

Research Article

Molecular epidemiology and viral load analysis of hepatitis C virus genotypes from Sindh, Pakistan

Afsheen Kazi¹, Shaista Bano¹, Sarfraz Ali Tunio^{1*}, Abdul Nabi Mirjatt¹, Faryal Ahmed Khushik¹ and Fawad Shabir Memon¹

1. Institute of Microbiology, University of Sindh, Jamshoro-Pakistan

*Corresponding author's email: sarfraz.tunio@usindh.edu.pk

Citation

Afsheen Kazi, Shaista Bano, Sarfraz Ali Tunio, Abdul Nabi Mirjatt, Faryal Ahmed Khushik and Fawad Shabir Memon. Molecular epidemiology and Viral load analysis of Hepatitis C virus genotypes from Sindh, Pakistan. Pure and Applied Biology. Vol. 10, Issue 1, pp341-347. <http://dx.doi.org/10.19045/bspab.2021.100037>

Received: 05/05/2020

Revised: 05/08/2020

Accepted: 25/08/2020

Online First: 28/09/2020

Abstract

The present study was aimed to assess the molecular epidemiology of Hepatitis C Virus (HCV) genotypes and viral load (VL) of HCV infected patients. A total of 261 anti-HCV positive patients were enrolled for VL analysis using COBAS AmpliPrep/COBAS TaqMan HCV and Abbott Real Time HCV Genotype II Assay Kit to determine the genotypes. The data demonstrated that 60.15% (n=157) samples were from females and 39.85% (n=104) from male patients. Serum markers such as Bilirubin, SGPT, PT, APTT, and α -fetoproteins were analyzed by commercial kits and Cobas-C 501, Sysmex Ca500 and Cobas-C 601 automatic analyzers as per manufacturer's guidelines. All patients were above 15 years age and their mean age was 39 years. Male to female ratio was 0.66% (104/157). Age-wise distribution revealed that 31-45 years age group comprised majority of the patients, whereas lowest number belonged to age group 61 and above. HCV genotyping data revealed five different genotypes/subtypes. Genotype 3a was most frequent accounting 76.24% (n=199) followed by Genotype 1a (2.29%), 1b (2.68%), 2a (4.98%). Moreover, 13.79% (n=36) samples revealed untypable genotype. Subtype 1b had highest mean VL of 6.84 log₁₀ IU/ml among all other genotypes. The data of serum markers analysis revealed a slight fluctuation but appeared under the normal range. However, Bilirubin was slightly lower in Genotype 1a as compared with other genotypes while elevated α -fetoprotein was found in patients infected with untypable genotypes. In summary, Genotype 3a was predominant genotype throughout Sindh and subtype 1b had highest viral load compared with subtypes 3a and 2a.

Keywords: Genotyping; Hepatitis C; HCV; Prevalence; Serum markers

Introduction

Hepatitis C virus (HCV) is major cause of hepatitis C with significant clinical problems in humans worldwide. Comprehensive knowledge of HCV genotypes is essential to understand the clinical relevance as the effectiveness of treatment and vaccine development are impacted by genotypes and subtypes. A great genetic variability in HCV has been

reported regionally [1]. Moreover, spread of HCV has been shown to occur through blood transfusion and parenteral exposures with contaminated medical equipments [2, 3]. HCV has a single stranded positive polarity genome of 9.6 kb. It has a single open reading frame encoding a polyprotein encompassing 10 mature proteins including three structural proteins annotated as C, E1, E2 and seven

nonstructural proteins including p7, NS2, NS3, NS4A, NS4B, NS5A, NS5B. Moreover, two highly structured untranslated regions are flanking the open reading frame which is responsible for regulating the translation and replication of the viral genome [4, 5]. At least six major HCV genotypes and hundreds of the subtypes have been reported across the world [6]. The clinical management of infection, response rate to anti-viral drugs and the vaccine development has been associated with the genetic variation in viral genome leading to distinct HCV genotypes and subtypes [7]. The patients infected with different genotypes respond differently to antiviral therapy especially alpha interferon, thus, both the genotype involved in infection and the viral load can be predictors of the therapeutic outcome and sustained virological response [8]. It has been shown that patients infected with HCV genotype-2 and genotype-3 demonstrated a sustained virological response to anti-viral treatment as compared with the patients infected with HCV genotype-1 [8]. Since the genotype has an important role in treatment and clinical management of infection, genotype must be determined prior to commencing standard interferon / antiviral therapy. Molecular epidemiological studies have demonstrated a great variation in genotype distribution by geographical location. Three HCV genotypes 1, 2, and 3 have worldwide distribution throughout Australia, Europe, USA and East Asia including Japan, Taiwan, Thailand, and China, while the geographic distribution of other genotypes is restricted to respective localities [9, 10, 11]. HCV-1a and 1b subtypes are predominantly distributed in the United States of America and Europe while HCV subtype 1b is most prevalent in Japan [12]. Genotypes 2a and 2b have been reported from North America, Europe, and Japan and genotype 2c is prevalent in northern Italy [12, 13]. Genotype 4 is the most prevalent in Central Africa, Middle East and Egypt [14, 15]. HCV-5 and HCV-

6 genotypes are predominantly found in South Africa and South East Asia, respectively [16]. Studies on molecular epidemiology and clinical relevance regarding HCV and its genotypes and subtypes in Pakistan are scarce especially from Sindh [17, 18]. A previous study by Pathan *et al*, 2019, demonstrated that genotype 3 was most prevalent followed by 1b subtype in Hyderabad, Sindh [19]. However, no recent study on clinical relevance of various HCV genotypes is available from Hyderabad, Sindh. Therefore, this study was designed to assess the clinical relevance and molecular epidemiology of HCV genotypes circulating in Sindh.

Materials and Methods

A cross-sectional prospective study was conducted at Institute of Microbiology, University of Sindh, Jamshoro for the period of 7 months from February 2017 to August 2017. This study was approved by the advanced studies and Research Board (AS&SB), University of Sindh, Jamshoro. The samples were collected from HCV patients belonging to different cities of Sindh who were attending the Civil Hospital Hyderabad and Diagnostic and Research Laboratory Hyderabad, Sindh. The information regarding age, sex, clinical history, and birth were obtained from each participating patient, with their consent. A total 261 samples were collected by convenient/standard sampling technique. All patients who yielded positive anti HCV antibodies were included in the study. HCV negative patients were excluded. HCV viral load was measured using COBAS AmpliPrep/COBAS TaqMan HCV Test. Briefly, 200µl of serum samples were used to extract HCV viral RNA using viral RNA extraction kit as per manufacturer's instructions (Qiagen). PCR positive HCV samples were further analysed for HCV genotyping using HCV genotyping protocol with Abbott Real Time HCV Genotype II Assay Kit as per protocol supplied by the manufacturers. Briefly,

three sets of PCR primers one set targeting the sequence within the 5'UTR, the second primer set used to amplify the NS5B region of genotype 1a and the third primer set to amplify the NS5B region of genotype 1b were used. Commercially available kits (SGPT Kit) and automatic chemistry analyzer (Cobas-C 501) were used to analyze biochemical markers such as SGPT and Bilirubin analysis and Automatic Analyzer Sysmex Ca 500 was used for PT and APTT. Analyzer 501 was used for albumin, Cobas – C 601 was used for α -Fetoprotein. The data were analyzed using the Microsoft Excel 2010. All presented quantitative data are means \pm standard deviations.

Results

A total of 261 samples were analysed for HCV viral loads and genotyping. The patients belonged to 13 different cities of Sindh, wherein highest number of samples was collected from Hyderabad (57.85%) and lowest from Pano Aqail (0.38%). All patients were above 15 years in age. All HCV positive samples were analysed based on age and gender (Figure 1). Majority of the samples 60.15% belonged to females whereas 39.85% were from male subjects. Females patients were more affected in all age groups. However, age group 31-45 years comprised highest number of patients followed by 46-60 while the age group 61 and above had lowest number of patients.

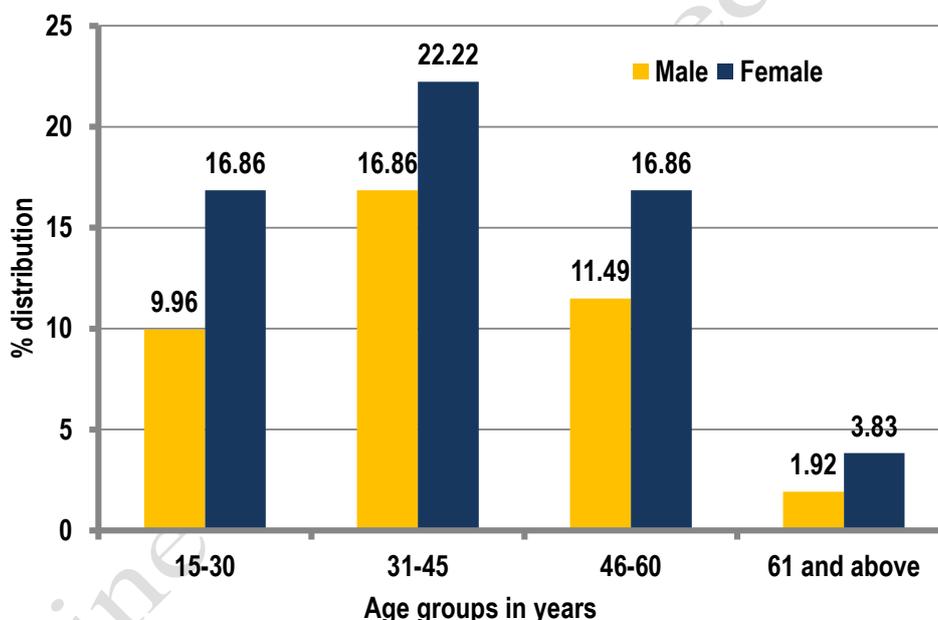


Figure 1. Graph demonstrating the age and gender wise distribution of samples

Distribution of HCV genotypes

The HCV genotyping data revealed five different genotypes/subtypes in which the genotype 3a was most frequent genotype infecting 76.24% (n=199) of the patients. The second most common variety found in this study was untypable 13.79% (n=36). The prevalence of other genotypes included: Genotype 1a (2.29%), 1b (2.68%), 2a (4.98%) (Figure 2). Whilst three genotypes namely 4, 5 and 6 and

their subtypes were not identified from any samples investigated in this study.

Association of HCV genotypes with viral load

Viral Load analysis revealed that patients infected with HCV subtype 1b had higher VL (mean VL 6.84 log₁₀ IU/ml) followed by subtype 3a and 2a (Figure 3). Generally, the genotype 1b has worldwide distribution. Previous studies have shown that genotype 1b is associated with higher viral loads, lower response to interferon

treatment and more severe clinical manifestations, In the present study, although Genotype 1b was not

predominant genotype, its mean VL was highest among all genotypes investigated which is consistent with published data.

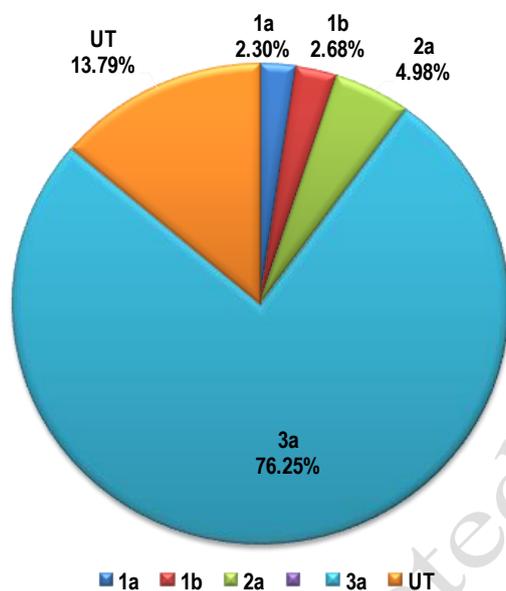


Figure 2. Graph demonstrating the prevalence of HCV genotypes/subtypes.

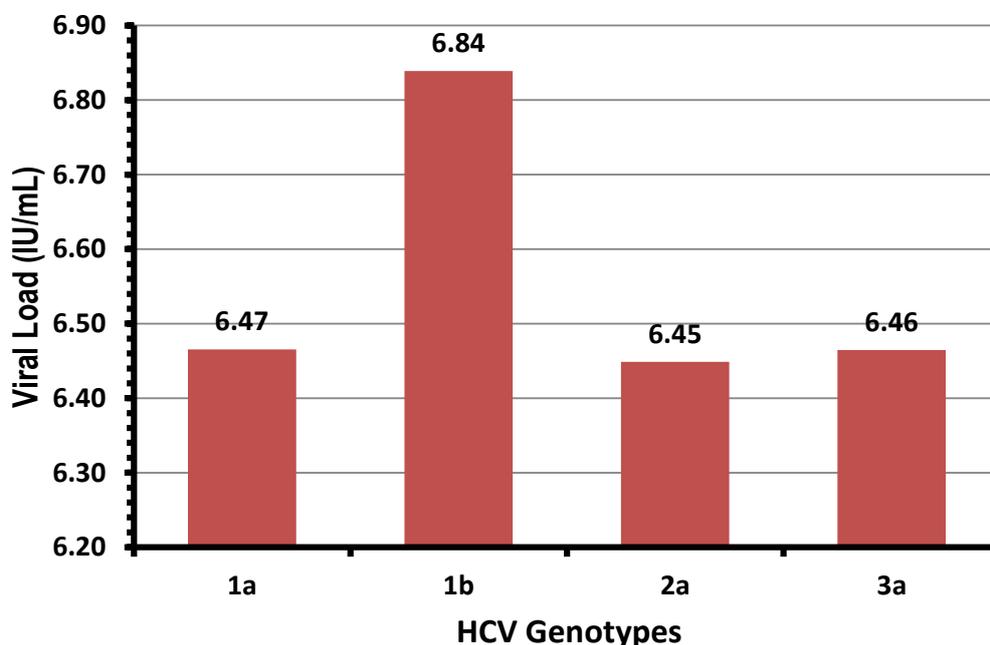


Figure 3. Graph showing the Genotype-wise Viral load analysis of the infected patients

HCV Genotype-wise serological marker analysis among HCV positive samples

Biochemical analysis of HCV positive serum samples was conducted to identify the association / clinical relevance with

HCV genotypes. Several biochemical parameters including Bilirubin, SGPT, PT, APTT, Albumin and α -fetoprotein were assessed and analyzed. The data revealed that almost all parameters demonstrated a

little fluctuation, but they were in the normal range. However, Bilirubin was slightly lower in Genotype 1a as compared with other genotypes while elevated α -

fetoprotein was found in serum of patients infected with untypable genotypes (Table 1).

Table 1. HCV genotypes and their association with serum markers among HCV patients

| Biochemical Markers | Genotype 1a (n=6) | Genotype 1b (n=7) | Genotype 2a (n=13) | Genotype 3a (n=199) | Genotype UT (n=36) |
|--|-------------------|-------------------|--------------------|---------------------|--------------------|
| Bilirubin | 0.42 ± 0.049 | 0.8 ± 0.662 | 0.7 ± 0.767 | 0.68 ± 0.592 | 0.6 ± 0.614 |
| SGPT | 37.5 ± 7.778 | 41.5 ± 1.732 | 36.5 ± 5.0479 | 38.2 ± 4.504 | 36.5 ± 4.116 |
| PT | 15.7 ± 2.217 | 14.2 ± 2.217 | 15.4 ± 1.988 | 15.3 ± 1.771 | 14.9 ± 1.910 |
| APTT | 33.5 ± 0.707 | 33.0 ± 1.414 | 33.8 ± 1.345 | 34.05 ± 1.820 | 34.0 ± 1.617 |
| Albumin | 3.48 ± 0.355 | 3.5 ± 0.350 | 3.0 ± 0.508 | 3.5 ± 0.410 | 3.7 ± 0.396 |
| α-fetoprotein | 5.6 ± 6.160 | 6.8 ± 6.965 | 2.11 ± 0.262 | 3.9 ± 3.461 | 9.4 ± 3.814 |

Discussion

The present study reports the molecular epidemiology of the HCV genotypes and their influence on viral loads among HCV infected patients from Sindh. Anti-HCV positive patient's serum was used for VL analysis followed by genotyping analysis to determine the frequency of genotypes circulating at Sindh. A few of the studies have been carried out in Sindh, regarding the genotype distribution; however, no recent study has focused on viral load analysis and its association with genotypes. The data demonstrated that females were higher (60.15%) than that of male (39.85%) patients. Among the HCV patients, Genotype 3a accounted for 76.25% of the total infected patients. These results are consistent with one of our recent report that genotype 3 was most prevalent genotypes circulating at Hyderabad, Sindh [19] as well as with other previously published reports across Pakistan demonstrating that the genotype 3 is more common in Pakistan. The second most common subtype in this study was 2a followed by 1b and 1a. Detection of HCV genotype and the viral loads have paramount importance as they aid in prediction of therapeutic outcomes among

patients treated with antiviral therapy i.e. interferon plus ribavirin. The patients infected with genotype 1 have been reported to demonstrate higher VL as compared to those patients infected with genotypes 2 and 3 [20, 21]. The present study has shown that the patients infected with genotype 1a yielded higher VL as compared to genotype 2 and 3. Our findings are in agreement with published data [22], however a Chinese study has reported that besides Genotype 1, the genotype 6 was also significantly associated with higher VL as compared to genotype 2 and 3 [23].

Conclusion

In summary, five different HCV genotypes/subtypes were found circulating in the Sindh. Females were higher than male patients infected with HCV and the most commonly affected age group was 31-45 years age. HCV genotype 3a was highly prevalent followed by 2a, 1b and 1a. Viral load analysis revealed highest VL in patients infected with subtype 1b. Periodical surveillance of HCV genotypes, VL analysis and effective therapeutic options for designing the best preventive strategies for HCV infections are needed for clinical management of HCV.

Authors' contributions

Conceived and designed the experiments: S Bano & SA Tunio, Performed the experiments: A Kazi, AN Mirjatt, Analyzed the data: A Kazi, FA Khushk & SA Tunio, Contributed materials/ analysis/ tools: A Kazi & FS Memon, Wrote the paper: SA Tunio & S Bano.

Acknowledgement

Authors would like to thank the management of Civil Hospital Hyderabad and Diagnostic and Research laboratory, LUMHS, Jamshoro for their cooperation and support during this work and the institute of Microbiology for providing laboratory space for the research work.

References

- Petruzzello A, Loquercio G, Sabatino R, Balaban DV, Ullah Khan N, Piccirillo M, Rodrigo L, di Capua L, Guzzo A & Labonia F (2019). Prevalence of Hepatitis C virus genotypes in nine selected European countries: A systematic review. *J of Clin Lab Anal* 33(5): e22876.
- Prati D (2006). Transmission of hepatitis C virus by blood transfusions and other medical procedures: a global review. *J Hepatol* 45(4): 607-16.
- Tunio S, Bano S, Pirzada Z, Rajput Z, Maheshwari M, Ahmed S, Soomro A & Mahraj S (2014). Prevalence of Hepatitis C and Hepatitis B co-infections in distric Hyderabad, Pakistan. *Sindh Uni Res J-SURJ (Sci Series)* 46(4): 511-514.
- Moradpour D, Penin F & Rice CM (2007). Replication of hepatitis C virus. *Nature Reviews Microbiology* 5(6): 453-463.
- Lindenbach B, Thiel H-J & Rice C (2007). Flaviviridae: The 693 Viruses and Their Replication. *Fields* 694: 1101-1152.
- Zein N & Persing D (1996). Hepatitis C Genotypes: current trends and future implications. *Mayo Clin Proc* 71: 458-462.
- Liew M, Erali M, Page S, Hillyard D & Wittwer C (2004). Hepatitis C Genotyping by Denaturing High-Performance Liquid Chromatography. *J Clin Microbiol* 42(1): 158 - 163.
- Dusheiko G, Schmilovitz H, Brown D, McOmish F, Yap P & Simmonds P (1996). Hepatitis C virus genotypes: an investigation of type-specific differences in geographic origin and disease. *Hepatol* 19: 13-18.
- Simmonds P (1997). Clinical relevance of hepatitis C virus genotypes. *Gut* 40(3): 291.
- McOmish F, Yap P, Dow B, Follett E, Seed C, Keller A, Cobain T, Krusius T, Kolho E & Naukkarinen R (1994). Geographical distribution of hepatitis C virus genotypes in blood donors: an international collaborative survey. *J of Clin Microbiol* 32(4): 884-892.
- Yu ML, Chuang WL, Chen SC, Dai CY, Hou C, Wang JH, Lu SN, Huang JF, Lin ZY & Hsieh MY (2001). Changing prevalence of hepatitis C virus genotypes: molecular epidemiology and clinical implications in the hepatitis C virus hyperendemic areas and a tertiary referral center in Taiwan. *J of Med Virol* 65(1): 58-65.
- Takada N, Takase S, Takada A & Date T (1993). Differences in the hepatitis C virus genotypes in different countries. *J Hepatol*. 17277 - 283.
- Nousbaum J, Pol S, Nalpas B, Landais P, Berthelot P, Brechot C & Group TCS (1995). Hepatitis C virus type 1b (II) infection in France and Italy. *Ann Intern Med* 122: 161-168.
- Abdulkarim A, Zein N, Germer J, Kolbert C, Kabbani L, Krajnik K, Hola A, Agha M, Tourogman M & Persing D (1998). Hepatitis C virus genotypes and hepatitis G virus in hemodialysis patients from Syria: identification of two novel hepatitis C virus subtypes. *Am J Trop Med Hyg* 59: 571-576.
- Chamberlain R, Adams N, Saeed A, Simmonds P & Elliot R (1997).

- Complete nucleotide sequence of a type 4 hepatitis C virus variant, the predominant genotype in the Middle East. *J Gen Virol* 78: 1341-1347.
16. Cha T, Kolberg J, Irvine B, Stempien M, Beall E, Yano M, Choo Q, Houghton M, Kuo G, Han J & Urdea M (1992). Use of a signature nucleotide sequence of hepatitis C virus for detection of viral RNA in human serum and plasma. *J Clin Microbiol* 29: 2528-2534.
 17. Idrees M (2001). Common genotypes of hepatitis C virus present in Pakistan. *Pak J Med Res* 40(2): 46 - 49.
 18. Idrees M (2001). Detection of Six Serotypes of HCV in anti-HCV Positive Patients and rate of ALT/AST abnormalities. *Pak J Microbiol* 2: 61-65.
 19. Pathan NL, Tunio SA, Bano S & Jatt AN (2019). Frequency distribution of hepatitis C virus genotypes circulating in Hyderabad, Sindh. *Pure and Appl Biol* 8(1): 133-138.
 20. Scott JD & Gretch DR (2007). Molecular diagnostics of hepatitis C virus infection: a systematic review. *Jama* 297(7): 724-732.
 21. Soriano V, Mocroft A, Rockstroh J, Ledergerber B, Knysz B, Chaplinskas S, Peters L, Karlsson A, Katlama C & Toro C (2008). Spontaneous viral clearance, viral load, and genotype distribution of hepatitis C virus (HCV) in HIV-infected patients with anti-HCV antibodies in Europe. *J of Infectious Dis* 198(9): 1337-1344.
 22. Berger A, Prondzinski MvD, Doerr H, Rabenau H & Weber B (1996). Hepatitis C plasma viral load is associated with HCV genotype but not with HIV coinfection. *J of Med Virol* 48(4): 339-343.
 23. Rong X, Lu L, Wang J, Xiong H, Huang J, Chen J, Huang K, Xu R, Wang M & Zhang X (2012). Correlation of viral loads with HCV genotypes: higher levels of virus were revealed among blood donors infected with 6a strains. *PLoS One* 7(12).