International Journal of Science and Technology Research Archive

ISSN: 0799-6632 (Online)

Journal homepage: https://sciresjournals.com/ijstra/

(Research Article)

Check for updates

Assessment of Hepatitis C virus genotypes in regular dialysis patients of Khyber Pakhtunkhwa, Pakistan

Imran Khan [1,*], Shaukat Ali Khan [2] and Noorul Akbar [3]

[1] *State Key Laboratory of Animal Nutrition, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, China 100193.*
[2] *School of Life Sciences Anhui Normal University.*
[3] *Department of Zoology, Kohat University of Science and Technology Kohat, 26000 Pakistan.*

International Journal of Science and Technology Research Archive, 2021, 01(01), 001–007

Publication history: Received on 18 May 2021; revised on 22 June 2021; accepted on 24 June 2021

Article DOI: https://doi.org/10.53771/ijstra.2021.1.1.0011

Abstract

Globally viral hepatitis is a major health problem. HCV is a causative agent of hepatitis and is responsible for acute and chronic hepatitis leads to cirrhosis and hepatocellular carcinoma. This study was carried out to know the HCV genotypes in Dialysis patients in NWFP (Pakistan). The age ranged from 15-65 years. During this study a total of 63 samples were collected and were analyzed for HCV genotypes. RNA was extracted from whole blood; reverse transcribed into cDNA and was subjected to multiplex PCR. Of these 63 samples, 14 were genotyped as genotype 3a was found in 9(64.28%) patients, followed by genotype 3b (21.42%) in 3 and 2a in 2(14.28%) patients. Three positive samples remained untyped. In age group 31 to 40 years, the number of positive patients were comparatively greater.

Keywords: Hepatitis C virus; Genotypes; Dialysis; PCR

1. Introduction

The virus causing hepatic inflammation or liver cirrhosis is known as Hepatitis C virus. It is a blood borne hepatotrophic virus [1]. A worldwide estimate of HCV prevalence shows that it is affecting 170 million people worldwide [2]. HCV belongs to the Flaviviridae family. HCV is divided into six genotypes with numerous subtypes. These genotypes can differ up to 30% from each other in nucleotide sequence. Within the genotypes there are many subtypes, with varying geographic distributions and modes of transmission is strongly associated with intravenous and percutaneous drug and needle use [3, 4].

In dialysis patients the rate of HCV infection is higher. In the last three decades 10-50% HCV prevalence has been reported in world most countries in hemodialysis patients with lower rates in such places as Ireland (1.7%) [5]. The transmission of the virus to hemodialysis patients is generally nosocomial with possible risk factors being failure to disinfect devices between patients, sharing of single-use vials for infusions, poor sterile technique, poor cleaning of dialysis machines, and poor distance between chairs [6]. Among kidney transplants, the prevalence is reported to be as high as 33.3%. Most of the kidney transplant patients underwent dialysis as well [7]. Individuals that are frequently exposed to blood, such as intravenous drug users, and hemodialysis patients, are at risk of acquiring HCV [8].

Prevalence of HCV infection is special prone groups found that the prevalence of HCV infections among hemodialysis patients in various countries is much higher than that among healthy blood donors, with the rate of 2 to 6% in northwestern Europe to more than 20% in Japan and over 60% in Saudi Arabia [9].

* Corresponding author: Imran Khan
State Key Laboratory of Animal Nutrition, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, China 100193.

Copyright © 2021 Author(s) retain the copyright of this article. This article is published under the terms of the Creative Commons Attribution Liscense 4.0.

In Pakistan the renal dysfunction is at the peak of health problems. There is no such study conducted ever before in this region where the HCV infection is determined at molecular level. In Pakistan there is no standard screening procedures which help reduce the freely transmission of diseases like HCV. The present study is designed to find out the active infection of HCV and HCV genotypes in dialysis patients as these are the highly risk group for blood borne disease. This study will help in the prevalence of HCV genotypes in this group of patients.

2. Material and methods

2.1. Sources of blood

Whole blood samples from patients attending the dialysis centers at Peshawar were collected and brought to the laboratory for further processing. During our sampling we were visiting three very well-known dialysis centers, i.e. dialysis ward Khyber Teaching Hospital Peshawar, National diagnostic dialysis center, and dialysis ward Hyatabad Medical Complex Peshawar. The entire collection consisted of whole blood samples of 63 dialysis patients. Our sampling was random selection of dialysis patients being transfused regularly as they are more sensitive to HCV. Then we processed the samples in laboratory. The procedure for RNA extraction and PCR are as under.

2.2. RNA extraction

For RNA extraction the Anagen RNA purification kit (superscript USA) was used. The protocol used was as the RNA was extracted by taking 900µl RRL Buffer in a 1.5µl tube and added 300µl of blood vortexed it. Then incubated it for 5 minutes at room temperature, and centrifuged the mixture for 5 minutes at 12000rpm. Two distinct layers were formed the supernatant was discarded and the pellet was left in the tube approximately 20µl. 300µl RCL was added to the tube vortexed properly and incubated at 65°C for 15 minutes and then cooled to room temperature, added 100µl RPD and vortexed then incubated on refrigerator for 5 minutes, then we centrifuged it for 10 minutes at 12000rpm.

New 1.5 µl tubes were labeled and added 300µl isopropanol as it works to precipitate RNA in a solution and added the supernatant and centrifuged it for 10 minutes at 14000rpm. The supernatant was discarded and added 300µl of 70% ethanol and centrifuged it at 14000rpm for 5 minutes. Again the supernatant was discarded and added 100% ethanol and centrifuged it at 14000rpm for 5 minutes.

Then the supernatant was discarded again, air dried the pellet which was actually RNA for 15 minutes. Then 40µl RRD solution was added to the tube and putted it for 30 minutes in refrigerator at −30 ^{o}C for using in further process.

2.3. Synthesis of complementary DNA (cDNA)

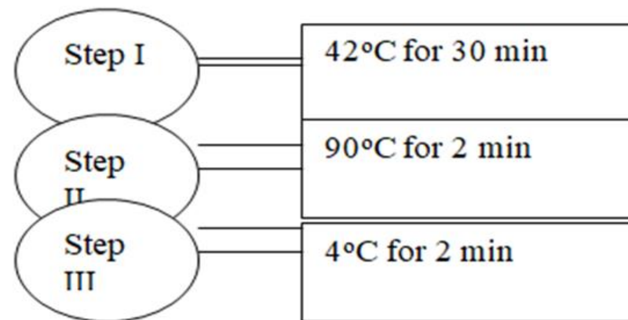

Figure 1 The cycling condition for RT-PCR

Synthesize 10 mircolitre from the extracted rehydrated RNA was used in reverse transcription into cDNA with molony murine leukemia virus reverse transcriptase (MMuLV RTase) (Fermentas Germany). The reverse transcription was carried out in thermal cycler for about 35 minute at 37 ^{o}C. The reaction mixture for the preparation of cDNA for a single reaction containing the following parameters:

- 5x buffers-------------------------4.0 µl
- dNTPs (10mM) ------------------2.0 µl
- primer-2(10pm) ----------------1.0 µl
- dH_2O (DEPC) ---------------------2.0 µl

- MMuLV (200U/µL) -------------1.0 µl
- Extracted RNA------------------10.0 µl

2.4. HCV Regular PCR

The first round of amplification was performed with 4 µl of cDNA by using one sense primer (Primer-1) and the other anti-sense primer (Primer-2). Reactions were carried out in a thermal cycler (Techne USA) with *Taq* DNA polymerase. The reaction mixture for a single reaction consisted of:

- 10x PCR buffer---------------------2.0 µl
- $MgCl_2$ (25mm) ---------------------2.4 µl
- dNTPs (500µM) -------------------1.0 µl
- P-1 (sense primer) ----------------1.0 µl
- P-2 (anti-sense primer) -----------1.0 µl
- dH_2O--------------------------------7.6 µl
- *Taq* DNA polymerase (5Uu/µL) -0.5µl
- cDNA from RT- PCR--------4.0 µl
- Step I 95^{0}C for 3 min
- Step II
  - 94° C for 45Sec
  - 64°C for 45 Sec
  - 72°C for 1 min

  35 cycles
- Step III 72 ^{o} C for 10 min

  4°C for 2 min

2.5. Genotype Specific PCR

Genotype with type specific primers from the core region of the HCV genome was performed for the five most common subtype and types of HCV which is the most common in such research area which are (1a, 2a, 3a, 3b, and 4) by type specific PCR program, as follow.

- 10X PCR Buffer---------------------------------2µl
- $MgCl_2$ (25mM) ----------------------------------.3 µl
- dNTPs (500 µM) ---------------------------------1.0 µl
- Sense primer (10 pM) --------------------------1 µl
- Anti-sense primer (19) (10pM) -----------------1 µl
- Anti-sense primer (2a) (10pM) ----------------1 µl
- Anti-sense primer (3a) (10pM) ----------------1 µl
- Anti-sense primer (3b) (10pM) --------------1 µl
- Anti-sense primer (4) (10pM) ---------------1.0 µl
- dH_20---4.1 µl
- TaqDNA polymerase-------------------------0.5 µl
- cDNA from regular PCR or PCR-2---------4 µl

The second round PCR or multiplex PCR was performed for each sample with genotype specific primers run the same program as for regular PCR.

2.6. Electrophoresis

PCR products were electrophoresed in 2% agarose gel prepared in 0.5 X TBE buffer (boiled for 2 min in a microwave oven and cooled to 50°C), adding ethidium bromide (1 µg/ mL) stained and evaluated under ultra violet UV light. The specific cDNA product of amplified product was determined by identifying the cDNA bands of a specific genotype (Key Below) comparing with 100-bp DNA ladder (Fermentas Germany), used as DNA size marker.

HCV Genotype	Product (bp)
1a	208
2a	139
3a	232
3b	176
4	99

3. Results

3.1. Sex wise distribution of HCV genotypes

Hepatitis C is a blood borne disease caused by HCV. Dialysis patients are more susceptible to infections like HCV because of the routine blood purification (Dialysis) and blood transfusions. Due to this reason dialysis population was selected for HCV detection and genotyping. In this study a total of 63 samples were investigated for HCV RNA and genotypes. Of these 63 samples 14 (23.33%) were genotyped while the remaining 49 (77.77%) were not typed.

Of the total 63 samples, 38 (60.31%) were males and 25 (39.68%) were females. The proportion of HCV genotypes in males were 19.44%, while in females the ratio was found 29.16%, table 1.

Table 1 HCV genotypes in male and female Dialysis patients

Gender	No of samples	No. of samples genotyped	%age
Male	38	7	18.42
Female	25	7	28.00

3.2. HCV genotypes in different age groups

The age range was from 15 to 65 years. Six classes of patients were designed having a difference of 10 years. Genotype 3a was most common in nearly all age groups while 3b and 2a were comparatively less frequent. The patients age of 35 to 45 are most frequently infected while the patients having age of 55 to 65 are less subjected to the infection of HCV. Further details of different age groups along with their corresponding genotypes are presented in the following table.

Table 2 HCV genotypes in different age groups

Age Group	No of patients	No: of samples typed (Genotype).	% Age
<10	0	0	0
11-20	7	1 (3a)	14.28
21-30	17	2 (3a, 2a)	11.76
31-40	12	5 (3a)	41.66
41-50	16	3 (3a, 3b)	18.75
>50	11	3 (3b,2a)	27.27

3.3. Proportion of different genotypes

There were three different genotypes detected in the samples. These were 2a, 3a and 3b. Out of these three, genotype 3a was found in greatest proportion 64.28%, while the remaining two (2a and 3b) were found in 14.28% and 21.42% of cases respectively. Three of the samples were HCV RNA positive but cannot typed by the prescribed system. Table 3

shows the percentage of differenc genotypes in the studied population. Genotype 3a is the most prevalent while some samples remained as false positive.

Table 3 Percentage of detected genotypes

Genotype	No. of samples	% Age
1a	0	0
2a	2	14.28
3a	9	64.28
3b	3	21.42
4	0	0
5a	0	0
6a	0	0
Untyped	3	17.64

3.4. Distribution based on number of transfusions

The number of transfusions was calculated for each positive patient from the first Dialysis date. To present these information five categories of transfusion number were made. The table indicates that patients Dialyzed for 5 to 10 times are greatest in number. Table 4 enlists the patients based on the transfusion numbers which is also a risk factor for blood borne diseases like HCV. Here the number of patients was low so the values are altered accordingly. Otherwise with more transfusion more risk is linked.

Table 4 No. of Transfusions Vs HCV Positive patients

No. of transfusions	No of HCV Positive patients	% Age
<5	4	28.57
5-10	5	35.71
10-15	1	7.14
15-20	0	0
>20	4	28.57

4. Discussion

Hepatitis C Virus (HCV) is a blood borne positive sense RNA virus. Patients of chronic dialysis are at increased risk of acquiring parentally transmitted hepatitis viruses from blood product transfusions or nosocomial transmission in hemodialysis units. Due to this reason dialysis patients were selected to be screened for HCV RNA and genotypes.

About 20% of the patients had anti-HCV antibodies and HCV-RNA was detected in 73% of the anti-HCV positive patients while studying the incidence and nosocomial transmission of HCV infection in haemodialysis patients [10]. In this region the HCV infection might be higher as in our study the HCV RNA was present in 22.5% of dialysis patients which may be due the small sample size.

In 11% Anti-HCV positive hemodialysis patients, HCV genotypes were investigated and genotype 1a was present in 75% of patients and 1b in 8.3% and 16.7% were infected with 3a [11]. But in our study the HCV genotype 3a was more prevalent in dialysis patients. Also in general population of Pakistan the HCV genotype 3a is common [8].

In USA. 50% of all infections in dialysis patients are due to genotype 1 [3]. But in this study genotype 3a was the most frequently found genotype.

In Chile 54% cases of HCV are due to blood transfusion in 54% versus just 5% with IVDU [12]. In this study it is found with the gradual increase in number of transfusion the risk for attaining HCV also ensures.

From 1980 onward the prevalence of HCV in dialysis patients were 50% greater than the normal population [5, 13].In this study the risk is less due to advancement in mechanization and medication of dialysis patients. The prevalence of HCV in Saudi Arabia is 9.24% [14]. The prevalence if HCV in Pakistan is greater than Saudi Arabia.

In 184 hemodialysis patients (110 males, 74 females) were examined for HCV-RNA by a reverse transcription-polymerase chain reaction method. The positive rate of HCV was found10.7% (20/184), greater than the general population. Using a second generation HCV antibody assay, the positive rate increased to 22%. HCV-RNA was detected in 15 of 184 patients (8.2%) positive patients (25%), and 10 of negative patients (6.1%). It was found that some hemodialysis patients have latent HCV infections that cannot be detected by currently available HCV antibody assays or routine biochemical liver function tests, and that the routes of transmission are not solely through blood transfusion [15]. In this study the prevalence rate can also be changed if we adopt an alternate methodology for HCV genotyping.

In a survey of dialysis patients the prevalence of anti-HCV was 11%. HCV RNA was detected in 12 samples: 75% were of genotype 1a and 8.3% 1b and two were of genotype 3, subtype 3a (16.7%) [11]. In our study the genotypic distribution was as 3a (64.28%), 3b (21.42%) and 2a (14.28%).

In a study of Dutch dialysis patients the genotypic analysis by reverse hybridization line probe assay revealed the presence of genotypes as 1a (23%), 1b (46%), 2 (3%), 2a (13%), 2b (1%), 3a (7%), and 4a (4%) [16]. Genotypes 3a, 2a, and 3b are more common in this area.

5. Conclusion

It is concluded from this work that dialysis patients are prone to HCV due to improper sterilization of dialysis centres in the concerned area. Here we found a significant number of patients were positive for HCV. Genotype 3a is the most frequently found genotype in this area.To address all these issues dialysis centres should be properly sterilized. HCV patients may be subjected to separate operational units to minimize the risk of the spread of the disease.

References

[1] Ryan KJ, Ray CG. Sherris Medical Microbiology, 4th ed.; McGraw Hill. 2004; 5512.

[2] WHO, Global surveillance and control of hepatitis C. Report of a WHO Consultation organized in collaboration with the Viral Hepatitis Prevention Board, Antwerp, Belgium. J Viral Hepat. 1999; 6: 35-47.

[3] Alter MJ, Kruszon-Moran D, Nainan OV, McQuillan GM, Gao F, Moyer LA, Kaslow RA, Margolis HS. The prevalence of hepatitis C virus infection in the United States, 1988 through 1994. N Engl J Med. 1999; 341: 556-62.

[4] Simmonds P. Genetic diversity and evolution of Hepatitis C virus -15 yers on. J Gen viral. 2004; 85: 3173-3188.

[5] Niu MT, Coleman PJ, Alter MJ. Multicenter study of hepatitis C virus infection in chronic hemodialysis patients and staff. Am J Kidney Diseases. 1993; 22: 568-573.

[6] Zampieron A, Jayasekera H, Elseviers M, Lindley E, De Vos JY, Visser R. Harrington M. European study on epidemiology and the management of HCV in the haemodialysis population—Part 1: centre policy. EDTNA ERCA J. 2004; 30(2): 84-90.

[7] Angelic M, Tisone G, Rapicetta M, Pisani F, Gandin C, Chionne P, Dettori S, Iaria G, Danese V, Orlando G, Casciani CU. Hepatitis C virus infection in Italian kidney graft recipients. Changing risk factors and hepatitis C virus genotypes. J Gastroenterology Hepat. 1997; 29: 448-55.

[8] Kuo GQ, Choo L, Alter HJ, Gitnick GL, Redeker AG, Purcell RH, Miyamura T, Dienstag JL, Alter MJ, Muhammad CE, Jan MA. Frequency of Hepatitis C in Buner, NWFP. JCPSP. 2005 Mohsen A.H., Trent HCV study group. An assay for circulating antibodies to a major etiologic virus of human non-A, non-B hepatitis. Science. 2001; 244: 362-364.

[9] Van-der-Poel CL, Reesink EP, Mauser-Bunschoten RH, Kaufmann A, Leentvaar-Kuypers RA, Chamuleau W, Schaasberg E, Bakker P. Prevalence of anti-HCV antibodies confirmed by recombinant immunoblot in different population subsets in The Netherlands. Vox Sang. 1991; 61: 30-36.

[10] Hmaied F, Mamou MB, Saune-Sandres K, Rostaing L, Slim A, Arrouji Z, Redjeb SB, Izopet J. J Med Viro. 2006; 78(2): 185-191.

[11] Freitas SZ, Da-Cunha RV, Martins RMB, Teles SA, Ibanhes ML. Motta- Castro, ARC. Memorias do Instituto Oswaldo Cruz. 2008; 103(4): 405-408.

[12] Soza A, Arrese M, Gonzalez R, Alvarez M, Perez RM, Cortes P, Patillo A, Riquelme A. Clinical and epidemiological features of 147 Chilean patients with chronic hepatitis C. Ann Hepatol. 2004; 3(4): 146-51.

[13] Hayashi J, Yoshimura E, Nabeshima A, Kishihara Y, Ikematsu H, Hirata M, Maeda Y, Kashiwagi S. Seroepidemiology of hepatitis C virus infection in hemodialysis patients and the general population in Fukuoka and Okinawa,. Japan. J Gastroenterol. 1994; 29: 276-281.

[14] Qadi AA, Tamim H, Ameen G, Bu-Ali A, Al-Arrayed S, Fawaz NA, Almawi WY. Hepatitis B and Hepatitis C virus prevalence among dialysis patients in Bahrain and Saudi Arabia: a survey by serologic and molecular methods. Am J Infect Control. 2004; 32(8): 493-5.

[15] Sakamoto N, Enomoto N, Marumo F, Sato C. Prevalence of hepatitis C virus infection among long-term dialysis patients: Detection of hepatitis C virus RNA in plasma. Journal of Medical Virology. 1993; 39(1): 11-15.

[16] Schneeberger P, Keur I, Van-Der Vliet W, Van, Hoek K, Boswijk H, Van Loon AM, Van Dijk WC, Kauffmann RH, Quint W, Van Doorn LJ. Journal of Clinical Microbiology. 1998; 36(6): 1711-1715.