

HEPATITIS C VIRUS GENOTYPES IN BAHAWALPUR

MASROOR A. QAZI, M. FAYYAZ, G. M.D. CHAUDHARY, AFTAB JAMIL
A. H. MALIK, A. I. GARDEZI AND M. H. BUKHARI
Department of Medicine, B. V. Hospital / Quaid-e-Azam Medical College, Bahawalpur

This study was conducted at Medical Unit-II Bahawal Victoria Hospital / Quaid-e-Azam Medical College Bahawalpur from May 1st, 2005 to December 31st 2005. The objective of this study was to determine hepatitis C virus (HCV) genotypes in Bahawalpur, Pakistan. In consecutive 105 anti-HCV (ELISA-3) positive patients, complete history and physical examination was performed. Liver function tests, complete blood counts and platelet count, blood sugar fasting and 2 hours after breakfast, prothrombin time, serum albumin, serum globulin and abdominal ultrasound were carried out in all the patients. True cut biopsy was performed on 17 patients. We studied HCV RNA in all these patients by Nested PCR method. HCV RNA was detected in 98 patients and genotyping assay was done by genotype specific PCR. Among total of 105 anti-HCV positive patients, HCV-RNA was detected in 98 patients. Out of these 98 patients there were 57 (58.2%) males and 41 (42.8%) females. Their age range was 18-75 years. The age 18-29 years 26 (26.5%), 30-39 years 35 (35.7%) and 40-75 37 (37.8%), while 10 (10.2%) patients were diabetics and 34 (34.7%) patients were obese. Liver cirrhosis was present in 10 (10.2%) patients. Forty two (43.9%) patients were symptomatic while 56 (57.1%) were asymptomatic. Out of 98 patients 11 (11.2%) were un type-able and 87 (88.8%) were type-able. 70/98 (71.4%) were genotype 3; 10/98 (10.2%) were genotype 1; 03/98 (3.1%) were genotype 2; 03/98 (3.1%) were mixed genotype 2 and 3; 01/98 (1%) were mixed genotype 3a and 3b. Genotype 3 is the most common HCV virus in our area which shows that both virological and biochemical response will be better. Because HCV genotype 3 is more frequent among the drug users which points towards unsafe injection practices in our area.

INTRODUCTION

Hepatitis C infection has been recognized as a major public health problem all over the world¹. Worldwide prevalence is 3%, while the prevalence in Pakistan is 4-7%². Hepatitis C infection is asymptomatic not only in chronic but also in acute stage³. It leads to chronic liver disease in 60% and liver cirrhosis in 10-20 years⁴. Hepatocellular carcinoma is an important complication of liver cirrhosis developing at the rate of 1-4% per year^{5,6}.

Hepatitis C infection has been recognized as a major public health problem all over the world. It is asymptomatic both in chronic and acute stage⁷. Worldwide prevalence is 3%, while the prevalence in Pakistan is 4-7%. Hepatitis C virus is an important cause of chronic liver disease and cirrhosis in Pakistan. Hepatitis C virus (HCV) is the major causative agent of non-A, non-B post-transfusional hepatitis, possessing a positive-stranded RNA genome. High variability of HCV genome appears not only in its highly variable region (HVR) of E2 protein, but also in the most highly conserved 5' NCR⁸. This has led to a classification of the virus into a series of genotypes that show distinct geographical distribution in various parts of the

world. On the basis of phylogenetic analysis of nucleotide sequences, multiple genotypes and subtypes of hepatitis C virus (HCV) have been identified⁹. Up till now, 6 major genotypes and 80 subtypes of hepatitis C have been identified. However, little is currently known about which HCV variants are present in Pakistan, India, Bangladesh and Burma. We have analyzed patients from Bahawalpur for HCV genotyping by using type-specific PCR.

PLACE AND DURATION OF STUDY

The study was conducted at Medical Unit-II, Bahawal Victoria Hospital / Quaid-e-Azam Medical College, Bahawalpur from May 1st, 2005 to December 31st 2005.

OBJECTIVES

To study frequency of hepatitis C virus (HCV) genotypes in Bahawalpur, Pakistan.

MATERIALS AND METHODS

In 105 anti-HCV (ELISA-3) positive patients, complete history and physical examination was carried out. Liver function test, blood complete

plus platelet count, blood sugar fasting and 2HPP, prothrombin time, serum albumin, serum globulin and abdominal ultrasound were carried out in all the patients. Liver biopsy was performed on 17 patients. We studied HCV RNA in all these patients by Nested PCR method which is intended to reduce the contaminations in products due to the amplification of unexpected primer binding sites. Nested PCR is based on five major processes: extraction of HCV RNA from serum sample, reverse transcription of target RNA to generate cDNA, two rounds of PCR amplification and detection (The sensitivity and specificity of this HCV RNA PCR assay is 97% and 99% respectively). And genotyping assay was done by genotype specific PCR in those cases where HCV RNA was detected. HCV RNA was isolated from 150µl serum of the patient and was reverse transcribed into cDNA with Molony-murine leukemia virus reverse transcriptase enzyme. The cDNA was subjected to two rounds of PCR amplification. The 1st round utilized the outer primers specific for the core region. The 2nd round was performed with one universal inner-sense and 11 genotype-specific anti-sense primers. The PCR products were electrophoresed on a 2% agarose gel, stained with ethidium bromide and evaluated on UV transilluminator. The HCV genotype for the sample was determined by identifying the genotype-specific cDNA band.

RESULTS

Among a total of 105 anti-HCV positive patients, HCV-RNA PCR was detected in 98 (93.33%). Out of these 98 patients, 57 (58.2%) were male and 41 (42.8%) were females. Ages range was 18-75

Table 1:

AGE	
Age range	Percentage
18-29 Yrs	26/98 (26.5%)
30-39 Yrs	35/98 (35.7%)
40-75 Yrs	37/98 (37.8%)
Genotypes	
Genotype	Total
III a	62 (63.3%)
III b	08 (8.2%)
I a	10 (10.2%)
Un-typified	11 (11.2%)
Mixed	04 (4.1%)
II a	03 (3.1%)
Total	98

Table 2:

GNOTYPE AND SEX			
Genotype	Male	Female	Total
III a	30	32	62 (63.3%)
III b	05	03	08 (8.2%)
I a	08	02	10 (10.2%)
Un-typified	07	04	11 (11.2%)
Mixed	04	-	04 (4.1%)
II a	03	-	03 (3.1%)
Total	57	41	98

years. Age between 18-29 years 26 (26.5%) patients, 30-39 years 35 (35.7%) patients and 40-75 years 37 (37.8%) patients, while 10 (10.2%) patients were diabetics and 34 (34.7%) patients were obese. Acute hepatitis was in 04 (4.0%) patients, while chronic hepatitis was present in 84 (85.7%) patients and 10 (10.2%) were having liver cirrhosis. Forty two (43.9%) patients were symptomatic while 56 (57.1%) were asymptomatic. Out of 98 patients 87 (88.8%) were typeable and 11 (11.2%) were untypeable .Among typeable patients 70 (71.4%) were genotype 3; 10 (10.2%) were genotype 1; 03 (3.1%) were genotype 2; 03 (3.1%) were mixed genotype 2 and 3; and 01 (1%) were mixed genotype 3a and 3b.

DISCUSSION

HCV infection is causing significant morbidity and mortality worldwide. In an infected individual the HCV genome population circulates as a quasi-species distribution of closely related yet heterogeneous RNA sequences. HCV evades host immune surveillance and establishes and maintains persistent infection because of these quasi-species^{10,11}. Genetic heterogeneity of HCV is great, and this may have important implications in diagnosis, pathogenesis, treatment, and vaccine development. The determination of HCV genotypes, subtypes and isolates has been helpful in understanding the evolution and the epidemiology of the virus, and is an important factor in the pre-treatment evaluation^{12,13}. Genotyping can be performed by different methods; direct sequence analysis, reverse hybridization, the use of restriction fragment length, the Trugene HCV 5'NC Genotyping Kit, the line-probe assay.¹ There are six major HCV genotypes¹⁴⁻¹⁶. Genotype 1 is most common (60 to 70 percent of isolates) in the United States and Europe¹⁷; genotypes 2 and 3 are less common in these areas, whereas genotypes 4, 5, and 6 are rare: Genotype 3 is most common in the Far East and Australia¹⁸⁻²¹. Genotype 4 is most

common in Africa and the Middle East. Genotype 5 is most common in South Africa and Genotype 6 is most common in Hong Kong²²⁻²⁴. Response to therapy with INF and Ribavirin depends not only upon host factors but also viral factors and among the viral factors HCV genotype is most consistently identified predictor. The sustained virologic response to pegylated interferon plus ribavirin ranges from about 40 to 50 percent with genotype 1 (including 1a and 1b) to as high as 70 to 80 percent with genotypes 2 and 3^{25,26}. More severe progression of chronic hepatitis C is seen in patients showing genotype 1b compared with those with genotype 2^{27, 28}. Patients who have a high body mass index and hepatic steatosis are at increased risk for the development of fibrosis; the risk of fibrosis in those with steatosis seems to be increased significantly with even moderate alcohol intake²⁹⁻³¹. Hepatitis C virus genotype 1 and a high baseline viral load are the major viral factors associated with lower response³².

The majority of the patients in our study (70/98) are having genotype 3 while genotype 1 is the second highest genotype. We did not detect genotype 4, 5 and 6. Majority of the patients were young below the age of 40. Genotype 1 was more common in males as compared to females. Diabetes was present in 10/98 cases. Hepatitis C Virus infection is said to be associated with diabetes mellitus³³⁻³⁷. Ten patients were having liver cirrhosis. Hepatitis C Virus genotype 3 seems to be high in Bangladesh, Pakistan, India and Thailand^{38,39}. HCV genotype 3 patients are easy to treat as compared to genotype 1 and 4⁴⁰. IRES (Internal Ribosome Entry Site) efficiency in vitro correlate with the treatment response with HCV genotype 3⁴¹. There is a strong evidence that HCV genotype 3 is a modulator of hepatic steatosis which appears to be a direct action on virus on hepatic lipid homeostasis⁴². Nonalcoholic fatty liver disease is now considered part of the metabolic syndrome, and, with alcohol and hepatitis C, is the most common cause of chronic liver disease in the United States⁴³⁻⁴⁶. Nonalcoholic fatty liver disease can also be caused by the HCV infection⁴². HCV genotype 3 is more common among the IV drug users and in our country it may be due to unsafe injection practices. High prevalence of HCV genotype 3 in Pakistan is a good hope for cure as well as control of Hepatitis C infection. HCV genotype should be determined in all HCV-infected persons prior to treatment in order to determine the duration of therapy and likelihood of response¹.

In **conclusion** it is our observation that genotype 3 is the most common HCV virus in our area which predicts not only short duration therapy but also better biochemical as well as

virological response. HCV genotype 3 is more frequent among the drug users in Europe and America but in our country it may be due to unsafe injection practices.

REFERENCES

1. Doris B. Strader, Teresa Wright, David L. Thomas and Leonard B. Seeff. AASLD Practice Guideline. Diagnosis, Management and Treatment of Hepatitis C. 2004.
2. 38th EASL, The European Association for the Study of Liver, July 2003.
3. Umar M, Bushra K, Amir R. Rawalians Research Forum on G.I & Liver diseases. Practice Guidelines, Hepatitis (2003).
4. National Institute of Health Consensus Development Conference Statement: Management of Hepatitis C: June 10-12, 2002. *Hepatology* 2002; 36: S1-S20.
5. Pakistan society of Gastrology, Guidelines for management of Hepatitis B and hepatitis C, 2003.
6. Gary LD, Schiff's diseases of the liver, eighth edition, edited by Eugene R. Schiff, Michael F. Sorrel, Willis C. Maddrey, Lippincott-Raven Publishers, Philadelphia, 1999.
7. Merican I, Sherlock S, McIntyre N, et al. Clinical, biochemical and histological features in 102 patients with chronic hepatitis C virus infection. *Q J Med* 1993 Feb;86 (2): 119-25.
8. Simmonds P. Genetic diversity and evolution of hepatitis C Virus-15 years on. *Journal of General Virology* (2004); 85: 3173-88.
9. Simmonds P, Bukh J, Combet C, et al. Consensus Proposals for a Unified System of Nomenclature of Hepatitis C Virus Genotypes. *Hepatology* 2005; 42: 962-973.
10. Farci P, Alter H, Govindarajan S, et al. Lack of protective immunity against re-infection with hepatitis C virus. *Science* 1992; 258: 135.
11. Farci P, Shimoda A, Wong D, et al. Prevention of hepatitis C virus infection in chimpanzees by hyperimmune serum against the hypervariable region 1 of the envelope 2 protein. *Proc Natl Acad Sci U S A* 1996; 93: 15394.
12. Analdi F, Torre F, Bruzzone BM, et al. Evaluation of a new hepatitis C virus sequencing assay as a routine method for genotyping. *J Med Virol.* 2001 Jan; 63 (1): 17-21.
13. Ross RS, Viazov SO, Holtzer CD, et al. Genotyping of hepatitis C virus isolates using CLIP sequencing. *J Clin Microbiol.* 2000 Oct; 38 (10): 3581-4.
14. Simmonds, P, Alberti, A, Alter, HJ, et al. A proposed system for the nomenclature of hepatitis C viral genotypes (letter). *Hepatology* 1994; 19: 1321.
15. Forns X, Maluenda MD, Lopez-Labrador FX, et al. Comparative study of three methods for genotyping hepatitis C virus strains in samples from Spanish patients. *J Clin Microbiol.* 1996 October; 34 (10): 2516-21.
16. Gargiulo F, De Francesco MA, Pinsi G, et al. Determination of HCV genotype by direct sequence analysis of quantitative PCR products. *J Med Virol* 2003 Feb; 69 (2): 202-6.

17. Blatt LM, Mutchnick MG, Tong MJ, et al. Assessment of hepatitis C virus RNA and genotype from 6807 patients with chronic hepatitis C in the United States. *J Viral Hepat*. 2000 May; 7 (3): 196-202.
18. Zuberi SJ, Arif A. Serotyping of the Hepatitis C in Pakistan. *J Pak Med Assoc*. May 2002; 52 (5): 218-9.
19. Shah HA, Jafri W, Malik I, et al. Hepatitis C Virus (HCV) genotypes and chronic liver disease in Pakistan. *J Gastroenterol Hepatol*. 1997 Nov; 12 (11) 758-61.
20. Moatter T, Hussain AS, Hamid S, et al. Comparative analysis of viral titers and histologic features of Pakistani patients infected with hepatitis C virus type 3. *Int J Infect Dis*. 2002 Dec; 6 (4): 272-6.
21. Khokhar N, Naila A, Omar SK. Hepatitis C Virus Serotype in chronic liver disease. *Pak J Med Sci*. 2002; 18 (2): 156-59.
22. Lau, JY, Davis, GL, Prescott, LE, et al. Distribution of hepatitis C virus genotypes determined by line probe assay in patients with chronic hepatitis C seen in tertiary referral centers in the United States. *Ann Intern Med* 1996; 124: 868.
23. Dusheiko G, Schmilovitz-Weiss H, Brown D, et al. Hepatitis C virus genotypes: An investigation of type-specific differences in geographic origin and disease. *Hepatology* 1994; 19: 13.
24. Domingo MJ, Fernandez I, Lumbreras C, et al. Use of DNA sequence hybridization with specific oligonucleotide probes to identify hepatitis C virus genotypes. *Enferm Infecc Microbiol Clin*. 1996 Aug-Sep; 14 (7): 433-5.
25. Anouk T, Rhonda M, Vijaya S, et al. Southeast Asian patients with chronic Hepatitis C: The impact of novel genotypes and race on treatment outcome. *Hepatology* 2002; 36: 1259-65
26. Poynard T, Bedossa P, Opolon P. Natural history of liver fibrosis progression in patients with chronic hepatitis C. The OBSVIRC, METAVIR, CLINIVIR, and DOSVIRC groups. *Lancet* 1997 Mar 22; 349 (9055): 825-32.
27. Kobayashi M, Tanaka E, Sodeyama T, et al. The natural course of chronic hepatitis C: a comparison between patients with genotypes 1 and 2 hepatitis C viruses. *Hepatology* 1996 Apr; 23 (4): 695-9.
28. Zein NN. Hepatitis C Virus Genotyping Clinical Significance of Hepatitis C Virus Genotypes. *Clin Microbiol Rev*. 2000; 13: 223-235.
29. Hourigan, LF, Macdonald, GA, Purdie, D, et al. Fibrosis in chronic hepatitis C correlates significantly with body mass index and steatosis. *Hepatology* 1999; 29: 1215.
30. Adinolfi LE, Gambardella M, Andreana A, et al. Steatosis accelerates the progression of liver damage of chronic hepatitis C patients and correlates with specific HCV genotype and visceral obesity. *Hepatology* 2001; 33: 1358.
31. Serfaty L, Poujol-Robert A, Carbonell N, et al. Effect of the interaction between steatosis and alcohol intake on liver fibrosis progression in chronic hepatitis C. *Am J Gastroenterol* 2002; 97: 1807.
32. Zeuzem S. Heterogeneous virologic response rates to interferon-based therapy in patients with chronic hepatitis C: who responds less well? *Ann Intern Med* 2004 Mar 2; 140 (5): 370-81.
33. Qazi MA, Fayyaz M, Chaudhary GM, et al. Frequency of Hepatitis C infection in Diabetes Mellitus. *Annals* 2005 Dec; 11 (4): 549-51.
34. Mason AL, Lau JY, Hoang N et al. Association of Diabetes Mellitus and chronic hepatitis C virus infection. *Hepatology*. 1999, Feb; 29 (2): 328-33.
35. Yang SQ, Chen HS, Jiang D et al. Relationship between chronic hepatitis C and type II diabetes mellitus. *Zhonghua Shi Yan He Lin Chuang Bing Du Xue Zhi*. 2003, Mar; 17 (1): 46-9.
36. Rafael S, Cristina H, Joan G, et al. High Prevalence of Hepatitis C Virus Infection in Diabetic Patients. *Diabetes Care* Volume 19, Number 9, September 1996, Page 998.
37. Gray H, Wreghitt T, Stratton IM, et al. High prevalence of hepatitis C infection in Afro-Caribbean patients with type 2 diabetes and abnormal liver function tests. *Diabet Med*. 1995 Mar; 12 (3): 244-9.
38. Ansari N, Ahmed A, Esmail J, et al. HCV Serotypes in Karachi: a Liaquat National Hospital Experience. *J Pak Med Assoc* May 2002; 52 (5): 219-20.
39. Dusheiko GM. Hepatitis C infection: from virology to a management. In: Decker R, Taroonon H, Hepatitis C 1997: essay and expert opinions on its natural history, epidemiology, diagnosis and therapy. Abbott Diagnostic Division. pp. 5-25.
40. Kobayashi M, Tanaka E, Sodeyama T, et al. The natural course of chronic hepatitis C: A comparison between patients with genotypes 1 and 2 hepatitis C Viruses. *Hepatology* 1996; 23: 695-99.
41. Yasmeen A, Hamid S, Granath FN, et al. Correlation between translation efficiency and outcome of combination therapy in chronic hepatitis C genotype 3. *Journal of Viral Hepatitis* 2005.
42. Kumar D, Geoffrey C, Garrell, et al. Hepatitis C Virus genotype 3 is cytopathic to hepatocytes: Reversal of hepatic steatosis after sustained therapeutic response. *Hepatology* 2002; 36: 1266-72.
43. Scheuer PJ, Ashrafzadeh P, Sherlock S, Brown D, Dusheiko G. The pathology of hepatitis C. *Hepatology*. 1992; 15: 567-71.
44. Moriya K, Yotsuyanagi H, Shintani Y, Fujie H, Ishibashi K, Matsuura Y, et al. Hepatitis C virus core protein induces hepatic steatosis in transgenic mice. *J Gen Virol*. 1997; 78: 1527-31.
45. Wong VS, Wight DG, Palmer CR, Alexander GJ. Fibrosis and other histological features in chronic hepatitis C virus infection: a statistical model. *J Clin Pathol*. 1996; 49: 465-9.
46. Hourigan LF, Macdonald GA, Purdie D, et al. Fibrosis in chronic hepatitis C correlates significantly with body mass index and steatosis. *Hepatology*. 1999; 29: 1215-9.