Short Communication

A Study on the Prevalence of HCV Genotypes and the Effect of Direct-Acting Antiviral Therapy on Clinical and Laboratory Parameters in HCV-Infected Patients at a Tertiary Care Center in North India

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Abstract

Background: The purpose of this study was to investigate the prevalence and distribution of different HCV genotypes, as well as to evaluate clinical and laboratory parameters in HCV-infected patients before and after DAA treatment. **Material and Methods:** An open-label prospective study was conducted on 50 HCV-infected individuals. The HCV-infected patients underwent a baseline evaluation with complete history, examination, and other clinical investigations. These patients received the appropriate DAA according to the genotype for 3 months. At the end of 3 months, these patients were again evaluated clinically. **Results:** The majority of instances were among younger age groups. Genotype 3 (66%) was the most common. There was a statistically significant difference found in clinical parameters regarding total bilirubin (p=0.008), SGOT (p=0.001), SGPT (p=0.035), ALP (p=<0.001) and Blood Urea Nitrogen (p = 0.004). When 1a vs 1b intragroup comparison was drawn, there was a significant mean difference found in SGOT (p value= 0.053) and Creatinine (p=0.050) parameters while rest shows no significant difference when associated. In the comparison of 1a vs 3 or 4, none of the parameters shows significant difference while; when 1b vs 3 or 4 comparison was laid out, SGOT and Creatinine was found near to significant. **Conclusion:** This study concludes that with the availability of DAAs, highly effective, short-duration, and safe regimens have created better outcomes for patients with HCV infection, especially in those groups where SVR was low with prior therapies or in those where IFN-based treatment strategies were contraindicated.

Keywords: Clinical, direct-acting antiviral therapy, HCV genotypes, India, laboratory, prevalence

Introduction

Hepatitis C is a liver disease caused by the hepatitis C virus (HCV), which can cause both acute and chronic hepatitis. The total global prevalence of HCV is estimated to be at an average of 1.6% (1.3-2.1%), corresponding to 115 (92-149) million viraemic infections. The majority of these infections, 104 (87-124) million, are among adults (defined as >15 years) with an anti-HCV infection rate of 2.0% (1.7-2.3%).[1] There are six major genotypes of HCV, which include many subtypes, and their distribution varies by region. Globally, genotype 1 (G1) accounts for 46% of all HCV infections among adults, making it the most common, followed by G3 (22%), G2 (13%), G4 (13%), G6 (2%), and G5 (4%). Currently, India harbors an estimated 10 -15 million chronic carriers of HCV, which is a major cause of liver-related mortality and morbidity. The prevalence of HCV infection in the general population is estimated to be around 0.5%–1.5%.[2] Overall, genotype 3 is the predominant genotype (63.85%), followed by genotype 1 (25.72%) in India.[3]

A significant number of those who are chronically infected will develop cirrhosis or liver cancer. Approximately 399000

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people die each year from hepatitis C, mostly from cirrhosis and hepatocellular carcinoma.[4] Antiviral medicines can cure more than 95% of persons with hepatitis C infection, thereby reducing the risk of death from liver cancer and cirrhosis, but access to diagnosis and treatment is low.[5] Acute HCV infection is usually asymptomatic and is only very rarely associated with life-threatening disease. About 15–45% of infected persons spontaneously clear the virus within 6 months of infection without any treatment. The remaining 60–80% of persons will develop chronic HCV infection. Of those with chronic HCV infection, the risk of cirrhosis of the liver is between 15–30% within 20 years.

All oral direct-acting antiviral (DAA) drugs for patients with chronic HCV infection have transformed treatment options.

This study was proposed to investigate the prevalence and distribution of different HCV genotypes in patients presenting to JNMCH, AMU, and Aligarh, as well as to evaluate the clinical and laboratory parameters in the HCV-infected patients before and after receiving treatment with DAA drugs.

Materials and Methods

After ethical clearance, an open-label prospective study was conducted at J.N. Medical College's Gastroenterology OPD, Medicine OPD, and IPD in Aligarh, Uttar Pradesh, India, from January 2019 to June 2020. The study included around 50 HCV-infected individuals aged 18 to 60 years after taking written informed consent. HCV-infected patients who have previously received antiviral treatment for HCV, HBV/HCV, or HIV/HCV co-infected patients, Patients in the age group of <18 years and >60 years, pregnant females, patients with diagnosed hepatocellular carcinoma, decompensated cardio-respiratory and renal dysfunction and those in whom the DAA drugs are contraindicated or had to be discontinued prematurely due to any reason are excluded from the study.

The HCV-infected patients underwent a baseline evaluation with complete history, examination, and the below-mentioned investigations while strictly maintaining the confidentiality of each patient. These patients received the appropriate DAA according to the genotype of the patient for three months. Appropriate DAA was used for different genotypes of HCV per the latest AASLD guidelines.[6] The patients were followed in Gastroenterology OPD during this period, and any significant events were recorded. At the end of three months, these patients were again evaluated clinically and with laboratory parameters to look for the effects of therapy with direct-acting antiviral drugs.

Investigation (before and after completion of 12 weeks of therapy with DAA drugs)

Complete blood count, erythrocyte sedimentation rate, liver function test, renal function test, blood sugar, USG abdomen, fibroscan, upper GI endoscopy, and any other investigation if required. HCV RNA should be performed 12 weeks after the completion of therapy.

NAT: detection of HCV RNA

Molecular virological techniques have a key role in the diagnosis and monitoring of treatment for HCV. The "gold standard" test for detecting active HCV replication is NAT. HCV NAT is useful in confirming the diagnosis of acute HCV infection, since RNA is detectable as early as 1 week after exposure and at least 4-6 weeks before seroconversion.

Qualitative NAT

Qualitative NATs have been considered as the confirmatory tool for HCV diagnosis. These assays commonly use conventional RT-PCR or transcription-mediated amplification (TMA). The present indication of qualitative NAT is to confirm viremia in patients with reactive anti-HCV results, and to screen blood donations for evidence of infection with HCV.

With the availability of more sensitive quantitative PCR that has a lower limit of detection (LOD) to as low as 30 copies/ml, qualitative assays as of now are less used.

Quantitative NAT

Quantification of HCV RNA can be performed by many methods. Commonly available formats include quantitative RT-PCR (qRT-PCR) and branched deoxyribonucleic acid (bDNA) technology. Owing to its good sensitivity (99%) and specificity (98-99%) quantitative PCR has replaced qualitative PCR.

HCV genotyping methods

The genotype of HCV for diagnosis is mostly determined by sequencing of a genomic nucleotide sequence or by kit-based assays, which employ complementary probes to report the genotype present in a specimen. Sequencing of highly conserved regions such as NS5, core, E1 and 5'UTR is the most recommended method used for genotyping of HCV.

The kit-based assays are easy to use and do not require expertise as is required for sequencing. Both TruGene 5'NC HCV Genotyping kit (Siemens Healthcare Diagnostics Division, Tarrytown, NY) and Versant HCV Genotype Assay LiPA (version I, Siemens Medical Solutions, Diagnostics Division, Fernwald, Germany) are based on 5'UTR sequence. Both these kits use reverse hybridization with genotype-specific oligonucleotide probes. Both kits claim to detect HCV genotypes 1 to 6. Abbott RealTime HCV Genotype II Kit claims to detect genotypes 1 to 5. HCV genotype kit cobas® HCV GT, manufactured by Roche, claims to identify HCV genotypes 1-6 and can discriminate between subtypes a and b of genotype 1. Fully automated system, cobas® 4800 (Roche, USA) has also been launched for HCV genotyping.

Statistical analysis

Categorical variables were presented in number and percentage (%), and continuous variables were presented as mean and SD. Quantitative variables were compared using a one-way analysis of variance with *post hoc* Tukey's test among groups. Baseline to after 12 weeks compared using Mc Nimar test. A P value of <0.05 will be considered statistically

significant. The data will be entered into an MS EXCEL spreadsheet, and analysis will be conducted using Statistical Package for Social Sciences (SPSS) version 23.0.

Results

The age of patients ranged from 15 to 75 years of age, with a mean age of 35.82 ± 13.55 years, the sex ratio of the population under study was equal (50% male to 50% female), and the mean age in male and females was 37 years and 34 years respectively. The studied group was differentiated on the basis of HCV genotype, 12 (24%) of the patients were genotype 1a, 4 (8%) with genotype 1b, 33 (66%) patients were diagnosed with genotype 3, and only 1 was genotype 4 (2%).

The laboratory changes of DAAs on clinical parameters were summarized from baseline to the 12th week following treatment [Tables 1-3]. Total bilirubin (*P* value = 0.008), SGOT (*P* value = 0.001), SGPT (*P* value = 0.035), ALP (*P* value = 0.001), and BUN (*P* value = 0.004) were shown to be significantly different in patients [Table 1].

24% of patients had leukocytosis before therapy, which was decreased to 2%; this difference was statistically significant. 22% of patients had increased bilirubin levels at baseline, which decreased to 4% 12 weeks after completion of therapy with a significant difference. There was significant improvement in SGOT and SGPT when compared within baseline and 12 weeks after the completion of therapy. ALP and BUN also show significant differences within the said period.

Table 4 depicts the intergroup comparison of clinical parameters with HCV genotype; there was a statistically significant difference found in two parameters, SGOT and creatinine, with *P* value equal to 0.055 and 0.053, respectively. The mean SGOT and mean creatinine was maximum in 1b (86.75 ± 155.47 and 0.19 ± 0.07) concerning the other two groups; the rest of the parameters were statically insignificant.

The intragroup comparison of clinical parameters in patients with the HCV genotype is concluded in Table 5. When 1a vs. 1b intragroup comparison was drawn, a significant mean difference was found in SGOT (*P* value = 0.053) and creatinine (*P* value = 0.050) parameters, while the rest shows no significant difference when associated. In comparing 1a vs. 3 or 4, none of the parameters showed a significant difference. In contrast, 1b vs. 3 or 4 comparisons were laid out, SGOT and creatinine were found near to significant (*P* values 0.059 and 0.061, respectively). Further, no parameter was significant in this comparison.

Discussion

An open-label prospective study was conducted about the prevalence of different HCV genotypes, the efficacy of DAAs, and their impact on various clinical and laboratory parameters. Most of the cases in this study were among younger age groups, 26-35 years, followed by 15-25 years, indicating that the prevalence is higher in the young adult group. Niu *et al*. (2016) found the greatest prevalence of HCV infection in the age range 50-59 years,[7] whereas Sood *et al*. (2012) reported 41-60 years[8] and Rajani *et al*. (2014) reported 11-20 years.[9]

In this study, genotype 3 (66%) was the most common, followed by genotype 1 (1a-24%, 1b-8%) and genotype 4 (only 2%). No cases of genotypes 2, 5, and 6 were reported. In India, according to Kumar *et al*. (2018),[10] genotype 3 is the most common (63.85%), followed by genotype 1 (25.72%). Genotype 2 is rarely reported, and genotype 5 has yet to be reported in India. HCV genotype 3 was shown to be the most prevalent (63.85%). Genotype 1 (25.72%), genotype 2 (0.002%), genotype 4 (7.5%), and genotype 6 (2.7%) were also found.[11]

There was a statistically significant difference found in clinical parameters of patients regarding total bilirubin (*P* value = 0.008), SGOT (*P* value = 0.001), SGPT (*P* value = 0.035), ALP (*P* value = <0.001) and blood urea nitrogen (*P* value = 0.004) in the current study. Liver function improved significantly after DAA treatment in the study conducted by González-Corvillo *et al*. (2019)[12] with ALT (52.5 to 17) and total bilirubin (0.61 to 0.50). AST (46.9 ± 1.9 to 24.1 ± 1.7) and ALT (45.5 ± 2.2 to 21.2 ± 2.1) lowered significantly after therapy with DAAs and follow-up after EOT. Total bilirubin decreased within the reference range from 0.78 mg/dL to 0.72 mg/dL, without

Table 1: Laboratory changes of clinical parameters before and 12 weeks after the completion of DAA therapy

	Baseline		After 12 week		Mean difference	% mean change	*P*
	Mean	SD	Mean	SD			
TLC	7867.00	2488.53	8221.40	1757.78	-354.40	-4.50%	0.131
Platelet (in lakh)	2.33	0.76	2.25	0.69	0.08	3.30%	0.142
Hb.	12.46	1.59	12.51	1.27	-0.04	-0.35%	0.660
Total bilirubin	1.22	1.26	0.75	0.24	0.46	38.12%	0.008
SGOT	54.52	58.77	27.46	17.69	27.06	49.63%	0.001
SGPT	86.38	192.02	28.00	17.57	58.38	67.59%	0.035
ALP	76.40	71.40	30.94	21.54	45.46	59.50%	<0.001
Creatinine	0.73	0.18	0.70	0.15	0.03	3.71%	0.198
Blood urea nitrogen	14.86	6.92	13.64	5.63	1.22	8.21%	0.004
RBS	97.26	16.20	94.74	11.27	2.52	2.59%	0.114

Table 2: Distribution of clinical parameters before and 12 weeks after the completion of DAA therapy according to their normality range

	Interpretation	Baseline		12 weeks after the completion of therapy		*P*
		n	%	*n*	%	
TLC	Normal	38	76.0%	49	98.0%	0.001
	Leukocytosis	12	24.0%	1	2.0%	
Platelet	Thrombocytopenia	3	6.0%	6	12.0%	0.250
	Normal	47	94.0%	44	88.0%	
Hemoglobin	Anemic	9	18.0%	8	16.0%	1.000
	Normal	41	82.0%	42	84.0%	
Total bilirubin	Normal	39	78.0%	48	96.0%	0.004
	Increased	11	22.0%	2	4.0%	
SGOT	Normal	39	78.0%	47	94.0%	0.021
	Increased	11	22.0%	3	6.0%	
SGPT	Normal	28	56.0%	47	94.0%	<0.001
	Increased	22	44.0%	3	6.0%	
ALP	Normal	44	88.0%	49	98.0%	0.050
	Increased	6	12.0%	1	2.0%	
Creatinine	Normal	50	100.0%	50	100.0%	NA
	Increased	0	0.0%	0	0.0%	
BUN	Decreased	8	16.0%	9	18.0%	0.017
	Normal	29	58.0%	36	72.0%	
	Increased	13	26.0%	5	10.0%	
RBS	Decreased	4	8.0%	2	4.0%	0.500
	Normal	0	0.0%	0	0.0%	
	Increased	46	92.0%	48	96.0%	

Applied McNemar test for significance

Table 3: The mean and standard deviation of the clinical parameters

Mean difference of parameters from baseline to 12 weeks	Mean	SD
TLC	-354.32	1632.91
Platelet	0.08	0.36
Hb	-0.04	0.70
Total bilirubin	0.46	1.19
SGOT	27.06	53.12
SGPT	58.38	190.77
ALP	45.46	68.75
Creatinine	0.03	0.15
Blood urea nitrogen	1.22	2.87
RBS	2.52	11.09

any statistical significance (Ridruejo *et al*., 2020l).[13] In a study, Bernuth *et al*. (2017)[14] found that serum activities for ALT (44.5–21.5 IU/L) and AST (45.0–30.0 IU/L) declined significantly from baseline to follow-up after EOT, and total bilirubin decreased without any statistical significance.

There was a statistically significant difference found in two parameters, SGOT and creatinine, with *P* value equal to 0.055 and 0.053, respectively. The mean SGOT and mean creatinine was maximum in 1b (86.75 ± 155.47 and 0.19 ± 0.07) concerning the other two groups; the rest of the parameters were statically insignificant.

When 1a vs 1b intragroup comparison was drawn, there was a significant mean difference found in SGOT (*P* value = 0.053) and creatinine (*P* value = 0.050) parameters, while the rest showed no significant difference when associated. In the comparison of 1a vs. 3 or 4, none of the parameters showed a significant difference, while 1b vs. 3 or 4 comparisons were laid out, SGOT and Creatinine were found near to significant (*P* values 0.059 and 0.061 respectively); further no parameter was significant in this comparison.

Conclusion

In the last two decades, the treatment of HCV has evolved from interferon (IFN)-based therapies with or without ribavirin (RBV) to pegylated-IFN (PEG-IFN) and RBV-based therapies that were better tolerated by patients. However, the introduction of oral drugs, which specifically target virus-specific proteins, has now revolutionized the treatment of chronic HCV. These agents are known as DAAs. These drugs have resulted in very high HCV cure rates even with reduced treatment duration and excellent tolerability by the patients compared to PEG-IFN- and RBV-based therapies. In India, sofosbuvir (SOF), daclatasvir (DCV), ledipasvir (LDV), and velpatasvir (VEL) are the most effective DAAs and have been made available at a compassionate price.

In this study, the prevalence of different HCV genotypes and the clinical and laboratory parameters in HCV-infected

Table 4: Intergroup comparison of laboratory parameters with HCV genotype

Mean difference of parameters from baseline to 12 weeks	HCV genotype						*F*	*P*
	1a (*n*=12)		1b (*n*=4)		3 or 4 (*n*=34)			
	Mean	SD	Mean	SD	Mean	SD		
TLC	11.67	1061.45	401.75	1184.24	-572.44	1813.59	1.04	0.363
Platelet	0.14	0.36	-0.15	0.59	0.08	0.34	0.96	0.389
Hb	-0.09	0.64	-0.17	0.85	-0.01	0.72	0.13	0.880
Total bilirubin	0.10	0.17	0.69	1.31	0.57	1.37	0.75	0.477
SGOT	16.17	21.79	86.75	155.47	23.88	36.73	3.08	0.055
SGPT	29.42	32.29	91.50	166.07	64.71	225.17	0.21	0.811
ALP	37.58	39.38	113.50	201.60	40.24	46.84	2.24	0.117
Creatinine	0.00	0.13	0.19	0.07	0.02	0.15	3.13	0.053
Blood urea nitrogen	0.50	2.78	3.50	1.73	1.21	2.93	1.68	0.197
RBS	2.17	6.98	9.25	13.60	1.85	11.98	0.80	0.456

Table 5: Intragroup comparison of laboratory parameters with HCV genotype

	HCV genotype					
	1a vs 1b		1a vs 3 or 4		1b vs 3 or 4	
	Mean diff.	*P*	Mean diff.	*P*	Mean diff.	*P*
TLC	-390.08	0.910	584.11	0.540	974.19	0.501
Platelet	0.29	0.358	0.06	0.876	-0.23	0.460
Hb	0.08	0.978	-0.08	0.941	-0.16	0.902
Total bilirubin	-0.59	0.673	-0.47	0.481	0.12	0.980
SGOT	-70.58	0.053	-7.72	0.894	62.87	0.061
SGPT	-62.08	0.845	-35.29	0.851	26.79	0.963
ALP	-75.92	0.133	-2.65	0.992	73.26	0.108
Creatinine	-0.20	0.050	-0.02	0.891	0.17	0.059
Blood urea nitrogen	-3.00	0.170	-0.71	0.740	2.29	0.286
RBS	-7.08	0.518	0.31	0.996	7.40	0.426

Tuckey's Post hoc test applied for significance between intragroup comparison

patients before and after treatment (DAAs) are investigated. Genotype 3 is the most prevalent genotype, followed by genotype 1a, while genotype 4 is the least prevalent. Genotypes 2, 5, and 6 were not reported. DAAs are highly effective treatment modalities for HCV infection with a good safety profile, which can be curative within a short duration. It was found to be very effective even in genotype 3, which is relatively difficult to treat. DAAs need to be made more accessible and affordable as an easy and effective treatment option for a large HCV-infected population in this part of the world.

Now, the guidelines prefer pangenomic drug combinations like glacapravir/pibrentasvir (not yet available in India) and sofosbuvir/velpatasvir. Thus, we can conclude that with the availability of DAAs, highly effective, short-duration, and safe regimens have created better outcomes for patients with HCV infection, especially in those groups where SVR was low with prior therapies or in those where IFN-based treatment strategies were contraindicated. Having SOF and other DAAs will definitely benefit Indian HCV patients, but efforts should be made to make DAAs accessible to all patients.

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Conflicts of interest

There are no conflicts of interest.

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