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Original Article

Prevalence and Detection of Genotyping of Hepatitis C Virus in Hemodialysis Patients

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ABSTRACT

Background: Hepatitis C virus is a significant global health distress, affecting millions of individuals worldwide. HCV is a disease that spreads through blood and mostly affects the liver. The condition may resolve on its own or progress to cirrhosis, fibrosis, or liver cancer. Chronic kidney disease patients on hemodialysis are at an increased risk for acquiring HCV infection due to cross contamination in dialysis units, blood transfusion, and kidney transplant. The aims of the study were to determine the genotypes among patients of HCV undergoing hemodialysis.

Objective: The objective of the study was to assess the prevalence and types of HCV genotypes in hemodialysis patients.

Methods: In this cross-sectional study, the HCV positive patients undergoing hemodialysis were enrolled. Samples were collected from the dialysis unit of Farooq Hospital Lahore. RT-PCR was performed to determine the HCV genotypes. This study was conducted on patients of both genders and all age groups. Chi-square test was performed to assess any significant correlation between HCV genotypes in all age groups and both genders.

Results: In this study out of a total of 80 hemodialysis patients, 19 (23.8%) were females and 61 (76.3%) were males. The most common genotype observed was 3 (72.5%) followed by 1a (12.5%), 1(10.0%) and 1b (5.0%).

Conclusion: The current study revealed that the genotype 3 of HCV as the most frequent genotypes in the hemodialysis patients of our population with most patients in the age group of <60 years.

Keywords: Hepatitis C virus, chronic kidney disease, hemodialysis, HCV genotyping

INTRODUCTION

Hepatitis C virus (HCV) is a blood borne infectious disease that primarily affects the liver. The condition may resolve on its own or progress to cirrhosis, fibrosis, or liver cancer. HCV is a major pathogen of the hepatitis group of liver cirrhosis and hepatocellular carcinoma (1). There are seven distinct genotypes of HCV, each of which contains a variety of subtypes, and they are typically found all over the world. Based on the genetic material in the RNA strands, a genotype is a classification of the virus. The chronic HCV is so difficult to treat because these quasi-species are capable of rapid mutation and develop resistance to available treatments (2).

Globally, genotype 1 of HCV is the most prevalent genotype while Subtype 1b is more frequent In Europe and Japan and subtype 1a in The United State of America (3). Genotype 1 is more prevalent in Pakistan than genotype 3a (4). Knowing the genotype of HCV is helpful in making a therapeutic recommendation for physicians (5). People have HCV genotypes 2 and 3 are showing more sensitivity alpha interferon and ribavirin as compared to people have HCV genotype 1. In addition, the recommended treatment duration for combination therapy is determined by genotype (6).

Chronic kidney disease (CKD) is one of the more common extra hepatic manifestations present in patients with chronic HCV (7). HCV spreads through parenteral route, primarily through percutaneous exposure to blood, making dialysis patients more prone to acquiring infection. In fact, nosocomial transmission is the main method of spread of HCV in dialysis units and patient to patient spread of HCV infection has been documented (8). The prevalence of HCV infection is higher in persons on chronic hemodialysis

compared with the general population. The prevalence rates of HCV range from 4% to 9% in high income countries, but is significantly higher and varies widely across different countries in the Middle East, North and Sub-Sahara Africa, Asia, and Eastern Europe (9).

Kidney transplantation, blood transfusion, and treatment within a dialysis unit are considered as the main risk factors for HCV infections in dialysis patients. At present, effective screening of blood and kidney donors for HCV antibodies has considerably reduced the risk of transmission of HCV through blood and transplanted organs (10). The present study was planned to find out frequency of HCV genotypes in hemodialysis. The overall objective of the study was the determination of different genotypes among patients of HCV undergoing hemodialysis.

MATERIAL AND METHODS

The cross-sectional study was conducted in Farooq Hospital Westwood Lahore for one year. Both male and female patients of all age groups having CKD and undergoing hemodialysis were included in this study. Similarly, the patients having co-infection of HBV or HIV and patients with any other disease and HCV negative patients were excluded from this study. The study was approved by the Ethical review committee. The questionnaire was designed as a data collection tool to collect information from individuals. After taking written informed consent, about 03 ml blood samples were collected from the hemodialysis patients in clotted vials (IMPROVE). Clotted samples were centrifuged to separate the serum at 3000 rpm for ten minutes. Serum that had been collected was kept at-20°C in the refrigerator before processing for DNA extraction.

The viral nucleic acid was isolated from serum sample by using a “Qiagen nucleic acid” extraction kit. In the manual nucleic acid extraction procedure, different steps were followed. Firstly, 25µL qiagenproteinase were added, then 200µL sample, 200 µL lysis buffer, 250 µL absolute ethanol, 500µL wash buffer 1, 500µL wash buffer 2, 500µL absolute ethanol, and elution buffer were added. The viral nucleic acid of each sample was eluted with 60µL elution buffer. The extracted viral nucleic acids were immediately subjected to a one-step RT-PCR reaction. The remaining nucleic acids were stored at-70 oC.

After the nucleic acid extraction, RT-PCR was performed to detect the HCV virus in hemodialysis patients by using ZEESAN kit. The master mix was prepared as per instructions (add (n+1)×19.0 µL of RT-PCR Mix and (n+1)×1.0 µL of Enzyme Mix into a 1.5 mL centrifuge tube). After that, 20µl mastermix was added in a 0.2mL PCR reaction tube. 05 µl extracted nucleic acid was added. Positive and negative controls were run with the test batch. All the tubes were placed in a thermocycler (Rotar Gene-Q 5plex). Denaturation was performed at 95 oC for 3 minutes and again for 15 seconds. Annealing was performed at 50 oC for 45 seconds and 50 cycles were used. The last step of the extension was done at 60 oC for 60 seconds and 50 cycles were used. The results were considered positive for HCV virus when cycler threshold (CT) value was twelve to nineteen and considered negative when CT value was more than thirty.

The genotyping of HCV was performed by BOSPHORE HCV GENOTYPING detection kit. The master mix was prepared as per kit instructions. After that, pipette 26µl of the master mix into each required well of an appropriate optical 96-well reaction plate according to the instrument mentioned. Then add 14µl of the sample or control. Positive and negative controls were run with the test batch. Thoroughly and carefully mix the samples and controls with the master mix by pipetting up and down and change tip for every sample. Close the 96-well reaction plate with optical adhesive film. Centrifuge the 96-well reaction plate in a centrifuge with a “microtiter plate rotor for 30 seconds at approximately 1000 x g (~ 3000 rpm). All the tubes were placed in a thermocycler (Light Cycler 480 II/Cobas Z-480 and Quant Studio5”. The amplification protocol is described in table 1. The CT values were automatically calculated by automated software and the results were interpreted according to table 2.

Data was analyzed using Statistical Package for the Social Sciences version 25.0 (SPSS 25.0). The quantitative variables like age were summarized as mean and standard deviation. The categorical value was expressed in the form of frequency and percentages. Chi-square test was used to find the association between variables.

Table 1: Real-time amplification protocols for HCV genotyping

	Stage	Cycle Repeats	Acquisition	Temperature (Degree Celsius)	Time
Reverse Transcriptase	Cycle Hold	1	-	50	30 minutes
Denaturation	Cycle Hold	1	-	95	14 minutes and 30 seconds
Amplification	Cycling	50	-	97	30 seconds
				54	01 minute and 20 seconds
			Yes	72	15 seconds

Cooling	Cycle Hold	1	-	22	01 minute

Table 2: Interpretations for HCV genotyping

Channel/ Detection Mix No	FAM	HEX	Cy5 (Internal control)	Result
1	+		-/+	Genotype 4
1		+	-/+	Genotype 1b
2	+		-/+	Genotype 1a
2		+	-/+	Genotype 2
3	none	+	-/+	Genotype 3
5		+	-/+	Genotype 6
5	+		-/+	Genotype 5

RESULTS

In this study out of a total of 80 hemodialysis patients, 19 (23.8%) were females and 61 (76.3%) were males. The mean age of hemodialysis patents was 46.96 + 14.80 years. All the enrolled patients were on dialysis. Mostly patients were on hemodialysis from less than one year. The frequency of hemodialysis in patients is shown in table 3. There are many causes which can lead to ESRD. The most common cause of ESRD was hypertension (30.0%) followed by diabetes (26.25%).

Table 3: Frequency and percentage distribution of dialysis in patients

HCV genotypes	Frequency (%)
<1 years	37 (46.25 %)
1–2.9 years	15 (18.75 %)
3–4.9 years	11 (13.75 %)
5–9.9 years	09 (11.25 %)
>10 years	08 (10.0 %)

Table 4: Causes of end stage renal disease in patients

Causes	Frequency (%)
Hypertension	24 (30.0%)
Diabetes	21 (26.25%)
Glomerulonephritis, vasculitis	10 (12.5%)
Other cause of ESRD	10 (12.5%)
Cystic/hereditary/congenital	06 (7.5%)
Interstitial nephritis	05 (6.25%)
Obstruction	04 (5.0%)

The genotyping of HCV was determined through PCR. The most common genotype observed was 3 (72.5%) followed by 1a (12.5%), 1(10.0%) and 1b (5.0%). Figure 1 shows HCV genotyping with band positions in hemodialysis patients. The association between HCV genotypes and study variables was determined by chi-square test (table 5). The p-value of <0.05 was considered significant. The present study shows no association between HCV genotypes and study variables. The p-value of gender and age was 0.80 and 0.90 respectively.

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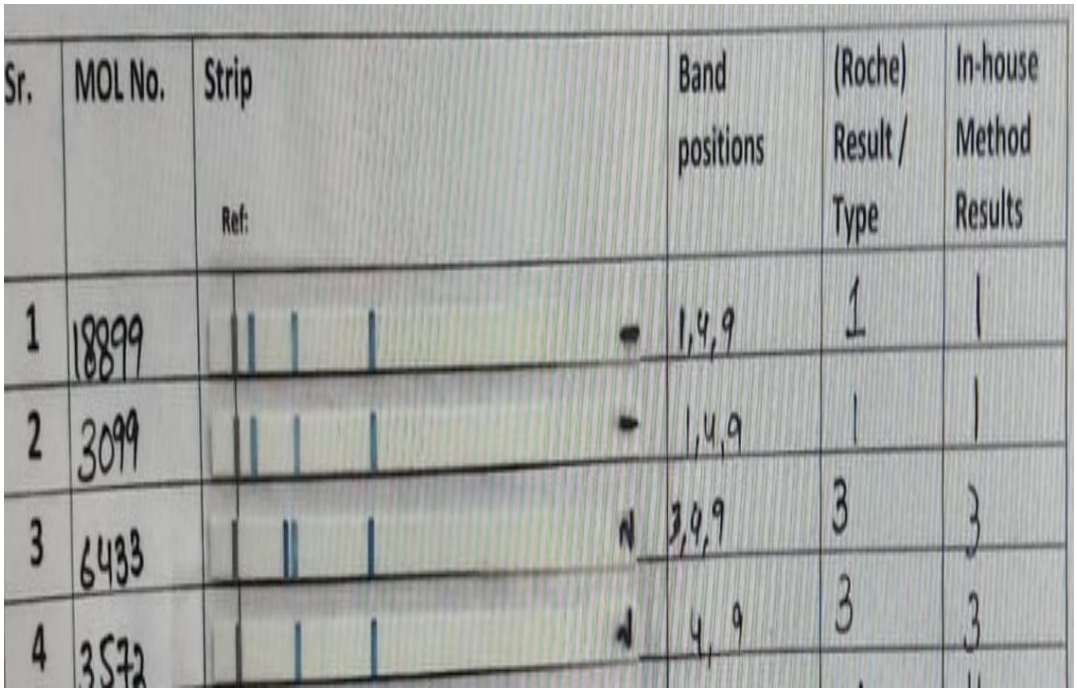

Figure 1: HCV genotyping with band positions

Table 5: Association of HCV genotypes with study variables

Variables	Genotype 1	Genotype 1a	Genotype 1b	Genotype 3	P-value
Gender					
Male	5 (62.50%)	8 (80.00%)	3 (75.00%)	45 (77.60%)	
Female	3 (37.50%)	2 (20.00%)	1 (25.00%)	13 (22.40%)	0.80
Age					
≤ 60	7 (87.50%)	9 (90.00%)	3 (75.00%)	50 (86.20%)	
> 60	1 (12.50%)	1 (10.00%)	1 (25.00%)	8 (13.80%)	0.90

P-value of <0.05 considered significant.

DISCUSSION

In the present study the RT-PCR used for the detection of HCV virus in hemodialysis patients. The prevalence of HCV varied among population. ELISA and RT-PCR techniques were used in many cities to determine the prevalence of HCV, including 6.6% in Kohat (11, 12), 9.2% in the Swat Region, 5.2% in Mardan, 9.3% in DI Khan, and 12.9% in Abbottabad (12, 13). The prevalence variations may be due to a variety of factors, including differences in the number of CKD patients in each study, genetic factors, immune status of patients, detection technique type, sample size, cultural knowledge of exposure to a risk factor for HCV transmission, and the diversity of the research that characterized the virus, which can contribute to a successful age group. Infection with the HCV was found to be more in males as compared to females. The consequences of the current study concurred with the study conducted in swat and Buner region. They were reported that the male had a statistically significant association with HCV positivity as compared to females (14, 15). While contradicted the study were accounted with different examinations which were found there are no contrasts between the two genders in HCV disease among hemodialysis patients in various urban areas like Kohat (14, 16).

A study was conducted in Iran in 2011. In this study 514 hemodialysis, patients were enrolled. From the total, 286 were male and 228 were female patients. In this study, the male population of hemodialysis is dominant as compared to the female population of hemodialysis patients (17). In the present study, 75 (50%) male and 75 (50%) female hemodialysis patients were observed. In the present study, the percentage and frequency distribution of gender was the same which is not related to the previously conducted study of Iran. The frequency of HCV was also observed in hemodialysis patients. 81 (54.0%) hemodialysis patients have non-reactive HCV but 69 (46.0%) hemodialysis patients have reactive HCV. The present study is also in agreement with a study conducted by Hussein NR et al, in 2015. In this study, they also showed that prevalence of HCV was more in hemodialysis patients (18). It is also in

agreement with a study conducted by Jadoul et al, in 2019. In this study, they concluded that HCV is a major risk factor in hemodialysis patients. They showed that hemodialysis patients were at high risk to infect with HCV. They also found high frequency of HCV in hemodialysis patients (19).

In this study, the most common genotype observed was 3 (72.5%) followed by 1a (12.5%), 1(10.0%) and 1b (5.0%). A study led to explore the prevalence of HCV in Iran, The prevalence of HCV was viewed as between 9-12% as per the strategy utilized for the determination (20). In another study exploring the HCV genotypes in patients with end stage kidney disease in Iraq, HCV genotype 1a was tracked down in all patients (21). The HCV genotypes 4 and 1 infections are the most difficult to treat. Recently, it was demonstrated that half of the people infected with HCV genotypes 1 and 4 in Iraq (22).

The present study showed no association of HCV genotypes and different age groups and gender. Ahmad et al. discovered a similar association, which is consistent with our findings (23). When further analyses of results were made regarding patient's age ≤60 years were more affected (86.3%), contrasted and other ordered factors old enough. Even though our findings agree with the study conducted by Ali et al., in which recurrence of HCV was greater in age group of 13 to 50 years. One possible explanation is the region's health department's awareness of HCV and its prognosis (23, 24). The present study showed that the genotype 3 (72.5%) were most common in our population. This study showed that the HCV genotype 3a (58.16%) was the most common in Pakistan, followed by 3b (9.05%), 2a (6.70%), 1a (6.22%), and 1b (2.39%) (25), however, the findings of the study were in consistent with our findings.

CONCLUSION

The current study revealed that the genotype 3 of HCV as the most frequent genotypes in the hemodialysis patients of our population with most patients in the age group of <60 years.

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