SMGroup

SM Journal of Biology

Research Article

Importance of Molecular Testing of Hepatitis C Virus Infection in Tertiary Care Hospitals

Matam Kavitha[1]*, KalalIravathy Goud[1], Adi Mahalakshmi[1], Ravi Vempati[1], Sargarika[1], Madhuri[1], Sunitha N[2], Lavanya[2], Abdulaziz A Alodhayani[3] and Imran Ali Khan[4]

[1]*Department of Molecular Biology and Cytogenetics, Apollo Hospitals, India*
[2]*Department of Infectious disease, Apollo Hospitals, India*
[3]*Department of Family and Community Medicine, College of Medicine, King Saud University, Saudi Arabia*
[4]*Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, King Saud University, Saudi Arabia*

Article Information

Received date: Sep 19, 2016
Accepted date: Nov 04, 2016
Published date: Nov 08, 2016

***Corresponding author**

Matam Kavitha, Department of Molecular Biology and Cytogenetics, Apollo Hospitals, India, Email: matamkavitha@gmail.com

Keywords Hepatitis C virus; Sequencing; Genotyping; Real time Polymerase chain reaction; Treatment; Prognosis

Abstract

The study shows the importance of currently available molecular diagnostic tests for detection, monitoring treatment and genotyping of the HCV, their clinical applications, and how these tests shed light on the natural history of HCV and the necessity of knowing the genotype for the treatment. To determine the distribution pattern of HCV genotypes in Hyderabad, South India and its time trend over last 6 years. The present study was undertaken in the Department of Molecular biology and Cytogenetics department, Apollo hospitals, Hyderabad from January 2010 to June 2016. The study was done on 300 patients. Real time PCR followed by nucleotide sequencing using automated nucleotide sequencer was used in HCV genotyping to understand the distribution pattern of HCV genotypes in Telangana. Among the 300 patients screened, 222(74%) were males, and 78(26%) are females. Among Genotype 1, 64(21.3%) were 1a, 53(17.6%) are 1b, 22(7.3%) are 1c. Genotype 2 in 19(6.3%). Among genotype 3, 31(10.3%) are Genotype 3a, 28(9.3%) are genotype 3b, 9(3%) are genotype 3h, 14 (4.6%) are genotype 3k. Among genotype 4, 33 are genotype 4a (11%), 24(8%) are genotype 4d, 4(1.3%) are genotype 5a. There were no reports of Genotype 6 in our study. A genotype test and quantitative HCV RNA test should be performed on all patients prior to therapy to best assess probability of response and to aid in selection of appropriate therapeutic regimen. Knowing the predominant genotypes is important to plan future prevention and treatment strategies as the treatment of different genotypes and prognosis differs considerably.

Introduction

Chronic HCV (CHC) infection is a global public-health problem, with approximately 170 million persons chronically infected [1] who are at an increased risk of morbidity and mortality due to liver cirrhosis, Hepatocellular Carcinoma (HCC), and extra-hepatic complications that develop [2]. India has 17 per cent of global population and 20 per cent of global disease burden. But less than 1.4 per cent of global clinical trials are done in India, according to Indian Society of Clinical Research (ISCR) [3]. The development of detection assays for HCV has paralleled the introduction of increasingly effective therapies with progressively decreasing adverse effects. Serologic and molecular assays for HCV have played major roles in the identification of those with the viral infection, in determining the severity of the disease, and in the response to therapeutic interventions [4]. A wide variety of genotyping methods are used, including PCR amplification followed by strip-based reverse hybridization, PCR followed by Sanger sequencing, and real-time PCR. Real Time PCR is more sensitive as compared to reverse transcriptase [5].

Molecular viremia, assays for detection and quantitation of HCV RNA levels, genotyping and subtyping are important tools to help physicians make treatment decisions. Prior treatment history, possibly including baseline resistance testing, is also helpful to guide the therapy. HCV isolates are classified into 6 genotypes that differ in their nucleotide sequence by 30%-35% and into multiple subtypes that differ in their nucleotide sequence by 20%-25% [6]. Genotypes 1a and 1b are the most prevalent genotypes in the United States and Western Europe, followed by genotypes 2 and 3. By contrast, genotype 4 is common in Egypt, genotype 5 in South Africa, and genotype 6 in Southeast Asia. This study focuses on the importance of molecular diagnostic tests for HCV, their clinical applications, and how these tests shed light on the natural history and optimal management of hepatitis.

Data on the prevalence of HCV infection and genotyping in Indian population is limited. The present study was undertaken to study the distribution pattern of HCV genotypes in HCV infected patients.

Materials and Methods

This is a prospective study conducted from June 2010 to May 2016 in the Department of Molecular Biology and Cytogenetics department, Apollo Hospitals, Hyderabad, India. The study was approved by the Institutional Ethical Committee. After informed consent to the patients 5 mL of peripheral venous blood samples were collected from the patients following standard procedure.

The Artus Hepatitis C QS-RGQ assay is a real-time PCR assay that uses a magnetic particle-based automated RNA extraction on the QIA symphony SP platform (Qiagen) followed by amplification of the HCV genome and detection on the Rotor-Gene Q platform (Artus Hepatitis C QS-RGQ assay). HCV RNA was extracted from plasma using QIAamp viral RNA extraction kit from plasma (Qiagen GmbH, Hilden, Germany). Real-time polymerase chain reaction (RTPCR) was performed in the department of Molecular biology using Rotor Gene Q Machine with Artus HCV RG RT-PCR Kit (Qiagen GmbH, Hilden, Germany). Hold 1 at 50 ˚C for 30 min, hold 2 15 ˚C for 15 min. Cycling at 95 ˚C for 30 Seconds, 50 ˚C for 60 Seconds, 72 ˚C for 30 Seconds for 50 cycles. HCV genotyping was done using direct DNA sequencing (THE TRUGENE® HCV Genotyping Assay, Siemens Erlangen, Germany) and bi-directional sequences where genotype and subtype characterization is determined by two fluorescently labeled DNA primers (for HCV genotypes 1-6).

Results

Three hundred consecutive newly diagnosed patients with chronic HCV infection, were included in the study.74% were males and 26% were females. The mean age of males is 49 and females is 51 (Table 1). Of the 300 patients, Among Genotype 1, 64(21.3%) were 1a, 53(17.6%) are 1b, 22(7.3%) are 1c. Genotype 2 in 19(6. 3%). Among genotype 3, 31(10.3%) are Genotype 3a, 28(9.3%) are genotype 3b, 9(3%) are genotype 3h, 14 (4.6%) are genotype 3k. Among genotype 4, 33 are genotype 4a (11%), 24(8%) are genotype 4d, 4(1.3%) are genotype 5a (Table 1). There were no reports of Genotype 6 in our study. The trend of HCV genotypes has not changed over the entire study period. Consistently, genotype 1(46.3%) is more prevalent followed by genotype 3(27.3%), Genotype 4(19%) and genotype 5(1. 3%). The distribution of the genotypes detected is represented in Table 2. Surprisingly, Genotype 5 is detected in 1.3% of the study population.

Discussion

The most recent estimates of HCV disease burden show an increase in seroprevalence over the last 15 years to 2.8%, equating to>185 million infections worldwide [6]. Prevalence of HCV infection in general population in India ranges from 0.1%-7.9% while among blood donors it ranges from 0.29%-1.85%. Chronic HCV infection is a major cause of liver related mortality and morbidity. HCV RNA quantification and genotyping are the important molecular diagnostic tests in managing chronic HCV patients undergoing anti-viral therapy. Clinicians determine treatment regimen and duration based on the HCV genotype.

Table 1: Demographic details.

N	Males	Females
300	222(74%)	78 (26%)
Age	49	51

There are Six HCV genotypes and more than 90 subtypes which are identified round the world on the basis of phylogenetic and sequence analyses of whole viral genomes [7-9]. Since there is significant influence on severity of disease, interferon therapy response, and its duration, HCV genotype identification before prescribing therapy is important. Patients infected with HCV genotype 1 and 4 will have to receive IFN and ribavirin for a period of 48 weeks. Persons with these genotypes show a poor sustained viral response when tested 24 weeks after completion of therapy [10]. On the contrary, patients infected with HCV genotype 2 and 3 are reported to have better response to therapy. This remarkable heterogeneity of HCV must be studied in order to develop strategies for designing a successful vaccine.

This is the largest prospective study in India reporting the results of HCV genotyping. The rate of prevalence of HCV genotypes 3 and 1 has already been reported [11]. This study reports that HCV genotype 1 is the predominant genotype (46.2%) followed by genotype 3 (27.2%) in Telangana region. Genotype 4 mainly seen in Egypt and Middle East, in our study is reported in (19%) of study group. Genotype 2 is reported in (6.3%) of our study population compared to the study by Christdas J, et al. 2013 seen in 0.43% of study population. None of our study group has genotype 6. Interestingly, Genotype 5 is reported in 1.3% of the study group (Table 2).

Despite the differences in the frequency of occurrence of different genotypes across the Indian sub-continent, there is also a pattern of distribution of HCV genotypes throughout the country. Besides this there appears a trend of increased occurrence of genotype 4 (19%) and genotype 5 within India, which is evident from our current study. Genotype 5 was found to be prevalent exclusively in patients from South Africa and central parts of France, but to our surprise was found in 1.3% of Telangana state.

Globally, genotype 1 (46.2%) is estimated to account for more HCV cases than any other with over one-third of genotype 1 cases located in East Asia. HCV genotype 3 is the next most accounting (30.1%) cases globally, approximately three-quarters of which occur in south Asia. Genotypes 2(9.1%), 4(8.3%), and 6 (5.4%) are responsible for the majority of the remaining cases of HCV worldwide, respectively.

Table 2: Genotype distribution.

Genotype	Percentage Distribution
1a	64(21.3%)
1b	53(17.6%)
1c	22(7.3%)
2	19(6.3%)
3a	31(10.3%)
3b	28(9.3%)
3h	9(3%)
3k	14(4.6%)
4a	33(11%)
4d	24(8%)
5a	4(1.3%)

Study conducted by Messina, et al. [12] computed relative genotype frequencies for each Global Burden of Disease (GBD) as defined by WHO which included 1,217 studies, with 117 countries, of which 15 studies were from India. This study indicated genotype 3(>50%) the most prevalent, followed by Genotype 1(25-50%), Genotype 2(.01-10%) and Genotype 4(.01-10%), Genotype 5(.01-10%), Genotype 6(<.01%) in India.

Genotype 1, 2 and 3 from our study is correlating with the global studies, but genotype 4 from our study is prevalent in 19% which is high as compared to global estimate (8.3%). North Africa and the Middle East have the largest number of genotype 4 cases. East Asia accounts for the greatest numbers of genotype 2 and genotype 6 HCV cases, the great majority of which occur in Southern and Eastern sub-Saharan Africa. Genotype 5 was seen in 1.3% of our study group which is slightly high unlike compared to global population (<1% of all HCV cases). This discrepancy may be due to our small sample size, may be larger sample study might give a better answer.

The distribution of HCV genotypes varies in different geographical regions of India. Genotype 3 is the predominant genotype in North India whereas genotype 1 in South India. Among 300 HCV genotype samples in our study, 139 patients were genotype 1a, while 82 patients were genotype 3 which is in concordance with the previously published studies from south India [13-15]. As only five genotypes were identified in our study, it is difficult to comment on the age and sex distribution of different genotypes. All the 1a genotypes were patients less than 59 years of age 1b less than 53 years of age, 1c less than 57 years of age. Genotype 2 is less than 56 years of age. Genotypes 3a, 3b, 3h, 3k are less than 58 years of age. Genotype 4 is less than 63 years and Genotype 5 is 52 years of age. In literature no significant differences have been found in genotype distribution with respect to age and sex. But a study by Idrees, et al. [16] from Pakistan reported that subtype 1a/1b were more common in younger patients and subtypes 2a/2b and 3a/ 3b were common in older patients. More extensive work needs to be done before conclusively commenting on the various genotypes prevalent in this region. Knowing the predominant genotypes is important to plan future prevention and treatment strategies as the treatment of different genotypes and prognosis differs considerably.

Molecular tests are useful for detection, monitoring the treatment and its response of hepatitis HCV. Qualitative nucleic acid tests have low limits of detection (<50 IU HCV RNA/mL) and are used for confirmation of HCV infection and for screening blood donations. Quantitative HCV RNA testing provides prognostic information regarding likelihood of treatment response and plays an important role in monitoring the antiviral response to treatment. The new HCV therapies have specific indications, including durations and drug combinations appropriate for use for treatment of a particular patient, which vary according to HCV genotype, a critical determinant of treatment response. It is likely that genotyping will continue to be performed even as more pan-genotypic regimens are developed.

It must be stressed however, that the reported distribution of the various genotypes can be expected to change with increasing migration of population and changes in high risk behavior and life-style. There is a need for public education in this country about routes of transmission of viruses such as HCV especially in this era where we still await a suitable HCV vaccine since HCV exhibits an extraordinarily high degree of genetic diversity.

Conclusion

Diagnostics have played an important role in numerous aspects of HCV, from its discovery to prevention of its transmission, treatment management, and, hopefully, eventual global eradication. Molecular assays are more sensitive and specific for detecting HCV and have proven effective. The information provided by the present study from this region provides valuable information to physicians in clinical decision making. It is interesting to note that some new trends have occurred in genotype distribution in our study group. Such studies and trend analysis are also important to document in the wake of licensure of the newer generation directly acting antivirals for HCV such as bocepravir and teleprevir. Knowing the predominant genotypes is important to plan future prevention and treatment strategies as the treatment of different genotypes and prognosis differs considerably.

Acknowledgment

The Authors acknowledge all the patients included in the study and the management of Apollo Hospitals for their support.

References

1. Lavanchy D. Evolving epidemiology of hepatitis C virus. Clinical Microbiology and Infection. 2011; 17: 107-115.
2. Vermeiren AP, Dukers-Muijrers NH, van Loo IH, Stals F, van Dam DW, Ambergen T, et al. Identification of hidden key hepatitis C populations: an evaluation of screening practices using mixed epidemiological methods. PloS one. 2012; 7: e51194.
3. Mareen J, Madhukara J. An interesting case of Lucio phenomenon triggered by activation of hepatitis C infection. Indian dermatology online journal. 2016; 7: 406-409.
4. Cloherty G, Talal A, Coller K, Steinhart C, Hackett J Jr, Dawson G, et al. Role of Serologic and Molecular Diagnostic Assays in Identification and Management of Hepatitis C Virus Infection. Journal of clinical microbiology. 2016; 54: 265-273.
5. Khan IA, Vattam KK, Jahan P, Hasan Q, Rao P. Importance of glucokinase -258G/A polymorphism in Asian Indians with post-transplant and type 2 diabetes mellitus. Intractable & rare diseases research. 2016; 5: 25-30.
6. Mohd Hanafiah K, Groeger J, Flaxman AD, Wiersma ST. Global epidemiology of hepatitis C virus infection: New estimates of age□specific antibody to HCV seroprevalence. Hepatology. 2013; 57: 1333-1342.
7. Hoofnagle JH. Course and outcome of hepatitis C. Hepatology. 2002; 36(5B).
8. Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, et al. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. The Lancet. 2001; 358: 958-965.
9. Simmonds P, Bukh J, Combet C, Deléage G, Enomoto N, Feinstone S, et al. Consensus proposals for a unified system of nomenclature of hepatitis C virus genotypes. Hepatology. 2005; 42: 962-973.
10. Hadziyannis SJ, Sette H, Morgan TR, Balan V, Diago M, Marcellin P, et al. Peginterferon-α2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. Annals of internal medicine. 2004; 140: 346-355.
11. Raghuraman S, Shaji R, Sridharan G, Radhakrishnan S, Chandy G, Ramakrishna B, et al. Distribution of the different genotypes of HCV among patients attending a tertiary care hospital in south India. Journal of clinical virology. 2003; 26: 61-69.
12. Messina JP, Humphreys I, Flaxman A, Brown A, Cooke GS, Pybus OG, et al. Global distribution and prevalence of hepatitis C virus genotypes. Hepatology. 2015; 61: 77-87.

13. Christdas J, Sivakumar J, David J, Daniel H, Raghuraman S, Abraham P. Genotypes of hepatitis C virus in the Indian sub-continent: A decade-long experience from a tertiary care hospital in South India. Indian journal of medical microbiology. 2013; 31: 349-353.

14. Panigrahi AK, Roca J, Acharya SK, Jameel S, Panda SK. Genotype determination of hepatitis C virus from northern India: identification of a new subtype. Journal of medical virology. 1996; 48: 191-198.

15. Valliammai T, Thyagarajan S, Zuckerman AJ, Harrison TJ. Diversity of genotypes of hepatitis C virus in southern India. Journal of general virology. 1995; 76: 711-716.

16. Idrees M, Lal A, Naseem M, Khalid M. High prevalence of hepatitis C virus infection in the largest province of Pakistan. Journal of digestive diseases. 2008; 9: 95-103.