ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 16, Issue 9, 2023

Online - 2455-3891
Print - 0974-2441

Research Article

ESTIMATION OF PREVALENCE OF HEPATITIS C VIRUS INFECTION BY SEROLOGICAL TEST AND REAL TIME RT-PCR TEST AMONG SUSPECTED VIRAL HEPATITIS CASES ATTENDING TERTIARY CARE HOSPITAL, KOLKATA

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Received: 24 December 2022, Revised and Accepted: 08 May 2023

ABSTRACT

Objective: Hepatitis C virus (HCV) infection is a major public health problem in India and worldwide. Majority remain asymptomatic until they develop serious complications like liver cirrhosis or hepatocellular carcinoma with fatal outcome. Hence, early diagnosis of active HCV infection and prompt initiation of treatment is important. Treatment with directly acting antivirals (DAAs) resulted in high sustained virological response (SVR) rates of >95% globally. This study was done to estimate seroprevalence and prevalence of active HCV infection among study population. After initiation of treatment, SVR rates were estimated.

Methods: This was hospital-based, cross-sectional, and observational study. Screening was done by third-generation Enzyme-linked immunosorbent assay to detect anti-HCV antibody, then confirmatory real-time reverse transcription-polymerase chain reaction (RT-PCR) test was done for detection of active cases and determining their viral load. Treatment of 12-week duration was initiated by DAAs and followed up to estimate SVR by doing real-time RT-PCR after 12 weeks of treatment completion.

Result: Among 17,752 consecutive non-repetitive blood samples, seroprevalence was 1.78%. The prevalence of active cases was 1.52%. HCV active infection was prevalent more among male (64.21%) and among 40–60 years age group. History of multiple blood transfusion (58%) was the most common risk factor, followed by multiple sex partners (13.3%). Coinfections with Hepatitis B virus and Human immunodeficiency virus were seen in 13.65% of cases. About 92% of patients completed their treatment. SVR was 97.87%.

Conclusion: High SVR of 97.87% is evidence-based data that support that proper treatment can eliminate HCV infection. Detection by real-time RT-PCR and highly effective DAA has made a paradigm shift to approach of HCV diagnosis and management in recent times.

Keywords: Hepatitis C, Seroprevalence, Real-time RT-PCR, Sustained virological response.

 DOI: http://dx.doi.org/10.22159/ajpcr.2023v16i9.48161. Journal homepage: https://innovareacademics.in/journals/index.php/ajpcr

INTRODUCTION

Hepatitis C virus (HCV) infection, a major cause of acute hepatitis (30%) and chronic hepatitis (70%), is a public health problem in India and worldwide. Global prevalence of HCV is estimated to be 2.5%, with 58 million chronic Hepatitis C infections and 1.5 million new infections per year as WHO report June 24, 2022, on Hepatitis C [1]. Majority of infected patients can remain asymptomatic both in acute and chronic phase, until they develop serious complication like liver cirrhosis or hepatocellular carcinoma [1]. Around 15–45% of infected individuals usually show a spontaneous clearance of HCV infection within 6 months of acute infection in the absence of treatment [1]. Rest of the infected individuals develop chronic infection, with risk of cirrhosis (15–30%) and hepatocellular carcinoma (1–4%) within 20 years of infection with fatal outcome like liver failure and death [1]. The prevalence varies among high-risk groups of patients. Studies from India showed higher prevalence among thalassemia patients with multiple blood transfusion which was as high as 13.04%, patients on hemodialysis was 13.23%, person who inject drugs (PWID) was 37.2% and female sex workers in N-E India was 9.6% [2-5].

Since availability of an effective vaccine against HCV is a distant dream, so prevention strategies to contain spread of HCV infection is essential. Hence, early diagnosis by proper screening and confirmatory tests with treatment of active HCV infection cases is most important. This will also reduce the burden of disease chronicity among the patients and eliminate the source of disease transmission. Hence, screening of HCV infection by detection of anti-HCV antibody has now become a common practice especially among patients of high-risk groups [6]. Moreover, early detection by both serological test (micro-Enzyme-linked immunosorbent assay [ELISA]) for the presence of anti-HCV antibody in blood, followed by molecular test (real-time reverse transcription–polymerase chain reaction [RT-PCR]) detecting viral load for confirmation has become increasingly crucial [6]. After diagnosis, treatment with a diverse group of directly acting antivirals (DAAs) with pan-genomic efficacy and excellent patient compliance resulted in high sustained virological response (SVR) rates (>95%) globally [1]. This has formed the basis of the noble dream of global elimination of HCV by 2030 [1].

Hence, this study was done to estimate the seroprevalence and prevalence of active HCV infection among targeted hospital-based study population. The sociodemographic and clinical profile and risk factors were studied among the active cases of HCV infection. The viral load of active cases before initiation of treatment was studied by doing real-time RT-PCR. Treatment response of these confirmed cases by follow-up viral load detection by real-time RT-PCR was also studied after 12 weeks of completion of treatment by DAAs (as per the specific guidelines of diagnosis and management in National Viral Hepatitis Control Program) [6].

METHODS

This hospital-based, cross-sectional, and observational study was done in the Department of Microbiology in collaboration with the School of Digestive and Liver Diseases (SDLD), for one and half years from January 2019 to June 2020 after taking proper institutional ethical clearance. Sample size was primarily calculated as 5000, using Raosoft-sample size calculator**,** considering ±2% as margin of error with 99% confidence level, considering a huge population (>20,000) and keeping a margin of 10% dropouts [7]. Suspected HCV-infected patients of all age groups, with clinical signs and symptoms of acute or chronic hepatitis, hepatic fibrosis, cirrhosis of liver, hepatocellular carcinoma, or high-risk patients attending outpatient department of SDLD or admitted indoor, referred to Microbiology department for HCV testing were included in our study after proper consent and counseling. Suspected high-risk groups include patients of thalassemia, on hemodialysis, with a history of multiple blood transfusion, injection by contaminated syringe or needle, PWID, having multiple sex partners, men doing sex with men (MSM), commercial sex worker, and with history of occupational exposure. Furthermore, asymptomatic patients before any operative or invasive procedure referred for HCV screening were included in the study.

HCV was diagnosed in two steps: First, the suspected and referred patients were screened by serological test by third-generation micro ELISA method to detect presence of anti-HCV antibody in blood. If test result comes reactive, patient was reported as HCV seropositive case, irrespective of patient's clinical condition. In the second step, for further confirmation and determination of active case, real-time RT-PCR was performed for quantitative assessment of HCV-RNA. (As per guidelines by National Viral Hepatitis Control Program, Govt. of India) [8]. If the quantity of HCV-RNA was found above detection level, then patient was regarded as active case and put on treatment.

ELISA was performed using testing kit "HCV ELISA Test Hepa-Scan" manufactured by Bhat Bio-Tech India (P) Ltd. The kit was designed to use for detection of antibodies to HCV Antigens from Core (structural) and three non-structural (NS) -NS3, NS4 and NS5 of viral genome with high degree of sensitivity (99%) and specificity (99.5%). The test procedures were followed as per kit literature. The test results were recorded and updated in the database of the study. The serum samples of all reactive cases were preserved and stored at 2–8°C when RT-PCR test was planned within 7 days or they were stored at –20°C to –70°C when test was done beyond 7 days.

For real-time RT-PCR test, first RNA extraction was done using QIAamp Viral RNA Mini Kit (QIAGEN, Hilden, Germany) following manufacturer's protocol. The extracted RNA was stored at –80°C for further processing by RT-PCR and also kept for re-testing if necessary and for quality control purpose. Quantitative RNA estimation by real-time RT-PCR was done using the kit- Artus® HCV RG RT-PCR Kit, QIAGEN which is an *in vitro* nucleic acid amplification test for quantitation of HCV RNA using Rotor-Gene Q PCR instrument and BIO-RAD PCR instrument (CFX96). The extracted RNA sample was mixed with master-mix solution in the separate master-mix room. The progress of amplification was observed in real-time. The test was assigned to quantitate HCV RNA over the range of 15 IU/ml. Hence, it is a quantitative assay and HCV-RNA when found >15 IU/mL, then patient was regarded as active case. Treatment was initiated among the active cases by DAAs among patients aged more than 18 years. For patients having no cirrhosis, two drugs combination of sofosbuvir and daclatasvir given for 12-week duration. For patients with cirrhosis-compensated, two-drug regimen of sofosbuvir and velpatasvir was given for 12-week duration and if patient had cirrhosis- decompensated, three-drug combination was given with sofosbuvir, velpatasvir, and ribavirin for 24 weeks [6]. These patients were followed up by repeat quantitative viral load detection by real-time RT-PCR after 12 weeks of completion of their treatment [6].

Data interpretation and statistical analyses were done using Excel spreadsheet (Microsoft Corporation) and descriptive biostatistics.

RESULTS

In this study 17,752 consecutive non-repetitive blood samples from suspected hepatitis C patients and patients whose blood samples were send for routine screening like pregnant women, before any surgical procedure and hepatitis patients intended for anti-HCV screening by ELISA methods during one and half year study period were included in the study, of which 316 patients were found reactive for anti-HCV antibody by HCV ELISA. Hence, the serological prevalence was estimated as 1.78%. Blood samples from these 316 seropositive patients were further tested for viral load detection by real-time RT-PCR. Among them, 271 patients showed detectable viral load above 15 IU/ml and were considered as active cases. Hence, the prevalence of active cases of HCV infection among the total study population was estimated as 1.52%. The prevalence of active cases among the seropositive cases was found to be 85.75% (271/316). Remaining 45 (14.25%) patients among 316 seropositive cases did not show any detectable viral load by real-time RT-PCR. Data on distribution of the sociodemographic profiles, risk factors, clinical conditions, and presence of any associated infections like Hepatitis B virus (HBV) or Human immunodeficiency virus (HIV) were collected from these 271 active HCV infection cases and analyzed as shown in Tables 1-4.

Pre-treatment viral load detected by real-time RT-PCR among 271 study subjects showed that a wide range of statistical dispersions (variability)

Table 1: Evaluation of data on distribution of sociodemographic parameters among active cases of HCV infection under this study (n=271)

Sociodemographic parameters studied	Frequency	Percentage
Sex		
Male	174	64.21
Female	97	35.79
Age		
1–10 years	5	1.85
11–20 years	10	3.69
21–30 years	42	15.49
31–40 years	53	19.56
41–50 years	61	22.51
51–60 years	69	25.46
61–70 years	23	8.49
>70 years	8	2.9
Occupation		
Housewife	66	24.4
Businessman	57	21
Farmer	44	16.2
Student	36	13.3
Service persons	18	6.6
Labourers	12	4.4
Drivers	11	4.1
Vendor	10	3.7
Retired	7	2.6
Healthcare workers	4	1.5
Community sex workers	2	0.7
Unemployed	2	0.8
Imprisoned	1	0.4
Pre-school	1	0.4

Table 2: Distribution of the active cases of HCV infection under this study as per different risk factors present and possible routes of transmission among them

Risk factors	Frequency	Percentage
History of blood transfusion	157	57.93
Multiple sex partners	36	13.28
History of surgery	30	11.07
History of injection	28	10.33
Person who injects drugs (PWID)	12	4.42
MSM	5	1.84
Occupational exposure	3	1.10

with maximum viral load was as high as 5, 42, 857, 14 I.U./mL to as low as 868 I.U./mL. Mean viral load was found to be 23,618,17.37 I.U./mL±2 standard deviation of 50,644,00.79 I.U./mL. Majority of active cases (262 out of 271) showed viral load below 1,00,00,000 I.U./mL and nine cases showed viral load above 1,00,00,000 I.U./mL.

Treatment with DAAs with two drugs combinations of 12-week duration was initiated among 256 patients and 15 patients whose age was < 18 years were not given this treatment. Out of 256 cases, 235 patients completed their treatment and were followed up by repeat quantitative viral load detection by real-time RT-PCR after 12 weeks of completion of their treatment. Rest 17 patients were lost to follow-up and four patients died during their course of treatment. Out of these 235 patients follow-up, viral load after 12 weeks was undetectable in 230 patients. Hence, these 230 patients achieved SVR. Five patients had treatment failure. Outcome is shown in Tables 5 and 6.

DISCUSSION

HCV is a blood-borne virus transmitted from person to person through blood transfusion, surgery, needle stick injuries, injections, sexual routes, and vertical transmissions affecting all age groups of patients. Estimated prevalence of HCV infection in India was between 0.5% and 1.5% in a study in 2014 by Puri *et al.* [9]. In our study, the seroprevalence was 1.78% which is higher because this study was done in a hospital setting in a tertiary care hospital among patients with suspected hepatitis and patients undergoing surgery as pre-operative screening tests. Our result is at par with a similar study done in Kolkata by Chakraborty *et al.* where prevalence was estimated to be 1.5% [10]. Similar study in Bangalore done by Rashmi *et al*. have estimated a much higher prevalence of 8.4% [11]. Studies done in North India by Jahan *et al.* and in Punjab by Jindal *et al.* showed much higher prevalence of 2.8% and 6.71%, respectively [12,13].

Table 3: Distribution of the active cases of HCV infection under this study with different clinical presentations

Clinical presentations and conditions	Frequency	Percentage
Asymptomatic cases	79	29.15
Chronic kidney disease with hemodialysis	72	26.57
Thalassemia	46	16.97
Chronic hepatitis	41	15.13
Cirrhosis	11	4.06
Compensated liver disease	7	2.58
TB	4	1.47
Asymptomatic antenatal	3	1.10
Acute hepatitis	2	0.73
Hepatocellular carcinoma	2	0.7
Kidney transplant	2	0.73
CA lung	1	0.37
Nephrotic syndrome	1	0.3

Table 4: Distribution of the active cases of HCV infection under this study as per presence of associated HBV and HIV infections

Associated infection	Frequency	Percentage
Only HIV	25	9.23
Only HBV	7	2.58
Both HBV and HIV	5	1.84
None	234	86.35

Out of 25 HCV/HIV coinfection patients, one had treatment failure. Out of seven cases of HCV/HBV coinfection, one patient died and out of five cases of HCV/HBV/HIV coinfections, one patient had treatment failure for HCV.
HCV: Hepatitis C virus; HBV: Hepatitis B virus

Table 5: Distribution of outcome of active cases of HCV infection who received treatment (n=256)

Outcome (n=256)	Frequency	Percentage
Completed treatment	235	91.79
Lost to follow-up	17	6.64
Death	4	1.56

A number of active HCV infections among 316 seropositive cases were 271, that is, 85.75%. Remaining 45 (14.25%) patients who did not show any detectable viral load by RT-PCR but were reactive for anti-HCV antibody by ELISA might be due to spontaneous clearance of HCV virus from their body in due course or due to false positive ELISA report. Similar result was produced in a study by Jindal *et al.* where HCV RNA was detected by quantitative RT-PCR in 84% of seropositive cases [13]. Hence, the prevalence of active HCV infections in our total study population confirmed by real-time RT-PCR was 1.52%.

Data were collected from these 271 active HCV infection patients and after analysis, it was seen that HCV infection was more prevalent among the male population (64.21%) similar to most of the studies [10,11] but unlike study by Qamar *et al.* in Pakistan where HCV infection was prevalent more in female population [14].

In our study, nearly 50% of active HCV infection patients belonged to 40–60 years age group and extremes of ages showed very less prevalence rate similar to most of the studies. Although our study showed maximum prevalence among housewives (24.4%), as most of the female patients were housewives, but among the male patients, prevalence was highest among businessmen (21%). In our study, the prevalence of active HCV infection among healthcare workers and commercial sex workers who are under high-risk population are found to be 1.5% and 0.7%, respectively.

Distribution of risk factors among these active HCV infection patients showed the most common risk factor to be a history of multiple blood transfusions in 58% of cases similar to studies by Chakraborty *et al.* who showed similar history in 80% of cases [10]. The study by Jindal *et al.* in North India showed major risk factor as unsafe medical procedures such as surgery, unsafe injections, and PWID [13]. Qamar *et al.* in their study also showed that PWID (intravenous drug abusers) was the major risk factor (21.06%), followed by puncture injury (11.73%), dental procedures (5.86%), and other surgeries (3.2%) but blood transfusion contributed only 0.8% [14]. In our study, history of past surgery, injections, and PWID together contributed as potent risk factors in 25% of the cases. Occupational exposure history was found in 1.1% of cases.

About 29% of patients were asymptomatic including three antenatal mothers. Blood transfusion history was a major risk factor in our study and patients requiring multiple blood transfusion like chronic kidney disease, patients undergoing hemodialysis and thalassemia, and patients were 26.5% and 17%, respectively, who eventually developed HCV infection. Similar studies showed HCV infection patients had a history of hematological disorders attending hematology OPD and requiring multiple blood transfusion [10]. About 15% of patients

Table 6: Outcome of active cases of HCV infection patients after completion of their treatment (n=235)

Total patients who completed treatment (n=235)	Frequency	Percentage
SVR (sustained virological response) (follow-up RT-PCR 12 weeks after completion of treatment showed viral load undetectable)	230	97.87
Treatment failure (follow-up RT-PCR viral load detectable)	5	2.13

presented with chronic hepatitis, 4% with cirrhosis, 2.5% with compensated liver disease, 0.73% with hepatocellular carcinoma, and 0.73% with acute hepatitis.

HCV and HBV coinfection was seen in 2.58% of cases and HCV with HIV were seen in 9.23% of cases. Similar results were seen in studies by Patel *et al.* and Chakraborty *et al.* where both the studies found around 10% HCV coinfection with HIV [10,15]. HCV, HBV, and HIV all three infections together were also seen in 1.84% of cases in our study. Out of five patients with treatment failure in our study, two had coinfections with HIV/HBV and out of four patients who died during this study, one had coinfection with HBV. Hence, coinfection with HIV/HBV can be a contributing factor for negative outcomes in these patients.

Hence, real-time RT-PCR test could detect viral load and confirm active HCV infection. Mean viral load was found to be as high as 23, 618, 17.37 I.U./mL ± 2 standard deviation of 50, 644,00.79 I.U./mL. These patients were immediately referred to Hepatology division of Gastroeneterology department for initiation of treatment following national guidelines for management of HCV infection [6]. After 12 weeks of completion of treatment, they were followed up by doing repeat real-time RT-PCR test and outcome was assessed [6]. About 92% of patients completed their treatment and did the repeat RT-PCR test. SVR was attained in 97.87% of cases who completed treatment, where no viral RNA was detected by RT-PCR which is evidence-based data that support the fact that proper treatment can eliminate HCV infection from the body. Treatment failure was seen in 2.13% of cases which is still the limitation and area to focus on. Coinfection with HIV/HBV (two cases), hepatic cirrhosis (two cases), and hepatocellular carcinoma (one case) were found to account for these five treatment failure cases. Four patients who died during this study period, one had coinfection with HBV, second one had chronic kidney disease undergoing hemodialysis, third case had hepatocellular carcinoma, and fourth one cirrhosis.

CONCLUSION

The serological prevalence was estimated to be 1.78% and prevalence of active cases of HCV infection detected by RT-PCR among the total study population was estimated as 1.52%. Early detection of detectable viral load by real-time RT-PCR in active cases and initiation of treatment with directly DAAs can attain Sustained Virologic Response and practically cure the patients which is the goal of treatment for Hepatitis C. In our study, SVR was attained in 97.87% of cases which is a ray of hope toward elimination of HCV infection by 2030. For this purpose, proper counseling of the patients for strict adherence to their treatment and follow-up is essential. Diagnosis by real-time RT-PCR and availability of highly effective DAA has changed the paradigm of HCV diagnosis and treatment in recent time.

ACKNOWLEDGMENT

The authors hereby take this opportunity to acknowledge the sincere help and support received from Prof Pallav Bhattacharryya, SNO, NVHCP, W.B., Prof Manimoy Banerjee, Director, IPGMER, Prof Abhijit Chowdhury, HOD, SDLD and last but not the least, Dr Ananya Pal, Technical Officer, NVHCP, IPGMER without whom this paper would not have seen the light of day.

AUTHORS CONTRIBUTION

1. Dr. Asis Kumar Ghosh: Design, Definition of intellectual content, Literature search, Acquisition of data, Analysis and interpretation of data, and Manuscript preparation
2. Prof Dr. Raja Ray: Concepts, Design, Definition of intellectual content, Acquisition of data, Analysis and interpretation of data, Manuscript editing, and Manuscript review.
3. Prof. Dr Kalidas Rit: Concepts, Design, Definition of intellectual content, Manuscript editing, and Manuscript review.
4. Dr Bipasa Chakraborty: Design, Definition of intellectual content, Literature search, Analysis and interpretation of data, Manuscript preparation, and Manuscript review.
5. Dr. SK MahiuddinAhammed: Design, Definition of intellectual content, Acquisition of data, and Manuscript review.

CONFLICTS OF INTEREST

Nil.

SOURCES OF SUPPORT

NHM funding.

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