



FREQUENCY OF HEPATITIC C VIRUS (HCV) GENOTYPES AND SUBTYPES

Farukh Imtiaz^{1*}, Shehzad Tarique², Safder Pervez³, Saba Faiz⁴, Zahid Hussain⁵, Sarmad Saeed⁶

^{1*}Associate professor community Medicine Khairpur Medical College Khairpur Mir's
Email. drfarukh.bhanbhro@gmail.com

²Demonstrator Community Medicine, Khairpur medical college Khairpur Mir's

³Associate Professor Medicine, Khairpur Medical College Khairpur Mir's

⁴Demonstrator Community medicine, Khairpur Medical College Khairpur Mir's

⁵Consultant Physician, Liaquat university of Medical and health Sciences, Jamshoro

⁶Medical officer, Department of Medicine, Khairpur Medical College, Khairpur Mir's

***Corresponding Author:** Farukh Imtiaz

*Associate professor community Medicine Khairpur Medical College Khairpur Mir's
Email. drfarukh.bhanbhro@gmail.com

ABSTRACT

Background: Viral hepatitis is still a major global public health concern; HCV is especially worrisome because it can develop chronic hepatitis that lasts a long time in vulnerable people. HCV is an encapsulated virus that was first discovered in 1989. It presents major clinical concerns as the second most common cause of viral hepatitis worldwide. Determining the HCV genotype is essential because treatment duration has been observed to differ according to genotype. While some genotypes react to treatment more quickly, others take longer to successfully eradicate the virus and achieve sustained virologic response (SVR). HCV infection is a major global health concern, because it is generally asymptomatic until clinical signs appear, it can be difficult to correctly estimate its incidence rate.

Objective: To assess the frequency of HCV genotypes and subtypes in people who are infected with hepatitis C Virus.

Study design: a cross sectional study

Place and Duration: This study was conducted at Khairpur Medical college teaching Hospital, Khairpur Mir's, Sindh, Pakistan from September 2022 to September 2023

Methodology: Participants in the study had to be 16 years of age or older and chronically infected with HCV. All the participants were positive for HCV RNA. These patients had 600 blood samples taken in order to ascertain their HCV genotypes. As directed by the Abbott Real Time HCV Genotype assay kit, RT-PCR was used for the genotyping analysis. Using fluorescent-labeled probes specific to each genotype, this kit detects the six major HCV genotypes (1-6) and their subtypes (1a and 1b). Primers target the 5'UTR region for genotypes 1-6 classification and the NS5B region for subtype detection of 1a and 1b.

Results: There were a total of 600 HCV positive samples assessed in this study subjected to genotyping. Out of 600 samples, 348 (58%) were males and 252 (42%) were females. This shows that the number of females in this research was lesser than males. The most frequent genotype was HCV genotype 3 affecting 85% of the total patients.

Conclusion: There were found to be eight genotypes and subtypes of hepatitis C virus, with genotype 3 being the most common.

Keywords: Adults, hepatitis C virus, genotypes, genotype 3

INTRODUCTION

Viral hepatitis is still a major global public health concern; HCV is especially worrisome because it can develop chronic hepatitis that lasts a long time in vulnerable people [1, 2]. HCV is an encapsulated virus that was first discovered in 1989 [3]. It is icosahedral in shape and contains single-stranded non-segmented RNA contained in a nucleocapsid. It presents major clinical concerns as the second most common cause of viral hepatitis worldwide [4]. Six of the eleven identified HCV genotypes are considered significant kinds, adding to the complexity of the infection [5].

Determining the HCV genotype is essential because treatment duration has been observed to differ according to genotype. While some genotypes react to treatment more quickly, others take longer to successfully eradicate the virus and achieve sustained virologic response (SVR) [6]. Thus, in order to guarantee good treatment outcomes and viral clearance, figuring out the genotype before starting treatment is strongly recommended. Patients with genotype 1 may require a lengthier treatment period, such as 48 weeks, but those with genotypes 2 and 3 normally require a 24-week course of treatment [7].

HCV infection is a major global health concern, because it is generally asymptomatic until clinical signs appear, it can be difficult to correctly estimate its incidence rate [8]. Changes in the global prevalence rates of HCV infection have resulted from awareness campaigns in numerous countries about important risk factors, including intravenous drug use and blood transfusions [9]. It is generally accepted that parenteral exposures involving infected needles and blood transfusions are the main ways that HCV is transmitted.

Globally, there are differences in HCV genotypes; genotypes 1, 2, and 3 are the most common [10]. Distinct regions are linked to distinct subtypes: 1a and 1b in North America and Europe, 1b in Asia and Europe, 2a in China and Japan, 2b in northern Europe, and 3a in South Asia and Australia [11]. Subtype 4a is found in Egypt, 4c in central Africa, and genotype 4 is prevalent throughout the Middle East [12]. Subtype 6a is more common in Macau, Vietnam, and Hong Kong, whereas subtype 5a is more common in South Africa. Other uncommon genotypes are 10a, 11a in Indonesia, 7a, 7b in Thailand, 8a, 8b, and 9a in Vietnam. Pakistan exhibits a significant frequency of untypable genotypes including genotype 3 [13]. Thus, this study was performed to assess the frequency of HCV genotypes and subtypes in people who are infected with hepatitis C virus.

METHODOLOGY

Participants in the cross-sectional study had to be 16 years of age or older and chronically infected with HCV. All the participants were positive for HCV RNA. These patients had 600 blood samples taken in order to ascertain their HCV genotypes.

A venipuncture was used to extract 5 milliliters of blood from each subject using a sterile syringe. The blood samples were processed in accordance with the manufacturer's instructions after being quickly placed into BD Vacutainer serum separation tubes. The gel tubes were to be gently inverted five times, left to coagulate for thirty minutes, and then centrifuged for ten minutes at 3000 rpm. During centrifugation, the gel in the tubes served as a barrier between the blood cells and the serum. After centrifugation before the serum that had separated was collected, and the samples were either used right away or kept for later examination at -20°C.

As directed by the Abbott Real Time HCV Genotype assay kit, RT-PCR was used for the genotyping analysis. Using fluorescent-labeled probes specific to each genotype, this kit detects the six major HCV genotypes (1-6) and their subtypes (1a and 1b). Primers target the 5'UTR region for genotypes 1-6 classification and the NS5B region for subtype detection of 1a and 1b.

RESULTS

There were a total of 600 HCV positive samples assessed in this study subjected to genotyping. Out of 600 samples, 348 (58%) were males and 252 (42%) were females. This shows that the number of females in this research was lesser than males. Table number 1 shows distribution of various HCV genotypes found circulating.

Table No. 1: distribution of various HCV genotypes

Genotypes	N	%
1	3	0.50
1,1a	5	0.80
1a	0	0.00
1b	2	0.30
1,1b	15	2.50
2	4	0.67
3	510	85.00
3,4	5	0.80
4	3	0.5
5	0	0
6	0	0
Untypable	53	8.93
<u>Total</u>	<u>600</u>	<u>100</u>

Table number 2 shows the distribution of HCV genotypes among men according to their age.

Table No. 2: distribution of HCV genotypes among men according to their age

Genotypes	Age groups in years (Male)			
	1-20	21-40	41-60	Above 60
1	-	3	-	-
1,1a	-	2	3	-
1a	-	-	-	-
1b	-	-	-	-
1,1b	-	3	7	-

2	-	-	1	2
3	13	87	162	19
3,4	-	2	-	2
4	-	-	2	-
5	-	-	-	-
6	-	-	-	-
Untypable	-	18	13	9
Total	<u>13</u>	<u>115</u>	<u>188</u>	<u>32</u>

Table number 2 shows the distribution of HCV genotypes among women according to their age.

Table No. 3: distribution of HCV genotypes among women according to their age

Genotypes	Age groups in years (Female)			
	1-20	21-40	41-60	Above 60
1	-	1	-	-
1,1a	-	-	3	-
1a	-	-	-	-
1b	-	1	-	-
1,1b	-	1	4	1
2	1	1	-	-
3	6	92	103	14
3,4	1	-	1	-
4	-	-	-	-
5	-	-	-	-
6	-	-	-	-
Untypable	-	9	11	2
Total	<u>8</u>	<u>105</u>	<u>122</u>	<u>17</u>

DISCUSSION

Many research carried out in Pakistan have consistently shown that genotype 3 is the most prevalent genotype [14]. According to Ahmed et al., genotype 3a is primarily common in Lahore [15]. Similar to this, a prior epidemiological study conducted in Baluchistan, Pakistan, indicated that genotype 3a

was the most common genotype [16]. These results are consistent with information from remote Asian nations like Nepal and nearby India, where genotype 3a is also the most common genotype across all genotypes and subtypes. On the other hand, this pattern of prevalence differs from that seen in the America, Europe, and Japan, where genotypes 1 and 2 are frequently documented.

Untypable was the second most common variant found, affecting 8.93% (n=53) of the patients. There was no genotype-specific PCR product obtained from these untypable genotypes. Other researchers in Pakistan have also reported significant prevalence of untypable genotypes, so this study is not the first to describe such findings [17]. Furthermore, a small percentage of patients had mixed genotypes. Notably, none of the patients in the study had HCV genotypes 1a, 5 or 6.

The least common genotype, 1b, was found in only 0.3% of patients. The study's participants were categorized into three age groups, and it was determined whether male and female patients had the same age-associated HCV genotypes. In all age groups, genotype 3 was consistently more prevalent. However, as the greatest number of positive cases were seen in the 41–60 age range, it seemed that this age group was more vulnerable to HCV genotype 3. Similar results have also been reported in other investigations [18, 19].

The genotype distribution differed slightly by gender, despite genotype 3 being the most common in both male and female patients. The genotype distribution of the two genders did not significantly differ from one another. There were seven genotypes that were shared by males and females in this study; however, genotype 4 was only found in males.

Only males were found to have genotype 4; females did not. On the other hand, genotypes 5 and 6 are more common in South Africa and Hong Kong, respectively, whereas genotype 4 is generally reported to be very abundant throughout the Middle East and North Africa. [20] Antiviral medication is available, but the increasing number of HCV genotype 3 patients is alarming and emphasizes the need for further precautions to stop the spread of infection. HCV genotype distribution patterns must be regularly monitored in order to guide treatment decisions and preventative actions.

CONCLUSION

According to the study, there is variation in the distribution of HCV genotypes by gender and age. Both genders are most impacted in the 41–60 year age range. There were found to be eight genotypes and subtypes, with genotype 3 being the most common.

Funding source

This research was conducted without receiving financial support from any external source.

Conflict in the interest

The authors had no conflict related to the interest in the execution of this study.

Permission

Prior to initiating this study, approval from the ethical committee was obtained to ensure adherence to ethical standards and guidelines.

REFERENCES

1. Pathan NL, Tunio SA, Bano S, Jatt AN. 15. Frequency distribution of hepatitis C virus genotypes circulating in Hyderabad, Sindh. *Pure and Applied Biology (PAB)*. 2019 Feb 27;8(1):133-8.
2. Khan TM, Mehr MT, Ullah H, Khan H, Iman NU. Frequency of hepatitis C virus genotypes in the north of Pakistan. *Gomal Journal of Medical Sciences*. 2014 Sep 17;12(2).
3. Khan S, Ali I, Badshah M, Khan QM, Haider ZN, Ali S, Khan IA, Ullah A. Molecular epidemiology of hepatitis C virus genotypes among chronically infected patients in Pakistan. *Jundishapur Journal of Microbiology*. 2019 Mar 31;12(3).
4. Waqar M, Khan AU, Rehman HU, Idrees M, Wasim M, Ali A, Niaz Z, Ismail Z, Rehman MU, Tariq M, Shah M. Determination of hepatitis C virus genotypes circulating in different districts

- of Punjab (Pakistan). *European journal of gastroenterology & hepatology*. 2014 Jan 1;26(1):59-64.
5. Butt S, Idrees M, Akbar H, ur Rehman I, Awan Z, Afzal S, Hussain A, Shahid M, Manzoor S, Rafique S. The changing epidemiology pattern and frequency distribution of hepatitis C virus in Pakistan. *Infection, Genetics and Evolution*. 2010 Jul 1;10(5):595-600.
 6. Khan N, Akmal M, Hayat M, Umar M, Ullah A, Ahmed I, Rahim K, Ali S, Bahadar S, Saleha S. Geographic distribution of hepatitis C virus genotypes in Pakistan. *Hepatitis monthly*. 2014 Oct;14(10).
 7. Inamullah I, Idrees M, Ahmed H, Ali M, Ali L, Ahmed A. Hepatitis C virus genotypes circulating in district Swat of Khyber Pakhtoonkhaw, Pakistan. *Virology Journal*. 2011 Dec;8:1-5.
 8. Ali A, Ahmed H, Idrees M. Molecular epidemiology of Hepatitis C virus genotypes in Khyber Pakhtoonkhaw of Pakistan. *Virology journal*. 2010 Dec;7:1-7.
 9. Attaullah S, Khan S, Ali I. Hepatitis C virus genotypes in Pakistan: a systemic review. *Virology journal*. 2011 Dec;8:1-6.
 10. Narahari S, Juwle A, Basak S, Saranath D. Prevalence and geographic distribution of Hepatitis C Virus genotypes in Indian patient cohort. *Infection, Genetics and Evolution*. 2009 Jul 1;9(4):643-5.
 11. Heathcote EJ (2007). Antiviral therapy: chronic hepatitis C. *J Viral Hepat* 1482-88.
 12. Rosen HR (2011). Chronic Hepatitis C Infection. *N Engl J Med* 364(25): 2429- 2438.
 13. Sy T & Jamal MM (2006). Epidemiology of hepatitis C virus (HCV) infection. *Int J Med Sci* 3(2): 41-6
 14. Afridi S, Zahid MN, Shabbir MZ, Hussain Z, Mukhtar N, Tipu MY, Akhtar F & Yaqub T (2013). Prevalence of HCV genotypes in district Mardan. *Virol J* 10(1): 90.
 15. Ahmad W, Ijaz B, Javed FT, Jahan S, Shahid I, Khan FM & Hassan S (2010). HCV genotype distribution and possible transmission risks in Lahore, Pakistan. *World J of Gastroenterol WJG* 16(34): 4321.
 16. Afridi S, Naeem M, Hussain A, Kakar N, Babar ME & Ahmad J (2009). Prevalence of hepatitis C virus (HCV) genotypes in Balochistan. *Mol Bio Rep* 36(6): 1511-1514.
 17. Singh B, Verma M & Verma K (2004). Markers for transfusion-associated hepatitis in north Indian blood donors: prevalence and trends. *Japanese J of Infec Dis* 57(2): 49-51.
 18. Rouabhia S, Sadelaoud M, ChaabnaMokrane K, Toumi W & Abenavoli L (2013). Hepatitis C virus genotypes in north eastern Algeria: A retrospective study. *World J of Hepatol* 5(7): 393.
 19. Anwar MI, Rahman M, Hassan MU & Iqbal M (2013). Prevalence of active hepatitis C virus infections among general public of Lahore, Pakistan. *Virol J* 10(1): 351.
 20. Waheed Y (2015). Effect of interferon plus ribavirin therapy on hepatitis C virus genotype 3 patients from Pakistan: treatment response, side effects and future prospective. *Asian Pacific J of Trop Med* 8(2): 85-89.