study from Vellore J. Christdas et al[22] had reported HCV genotype 3 was found to be the most predominant (63.85%) and most common amongst patients from East India followed by genotypes 1,6,4.

Two other patients were infected with recombinants of genotype 1 and 2. Subtype B was the most prevalent, forming 47.7% among the population in southern India.

In our study the predominant HCV genotype is 3a (61.1%) which is correlating with the study of Khan et al[23] which reported 3a as the predominant HCV genotype in HD patients.

In our study three samples had a mixed pattern of subtypes 16.6% (1b+3a (2), 1b+1a (1) 3/18), which is again on par with Khan et al who reported genotypes in 12.12%. Mixed genotypes may be due to mutations in the viral genome or co-infection.

Distribution of HCV Genotypes by Age and Sex: The frequency distribution of genotype 3a was most common in age group 20-40yr & 41-60yr while 2a, 2b were common in age group 41-60yr. The prevalence of genotypes in our study was more in males than females, while Untypable genotype was present more in female. Genotype 3a is prevalent in both males &females where as 2a,2b in males

Distribution of HCV Genotypes with Risk Factors: The risk factors of HCV infection in HD patients duration of dialysis, blood transfusion and no. of dialysis centers were compared among genotypes. In our study duration of dialysis was less than 2 years in untypable genotype and more than 2 years in 3a(18.8%), 2a, 2b and mixed genotypes were with history of Blood transfusion was present in 72.2% of genotypes.

To prevent HCV infection observation of strict aseptic precautions are necessary prior to dialysis.

Determination of genotypes of HCV is part of the pretreatment evaluation, development of newer diagnostic methods and vaccine production is a necessity.

Conclusion

1. The prevalence of hepatitis C infection in hemodialysis patients was found to be 16.8%.
2. The risk factors associated with HCV infection among hemodialysis patients were :

a. Duration of hemodialysis more than 2 years 30.43% (p value<0.0001) 2)
b. Undergone dialysis in more than 1 center 25.8% (p value = 0.0003)
c. Blood transfusion p value=0.475 (however it is insignificant)

3. Seroprevalence of hepatitis C infection was 76.3% in the age group 41-60yr. Seroprevalence among males was 17.8% and among females was 14.8%.
4. Patients on hemodialysis treatment are at high risk for HCV infection. Inspite of various measures adopted to curb nosocomial infections.

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