Int.j.pathol.2018;16(3)101-105

Original Article

Frequency of Hepatitis C Virus genotypes among non-responders to combination therapy

Amina Gul*, Naheed Gul**, Syed Luqman Shuaib*, Shahina Mumtaz*, Ijaz Ali***
and Jawad Ahmed****
*Department of Pathology, Khyber Medical College, Peshawar, **Department of Medicine. Shifa College of Medicine, Islamabad, ***Department of Biosciences COMSATS Institute of Information Technology, Islamabad,****Institute of Basic Medical Sciences (IBMS), Khyber Medical University (KMU), Peshawar

Abstract:
Background: Hepatitis C Virus has been characterized into seven major genotypes and multiple subtypes which display variable distribution across the globe. Accurate identification of Hepatitis C Virus genotypes prior to initiating antiviral therapy plays a vital role in the management of chronic Hepatitis C Virus infected patients. Diverse genotypes of Hepatitis C Virus differ with respect to treatment response, nature as well as duration of therapy. Due to treatment associated side effects and high costs, Hepatitis C Virus genotype determination especially among non-responders is required to help these patients in selection of appropriate antiviral therapy.
Objectives: The aim of this study was to find out the frequency of HCV genotypes among non-responders to combination therapy.
Patients and Methods: This descriptive study was carried out at Institute of Basic Medical Sciences, Khyber Medical University Peshawar from January 2016 to June 2016 after institutional ethical approval. Serum samples were collected from 110 chronic HV infected patients who failed to respond to either Conventional or Pegylated Interferon therapy. Identification of HCV genotype was performed using two sets of newly designed primers in a modified type specific nested PCR based genotyping assay. Agarose gel electrophoresis was carried out for identification of genotype specific PCR product.
Results: Among 110 actively infected samples, majority (53.4%) of the infections were attributed to HCV genotype 3a. Among other subtypes, HCV 1b (17.1%) and 1a (9.4%) genotypes were predominantly observed. HCV 3b, 2a and 2b accounted for infection in 8.2%, 5.4% and 3.3% of the patients respectively. Mixed infections with more than one type in a single specimen were found in 2.1% of the isolates, while 1.1% of the samples remained untypeable.
Conclusion: The current study reports a high frequency of Hepatitis C Virus 3a among studied isolates. Emerging resistance against antiviral therapy might be attributed to HCV 1b, 1a and other subtypes observed among non-responders.
Key Words: Hepatitis C Virus, HCV Genotype, Antiviral therapy

Introduction

Hepatitis C Virus(HCV) commonly causes cirrhosis and liver cancer.[1] In Pakistan, significant mortality and morbidity has been reported mainly due to complications of end stage liver diseases.[2] HCV is known to have marked genetic heterogeneity, due to RNA dependent RNA polymerase (RdRp) which lacks proofreading ability thereby resulting in rapidly evolution in its genome.[3,4] In each cell infected, the calculated rate of mutation for HCV is 1.2×10^{-4} substitutions per site.[5]Due to high nucleotide substitution rate, HCV genome has been classified into seven different genotypes and a series of subtypes.[6] These viral types and subtypes show differing distribution in different geographic regions.[7] Accurate knowledge of regional distribution of circulating HCV genotypes in our community is imperative for future research into vaccine development, correct formulation of healthcare policies and allocating resources accordingly. Currently, the clinical management of HCV is dependent on HCV genotype

Author for Correspondence:
Dr. Amina Gul, PhD Microbiology
Position: Assistant Professor Department of Pathology.
Institution: Khyber Medical College, Peshawar, Pakistan.
E. mail: dr.aminagul@gmail.com

which is the strongest predictive parameter of Sustained Virological Response (SVR).[8]Although considerable research has been conducted as far as the management of chronic HCV infection is concerned; the optimum treatment is not yet established. Recently, the development of Direct Acting Antivirals (DAAs) has significantly improved treatment outcomes for patients with HCV infection.[9] A careful investigation of previous studies from Pakistan regarding response to conventional INF/RBV combination therapy showed variable response rates ranging from 50-70%.[10,11]Peg-INF based therapies showed better outcomes with SVR rates reaching 80% and with DAAs an SVR of 93% has been reported.[12,13]In the present study we attempted to determine the distribution of HCV genotypes among non-responders. Identification of HCV genotype before or at least soon after initiating antiviral therapy will help in proper management of chronic HCV infected patients in terms of selection of appropriate antiviral therapy, prevention of various treatment related side effects and cost of therapy.

Patients and Methods

The present descriptive study was conducted from January 2016 to June 2016 after approval by the ethical review committee at Institute of Basic Medical Sciences, Khyber Medical University (IBMS, KMU), Peshawar. Both male and female patients who turned out to be non-responders after 24 weeks of therapy with Conventional or Pegylated Interferon were included in the present study. Serum samples were collected using a non-probability consecutive sampling technique from 110 chronic HCV infected patients after taking their consent. Patients with detectable viral RNA at 24 weeks of therapy were defined as Non-Responders (NRs).

Initially cDNA was synthesized by reverse transcription Polymerase Chain Reaction (PCR) using M-MuLV reverse transcriptase enzyme (Thermo Fisher scientific, USA). Isolated cDNA was used in two rounds of a Qualitative nested PCR based genotyping assay using newly designed primers from recently evolved HCV sequences that were submitted to various genome repositories. The amplified DNA fragments were separated using 2% agarose gel (Thermo Fisher Scientific, USA)at 110 volts for 40 minutes. A 50-bp DNA ladder (Thermo Fisher Scientific, USA) was kept as DNA size marker for identification of genotype specific PCR product and observed under UV transilluminator.

Results

Among 110 chronic HCV infected patients included in the present study 47 (43%) were female and 63 (57%) were male patients. Mean age of all the patients were 47 ± 9.5 (Mean ± SD). About 76 (69%) of the patients have a viral load of >800000 IU/ml, while only 34 (31%) patients have a viral load of <800000 IU/ml. Mean Alanine aminotransferase levels observed were 63 ± 10.3(Mean ± SD).

Gel electrograph of amplified PCR product is shown in Figure 1. HCV genotype 3a was the most frequently observed type present in 59 (53.4%) of the isolates. Among other subtypes, a high percentage of HCV 1b 18 (17.1%) and 1a 11 (9.4%) genotypes were observed. HCV 3b, 2a and 2b accounted for infection in (8.2%), 6 (5.4%) and 4 (3.3%) of the patients respectively. Mixed infection with more than one type in a single specimen were found in 2 (2.1%) of the isolates, while 1 (1.1%) of the samples remained not untypeable. Distribution of HCV genotypes among non-responders is illustrated in Figure 2.

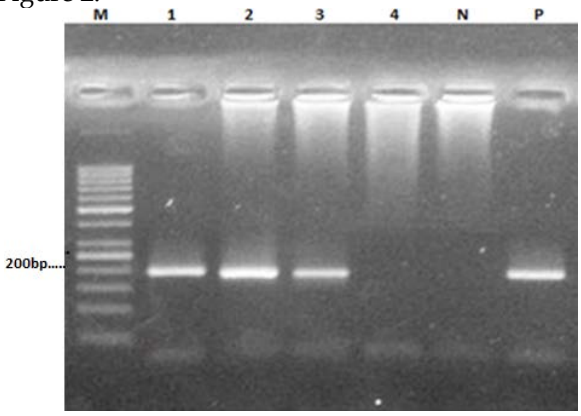

Figure 1: Gel electrograph of HCV PCR amplimer for detection of active HCV infection using qualitative PCR. Lane M shows 50 bps DNA ladder; Lane 1-3: Positive samples; Lane 4: Negative sample; Lane N: Negative control; Lane P: Positive control

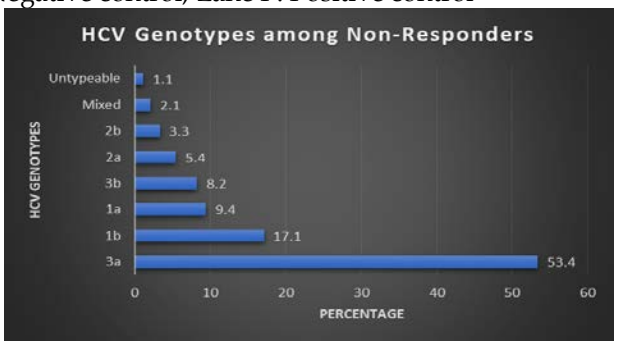

Figure 2: Represents the distribution of HCV genotypes among chronic HCV infected patients with non-response to antiviral therapy

Discussion

Epidemiological pattern of HCV genotypes has been reported to change over times due to a variety of reasons.[14,15] HCV genotypes demonstrates not only diverse distribution patterns but influences management strategies including type of therapy administered, duration of therapy and response rates. The present study was conducted to identify HCV genotypes among patients with non-response to combination therapy. Consistent with earlier reports from Peshawar and Pakistan we observed that HCV 3a was the most abundant genotype circulating among chronic HCV infected patients.[16] HCV 3a is also the most frequently encountered genotype in the neighboring countries like China, India and Iran.[7,17,18] High prevalence of HCV 3a in the neighboring countries points towards the possible transmission routes. Authentic data on HCV genotypes distribution in Afghanistan and the Tribal region of Pakistan is lacking because of poor law and order situation. A careful analysis of previous studies from Pakistan indicates that the percent prevalence of HCV 3a has dropped over times in this region and is being replaced by other HCV types including HCV 1b and HCV 1a infections. HCV 1b and 1a were observed in 17.1% and 9.4% of the non-responder isolates respectively. High frequency of these genotypes could be an important consideration in predicting response to antiviral therapy. A number of studies from Khyber Pakhtunkhwa (KP) and other provinces of Pakistan reported genotype 3b to be the second frequent genotype after 3a.[19,20]In another study from the Punjab province and KP province, HCV genotype 1a was claimed to be the emerging type as compared to HCV 1b in the present study.[15,21] This difference could be explained by the estimated high mutation rate of 1.2 X 10^{-4}substitutions per in each infected cell. Due to rapid genetic evolution, it is more likely that type-specific assays developed more than a decade and a half ago may not efficiently and specifically amplify various types and subtypes of HCV. The results for various types in this work have been validated by the use of new type specific primers which were designed using the recently evolved HCV sequences which indicates that the spectrum of HCV genotype distribution among non-responders in Peshawar might be a contributing factor in therapeutic failure. Possible risk factors for transmission of HCV in KP have been reported as intravenous drug abuse, transfusion of blood and blood products, contaminated needles and syringes, surgeries and dental extractions.[20] In Pakistan, lack of awareness programs at the public level and unsatisfactory health conditions with paucity of essentials for screening and sterilization and immigration of people, all have affected the epidemiology of HCV recently.[21] HCV genotype 1b is reported to be the predominant genotype in Japan.[22] Genotype 1b has been correlated not only with a poor response to therapy but with a high probability of developing cirrhosis and carcinoma of the Liver.[23] This rising prevalence of genotype 1b together with HCV 3b, 2a and 2b in Peshawar will further complicate HCV management due to poor response against the low cost of combination therapy for these resistant subtypes. SVR to INF and RBV therapy in patients infected with HCV genotype-2/3 and HCVgenotype-1 genotypes are 80% and 40%, respectively.[24] In KP, identification of genotype before introducing therapy is not carried out routinely and the common practice is to switch over NRs to long term combination therapy utilizing either qualitative or quantitativedetection of HCV RNA.[25]Patients infected with less responsive genotypes circulating among general population might experience therapy resistance as well.Mixed infection with more than one type in a single specimen were found in 2.1% of the isolates, while 1.1% of the samples remained untypeable. Mixed infections with a maximum of four different types in a single specimen was earlier being observed in viral strains from other countries like Iran, India, China, Egypt and Serbia including Pakistan. Possible risk factors responsible for causing mixed infections included reused syringes and razors at Barber shops, major and minor surgeries and multiple blood transfusions.[26] This might have resulted in repeated exposure to multiple HCV strains.

Conclusion: HCV genotype 3a still represents the major type present in Peshawar. Non-response to antiviral therapy could be attributed to emergence of HCV genotypes other than 3a or genetic mutations in circulating HCV 3a strains.

Conflict of interest statement

All the authors declare that we have no conflict of interest

Funding Source:

None

References

1. Finkelmeier F, Dultz G, Peiffer KH, Kronenberger B, Krauss F, Zeuzem S, et al. Risk of de novo Hepatocellular Carcinoma after HCV Treatment with Direct-Acting Antivirals. Liver cancer. 2018;7(2):190-204
2. Bartoletti M, Giannella M, Tedeschi S, Viale P. Opportunistic infections in end stage liver disease. Infect Dis Rep. 2018;10(1):7621.

3. Tsukiyama-Kohara K, Kohara M. Hepatitis C Virus: Viral Quasispecies and Genotypes. International journal of molecular sciences. 2017;19(1)
4. Sesmero E, Thorpe IF. Using the Hepatitis C Virus RNA-Dependent RNA Polymerase as a Model to Understand Viral Polymerase Structure, Function and Dynamics. Viruses. 2015;7(7):3974-94
5. Bull RA, Luciani F, McElroy K, Gaudieri S, Pham ST, Chopra A, et al. Sequential bottlenecks drive viral evolution in early acute hepatitis C virus infection. PLoS Pathog. 2011;7(9):e1002243
6. Smith DB, Bukh J, Kuiken C, Muerhoff AS, Rice CM, Stapleton JT, et al. Expanded classification of hepatitis C virus into 7 genotypes and 67 subtypes: updated criteria and genotype assignment web resource. Hepatology. 2014;59(1):318-27
7. Yan Z, Fan K, Wang Y, Fan Y, Tan Z, Deng G. Changing pattern of clinical epidemiology on hepatitis C virus infection in southwest china. Hepatitis monthly. 2012;12(3):196-204
8. Yan Z, Wang Y. Viral and host factors associated with outcomes of hepatitis C virus infection (Review). Mol Med Rep. 2017;15(5):2909-24
9. Asselah T, Marcellin P, Schinazi RF. Treatment of hepatitis C virus infection with direct-acting antiviral agents: 100% cure? Liver international : official journal of the International Association for the Study of the Liver. 2018;38 Suppl 1:7-13
10. Waheed Y. Effect of interferon plus ribavirin therapy on hepatitis C virus genotype 3 patients from Pakistan: Treatment response, side effects and future prospective. Asian Pac J Trop Med. 2015;8(2):85-9
11. Idrees M, Riazuddin S. A study of best positive predictors for sustained virologic response to interferon alpha plus ribavirin therapy in naive chronic hepatitis C patients. BMC Gastroenterol. 2009;9:5
12. Gill U, Aziz H, Gill ML. Rapid virological response tailors the duration of treatment in hepatitis C virus genotype 3 patients treated with pegylated interferon alfa-2a and ribavirin in Pakistan. Int J Infect Dis. 2013;17(11):e1017-21
13. Yek C, de la Flor C, Marshall J, Zoellner C, Thompson G, Quirk L, et al. Effectiveness of direct-acting antiviral therapy for hepatitis C in difficult-to-treat patients in a safety-net health system: a retrospective cohort study. BMC Med. 2017;15(1):204.
14. Lavanchy D. Evolving epidemiology of hepatitis C virus. Clin Microbiol Infect. 2011;17(2):107-15
15. Waqar M, Khan AU, Rehman HU, Idrees M, Wasim M, Ali A, et al. Determination of hepatitis C virus genotypes circulating in different districts of Punjab (Pakistan). Eur J Gastroenterol Hepatol. 2014;26(1):59-64
16. Al Kanaani Z, Mahmud S, Kouyoumjian SP, Abu-Raddad LJ. The epidemiology of hepatitis C virus in Pakistan: systematic review and meta-analyses. Royal Society open science. 2018;5(4):180257
17. Chakravarti A, Ashraf A, Malik S. A study of changing trends of prevalence and genotypic distribution of hepatitis C virus among high risk groups in North India. Indian J Med Microbiol. 2013;31(4):354-9
18. Jahanbakhsh Sefidi F, Keyvani H, Monavari SH, Alavian SM, Fakhim S, Bokharaei-Salim F. Distribution of hepatitis C virus genotypes in Iranian chronic infected patients. Hepatitis monthly. 2013;13(1):e7991
19. Khan S, Attaullah S, Ali I, Ayaz S, Naseemullah, Khan SN, et al. Rising burden of Hepatitis C Virus in hemodialysis patients. Virol J. 2011;8:438
20. Idrees M, Riazuddin S. Frequency distribution of hepatitis C virus genotypes in different geographical regions of Pakistan and their possible routes of transmission. BMC Infect Dis. 2008;8:69
21. Afridi SQ, Zahid MN, Shabbir MZ, Hussain Z, Mukhtar N, Tipu MY, et al. Prevalence of HCV genotypes in district Mardan. Virol J. 2013;10:90
22. Takada N, Takase S, Takada A, Date T. Differences in the hepatitis C virus genotypes in different countries. J Hepatol. 1993;17(3):277-83
23. Zein NN. Clinical significance of hepatitis C virus genotypes. Clin Microbiol Rev. 2000;13(2):223-35
24. Akram M, Idrees M, Zafar S, Hussain A, Butt S, Afzal S, et al. Effects of host and virus related factors on interferon-alpha+ribavirin and pegylated-interferon+ribavirin treatment outcomes in chronic Hepatitis C patients. Virol J. 2011;8:234.
25. Ali S, Ali I, Azam S, Ahmad B. Frequency distribution of HCV genotypes among chronic hepatitis C patients of Khyber Pakhtunkhwa. Virol J. 2011;8:193
26. Inamullah, Idrees M, Ahmed H, Sajid ul g, Ali M, Ali L, et al. Hepatitis C virus genotypes circulating in district Swat of Khyber Pakhtoonkhaw, Pakistan. Virol J. 2011;8:16

HISTORY	
Date Received:	13-08-2018
Date sent for Reviewer:	15-11-2018
Date Received Reviewer's Comments:	28-11-2018
Date Received Revised Manuscript:	18-12-2018
Date Accepted:	19-12-2018

CONTRIBUTION OF AUTHORS	
Author	**Contribution**
Amina Gul	A,B,C,D
Naheed Gul	A,C,D,E
Syed Luqman	A,B,D
Shahina Mumtaz	A,B,E,F
Ijaz Ali	A,E
Jawad Ahmed	D,E

KEY FOR CONTRIBUTION OF AUTHORS:

A. Conception/Study Designing/Planning
B. Experimentation/Study Conduction
C. Analysis/Interpretation/Discussion
D. Manuscript Writing
E. Critical Review
F. Facilitated for Reagents/Material/Analysis