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Original Research Article

Prevalence of HCV Infection in Adult Patients and Viral Response to Antiviral Treatment at Tertiary Care Center

Rutu Chapla[1], Hiral Gadhavi[2], Hitesh Shingala[3]

[1]Senior Resident, Department of Microbiology, GMERS Medical College Sola, Ahmedabad, Gujarat, India

[2]Assistant Professor, Department of Microbiology, Shri M.P. Shah Government Medical College and Hospital, Jamnagar, Gujarat, India

[3]Professor and Head, Department of Microbiology, Shri M.P. Shah Government Medical College and Hospital, Jamnagar, Gujarat, India

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Corresponding author: Dr. Rutu Chapla

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Abstract:

Background: Hepatitis C virus (HCV) infection is currently the most significant public health problem globally and also in India. HCV is one of the leading causes of chronic hepatitis worldwide and an important risk factor for progression to advanced entities such as liver cirrhosis, hepatic decompensation, and Hepatocellular Carcinoma (HCC).

Aims and Objectives: The present study deals with prevalence of HCV infection in adult patients and determines the effectiveness of treatment with Directly Acting Anti-viral Drugs (DAA) by undetectable HCV RNA in blood at the end of treatment.

Material and Methods: A total of 10,000 patients whose serum samples underwent the anti HCV ELISA testing were enrolled in the study. Anti HCV antibody production was tested by using 3rd generation ELISA and HCV RNA detection was performed by RT PCR.HCV RNA positive patients were given anti-viral treatment for 3 months. After 12 weeks of completion of treatment for HCV infection, patient's samples were tested again for HCV RNA in plasma by RT-PCR for monitoring effectiveness of treatment.

Results: The seroprevelence of HCV infection was 0.8%.The positive rate of anti –HCV antibodies were higher among males of 18-30 years of age. The most probable risk factor for HCV transmission was blood transfusion due to thalassemia which was seen in 31.25%.Among the anti-HCV positive patients, the HCV RNA positive rate was 66.25%. After the treatment with DAA in HCV RNA positive patients Sustained Virological Response (SVR) was achieved in 91% of patients.

Conclusion: The seroprevelence of HCV infection was 0.8%.Approximately 66.25% of the anti-HCV positive patients also tested positive for HCV PCR. This positive correlation between serum anti-HCV and HCV PCR improve screening and facilitates timely intervention to prevent the spread of infection. And HCV PCR is highly sensitive and specific for detecting active infection and monitoring viral response after treatment with DAA.

Keywords: Hepatitis C virus, Anti-HCV antibody, HCV RNA, Viral response.

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Introduction

Hepatitis C virus (HCV) infection is one of the main causes of chronic hepatitis worldwide and also an important risk factor for progression of the disease to advanced entities such as liver cirrhosis, decompensation, and Hepatocellular Carcinoma (HCC) [1,2].

Globally, approximately 58 million people have chronic hepatitis C virus infection; with about 1.5 million new infections occur each year. There are an estimated 3.2 million adolescents and children with chronic hepatitis C infection [3]. Currently there are 12.5 million carriers in India. Chronic HCV infection accounts for about 12-20% of chronic liver diseases and 12-32% of hepatocellular carcinoma [4]. It is the most common cause of post-transfusion hepatitis and also one of the common reasons for liver transplants worldwide. It is a leading cause of death in men in their forties and fifties. [5]

Hepatitis C virus is mainly a blood-borne virus and blood transfusion, intravenous drug abuse and healthcare-related procedures are common modes of spread of hepatitis C virus; less common routes are vertical and sexual transmission. [6] Common

routes of HCV transmission in India are blood transfusion and unsafe therapeutic procedures. [7] Screening and diagnosis of HCV infection in high-risk groups is very important, so that we can treat them early with antiviral therapy.

A model was commissioned by the World Health Organization (WHO), which suggested that HCV could be eliminated as a public health threat by 2030 if the response met targets for key interventions. For this on the occasion of world hepatitis day, 28th July 2018, National Virus Hepatitis Control Program (NVHCP) has been launched by the Ministry of Health and Family Welfare, Government of India. In order to achieve the Sustainable Developmental Goal (SDG) 3.3 which aims to end viral hepatitis by 2030, it is an integrated initiative for the prevention and control of viral hepatitis in India. [4]

The first step used for the detection of hepatitis C virus is serological tests like Enzyme-Linked Immunosorbent Assay (ELISA) and the Recombinant Immunoblot Assay (RIBA). These serological rapid tests may have sensitivity and specificity for the detection of HCV antibodies and have the added value of providing the result quickly to the patients. However these tests for HCV cannot differentiate people who had spontaneous resolution from those who are chronically infected, so for confirmation of active infection and quantification of HCV RNA and for monitoring the response to treatment molecular diagnostic techniques are the most sensitive and specific tests. Quantitative assays are used to measure the baseline viral load before initiation of therapy and also for monitoring prognosis. Hence the present study deals with the prevalence of HCV infection in adult patients and to evaluate the HCV viral load in HCV ELISA positive patients and to determine the effectiveness of treatment with anti-viral drugs by undetectable HCV RNA in blood at the end of treatment.

Aims and Objectives

1. To estimate the seroprevalence of HCV infection in adult patients by ELISA and detect the HCV viral load in HCV ELISA positive patients.
2. To study the effectiveness of anti-viral treatment in HCV viral load positive patients after 12 weeks of treatment as undetectable HCV RNA in blood.

Material and Methods

This retrospective study was conducted in the Department of Microbiology, for a period of 13 months from January 2021 to January 2022. Blood samples of adult patients received from various departments of the hospital were screened for HCV infection. 3-5 ml of blood was withdrawn aseptically by venepuncture and transferred to a plain tube (clot activator) for anti-HCV antibody ELISA and received in the laboratory. After the separation of serum, all the samples were tested for the presence of anti-HCV antibodies by 3rd generation ELISA. All HCV seropositive patients are further referred by the physicians to a treatment centre for registration under NVHCP, for baseline investigation and HCV viral load testing and treatment initiation according to NVHCP guidelines.

All the HCV seropositive samples were tested for HCV RNA by quantitative Real -Time Polymerase Chain Reaction (RT-PCR). For that 10 ml of blood was withdrawn for plasma in an EDTA tube and received at the laboratory.

The tube was centrifuged for the separation of plasma. This was stored at –20°C deep freezer until tested for PCR.HCV RNA positive patients were given anti-viral treatment for 3 months. After 12 weeks of completion of treatment for HCV infection, the patient's samples were tested again for HCV RNA in plasma by RT-PCR to monitor the effectiveness of treatment.

Results

A total of 10,000 samples were received and tested in the Department of Microbiology for anti-HCV antibody by ELISA during the study period. Out of 10,000 patients tested for anti-HCV antibodies by ELISA, 80(0.8%) were positive. (Table 1)

Table 1: Seroprevalence of Hepatitis C infection among patients in a tertiary care hospital

Total no. of samples received	Total no. of positive samples	Percentage of total no. of positive samples
10000	80	0.8%

Age-wise analysis in the present study showed high seropositivity among individuals in the age group of 18-30 years (41.25%) followed by the age group 31-40 years (17.5%) and 51-60 years (16.25%). The Lowest prevalence was observed in age group greater than 60 years (10%). Among the 80 anti-HCV positive patients, 46(57.5%) and 34(42.5%) were males and females respectively which is shown in Table 2.

Table 2: Gender-wise Distribution of Anti-HCV Positive Cases

Gender	No. of ELISA positive cases	Percentage
Male	46	57.5%
Female	34	42.5%
Total	80	100%

In the present study, the probable risk factor for HCV transmission was observed as blood transfusion due to thalassemia in 25 cases (31.25%), haemodialysis in 21 cases (26.25%), surgery and blood transfusion in 10 cases (12.5%), and IV drug abuse in only two cases (2.5%).No risk factors were identified in other anti-HCV positive cases (27.5%).

Table 3: Distribution based on probable History of exposure to HCV infection

Risk factor	No. of Anti HCV positive cases
Blood transfusion due to thalassemia	25(31.25%)
Haemodialysis	21(26.25%)
Surgery and Blood transfusion	10(12.5%)
IV drug abuse	2(2.5%)
Unknown	22(27.5%)
Total	80(100%)

Out of 80 samples tested for the presence of HCV RNA by real-time RT-PCR, 53 (66.25%) were detected to have HCV RNA and 27(33.75%) were tested negative by PCR which is seen in the following table.

Table 4: Result of HCV PCR in Anti-HCV Positive Cases

HCV PCR	No. of cases	Percentage
Positive	53	66.25%
Negative	27	33.75%
Total	80	100%

Table 5: Comparison between positive and negative PCR patients as regards Liver enzymes

	Positive PCR	Negative PCR	P value
Liver Enzyme			
(ALT) Mean(SD)	113.9(88.5)	91.5(68.2)	0.008
(AST)Mean (SD)	110.5(86.1)	89.2(61.5)	0.012

***ALT-Alanine Aminotransferase, AST- Aspartate Aminotransferase**

There was a statistically significant difference in AST and ALT in positive PCR and negative PCR cases.

Significant positive correlation of HCV RNA viral load was found with ALT ($P < 0.008$) and AST ($P < 0.012$).AST and ALT levels higher in positive HCV PCR cases compared to negative PCR cases. Of the 53 patients positive for HCV viral load, 44 patients started treatment for HCV infection, and 9 patients died before starting treatment. So we have evaluated the virological response in 44 patients after 12 weeks of treatment.

Out of 44 patients, 40 (91%) patients showed a sustained virological response after the completion of anti-viral treatment for 12 weeks, while four patients showed virological failure.

Table 6: Virological response after 12 weeks of treatment

No. of cases treatment started	SVR achieved	Virological Failure
44(100%)	40(91%)	4(9%)

Discussion

Hepatitis C virus infection is a serious threat to the health care system. It can cause varying clinical conditions ranging from acute infection to chronic hepatitis and hepato- cellular carcinoma. The seroprevalence of HCV varies in different parts of the country. The complex and uncertain nature of HCV infection and its chronicity emphasises the difficulties in the prevention and control of HCV.

The information about the seroprevalence of HCV among adult patients and viral response to anti-viral treatment help to start effective strategies to prevent cirrhosis development and its sequels. In this study, 10,000 patients were screened for anti-HCV antibodies. Out of these 80(0.8%) tested positive. So, the seroprevalence of HCV infection is 0.8%. This finding agrees with similar studies done by Hulinaykar et al [8] (0.82%) and Shaiji PS et al [9] (0.72%). The study done by Jahan et al [10] showed a higher prevalence (1.2%) as compared to our study. Concerning variation of HCV sero-prevalence, the reasons cannot be completely discerned. These discrepancies might be explained by various reasons like the difference in demographic characteristics of the study population, the difference in hepatitis epidemiology, awareness of the routes of HCV transmission, efforts made to implement universal precautions by health professionals and the mandatory HCV screening prior to blood donation and before any surgical procedure [11]. Among the study population, patients in the age group of 18-30 years (41.25%) were most commonly affected. This finding goes in correlation with the study done by Qamar Z et al [12] where the prevalence was seen

more in age groups 21-30 years. And another study done by Raheem T et al [13] also shows a similar results.

The socio-economic implication of this finding more in the 21-30 years of age group is imperative. This age group is an economically and sexually active age group. Thus, there are greater chances of getting infected and the productivity due to liver dysfunction are reduced. [13] Regarding the gender of hepatitis C positive patients, this study has shown that there were 46 (57.5%) males and 34 (42.5%) females showing a predominance of male gender. Similar findings were reported by Parveen M et al [14]. It showed a significantly higher prevalence in male (65%) patients in comparison with the females (35%). Malhotra R et al [15] also have shown male predominance in their study. This higher proportion of males than females in HCV infection could be a reflection of more males coming for treatment in our setting. It could be due to more social mobility in males than females and thus greater vulnerability to being infected. [15]

The most frequent risk factor for HCV transmission in this study was blood transfusion observed in 31% of cases followed by haemodialysis in 26%. Blood transfusion history of thalassemia was elicited in 31% of sero-positive cases. This finding correlates well with studies done by Bhattacharya KK et al [16] which showed 25% and Akhtar S et al [17] which showed 36.21% of patients having blood transfusion history. This could be attributed to the fact that blood transfusion allows a large quantum of infective virions into the susceptible patient. [18] In the present study, 26% of patients with chronic renal failure on long-term haemodialysis were found to be positive for anti-HCV antibodies. A similar finding was observed by Divya Soin et al [19] and Malhotra R et al [20]. This could be explained by the fact that patients on haemodialysis are at an increased risk for acquiring HCV as a result of multiple blood transfusions and cross-contamination from the dialysis circuit. Stringent blood screening and strict infection practices in dialysis units are required for reduction of transmission. [18]

Anti–HCV antibody ELISA test is used as screening test for the detection of HCV infection. RT-PCR is used for quantitative measurement of viraemia for diagnosis in acute HCV infection, for evaluation of HCV viraemia in asymptomatic patients with normal liver enzymes, for assessing as well as predicting treatment response and also assessing the severity of disease. Among 80 anti-HCV ELISA positive cases, 53(66.25%) tested positive for HCV-RNA. This is consistent with the study done by Yurong Li et al [21] which shows HCV RNA detection rate of 64.26% and a 63.6% detection rate observed by Chhina D et al. [22]

Sero-positivity among PCR negative patients may be due to spontaneous resolution of infection, false positive result of ELISA which occurs in patients with autoimmune diseases like autoimmune hepatitis, Sjogren syndrome, Lichen planus, polyarthritis nodosa etc. or due to non-specific antibodies detected by ELISA [23]. Hence, all the anti-HCV positive results should be confirmed by testing for HCV RNA. Studies showed that AST and ALT were significantly higher in HCV PCR positive patients than in HCV PCR negative patients; there is a significant positive correlation between liver enzyme value and HCV viral load (p value < 0.01). Rajvanshi C et al [24] also found a positive correlation between HCV viral load and different aminotransferases (AST and ALT). Ahmad et al [25] emphasize the significant relationship between elevated levels of liver enzymes with hepatitis C viral load in their study.

The hepatitis C virus causes liver inflammation, an increase in viral load means that there is an increase in liver inflammation. A rise in ALT is usually an indicator of a damaged liver or inflamed liver. In combination with other tests, AST is often used to monitor liver inflammation and damage. So the liver enzymes were correlated with viral load. [25] They are used as surrogate markers to monitor the response to therapy along with HCV PCR. However, SVR should be decided based on viral load tests only.

In our study, after completion of treatment sustained virological response was seen in 91% of patients which is in concordance with a study done by Welzel TM et al [26] in which SVR was seen in 91% of patients. Another study by Sacco R et al [27] shows that 98% of SVR. 4 patients (9%) failed to achieve SVR due to discontinuation of treatment in between and irregularity in taking drugs over a time. There are many reasons for treatment failure like patients was slow responder, early treatment discontinuation and irregularity or patients have more advanced liver disease. [28]

Conclusion

In the absence of a vaccine, primary prevention of hepatitis C should be targeted to reduce transmission of the virus. For these early diagnosis and treatment is necessary. Anti-HCV antibody detection by ELISA and HCV PCR improve screening and facilitates timely intervention to prevent the spread of infection. HCV PCR is highly sensitive and specific for detecting active infection and monitoring viral response after treatment with DAA.

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