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Original Research Article

Co-relation of hepatitis C RNA load with antiviral therapy and risk factors among hepatitis C seropositive patients

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ABSTRACT

Background: Hepatitis C virus is a bloodborne virus and the major cause of chronic hepatitis, cirrhosis and hepatocellular carcinoma. Viral load is the prognostic marker of the disease progression. The aim of this study is to determine the correlation of hepatitis C RNA load with antiviral therapy and its risk factors among the HCV seropositive patients. This study will be helpful in early assessment of the disease progression and its complications.
Materials and Methods: The blood samples were collected over a period of one year from April 2022-March 2023. The serum was subjected to ELISA for Anti HCV Ab. Viral load quantification was done by MylabPathoDetect HCV Quantitative PCR Kit in HCV seropositive patients.
Results: About 18,882 patients were tested for HCV infection over a period of one year. 75 patients were positive for HCV infection. Prevalence of HCV was 0.39. The mean viral load was reduced from 3.08×10^5 IU/ml to 1.98×10^4 IU/ml and Sustained Viral Response (SVR12) was achieved in 63(84%) patients after effective treatment with direct acting antiviral agents. High risk sexual behavior was the most common risk factor observed in seropositive patients.
Conclusion: The current study determines the reduction in mean viral load and achieving sustained viral response after the effective antiviral therapy at the earliest, which is helpful in prevention of disease progression and its complications.

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1. Introduction

Hepatitis C is an inflammation of the liver caused by the Hepatitis C Virus.[1] Globally, an estimated 58 million people have chronic hepatitis C virus infection, with about 1.5 million new infections occurring per year.[1] There are an estimated 3.2 million adolescents and children with chronic hepatitis C infection.WHO estimated that in 2019, approximately 2,90,000 people died from hepatitis C, mostly from cirrhosis and hepatocellular carcinoma (primary liver cancer).[1]

National seroprevalence of Hepatitis C was 0.32% and in Chhattisgarh it was 0.34.[2] The Hepatitis C virus is a bloodborne virus and most infection occur through exposure to blood from unsafe injection practices, unsafe health care, unscreened blood transfusions, injection drug use and unsafe sexual practices that lead to exposure to blood.[1,3] Hepatitis C transmitted predominantly by percutaneous or mucosal fluids, exposure to infected blood and various body fluids lead from asymptomatic infection to acute, chronic infection and progress to cirrhosis and hepatocellular carcinoma.[2,4,5]

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Direct-acting antiviral agents (DAAs) targeting the HCV NS3/4A protease, the NS5A serine protease, and the NS5B RNA-dependent RNA polymerase have been used in clinical practice since 2010. DAAs have achieved high SVR (sustained viral response) rates in most populations, with fewer adverse effects. Hence, DAAs have replaced IFN-based regimens in international guidelines for most patients with chronic hepatitis C infection.[6] DAAs can cure more than 95% of persons with hepatitis C infection, but access to diagnosis and treatment is low. The effect of DAA treatment on the liver-related complications is crucial for prognosis and future management. Liver fibrosis is a key predictor of hepatic and extrahepatic outcomes. Although patients receiving DAA-based therapy in terms of reversal of fibrosis is unclear.[6]

Hepatitis C virus (HCV) RNA levels have been a predictive on-therapy marker of treatment outcome.[7] DAA-only treatment regimens are replacing interferon-containing regimens as the standard of care. As a result, it has become essential to reevaluate the utility of HCV RNA levels in predicting treatment outcome and in guiding clinical decision making.[7]

The aim of this study was to determine the correlation of HCV RNA load to predict treatment response in patients treated with DAA regimens and to analyze the potential risk factors among the study population. This study will be helpful in early assessment of the disease progression and prevent its complications.

2. Materials and Methods

2.1. Study design and population

A Hospital based prospective cross-sectional study was conducted by Department of Microbiology amongst the patients attending IPDs and OPDs of Dr. B.R. A. M. Hospital, Raipur, C.G over a period of one year from April 2022-March 2023. This study was conducted after getting approval of the Institutional Ethical committee of Pt JNM Medical college and Dr. B. R. A. M Hospital, Raipur, C.G. During the study total number of 18,882 patients were tested for Anti HCV Antibody among them 75 seropositive patients for HCV who had given written consent were included in this study. All patients were naive for antiviral therapy for HCV at the time of enrolment. All the Clinical history, patient's baseline test of liver function and socio-demographic details such as gender, age, medical history and co-morbidities of the patients were collected in a pre-defined case format at the time of enrollment.

2.2. Sample collection and processing

3-5ml of venous blood was collected by venipuncture in a sterile dry and labeled ethylene diamine tetra-acetic acid (EDTA) containing tubes vial. To avoid degradation of viral nucleic acid in the specimen, serum was removed from clotted blood within 4hrs of collection and stored at -70°C. Serum samples was kept at 4-8°C for maximum for 7days and for longer duration, it was frozen at -70°C or lower and transported to testing lab on frozen ice pack.

The specimen was centrifuged at 3000 rpm for 15 mins as the serum got separated. Then serum was subjected to ELISA (Enzyme Linked Immunosorbent assay) for serological markers (Anti HCV Ab) as per kit literature. The ELISA kit used was HCV MERILISA kit of Meriline diagnostic Pvt. Ltd. (Gujarat, India). The ELISA is of third generation Sandwich immunoassay. Known serum specimens which had tested positive & negative were included as external positive & negative controls for quality control.[8]

2.3. Hepatitis C virus RNA quantitation

HCV RNA was extracted using operating manual of MylabPathodetect HCV Quantitative PCR kit (Pune, India). HCV quantitative polymerase chain reaction (PCR) was done by MylabPathodetect HCV Quantitative PCR kit (Pune, India), an in vitro diagnostic test based on real-time PCR technology utilizing reverse transcriptase (RT) reaction to convert RNA into cDNA, PCR for the amplification of specific target sequences and target specific probes for the detection. The test can quantitate HCV RNA in the linear range of 40 IU/ml to 7×10^7 IU/ml.

2.4. Non invasive assessment of liver fibrosis

APRI Score: Aspartate aminotransferase (AST)-to-platelet ratio index (APRI) is a simple index for estimating hepatic fibrosis based on a formula derived from AST and platelet concentrations. Based on the available data from literature review, the lower and upper cut-off values for APRI is 1.45 and 3.25, respectively. APRI were used to predict patients with significant fibrosis.[9] To detect cirrhosis, 1.0 and 2.0 are the lower and upper cut-off values respectively for APRI.[9]

APRI = [{AST (IU/L)/Upper normal limit of AST (IU/L)}/ Platelet count (10^9 /L)].[9,10]

2.5. FIB-4 score

It is a simple index for assessing liver fibrosis calculated in each patient. Based on literature review, the lower and upper cut-off values for FIB-4 score is 0.5 and 1.5 respectively.

FIB-4 = Age (years) × AST (IU/L)/ Platelet count (10^9/L) × ALT (IU/L)$^{1/2}$.[9,11]

2.6. Statistical analysis

All the data such as patient's clinical, socio-demographic data and laboratory parameters were entered in to Microsoft Excel 2007 version. Statistical analysis was performed using Epi Info 7.0 software by CDC. The data are presented as the frequency, percentage and mean. Correlation of HCV

RNA load with DAAs and risk factors was analyzed by using Linear regression. In all tests, the values of $P < 0.05$ were considered as statistically significant.

3. Result

Table 1: Prevalence of Hepatitis C virus (n= 75)

S. No.	Gender	Tested	HCV Positive (%)
1	Male	9286	41(0.21)
2	Female	9577	33(0.17)
3	TG	19	1(0.005)
4	Total	18,882	75(0.39)

In our study out of 18,882 patients who were tested for HCV (Anti HCV Ab) during the study period 75 patients were positive for Anti HCV Ab. The prevalence of Hepatitis C virus infection was 0.39. Prevalence among the males was 0.21, females 0.17 and transgender 0.005. (Table 1). Males to Females ratio was 1.2:1.

Age and gender wise distribution in Table 2 showed that most common age group was 41-50 years of age group where 19 (25.33%) patients were positive for HCV. Above 70 years 3(4%) of patients were positive for HCV. Mean ± SD was 46.68 ±14.26.

In our study among the 75 HCV positive patients the risk factors analysis showed that the high risk sexual behavior was the major risk factor found among in 41(54.66%) patients and Family history/past exposure history was found in 1(1.33%) patient (Table 3).

In our study 75 HCV seropositive patients were treated with 3 types of DAA drug regimens. Regimen-1 included Sofosbuvir and Declastavir prescribed in 12(16%) patients. Regimen-2 included Sofosbuvir and Velparasvir prescribed in 43(57.33%) patients. Regimen-3 included Sofosbuvir, Velparasvir and Ribavirin prescribed in 2(2.66%) cases. Regimen-2 was prescribed in maximum number of patients for duration 12 weeks. All the HCV positive patients underwent for RNA Load quantification before and after starting the treatment regimen of duration 12 weeks. 100% SVR (sustained virological response) was observed in SOF+VEL+RIB regimen (Table 4).

In the current study we had compared the pre-treatment and post-treatment RNA load of the patients treated with DAA for duration of 12 weeks.(Table 5) The sustained Viral Response (SVR) i.e RNA load less than 15 IU/ml were observed in 63(84%) patients in our study after the completion of treatment with DAAs for duration of 12 weeks.

In this current study we had observed the correlation of HCV RNA load with the abnormal laboratory parameters such as AST, ALT, Serum Bilirubin level, PT INR level, platelet count. We had found the viral load was not significantly associated with elevated ALT, AST and Serum Bilirubin level and p-values were shown in Table 6.

Table 6: Correlation of laboratory parameters with viral load (n=75)

Laboratory parameters	Mean ± SD	P-value
ALT	46.83±31.14	0.473
AST	49.87±31.49	0.955
Platelet Count	18693±104399.8	0.090
PT INR	0.16±0.41	0.318
Serum Bilirubin	2.0±4.8	0.638

4. Discussion

In this study we assessed co-relation of HCV RNA load with anti-viral therapy with DAAs and risk factors among the study population. The seroprevalence of HCV was found to be 0.39. The male to female ratio is 1.2:1 in our study. Male preponderance may be due to higher chances for males to be exposed to the risk factors. 19 (25.33%) patients were affected by HCV infection belonged to 41-50 years of age group and mean age ± Standard deviation (SD) was 46.68±14.26 The higher frequency in elderly persons may be due to longer exposure to risk factors for HCV transmission such as their frequent visits to dental clinics, past blood transfusion, chronic diseases, past hospitalization, iatrogenic transmission resulting from inadequately sterilized equipments, inappropriate reuse of supplies.

Similar finding was found in a recent study Ahmed et al was conducted in 35 HCV positive patients (27 were treatment naïve and 8 were treatment failure),[12] Male to female ratio was 2:1 and most common age group was 51-60 years, 40% of patients belong to this age group. M. E. Cardaba -Garcia et al was conducted in 330 patients (247 patients were treatment naïve patients) and male to female ratio was 1.7:1 and the mean age ± SD was 53.8±11 years.

Risk factors analysis showed that major risk factor was high risk sexual behavior was observed in 41(54.66%) patients followed by the patients with chronic diseases (chronic liver disease=06, chronic kidney disease = 12) observed in 18(24.00%) patients. Unprotected sex and sexual affairs with multiple partners are the major cause of spread. Extramarital affairs can also be one of the risk factors for HCV infection. Lack of awareness and transmission through sexual contact were positively related to hepatitis C virus infection. A similar study Ahmed et al. observed the major risk factor was Dentistry found among in 28(80%) patients, followed by history of hospitalization 23(65%).[12]

In our study, all patients were treated with three regimens. Regimen-1,2,3 includes SOF+ DEC, SOF + VEL, SOF+ VEL+ RIB respectively. Sustained Virological Response 12 (SVR) was achieved in 63(84%) of patients after 12 weeks successful treatment with DAAs. SVR in

Table 2: Age & Gender wise distribution of HCV (n=75)

S. No.	Age group	Male (%)	Female (%)	TG (%)	Total (%)
1	0-10	0	0	0	0
2	11-20	01(1.33)	0	0	01(1.33)
3	21-30	09(12.00)	03(4.00)	01(1.33)	13(17.33)
4	31-40	06(8.00)	07(9.33)	0	13(17.33)
5	41-50	13(17.33)	06(8.00)	0	19(25.33)
6	51-60	08(10.60)	10(13.33)	0	18(24.00)
7	61-70	03(4.00)	05(6.66)	0	08(10.60)
8	71-80	01(1.33)	02(2.66)	0	03(4.00)
9	Total	41(54.66)	33(44.00)	01(1.33)	75(100)

Table 3: Distribution of risk factors of HCV seropositive patients (n=75)

S. No	Risk factors	Total (%)	P-value
1	High risk sexual behavior	41(54.66)	0.632
2	Blood transfusion	05(6.66)	0.056
3	PLHA	08(10.66)	0.335
4	Chronic disease	18(24.00)	0.966
5	Therapeutic drug users	02(2.66)	0.427
6	Tattooing/ body piercing	06(8.00)	0.651
7	Family H/O or Past H/O	01(1.33)	0.957
8	H/O Hospitalization	02(2.66)	0.987
9	H/O surgery	06(8.00)	0.678

Table 4: Combined DAAs regimen prescribed in HCV positive patients (n=75)

Regimen	Drugs	12wks	SVR 12	P- value
Reg-1	SOF+DCV	12(16.00)	11/12(91%)	0.419
Reg-2	SOF+VEL	44(57.33)	39/44(88%)	0.440
Reg-3	SOF+VEL+RIB	02(2.66)	2/2(100%)	0.399
No treatment	-	17(22.66)		

Table 5: Viral load pre and post treatment with DAAs (n=75)

S. No	Parameters	Pre-treatment	Post- treatment with DAAs
1	Viral load $>10^5$(n%)	24(32%)	03(4%)
2	Above Mean Viral load (n%)	17(22.66%)	12(16%)
3	Mean Viral Load (IU/ml)	308170.20	19889.37
4	SVR achieved (n%)	-	63(84%)

SOF+ DEC was 91%(11/12), SOF + VEL was 88%(39/44), SOF+ VEL+ RIB was 100%(2/2). The p-values of these regimens were 0.419, 0.440, 0.399 respectively. Although the regimens were good at achieving SVR but their correlation statistically insignificant with the HCV RNA load. Combined regimen with Ribavirin enhances the overall efficacy of treatment by broadening antiviral activity, improving synergy, reducing resistance. In Sidharthan et al 4 types of DAA regimens were given.[13] Among them SVR12 achieved in SOF+RIB 69%(38/55), SOF+ Ledipasvir 100%(20/20), SOF+ Ledipasvir + GS-9669 95%(19/20), SOF+ Ledipasvir + GS-9451 100% (19/19). In C. M. Hong et al SVR12 was achieved in SOF+ RIB 98.2%, SOF+ Ledipasvir + RIB 97% (P value- 0.0117), SOF+DEC+RIB was 100%.[6]

We had found the pre -treatment mean RNA load was 3.08×10^5 IU/ml which was reduced to 1.9×10^4 IU/ml after treated with DAAs for 12weeks. 24(32%) patients had their pre treatment HCV RNA load more than 10^5IU/ml and post treatment with DAAs their number was reduced to 3(4%). Due to targeted inhibition of critical viral protein, rapid suppression of viral replication, broad spectrum antiviral activity against HCV, high rate of achieving SVR the DAAs more effective for HCV infection. Similarly, In Ahmed et al 11(31.4%) patients had viral load more than 10^5 IU/ml post treatment with DAAs.[12] In Sidharthan et al after 24 weeks treatment with DAA 60% (33/55) patients had viral load more than 8×10^5 IU/ml (p-value=0.53).[13] In C. H. Cheng et al 74% (164/220) patients had viral load more than 10^5IU/ml after the treatment with DAAs.[14]

We had co-related the Laboratory parameters such as ALT, platelets count, PT INR with viral load and had p-values of 0.473, 0.09, 0.318 respectively. HCV RNA load measures the level of active viral replication and liver enzymes only reflect the liver's response to injury and over all function. There is no straightforward correlation between viral load and laboratory parameters observed in our study. Similar finding observed in Ahmed et al. compared the HCV viral load correlations with liver function abnormalities showed that ALT, platelet abnormality, prothrombin activity abnormality p-values were 0.152, 0.154, 0.087 respectively. In C. M. Hong et al the AST, ALT, Serum Bilirubin p-values were 0.996, 0.55, 0.766 respectively.[6]

We assessed the liver fibrosis of each patient by Non invasive tests such as APRI and FIB-4 score in our study. For APRI Score mean & standard deviation was 36.85±178.2 and p-value= 0.776. FIB-4 mean & standard deviation was 171.1±833.2 and p-value=0.779. These scores are only assessment of liver fibrosis. They help to identify fibrosis and cirrhosis, guide treatment decision, and reduce the need of invasive procedures. In C. H. Cheng et al they also assessed the liver fibrosis by these two methods. APRI score Mean ± SD was1.6 ± 1.71 and p-value=0.005, and FIB-4 score Mean±SD was4.76 ± 4.24 and p-value = 0.91.[14]

Non -invasive assessment of Liver fibrosis by APRI Score and FIB-4 Score showed that 8(10.66%) were complicated as they had APRI score more than the reference value. 67(89.33%) patients were uncomplicated. Among all the study subjects the adherence to the treatment was found to be more than 60% after 4 weeks of the starting of DAA regimens and increased to more than 80% after 12 weeks of the regimen was observed in our study.

In our study we observed that more 63(84%) patients achieved the SVR at 12 weeks of the DAA therapy. We had observed 1(1.33%) patient with co-infection of HIV and was known diabetic had abnormal liver functions test high APRI score and High FIB-4 score the DAA was started, but the patient did not survive.

In this current study we had analyzed the co-relation of HCV RNA load with antiviral therapy. The reduction in the mean RNA load and the number of patients above viral load reduced after the successful treatment with DAAs duration of 12 weeks, but the co-relation was statistically insignificant. The risk factors analysis showed high risk sexual behavior was major risk factor for HCV infection. We had correlated the HCV RNA with potential risk factors this also showed that the correlation was statistically insignificant. This finding of our study was similar to findings of the study which we had discussed in above.

5. Conclusion

In conclusion our study demonstrates that the co-relation of HCV RNA load with anti viral therapy with DAAs and risk factors in HCV seropositive patients. The combination therapy of DAAs reduces the treatment duration and increases the SVR12 rate and improves outcome of the patient. The HCV RNA load monitoring predicts the treatment outcome and guide the DAA therapy. We believe that although there are no obvious baseline host and virological factor to predict the regression of the liver fibrosis and inflammation after antiviral therapy but high SVR rate may improve the outcome of the patient. Further research on this context is required for early assessment of the disease progression and to prevent its complications.

6. Sources of Funding

None.

7. Conflict of Interest

None.

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