MODULATION OF TOLL LIKE RECEPTOR 3, 7 AND 8 EXPRESSION IN HEPATITIS C VIRUS INFECTED PATIENTS

Rushna Firdaus*, Aritra Biswas*, Kallol Saha*, Anirban Mukherjee*, Sujit Chaudhuri†, Asokananda Konar‡, Alok Chandra§, Provash Chandra Sadhukhan*

ICMR Virus Unit, Kolkata, West Bengal, India, †Department Of Gastroenterology, Amri Hospital, Saltlake, Kolkata, West Bengal, India, ‡Department Of Gastroenterology, Peerless Hospital And B.K. Roy Research Centre, Kolkata, West Bengal, India, and §Department Of Gastroenterology, Command Hospital, Kolkata, West Bengal, India

Background: Hepatitis C virus (HCV) is the major cause of chronic hepatitis worldwide which finally leads to the development of hepatocellular carcinoma (HCC). Due to genetic heterogeneity, HCV evades the immune system and establishes chronic infection within the host. Toll like receptors (TLR) has been shown to play an important role in the course of many viral infections, but the role of TLRs in HCV pathogenesis has not been well elucidated so far. In the Indian context there is not much evidence of the toll like receptor and how it regulates the pattern of regulation amongst the different regulatory pattern within the genotype 3 infected patients, the major circulating genotype in this country.

Objective: Our aim was to analyse the mRNA expression of TLR 3, 7, 8 and 9 in HCV infected interferon (IFN) treated patient's to see the modulation of TLR expression in this group of patients.

Methodology: Serum samples from HCV infected individuals from different population groups including chronic HCV and cirrhosis, IFN-treated resolved and relapsed cases were assessed in this study. Viral RNA was isolated from serum and subjected to real time PCR and nested RT-PCR for HCV viral load, genotyping assay respectively. Total RNA from whole blood was extracted and mRNA expression of TLR3, TLR7, TLR8 and TLR9 gene was analyzed by quantitative real time RT-PCR using housekeeping ß-actin gene as the internal control.

Result: A total of 57 HCV sero-reactive patient samples were studied. The quantitation data shows that viral load of the group of patients who had been induced with interferon therapy showed a viral load in the range 2.5×10^6 to 9.8×10^3 IU/ml. Patients who have been cleared of HCV infection showed a viral load at Below detection level whereas the viral load of chronic HCV and cirrhosis patients ranged from 2.2×10^3 to 1.88×10^7 IU/ml. Genotyping analysis reveals that genotype 3 was the major genotype variant. Analysis of the mRNA expression levels of TLR3, TLR7 and TLR 9 shows that chronic patients with cirrhosis of liver showed a significant amount of change in mRNA expression compared to healthy control. Patients with relapsed HCV infection showed a significant amount of change in mRNA expression of TLR7 and TLR8 compared to healthy control whereas, TLR3 expression was not that much significant.

Conclusion: The results obtained could be in an important indicator in predicting future trends of the regulation of the TLR, which could also aid in predicting a molecular target as HCV vaccine or vaccine adjuvant.

Corresponding author: Rushna Firdaus.
Email: rushna2011@gmail.com

GENOTYPE DISTRIBUTION AND TIME TREND OF HEPATITIS C IN SOUTH INDIA

E. H. Rooby, George Peter, P. V. Mashhood, Anil P. John, Yamuna R. Pillai, N. Premalatha, K. R. Vinayakumar

Department of Medical Gastroenterology, Government Medical College, Trivandrum, Kerala, India

Background: Hepatitis C virus (HCV) shows considerable genetic heterogeneity among isolates from all over the world. Six main groups of sequence variants are recognized. Genotype 1,2 and 3 are most common in North America and Europe, genotype 4 is most common in Middle East, genotype 5 and 6 are common in South East Asia. In India, genotype 3 is most common and genotype 2 is least common. The knowledge about the genotype distribution of HCV in a geographical area may help for treatment plan and also provide clues about the outcome of HCV related liver disease in that area.

Objective: To determine the distribution pattern of HCV genotypes in Kerala, South India and its time trend over last 5 years.

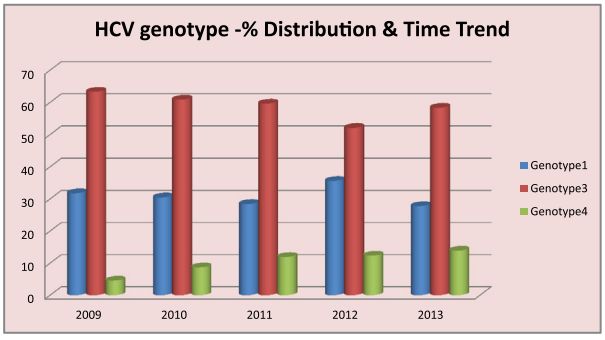
Material and Methods: Data was collected from all hepatitis C patients attending Department of Medical Gastroenterology, Trivandrum from January 2009 to November 2013. All the newly diagnosed HCV patients were included. Those patients whose HCV genotype could not be ascertained were excluded. Nested RNA PCR followed by nucleotide sequencing using automated nucleotide sequencer was used in HCV genotyping.

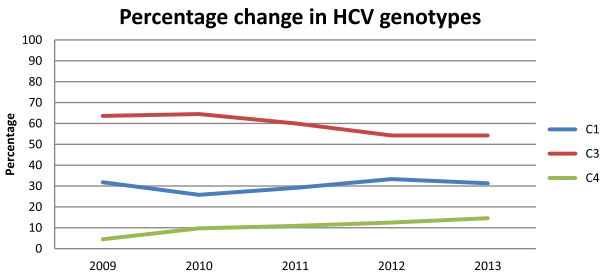
Results: Two hundred and sixty three consecutive newly diagnosed patients with chronic HCV infection, were included.71% were male and 29% were female. Of the 263 patients, genotype 3 was found in 152 (57.79 %), Genotype 1 in 81 (30.70%), genotype 4 in 31 (11.78 %). None were infected with genotype 2. Among genotype 3,72.4% were 3b and 27.58% were 3a. Among genotype 1,75% were 1b, 12.5% were 1a and 12.5% were 1c. Among genotype 4,45.45% were 4a, 45.45% were 4d and 9.09% were 4b. A rising trend of Genotype 4 from 2009 to 2013 was noted. The

percentage of genotype 4 is 4.54% in 2009, 8.69% in 2010, 11.92 % in 2011, 12.32% in 2012 and 13.88 % in 2013. This rising trend of genotype 4 is statistically significant (P = 0.014).

Conclusion: Genotype 3 is the most prevalent HCV genotype in South India (with maximum of 3b followed by 3a) followed by genotype 1 (commonest is 1b followed by 1a and 1c) and then genotype 4 (commonest are 4a and 4d, with 4b as least common). A rising trend of Genotype 4 is noted from 2009 to 2013.

Discussion: HCV genotype 4, which is resistant to therapy, traditionally considered to be confined to the Middle East and Africa, now shows a rise in trend in South India.





Corresponding author: Rooby E.H.
Email: roobyshaheer@gmail.com

AN INCREASING TREND OF HEPATITIS C VIRUS GENOTYPE 1 AMONG HIGH RISK GROUP POPULATIONS IN EASTERN INDIA

Provash Sadhukhan, Kallol Saha, Rushna Firdaus, Aritra Biswas, Anirban Mukherjee

ICMR Virus Unit, Kolkata, West Bengal, India

Background: Hepatitis C Virus (HCV) is increasingly a huge burden on health care systems throughout the world with large number of infected people worldwide. Genotyping and evaluation of viral load in HCV infected patients is important for clinical evaluation and therapeutic interventions. Thus, the present study was designed to determine the distribution pattern of HCV genotypes in high risk group patients and establish their relationship with viral load.

Objectives: The major aims of this study were to ascertain the predominant circulating strain from eastern India among the high risk group populations and to correlate the viral load within these HCV patients.

Methods: A total of 444 HCV sero-positive samples from different high risk population groups like history of blood transfusion (HBT), dialysis (D), hemophilia (H), intra venous drug users (IVDUs) and thalassemia (T) patients were included in this study. The detection of HCV RNA was performed using nested RT-PCR assay of the 5′ untranslated region. For genotyping, five prime untranslated region (5′ UTR) positive samples were subjected to core region amplification by nested RT-PCR assay. The HCV viral load was measured based on 5′ UTR of HCV genome using AgPath-ID™ One Step RT-PCR kit.

Results: Of the total 444 sero-positive samples 315 (70.94%) were RNA positive among them 228 were genotyped. Genotype 3 was found to be 52.24% (3a: 62.5%, 3b: 36.72%, 3k: 0.78%) followed by 47.34% genotype 1 (1a: 34.48%, 1b: 63.8%, 1c: 1.72%), genotype 6h was 0.42% of our study population. Genotype 1b was the predominant genotype within the population groups of HBT (33.34%), D (35.71%) and IVDUs (38.46%) and genotype 3a was significantly high only in the populations comprising of T (63.43%) individuals. The mean viral load among the different patients groups were 6.28 ± 0.89 log10 IU/ml. The viral load observed among the three genotypes were 6.43 ± 0.75 $\log_{10}$ IU/ml, 6.31 ± 0.77 $\log_{10}$ IU/ml and 5.12 ± 0.87 $\log_{10}$ IU/ml for genotype 1, 3 and 6 respectively. Genotype 1 was associated with significantly higher (P < 0.0001) viral load compared to genotype 3 and 6.

Conclusion: The major circulating strain prevalent within the high risk group of eastern region is found to be genotype 3 closely followed by genotype 1 and with a small fraction of genotype 6. Increase in overall percentage of genotype 1 is being observed among high-risk group population. The viral loads were also significantly associated with the genotypes and genotype 1 showing statistically higher viral load compared to that of genotype 3 and 6.

Corresponding author: Provash Sadhukhan.
Email: provash2000@gmail.com

ELEVATED LEVEL OF LIVER ENZYMES IS NOT A SERUM MARKER FOR HEPATITIS C VIRUS INFECTION AMONG ß-THALASSEMIC INDIVIDUALS

Provash Sadhukhan*, Rushna Firdaus*, Kallol Saha*, Anirban Mukherjee*, Prosanto Chowdhury†, Maitreyee Bhattacharyya‡