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Molecular Identification And Prevalence Rate Of Hepatitis C Virus Among Hemodialysis Patients In Peshawar Kp Pakistan

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Abstract

Hepatitis C Virus remains a significant risk for both patients and medical staff in hemodialysis centres. The current study aims to determine the infection rate of hepatitis C virus, and risk factors associated with hepatitis C virus in hemodialysis patients admitted in Hayatabad Medical Complex Peshawar. A cross-sectional study was based on the processing of blood samples from 306 hemodialysis patients with routine hemodialysis were collected during the period16thSeptember 2020 till 16th December 2020 at Hayatabad Medical Complex Peshawar. After preparation of samples, enzyme-linked immunosorbent assay test was performed to detect on hepatitis C virus –antibody (Immunoglobulin G), then the seropositivity samples confirmed by real-time polymerase chain reaction technique. The rate of hepatitis C virus infection among hemodialysis patients in Hayatabad Medical Complex Peshawar was 7.8% (24/306), and there were no differences between males and females (12 cases) for each case. Age between 61-70 years was the most   likely 9(37.5%). There were no Environmental differences between the patients of

Peshawar or around Peshawar (50%) for each one, and 22 married patients (91.70%) more than unmarried 2(8.30%). Patients with primary education were more frequent 9 (37.50%),hemodialysis duration ranging from 1-4 years showed more frequent than other periods 21 (87.5%). The rate of hepatitis C virus infection was moderate among hemodialysis patients in Hayatabad Medical Complex Peshawar and there are no differences between gender and residence and the infection increases with the duration of dialysis.

Keywords: Hepatitis C, Hemodialysis, Frequency Rate of HCV, Elisa.

Introduction

Liver disease due to the hepatitis C virus (HCV) is a major public health concern that affecting millions of people globally (1). Lead to significant morbidity and mortality in developing and undeveloped countries. The clinical manifestation of viral hepatitis varied from subclinical to a life-threatening infection (2). The virus exists as an enveloped which is 50 nm in size, positive stranded RNA virus is made up of 9.6 nucleotide bases and is covered by an icosahedra nucleocapsid which is further surrounded by a lipid bi layer containing two viral glycoproteins, envelop one (E1) and envelope two (E2) proteins (3) HCV is mostly dangerous among all hepatitis strains because its morbidity rate is high (3). HCV is enveloped, small circular, positive-sense and single stranded ribonucleic acid (RNA) virus from genus Hepacivirus, family Flaviviridae with a diameter of 50 nm (4) The total length of RNA genome is about 9.6 kb with one open reading frame (ORF) and 5' and 3' untranslated regions (UTRs) at both edges (5) HCV has six main genotypes (1–6). Genotyping is most important for organization of HCV treatment and helps to cure HCV infections (6) HCV G-1, HCV G-2 and HCV G-3 are distributed worldwide, whereas HCV G-4, HCV G5 and HCV G-6 are present in certain areas of the globe (7).Genotype 1 (G1) is the most predominant in the world (~83.4 million people) followed by G3 (~54.3 million), then G2, G4 and G6 (~15.6 million), and G5 (~1.4 million) (Messina et al., 2015). Transmission of HCV infection is mainly by exposure to infected devices and tools despite rigid hygienic control, infected blood or blood products, hemodialysis, intravenous (IV) drug abuse, and organ transplantation (8). In hemodialysis centers, HCV infection remains a major concern. Blood transfusions as well as nosocomial infection continue to play important roles in the transmission of HCV (9) the high incidence of persistent infection is related to the high genetic diversity of HCV genotypes Lack of an effective hepatitis C vaccine (10). Hemodialysis(HD)is the chosen treatment for end-stage hemodialysis patients and at the same time the patients at risk of developing blood infection infections, including hepatitis viruses synergy scale in accelerating progress to hepatic malformations(11). The frequency of HCV in hemodialysis units was detected all over the world. Risk factors include suppressed immunity for patients, for long periods exposure to blood vessels and multiple blood transfusions, invasive medical procedures, and share contribution to the environments of infected patients for viral hepatitis C prevalent between hemodialysis patients (7,8). Patients undergoing hemodialysis should be tested when they first start dialysis or when transferred from another dialysis facility (12). Initial testing is suggested with either an enzyme immunoassay (EIA) or a nucleic acid test, depending on the low or high prevalence of the virus in the country and the particular hemodialysis unit (13). Prevalence of HCV is different in different region of the world. Like Western Europe, the Americas and Australia are considered regions of low HCV prevalence (<2%) (14)Mongolia has also higher HCV prevalence (above 10%), followed by Uzbekistan and Pakistan where, according to some reports, around 6% of the total population is infected with HCV (15). The aim of the current study to determine the infection rate of hepatitis C virus, and risk factors associated with

hepatitis C virus in hemodialysis patients admitted in Hayatabad Medical Complex Peshawar. Although previous studies exist that have revealed HCV prevalence in different time frames (16) but as prevalence differs in respect to time, that's why to report the recent HCV prevalence in in hemodialysis patients in Peshawar regions of KPK, We have conducted this study

Material and Methods

Study design and samples

A cross-sectional study was conducted on hemodialysis patients who were admitted to the Hayatabad Medical Complex Peshawar, during the period from 16thSeptember 2020 until 16th December 2020**.**

Inclusion criteria

The included blood sample was taken from hemodialysis patients from both genders,

Sample Collection

Approximately (5 ml) of blood was collected from (306) hemodialysis patients (177 were males and 129 were females), the samples were put into a test tube containing an anticoagulant, ethylene amine acetic acid (EDTA), and expelled at 1500 rpm for ten minutes, the produced plasma was collected in Eppendorf tubes and stored in deep freezing at (-20 ° C) until it was used to identify IgG antibodies to hepatitis C virus by enzyme immunoassay, then samples were tested positive for IgG from HCV confirmed by using RT-PCR to detect the viral RNA in each sample. Data were collected through an interview with each patient after taken the permission from each one to perform research, through a structural questionnaire sheet which includes age, gender, marital status, residence, level of education, duration of dialysis.

Enzyme Immune Assay for Hepatitis C Virus

All patients were screened for anti-HCV IgG antibodies by third-generation commercial ELISA test according to the manufacturer's instructions of Foresight EIA test kit (Cat. No. 1231-1031, USA) in the laboratory of Hayatabad Medical Complex Peshawar used for the qualitative detection of IgG antibodies to HCV in human plasma.

HCV-RNA Extraction and Hepatitis C Virus Quantification

All positive samples are extracted for HCV-RNA by using Exi Prep TM Dx Viral DNA\RNA Kit (Cat. No K-4471/ K-4472/ K-4473, Korea), from the plasma according to the manufacturer's instructions. RNA was isolated from 400µl plasma using an automated system depending on the magnetic beads method in the isolation of the RNA. After the extraction of HCV-RNA, Anatolia Bosphore HCV quantification kit (Cat. No. ABHCQ1, Anatolia Gene works, Turkey) according to the manufacturer's instructions used for detecting and quantities HCV nucleic acid from all samples depending on the protocol of RT-PCR technique.
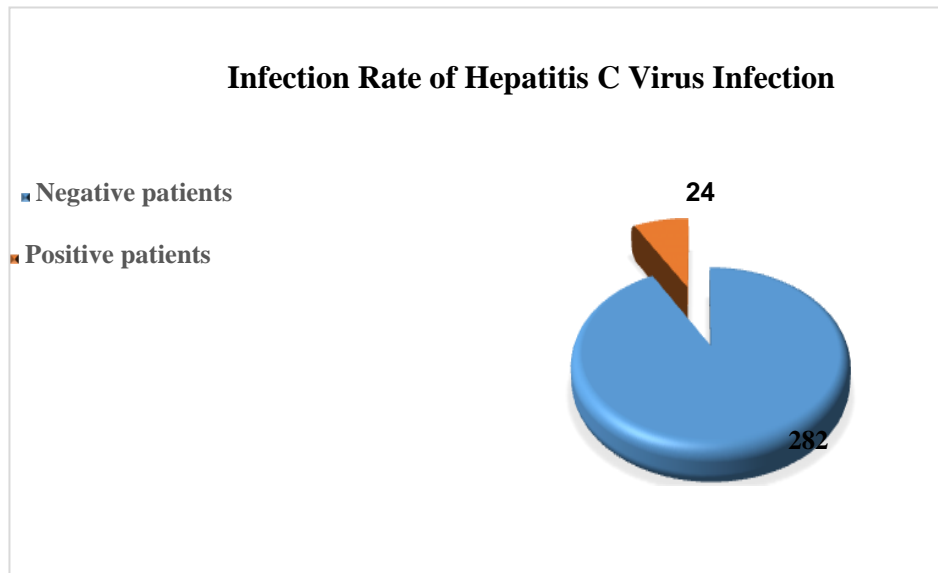
Statistical Analysis

All data were analyzed using the Statistical Analysis Program (SAS) - 2012, version 22, number, percentage, and proportion, and chi-square was used to test the effect of different factors in the study, for a significant comparison of the percentage (0.05 and 0.01 probability) in this study

Results

The rate of infection of hepatitis C virus infection among hemodialysis patients in this study was 7.8% (24 of 306) according to the results of enzyme immunosorbent assay and RT-PCR techniques, as shown in Figure1

Fig1. The rate of infection of hepatitis C virus infection among hemodialysis patients

Among 24 hemodialysis patients, 12 (50%) males and 12 (50%) females were positive for hepatitis C virus with statistically significant differences (P = 0.0001) between positive and negative patients. The infection was more frequent in the age group (61-70 years old) was 37.5% with highly significant differences (P=0.0087) and there was an increase in HCV infection with increasing age with a mean age of 51.6 ± 14.16(SD) years. Half (50%) of the hepatitis C virus infection among dialysis patients lived in Peshawar district and 50% around Peshawar district and there were statistically significant differences (P = 0.0001). Regarding marital status, the highest incidence of viral hepatitis was observed in 22 married patients (91.7%) with statistically significant differences (P = 0.0074), followed by unmarried 2 (8.3%) with significant differences (P = 0.0461). Also, most of the negative cases were 196 (69.5%) married and 50 (17.7%) unmarried. Concerning educational levels in hemodialysis patients with HCV infection, 9 (37.5%) had primary education with significant differences (P = 0.0288), 8 (33.3%) had secondary education with statistically significant differences (P = 0.0081), While low cases 6 (25%) had illiterate education and 1 (4.2%) had higher education (had institute or college degree) without statistically significant differences, as shown in Table1.

Table 1 Demographic Characteristic among Hemodialysis Patients.

Variable factors		Positive No. (%)	Negative No. (%)	Chi-square*P-value **P-value
Gender type	Male	12(6.80%)	165(93.20%)	132.25 **(0.0001)
	Female	12 (9.30%)	117(90.70%)	85.465 **(0.0001)
Age groups (years)	1-12	1 (4.20%)	20 (7.10%)	3.026 NS (0.375)
	13-24	1 (4.20%)	30 (10.64%)	2.691 NS (0.331)
	25-36	5 (20.80%)	30(10.64%)	4.095 * (0.0466)
	37-48	5(20.80%)	50 (17.70%)	0.772 NS (1.026)
	49-60	3(12.50%)	70(24.80%)	4.821 * (0.0398)
Residence	Rural	9 (37.50%)	60(21.29%)	6.259 ** (0.0087)
	Urban	0(0.00%)	22(7.80%)	6.802 ** (0.0079)
Marital Status	Single	2 (8.30%)	50 (17.70%)	4.772 * (0.0461)
	Married	22(91.70%)	196 (69.50%)	7.205 ** (0.0074)
	Divorced	0 (0.00%)	5 (1.80%)	0.084 NS (0.933)
	Widow	0 (0.00%)	31 (11.00%)	4.659 * (0.0452)
Educational levels	Illiterate	6(25.00%)	68 (24.10%)	0.561 NS (0.692)
	Primary school	9 (37.50%)	148 (52.50%)	5.038 * (0.0288)
	Secondary school	8 (33.30%)	45 (16.00%)	6.502 ** (0.0081)
	High education	1 (4.20%)	21 (7.40%)	1.064 NS (0.329)
Total		24(100%) 24 (100%)		282(100%)

The maximum duration of dialysis in positive HCV-infected patients was (7 years) while the minimum duration was (1 year) with a mean of 3.38 ± 1.3 (SD) years. Hepatitis C virus was more common in patients on hemodialysis for (1-4 years), approximately 21 (87.5%) with statistically significant differences (P = 0.0054), and less common in patients on hemodialysis duration (5-7 years) were 3 (12.5%) with non-significant differences (P = 0.479), as shown in Table 2.

Table 2: Distribution of Positive and Negative Hepatitis C Virus According to Duration of Dialysis

Duration of Dialysis	Positive No. %	Negative No. %	Chi-square *P-value
Less than one year	0 (0.0%)	72(25.5%)	8.61 ** (0.0037)
1-4 years	21(87.5%)	173 (61.3%)	7.26 ** (0.0054)
5-7 years	3(12.5%)	30 (10.6%)	0.594 NS (0.479)
8-10 years	0(0.0%)	7 (2.5%)	0.558 NS (0.572)
Total	24 (100%)	282(100%)	---

Discussion

According to the results of the ELISA and RT-PCR techniques, the rate of HCV infection among dialysis patients at Hayatabad Medical Complex Peshawar was 7.8%. The result of this study is comparable to several Pakistani studies conducted in different cities such as 6.6% in Kohat by using ELISA and RT-PCR techniques (17,18), 9.2% in the Swat Region of by ELISA and PCR technologies were distributed in 5.2% in Mardan, 9.3% in DI khan, and 12.9% in Abbottabad (19,20). The similarities in the results of the current study and others due to geographical conditions, as all relevant studies were conducted in countries with similar genetic and ethnic factors, or they could be related to similarity of transmission or risk factors for the disease as well as the use of the same technique in detection. The Prevalence variations can be because the virus differs from region to region and year to year, or to the diversity of the research that characterized it, which can contribute to a successful age group, differences in the number of chronic kidney patients in each study, genetic factor, immune status of patients, detection technique sort, sample size, and cultural knowledge of exposure to a risk factor for transmission of the hepatitis C virus. According to gender, it was found that infection with hepatitis C virus is equal in males and females 12 (50%) with statistically significant differences (P =0.0001) between positive and negative cases. The results of the current study agreed with other studies which were found there are no significant differences between both genders in viral hepatitis C infection among hemodialysis patients in different cities such as Kohat (21,22).While disagreed with the study were reported a male had a statistically significant association with anti-HCV positivity than females such swat and Buner region (23,24). In this study, the infection rate was highest at ages (61-70 years) with significant differences. This may be attributed to the higher rate of renal disorders in the elderly, which has led to dialysis treatment, which is one of the high-risk factors for infection. According to the residence of patients in the study population, Hepatitis C virus infections in Peshawar and around Peshawar were the same (50% for each one) with highly significant differences (P=0.0001). This is due to the similarities between these areas of the viral pattern and the nature of the area, as well as the mode of transmission and exposure among members of these communities to the same risk factors for this disease and health customs and traditions. the highest infection rate of HCV was noticed with married patients 22(91.7%), followed by single patients 2(8.3%) with significant differences among these groups. These results agreed with several studies conducted in different areas of Pakistan a significant relationship between marital status and HCV infection in hemodialysis patients. The results relate to the fact that the virus is transmitted through sexual or intimate contact, that is, about 0.4-3% of its transmission (25). HCV caused by prolonged or short exposure to infection risk factors, such as the use of the same dialysis machines in patients with hepatitis C virus antibodies, insufficient disinfection and disinfection of environmental surfaces, and misuse of intravenous drugs.

Conclusion

The rate of hepatitis C virus infection was moderate among hemodialysis patients in Peshawar Region and there are no differences between gender and residence and the infection increases with the duration of dialysis.

Approval from ethical committee.
The study was approved by the Ethical Committee of the Department of Genetics, Hazara University, Mansehra kp Pakistan

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