

Correlation Study Between HCV Genotypes Distribution Pattern and Viral Load in a Tertiary Care Hospital in Kolkata, India

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ABSTRACT

Background: Hepatitis C virus infection is a leading cause for chronic liver disease. It has wide population specific genotype variability. Genotype knowledge and viral load assessment are equally important for designing therapeutic strategies and as predictors of treatment outcome among hepatitis C (HCV) infected patients.

Materials and Methods: Between June 2012 and 2013 an observational study was conducted among 350 chronic hepatitis patients visiting Calcutta National Medical College, Kolkata, India. Among them, 110 anti-HCV antibody positive cases were diagnosed and subjected to viral RNA extraction, viral genotyping and viral load quantification using polymerase chain reaction (PCR) based techniques.

Statistical Analysis: Statistical analysis was done with IBM SPSS Statistics software, version 20. p-value <0.05 was regarded as statically significant.

Results: Among 66 HCV RNA positive cases, genotypes 1a, 3a and 3b were observed among 18 (27%), 44(67%) and 4(6%) cases respectively. Genotype 3a had higher viral load than patients infected with genotypes 1a and 3b. However, no statistical significance was observed for viral load among the various HCV RNA genotypes.

Conclusion: Genotype 3a accounted for the highest number of cases with positive HCV RNA. However, no statistically significant difference existed for viral load among the various HCV RNA genotypes in this study.

Keywords: PCR, Viral Genotype

INTRODUCTION

Hepatitis C virus infection has emerged in recent times as a leading cause of chronic hepatitis, liver cirrhosis and hepatocellular carcinoma [1]. The progression of Hepatitis C virus within the liver hepatocytes more often or not is quite protracted requiring timely diagnostic interventions like serology, biochemical tests and radiological examinations for proper detection [2]. Six major HCV genotypes have been identified across the world [3]. HCV genotypes variations are important factors for patient management for these are associated with different responses to the current standard anti-HCV regimen consisting of pegylated interferon (PEG) plus rivabarin [4]. Genotypes 1 and 4 are more resistant compared to genotypes 2 and 3 to this therapy [5]. Patient viral load also affects treatment duration and responses [6]. Patients infected with genotype 1 had higher viral loads in comparison to those with genotypes 2 or 3 [7]. However, correlation between viral loads and other HCV genotypes have not been described. The study was conducted in this background to determine the distribution pattern of HCV genotypes in plasma samples of HCV viremic individuals and their association with viral load.

MATERIALS AND METHODS

The hospital-based study was conducted upon 350 patients with chronic hepatitis attending the medical outpatient department or admitted in Calcutta National Medical College, Kolkata, India in collaboration with National Institute of Cholera and Enteric Diseases (NICED), Kolkata, India during June 2012-2013. Diagnosis of cases were confirmed based upon clinical features, liver function tests, ultrasonography findings, endoscopy and wherever indicated by liver biopsy. Proper ethical committee approval was obtained from the institutional ethical committee prior to the study. Informed consent was obtained from individual patients. All the patients were

diagnosed positive for HCV antibodies using 3rd generation ELISA method (J. Mitra & Co., Pvt. Ltd., New Delhi, India). Patients on immunosuppressive drugs and history of alcohol intake, evidence of HBsAg or HIV were excluded from the study.

The ELISA positive subjects were subjected to RNA extraction using QIAamp Viral RNA mini kit (Qiagen, Hilden, Germany) according to manufacturer's protocol.

For detection of HCV RNA, Nested RT-PCR was done based upon the 5' Un Translated Region (UTR) employing primers of Bukh et al., [8]. The positive samples gave a band at 256bp in ethidium bromide stained 1.5 % Agarose gel under a gel documentation system. HCV RNA quantification was done using ABI real time Q-RT-PCR kit (AgPath-IDTM One Step RT-PCR kit). The HCV primer and probe sequences were directed against the 5' non-coding region of the HCV genome. HCV standards were obtained from Genome Diagnostic Pvt. Ltd., India. The HCV load in plasma was expressed as log₁₀ international units per millilitre (log₁₀ IU/ml). HCV genotyping was done with reverse transcriptase PCR (RT-PCR) according to Bukh and Cantaloube et al., respectively for core and NS5B in a Veriti 96 well Thermal Cycler (ABI, Foster City, USA) [9]. The nested round was performed using 2μL of the first round product.

The bands were electrophoresed using 1.5% agarose (Sigma-Aldrich, St. Louis, USA) gel and documented under gel documentation system (Bio-Rad, USA). 5' UTR, Core and NS5B bands at 256, 405 and 389bp were excised and gel purified using QIA quick Gel extraction kit using manufacturer's protocol [Table/Fig-1].

The sequencing reaction is performed using ABI big dye 3.1 sequencing kit with the manufacturer's protocol. The reaction is performed in 10μL reaction volume with 1μL of the gel extracted product. The Sequencing PCR product is then cleaned up and loaded into the Automated DNA sequencer ABI 3130XL.

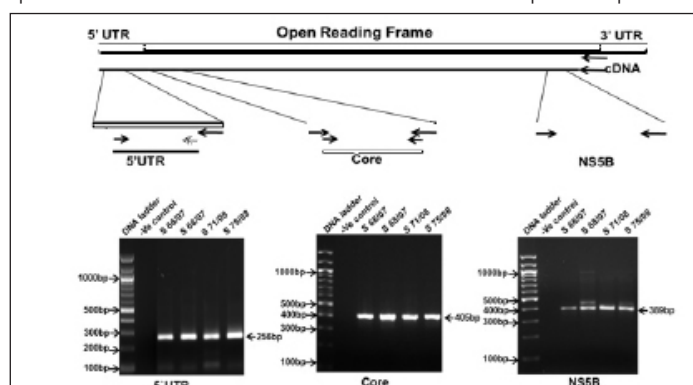
STATISTICAL ANALYSIS

Statistical analysis was done with IBM SPSS Statistics software, version 20. p-value <0.05 was regarded as statically significant.

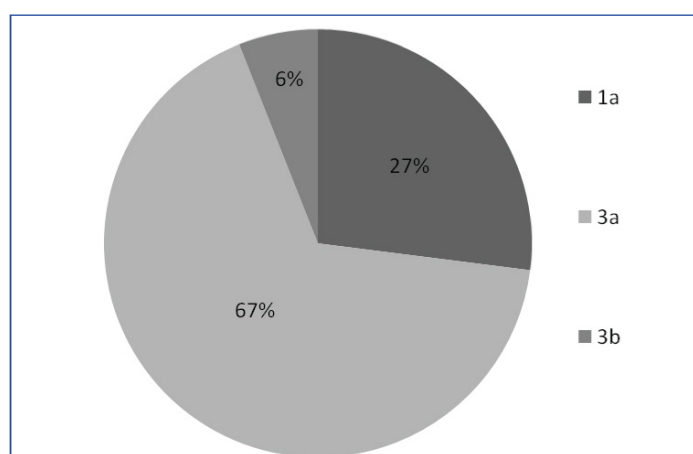
RESULTS

Of the 350 chronic liver diseases patients screened for the presence of anti-HCV antibodies, 110 were positive. These HCV antibody positive patients were tested for the presence of HCV RNA and 66 patients were found to be HCV RNA positive. All HCV RNA positive samples were subjected to genotype determination. The analysis revealed the presence of genotypes 1, 3a and 3b using RFLP and type specific PCR followed by direct sequencing. Genotype 1a was seen in 18 (27%) patients. Genotype 3 was observed in remaining 48 (73%) patients. Of these, 44 showed infection with subtype 3a (67%) while 4 had subtype 3b (6%) [Table/Fig-2].

Cases with mixed genotype infection were not found. Viral load quantification was carried out in all 66 HCV RNA positive patients



[Table/Fig-1]: Nested RT-PCR amplification of 5'UTR, Core and NS5B regions of HCV genome from archived samples



[Table/Fig-2]: Percentage of various HCV genotypes
HCV Genotypes : 1a, 3a, 3b

Hcv Genotypes	Number of Cases	Viral Load (Log ₁₀ Iu/ML) (Mean ± Standard Deviation)
1a	18	13168.89 ± 2694.5
3a	44	28733.57 ± 7016.23
3b	4	4774.5 ± 127.34

[Table/Fig-3]: Mean viral load among HCV genotypes

Bonferroni						
(I) Variable 1 (HCV- genotypes)	(J) Variable 2	Mean Difference (I-J)	Std. Error	Signi- ficance p value *	Iue	
					Lower	Upper
					Bound	Bound
3a versus	1a	14923.38	23348.16	1.000	-44281.53	74128.30
3a versus	3b	23317.77	43579.65	1.000	-87188.98	133824.52
3b versus	1a	-8394.38	46128.00	1.000	-125363.08	108574.30

[Table/Fig-4]: Post-hoc annova tests of multiple comparisons for viral load among HCV genotypes
* p-value significant at < 0.05

(expressed in mean ± standard deviation) and was compared between the three groups of genotypes. The average viral load of the patients infected with genotype 3a was significantly higher than average viral load of the patients infected with genotypes 1 and 3b [Table/Fig-3]. However, no statistical significance was observed for viral load among the various HCV RNA genotypes [Table/Fig-4].

DISCUSSION

HCV genotype distribution pattern vary widely from one continent to the other. Prevalence of genotypes 1, 2 and 3 are known to be distributed unequally throughout the world [10,11]. While Subtype 1a is prevalent in the American continents, Europe and Australia in subtype 1b prevails in North America, Europe and certain regions of Asia only [12,13]. Genotype 2 infection however is not so prevalent. [14]. HCV genotypes 1, 2 and 3 have been detected in North India, with 3 being the predominant one [15,16] while studies from South India reported high occurrence of genotype 1 followed by 3 [17,18].

The present study results showed that type 3a (66.67%) was the most common genotype followed by type 1 (27.27%) and type 3b (6.06%). No regional difference existed for HCV genotypes distribution pattern in vast regions of South Asian countries like in Iran and Pakistan where 3 was the predominant HCV genotype. However, results of given study were different from Japan and Thailand where genotype 1 was the common HCV genotype [19-21]. In our study HCV genotypes 4, 5 and 6 were not detected. These observations were similar to those reported previously regarding the near absence of these genotypes from this region [22]. These findings were similar to the conclusions obtained from other study reports from India that the most prevalent genotype is 3a followed by genotype 1a. Apart from viral genotype, viral load prior to antiviral therapy is regarded as an important prognostic sign and a valuable predictive sign for outcome of antiviral therapy. High base line viral load in terms of HCV RNA copy numbers was associated with low response to standard interferon therapy and higher probability of relapse compared to those with low-level viraemia [23]. The detected HCV genotypes and viral loads had both been extensively analysed. Many previous case reports had suggested that mean HCV RNA were higher in patients infected with genotype 1 were more likely to have higher viral loads than those infected with genotype 2 and 3. More efficient viral replication machinery of genotype 1 as compared to the others has been assumed to responsible for this [24]. However, the correlation between HCV genotypes and viral load remains controversial. In the present study the mean viral load in patients with genotype 3 was significantly higher than those with genotypes 1 and 2 but the correlation was not statistically significant (p-value>0.5). The results differ from a similar Pakistan based study where a high viral load was associated with genotypes 1a and 1b compared to other genotypes [25]. Our findings carry some important implication as timely detection and treatment are significant to achieve a high level of sustained virological response (SVR) [26]. Early time detection involves the identification of low HCV RNA level [5]. As determined by Von et al., and Dalgard et al., shorter therapy schedules for genotype 3 HCV infected patients with low baseline viral load could attain a SVR as compared to those with a high viral load [27,28]. Studies have shown that mixed HCV genotypes are more frequent in cases due to blood transfusion, especially in thalassaemic patients [26]. Franciscus had stated that mixed genotypes in a single patient may affect the antiviral therapy response and disease succession [29]. In the present study there were thalassaemic patients who had received unsafe blood in past. Fortunately, however no mixed genotype affected HCV cases were detected.

CONCLUSION

The present study results highlighted that genotype 3 is the predominant genotype in this geographical region followed by

genotype1. However, no significant correlation was found between HCV genotypes and viral load in this study. The limitation of the study includes consideration of cases admitted only in a tertiary care hospital whereas a large number of chronic hepatitis patients are admitted in district and sub divisional hospitals. Multicentric study upon a larger population spanning over several years would have probably been helpful to further ascertain the correlation between viral genotypes and load. Our findings recommend that prior information about HCV genotype and basal RNA viral load should be an integral part of national planning strategies against HCV level at the therapeutic level. These results should help to individualize antiviral therapy, reduce side effects of antiviral therapy, economic burden and promote optimum response rates.

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