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Characterization of Circulating HCV Genotypes: A Cohort Study in Uttar Pradesh, India

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Abstract

Hepatitis C virus infection is one of the main causes of liver disease that can lead to liver cirrhosis and finally to the chronic development of Hepatocellular Carcinoma (HCC). With 8 different HCV genotypes circulating, it becomes necessary to characterize the pathogenesis caused by each one of the HCV genotypes infecting population of a particular setting. Present study aims to identify the circulating Hepatitis C virus (HCV) genotypes in the different areas of State of Uttar Pradesh, India and understand their clinical etiology and related co-morbidities. The patients reporting to Sharda Hospital, Uttar Pradesh, India who were diagnosed positive for HCV were included in the study. The informed consent was obtained and then blood was drawn. In-house primers, for all the 8 genotypes were prepared and RT-PCR was performed. The amplified product was subjected to Agarose gel electrophoresis to identify the genotypes present in individual samples. Total 30 serum samples were taken. Of these, 21 (70%) showed presence of multiple genotypes, while 7 (23.33%) showed positivity for single genotype and two did not show any bands. Presence of multiple genotypes in individual patient has been reported for the first time. The pathological consequences of multiple genotypes within one host need to be studied further in terms of increased risk of developing a chronic liver disease. Further studies are being undertaken to study association of specific HCV genotype and multiple genotypes, Core, NS3 and NS5 viral proteins with the disease progression to develop predictors of liver cirrhosis and HCC.

Keywords: Genotypes, Hepatitis C virus, Hepatocellular carcinoma, India

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INTRODUCTION

The Hepatitis C virus (HCV) infection is increasing in different regions of the world at the rate of 58 million infections per year and has become one of the main reasons of mortality due to liver infections.[1-7] Every year almost 6-12.5 million people in India get infected due to the HCV infection.[8] Out of this, around 27% are reported cases of cirrhosis and 25% of patients are reported cases of Hepatocellular Carcinoma (HCC).[9] There is inflammation of the liver initially and then the patients eventually end up with the development of chronic liver cirrhosis, i.e. scarring of the whole liver and other complications related to it. Studies have reported that it takes almost sixteen years to develop fibrosis after acquiring HCV infection.[10] It was found that the rate of fibrosis was less below the age 30 and higher in older patients.[10] Only few patients are able to eliminate the virus during the initial stage, however the chances that virus may still remain active in the infected cells is also there. Till date, eight types of HCV genotypes have been reported which when sequenced showed 30% difference.[11] The main genotypes reported are HCV genotype 1 and HCV genotype 3 followed by Genotype 2, 4 and 6.[12-16] The other HCV genotypes 5 and 7 are also reported but are limited to certain geographical regions.[16] Recently, Genotype 8 has been identified from Punjab, India in the year 2018.[12]

Though the different genotypes have been reported, yet the possibility of multiple types in an individual patient has not been much reported. One study has indicated 2-10% presence of multiple infections of HCV genotypes.[17] The present study has been undertaken to study the occurrence of multiple genotypes and then correlate it with the clinical symptoms to address to the severity of this disease.

MATERIALS AND METHODS

The patients who were referred to Sharda Hospital with liver inflammation during the period of March 2022 to July 2023 were taken for the study. Once the Ab-HCV positivity was confirmed in their blood samples, the blood was brought to the laboratory under strict biosafety protocol and then serum was separated and stored at -80°C until further experimentation. The consent for the study was taken both orally/verbally from each individual patient and for patients with older age admitted in ICU, the consent was taken by thumb impression. The serum was then subjected to viral RNA extraction employing the QIAamp viral RNA mini kit (m/s Qiagen, CA) following the manufactures instructions. The RNA was then subjected for amplification using the One step RT-PCR kit (m/s Ambion, USA). For the amplification, In-house primers were designed for all the eight genotypes. These were then commercially synthesized by m/s Eurofins, India. The cycling conditions are as follows: Reverse transcription at 55°C for 30 minutes, Primer Annealing and extension of 50 cycles, each of 95°C for 15 seconds, 95°C for 35 seconds, 50°C for 60 seconds. The final extension at 72°C for 30 seconds. The PCR products were then run on 2% agarose gel, and then observed in Gel Documentation System.

RESULTS

Table 1 shows the primers designed in-house (Centre of Excellence in Virology & Immunology, Sharda University, U.P., India) for all the eight genotypes. The NCBI was searched for the reference HCV genomes for all the 8 genotypes. The following were selected: For Genotype 1: NC_004102.1, for Genotype 2: NC_009823.1, for Genotype 3: NC_009824.1, for Genotype 4:

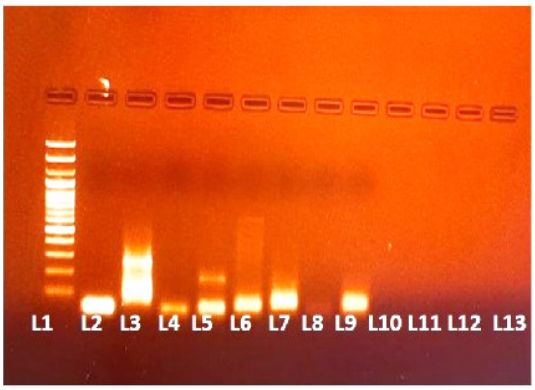

Figure 1. Agarose Gel showing bands for HCV patient ACVI/21/07 (Bands visible in G2, G4)

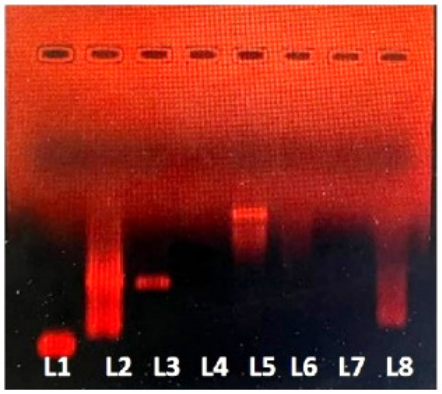

Figure 2. Agarose Gel showing bands for HCV patient ACVI/06/01 (Bands visible in G2, G3 and G5)

NC_009825.1, for Genotype 5: NC_009826.1, for Genotype 6: NC_009827.1, for Genotype 7: NC_030791.1 and for Genotype 8 - MH590698.1. Using the NCBI primer designing software, the region of 7400-7600 bp which encodes for NS5 protein was selected.

A total of 30 HCV positive samples were collected during the period. The amplified template when run on Agarose Gel Electrophoresis indicated presence of multiple bands in 70% (21 patients) of the patients. (Table 2). While only 7 (23.33%) showed positivity for single genotype. There were 2 which did not show any bands. The gel images are shown in the Figures 1 to 4.

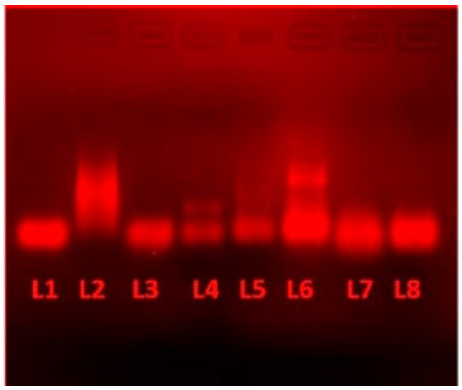

Figure 3. Agarose Gel showing bands for HCV patient ACVI/28/13 (Bands visible in G4 and G6)

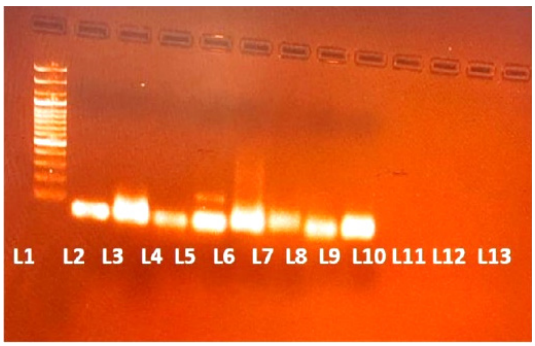

Figure 4. Agarose Gel showing bands for HCV patient ACVI/22/08 (Bands visible in G4)

Table 1. Showing the In-house primer designing of different HCV genotypes

HCV genotypes	Primer type	Sequences 5' to 3'	Expected size (in bp)
Genotype 1	Forward primer	CAGCACACGACATCTTCCGT	184
	Reverse primer	GCATTACGGGCGACAATACG	
Genotype 2	Forward primer	GTTGGGGCTCTACCTGCTC	161
	Reverse primer	CGATTCAGGCCTTTCCAC	
Genotype 3	Forward primer	TGTCACTAACGGTGGACCAA	249
	Reverse primer	TTCAGCTGGACGGCTCTAAT	
Genotype 4	Forward primer	GGCAGCTTTGATTCTTCAGC	178
	Reverse primer	CCGATGACGGATCTTATTCG	
Genotype 5	Forward primer	AGAGCAAGGGGTGATGAGC	156 or 1212
	Reverse primer	GACGCGGCTTCATATTCTTC	
Genotype 6	Forward primer	AACAGTGGACCAGGAACCAG	199
	Reverse primer	GGCTGATAAGGTGTTTGTGGA	
Genotype 7	Forward primer	TCTGGTGCTGACTGTTGACC	153
	Reverse primer	TCCGCCATTAGTCTGGATTC	
Genotype 8	Forward primer	CCATGAGCCAGAGTCCAAGT	175
	Reverse primer	GTCTTTTCCCCCATCATCTG	

Table 2. Analysis of the Genotyping present in the HCV patients

No.	Sample code	HCV G1	HCV G2	HCV G3	HCV G4	HCV G5	HCV G6	HCV G7	HCV G8	Total Genotypes observed
1.	ACVI/06/01		√	√	√					3
2.	ACVI/07/02				√	√	√			3
3.	ACVI/11/03				√	√	√			3
4.	ACVI/12/04				√	√				2
5.	ACVI/18/05						√			1
6.	ACVI/20/06	-	√	-	-	-	-	-	-	1
7.	ACVI/21/07		√		√					2
8.	ACVI/22/08				√					1
9.	ACVI/23/09				√	√	√			3
10.	ACVI/24/10						√			1
11.	ACVI/25/11		√		√	√				3
12.	ACVI/27/12		√				√			2
13.	ACVI/28/13				√		√			2
14.	ACVI/36/14		√			√				2
15.	ACVI/42/15		√				√			2
16.	ACVI/44/16		√		√	√	√			4
17.	ACVI/51/17		√		√					2
18.	ACVI/58/18		√		√	√				3
19.	ACVI/62/19		√		√					2
20.	ACVI/80/20		√		√					2
21.	ACVI/81/21						√			1
22.	ACVI/82/22		√							1
23.	ACVI/83/23					√	√			2
24.	ACVI/88/24	-	-	-	-	-	-	-	-	0
25.	ACVI/89/25	-	-	-	-	-	-	-	-	0
26.	ACVI/100/26				√					1
27.	ACVI/105/27		√				√			2
28.	ACVI/107/28		√				√			2
29.	ACVI/108/29		√				√			2
30.	ACVI/109/30		√				√			2

The clinical symptoms presented by these HCV patients infected with multiple genotypes were compared with those with individual genotypes (Table 3). It was observed that 33.33% (7) of the patients with multiple genotypes had Grade I fatty liver compared to presence of only 1 such case in single genotype infected patients. Hepatomegaly 19.04% (4), Splenomegaly 9.52, abdominal pain 9.52% (2) and Liver cirrhosis 4.76% (1) was also observed in multiple genotype infected HCV patients compared to single genotype infected HCV patients (Hepatomegaly 0, Splenomegaly 1, abdominal pain 1 and Liver cirrhosis 1 case respectively.) Two cases each of Blood transfusion were seen in both the categories while one case of Intravenous drug use was observed in patients with multiple genotypes. Other complications observed in patients with multiple genotype infection were Pleural effusion, Transaminitis, Renal calculi, angina pectoris, Pyrexia, Diabetes, Uterus cyst, Steatosis, and alcohol intake (Table 3).

Table 3. Clinical conditions of HCV infected patients taken for the study

No.	Patient Code	Age	F/M	Complaints/Symptoms
1.	ACVI/06/01	50	M	Not identified
2.	ACVI/07/02	53	F	Grade I fatty liver, right hepatic simple cyst, palpitation
3.	ACVI/11/03	60	F	Grade I fatty liver
4.	ACVI/12/04	51	M	Liver cirrhosis, alcoholic, Jaundice, mouth bleeding
5.	ACVI/18/05	72	M	Liver inflammation, alcoholic, smoker, bloating after meals, burning sensation on urination, vomiting after meals, renal problems, lower back pain, pain during micturition
6.	ACVI/20/06	75	F	Hepatitis C related chronic liver disease with ascites and splenomegaly, umbilical hernia, severe anemia, dyspnea, abdominal pain
7.	ACVI/21/07	50	F	Abdominal pain, Joint pain, lower back pain, Tobacco use
8.	ACVI/22/08	55	M	Pyrexia, dyspnea, Alcoholic, allergic symptoms
9.	ACVI/23/09	55	M	Swollen neck, Grade I prostatomegaly
10.	ACVI/24/10	32	F	Low BP, typhoid, back pain, body aches, weakness, heart palpitations after eating, fatigue
11.	ACVI/25/11	51	M	Grade I fatty liver with hepatomegaly, splenomegaly, B/L pleural effusion
12.	ACVI/27/12	45	F	Grade I fatty liver, mild hepatomegaly, Radiating pain, Abdominal pain, nausea after meals, intestinal infection
13.	ACVI/28/13	30	F	Loss of appetite, Blood transfusion case, skin allergy, anxiety, chronic diarrhea
14.	ACVI/36/14	40	M	Abdominal pain, Alcohol use, shooting pain on right side, Beer on daily basis
15.	ACVI/42/15	40	M	Grade I fatty liver, Bloating, fatigue, loose motions
16.	ACVI/44/16	30	M	Transaminitis
17.	ACVI/51/17	45	M	Liver related problems, constipation, foot swelling, Hookah use, Bidi use
18.	ACVI/58/18	64	M	Alcohol use, bloating, B/L renal calculi, angina pectoris, BPH
19.	ACVI/62/19	65	F	Grade I fatty liver, loss of appetite, bloating, insomnia
20.	ACVI/80/20	37	M	Past case
21.	ACVI/81/21	50	M	Fatty liver Grade I, Abdominal pain, Headache, Head lump, dizziness, High BP, HTN, Cholesterol
22.	ACVI/82/22	30	F	Blood transfusion case, Thyroid, Hysterectomy
23.	ACVI/83/23	40	M	Accidental Case (23 yrs. ago), Bed sores with continuous bleeding, Pyrexia
24.	ACVI/88/24	42	F	Diabetes, BP patient, Cholesterol, Abdominal pain, liver inflammation, chills at night with fever, Dizziness
25.	ACVI/89/25	52	F	Uterus Cyst, bleeding from past 2 1//2 months, Back pain, Fatigue, Bloating, Hookah use, Blur vision
26.	ACVI/100/26	33	F	Blood transfusion, Abdominal pain because of gallstones
27.	ACVI/105/27	35	M	Abdominal pain, indigestion, Bloating, loose motions after meals, Steatosis, Pain upon micturition
28.	ACVI/107/28	35	M	Subacute intestinal obstruction, Hepatomegaly, Splenomegaly, Bidi use, Alcoholic, Abdominal pain, Bloating, Intravenous drug user
29.	ACVI/108/29	47	M	Dyspnea, Pyrexia, Bidi use, Alcohol, Blood transfusion, cystectomy
30.	ACVI/109/30	45	F	Grade I fatty liver with borderline hepatomegaly, Fever, Joint pain, Bloating, Shoulder pain

DISCUSSION

Hepatitis C viral infection is now considered a cause of serious global concern due to the rise in the liver related morbidity and mortality.[18-20] The serious complications arise in the form of cirrhosis followed by carcinoma of the liver, then liver failure and finally death.[21] With the increase in the advancement of the disease, 8 different genotypes have been identified.[12] Since the virus is a blood borne disease, hence chances of varied genotype infections are possible.[22-24] However, this has not been explored fully in case of Hepatitis C virus, as observed in another

member of the Flavivirus family i.e. Dengue virus.[25] There have been some studies in the past wherein technique to detect multiple genotypes and subtype have been attempted but many limitations have been found.[11]

The present study has been conducted to identify the presence of multiple genotypes in patients infected with HCV infection in a cohort of population of Uttar Pradesh, India. The study indicated that there are chances that multiple genotypic HCV infection can occur and that patients with severity of disease, tend to have multiple genotypic infection of HCV virus. This can be justified by the fact that Grade I fatty liver was observed to be more in patients with multiple genotypes as indicated in our studies. A study done by Wu and co-workers have found presence of Genotype 3 and correlating with presence of fatty liver.[26] Another study has reported Genotype 3 present in patient with steatosis condition.[27]

CONCLUSION

To conclude, we hereby report occurrence of multiple genotypes in HCV infected patients and also association of a specific genotype with the disease severity. The presence of more than one genotypes with close proteomic similarities could lead to poor adaptive immune responses against specific HCV proteins. Further studies are in progress to address crucial issue of translation of HCV into HCC.

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None.

CONFLICT OF INTEREST

The authors declare that there is no conflicts of interest.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct, and intellectual contribution to the work, and approved it for publication.

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DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

This study was approved by the Institutional Ethical Committee, Sharda University, India (File No. SU/SMS&R/76-A/2021/40 dated 11/02/2021).

INFORMED CONSENT

Written informed consent was obtained from the participants before enrolling in the study.

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