

Study of Co-Relation between Hepatitis C Viral Load with Clinical Presentations, Laboratory Investigations and Sofosbuvir Combination Therapy

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Abstract

Hepatitis C virus is a causative agent of both acute and chronic liver disease and is associated with varied clinical presentations. Aims & Objectives: The study was undertaken co-relate the various clinical manifestations and derangement of liver and endocrine function with HCV viral replication and to find out the impact of Sofosbuvir combination therapy. Result: Derangement of liver function as indicated by Bilirubin, AST and ALT has significant correlation with active viral replication as indicated by viral load assay. Derangement of Thyroid function is also found in a third of HCV infected patients. The study also found Sofosbuvir combination therapy induces an early virologic response in both Genotype 1 and Genotype 3 infection.

Keywords: Hepatitis C; Viral Load; Sofosbuvir; Liver Function Test; AST; ALT; Gen expert

Introduction

Hepatitis C virus, a member of genus Hepacivirus, is a single-stranded positive sense RNA virus recognized first in the year 1989. It is a globally recognised pathogen causing a significant number of both acute and chronic liver disease [1]. Around 80% of individuals exposed to HCV develop chronic infection; 3–11% of those with chronic HCV infection will develop liver cirrhosis within 20 years which could lead to associated risks of liver failure and hepatocellular carcinoma [2].

The high-risk groups of Hepatitis C infection includes those patients who are multi-transfused (e.g. thalassemia major), patients on maintenance haemodialysis, intra-

venous drug abusers followed by persons with chronic alcohol intake, tattooing, perinatal transmission, homosexual persons, persons with HIV and unsafe medical procedures (for example needle stick injury) [3,4].

An understanding of the associated risk factors and natural history of Hepatitis C infection with proper screening would help the clinicians in treatment of such patients and thereby significantly decrease the morbidity and mortality associated with the infection and improves their general life-style. The present study was carried out to determine the co-relation of clinical and laboratory parameters with Hepatitis C viral load which could help in

rendering adequate management measures of such patients.

Aims and Objectives

1. To find out the prevalence of active disease among Anti HCV Antibody positive patients.
2. To find out if there is any relationship between active viral replication as indicated by viral load assay with various clinical presentations, including immune-complex mediated manifestations.
3. To study the relationship between active viral replication and derangement of liver function tests (i.e. bilirubin, AST, ALT, Al. Phosphatase etc.)
4. To study the prognosis of anti-viral therapy by measurement of viral load and comparing the same with patients not treated by anti-viral therapy.

Materials and Methods

Study Type

The study is a descriptive cross-sectional study.

Study Area

The study was done in Department of Microbiology, Medical College, and Kolkata which is a tertiary care hospital in West Bengal.

Study Population

Patients from Kolkata and adjoining districts of West Bengal, of different age groups and both genders who came to Medical College & Hospital, Kolkata with a history of blood transfusion, jaundice, easy fatigue and were clinically suspected to be suffering from liver disorder were included in the study. Sample size was not determined *a priori* since the study enrolled all patients who had Anti HCV positive by ELISA or Rapid test and were referred to Department of Microbiology for laboratory confirmation.

Study Period

1. Samples were collected from September 2018 to August 2019
2. Result analysis was done in the month of September 2019.

Subject Selection Criteria

Inclusion Criteria

Blood samples from all patients with jaundice, easy fatigue, fever, joint pain with or without rash, ascites

receiving blood transfusion, intravenous drug use was taken. Patients with history of needle stick injury, chronic alcohol intake, tattooing, perinatal transmission, high risk sexual behavior were taken. Only those patients who were Anti HCV positive either by ELISA test or Rapid test coming to Department of Microbiology, Medical College and hospital, Kolkata were included in this study.

Exclusion Criteria

The following patients were excluded from the study:

1. Patients unwilling to give consent or blood.
2. Patients who were serologically negative for Anti-HCV antibody.

Patient details

Relevant history of patients suffering from jaundice, easy fatigue, joint pain, fever, rashes etc. were taken. History of blood transfusion, intra-venous drug use, unsafe medical practice, needle stick injury, alcohol intake, sexual behaviour, etc. were also taken. Clinical examination was performed. Investigations such as haemoglobin (Hb), the total and the differential leucocyte counts (TLC and DLC), platelet count, haematocrit (Hct), liver function tests (LFT), Urea, Creatinine, Thyroid function tests and Ultrasonography of abdomen were done in all the patients.

Other relevant investigations were performed according to the clinical conditions of the patients.

Collection of blood and its processing

With aseptic precautions blood was collected from clinically suspected cases and serum was separated by centrifugation. It was stored at -20°C until further testing. HCV Viral load is estimated using Gene Xpert Diagnostic system which manufactured by Cepheid. Following centrifugation of whole blood samples, 1.3mL of plasma can be pipetted directly into the Gene Expert PCR cartridge. Absence of red cells, bubbles, any particulate matter must be ensured. Cartridge barcode is scanned and patient details and identity number is provided. Cartridge is placed in the thermo cycler and is processed. After 1hr 48min of processing, the result is displayed as HCV DETECTED / NOT DETECTED and if detected its viral load. The viral titer was displayed as a log value as well as an E value, whose numerical values were obtained from the [online link](http://www.healthcare.uiowa.edu/path_handbook/Appendix/Calculators/TenExponential.html) http://www.healthcare.uiowa.edu/path_handbook/Appendix/Calculators/TenExponential.html

Data analysis

For statistical analysis data were enrolled into a Microsoft Excel spread sheet and then analysed by EpiInfo

7 and Graph Pad Prism version 5. Rate of positivity was estimated by analysing data for the results of Hepatitis viral load by Polymerase chain reaction and any variations in disease reporting by gender, age, clinical features and laboratory parameters were assessed.

Data were summarized as mean and standard deviation for numerical variables and counted and percentages for categorical variables were enumerated. The categorical values were compared by Chi-square test and the non-categorical values were compared by student unpaired T-test. The p-value was calculated wherever required and value ≤ 0.05 was considered to be statistically significant.

Results

Out of the Anti-HCV antibody positive 53 patients who came to the Department of Microbiology, Medical college and hospital, Kolkata for detection of active Hepatitis C virus infection by Polymerase chain reaction method, 49 patients were both ELISA and Immune chromatographic test positive for Anti-HCV antibody and 4 patients were only ELISA test positive for Anti-HCV antibody. Out of the

49 patients who were both ELISA and ICT positive for Anti-HCV antibody, 29 patients were having active Hepatitis C virus infection which includes 5 patients who were previous HCV PCR positive but became negative after treatment and 20 patients were HCV PCR negative. Also the 4 patients who were ELISA test positive for Anti-HCV antibody were Hepatitis C virus PCR negative.

	PCR Positive	PCR Negative
ELISA and ICT positive	29	20
Only ELISA positive	Nil	4
Total	29	24

Table 1: Distribution of Anti-HCV antibody patients by ELISA and ICT method with regards to HCV PCR testing.

Age-wise distribution table shows that the prevalence of Hepatitis C infection was more in the 11-20 years age group (17 patients- 12 newly diagnosed and 5 previously treated for HCV) followed by 0-10 year's age group which corresponds to increased demand of blood transfusion in splenomegaly patients.

	0-10 yrs	11-20 yrs	21-30 yrs	31-40 yrs	41-50 yrs	>50 yrs	Total number of cases
PCR +	6	17*	Nil	Nil	4	2	29
PCR -	9	10	Nil	Nil	1	4	24
Total number of patients	15	27	Nil	Nil	5	6	53

Table 2: Age-wise distribution.

*Out of 17 patients belonging in the age group 11-20 yrs who were HCV PCR positive, 5 patients were previous HCV PCR positive who became negative with treatment.

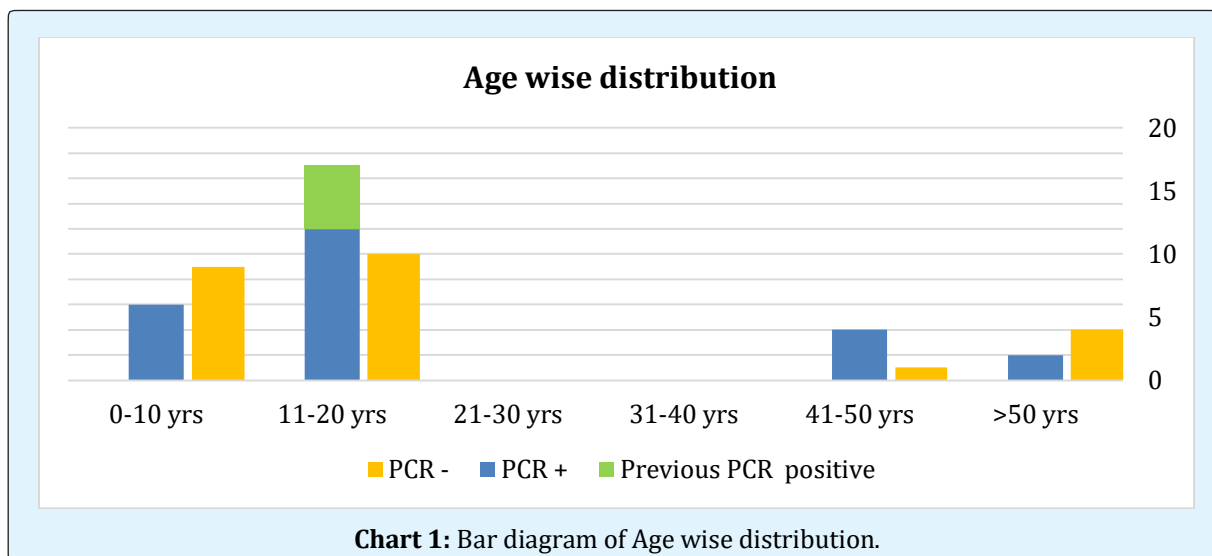


Table 3 shows the distribution of Hepatitis C infection among patients with or without jaundice. Hepatitis C virus RNA was detected in only 18 patients with jaundice

(14 newly diagnosed and 4 previous HCV PCR positive) and 11 patients (10 newly diagnosed and 1 previous HCV PCR positive) who did not have jaundice.

	Jaundice present	Jaundice absent	Total number of cases
PCR +	18	11	29
PCR -	15	9	24
Total number of cases	33	20	53

Table 3: HCV Distribution based on Presence/ Absence of Jaundice.

It was noted that among the 29 patients who were tested positive for Hepatitis C virus, few had the clinical findings like hepatomegaly (10 patients), arthritis (10 patients), vasculitis (3 patients), ascites (5 patients), and

splenomegaly (7 patients) and splenectomised (7 patients). No significant co-relation was found between clinical presentations and HCV viral load.

	Hepatomegaly present	Hepatomegaly absent	Total number of cases	Arthritis present	Arthritis absent	Total number of cases	Vasculitis present	Vasculitis absent	Total number of cases	Ascites present	Ascites absent	Total number of cases	Splenomegaly present	Splenomegaly Absent	Splenectomised	Total number of cases
PCR +	7+3*	17+ 2*	29	7 + 3*	17+ 2*	29	2 + 1*	22+ 4*	29	3 + 2*	21+ 3*	29	5 + 2*	15	4 + 3*	29
PCR -	5	19	24	3	21	24	1	23	24	3	21	24	6	18	0	24

Table 4: Distribution of PCR results based on Clinical findings.

The * marked number of patients were previous HCV positive patients who became HCV PCR negative after treatment.

Table 5 shows co-relation between Hepatitis C infectivity and Investigations which includes liver function tests like Bilirubin, AST, ALT, ALP, Prothrombin time, APTT, total protein and albumin; renal function tests, random blood sugar and thyroid function tests. Majority of the Hepatitis C virus PCR positive patients

showed deranged liver function tests and AST, ALT, ALP, Conjugated and Unconjugated bilirubin showed significant co-relation with active HCV infection. Around 8 patients out of 29 HCV PCR positive patients had Hypothyroidism. However no correlation was noted with renal function tests and blood sugar.

	Total Bilirubin		Conjugated Bilirubin		Unconjugated Bilirubin		AST		ALT		ALP		Prothrombin Time		APTT	
	PCR +	PCR -	PCR +	PCR -	PCR +	PCR -	PCR +	PCR -	PCR +	PCR -	PCR +	PCR -	PCR +	PCR -	PCR +	PCR -
↑	19 + 5*	12	20 + 5*	11	20 + 5*	11	18 + 4*	10	15 + 4*	6	14 + 3*	10	4 + 2*	5	2 + 1*	3
↓	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	1
Normal	3	9	2	10	3	9	3 + 1*	10	6 + 1*	15	7 + 2*	10	1	2	1*	1
NA	2	3	2	3	2	3	3	4	3	4	3	4	19 + 3*	17	22 + 3*	19
Total no. of cases	29	24	29	24	30	23	29	24	29	24	29	24	29	24	29	24

	Total Protein (6-8g/dl)		Albumin (3.5-5 g/dl)		A:G (1.2:1 to 2.5:1)		Urea (15-40 mg/dl)		Creatinine (0.68-1.36 mg/dl)		RBS		Thyroid profile		
	PCR +	PCR -	PCR +	PCR -	PCR +	PCR -	PCR +	PCR -	PCR +	PCR -	PCR +	PCR -		PCR +	PCR -
↑	6 + 2*	Nil	2	Nil	Nil	Nil	2	Nil	Nil	Nil	Nil	Nil	Hypothyroid	5 + 3*	1
↓	Nil	1	Nil	1	2	5	Nil	Nil	Nil	Nil	Nil	Nil	Hyperthyroid	Nil	Nil
Normal	13 + 3*	17	20 + 5*	16	17 + 5*	13	19 + 5*	18	21 + 5*	18	12 + 5*	13	Normal	5 + 2*	11
NA	5	6	4	5	5	6	3	6	3	6	12	11	NA	14	12
Total no. of cases	29	24	31	22	29	24	24	29	29	24	29	24		29	24

Table 5: Distribution of PCR results based on Investigations.

The * marked number of patients were previous HCV positive patients who became HCV PCR negative after treatment.

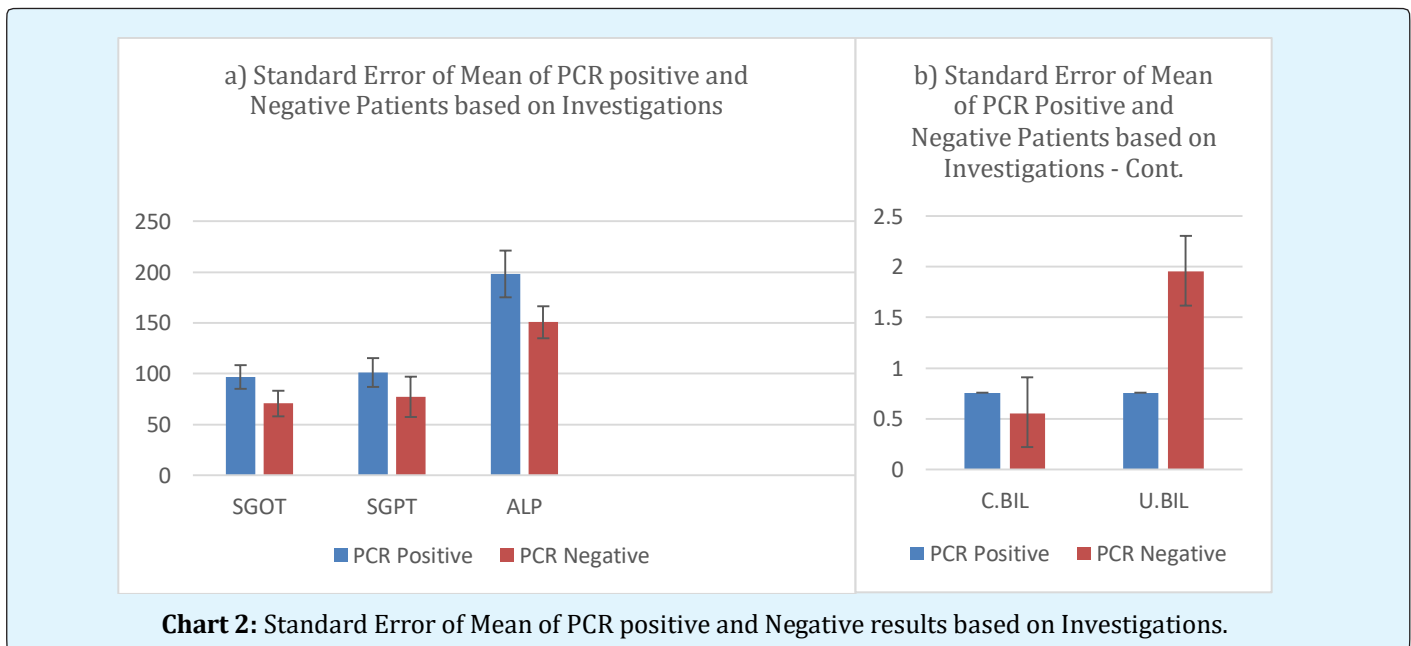


Table 6 gives the details of five patients who were previously Hepatitis C virus PCR positive; all five of them have completed treatment with Sofosbuvir combination therapy. One patient who did not receive any treatment initially and showed an increase in the viral load 4 years

later, were successfully treated with Sofosbuvir plus Ledipasvir. Among the treated patients, 3 patients reported genotype 3 and 2 patients reported genotype 1 and were treated accordingly.

S. no.	Pretreatment viral load	Genotype	Treatment	Duration	Viral Load 3 months after therapy
1	22,07,000IU/ml	1	Sofosbuvir plus Ledipasvir	3 mths	HCV Not Detected
2	19,74,883 IU/ml Initially and 61,65,950IU/ml after 4 years	1	Sofosbuvir plus Ledipasvir	3 mths	HCV Not Detected
3	54,954 IU/ml	3	Sofosbuvir plus Daclatasvir	3 mths	HCV Not Detected
4	27,68,488 IU/ml	3	Sofosbuvir plus Daclatasvir	3 mths	HCV Not Detected
5	2,630 IU/ml	3	Sofosbuvir plus Daclatasvir	3 mths	HCV Not Detected

Table 6: Patient on Anti-HCV treatment- New and Old viral loads.

Discussion

Out of the 53 Anti-HCV antibody positive patients who came to the Department of Microbiology, Medical College and Hospital, Kolkata, and who consented for PCR testing, 92.45% were Anti-HCV reactive by both ELISA and Immuno chromatographic method whereas rest were reactive only by ELISA method.

The risk factors of HCV transmission include blood and blood product transfusion, unsafe sexual practices, Intravenous drug abuse and unsafe injection practices, tattooing and acupuncture [5]. In our study, blood transfusion was found to be the most common mode of HCV transmission.

Age-wise highest prevalence of antibody against Hepatitis C virus was found in the age group 11-20 years

	No. of Patients	RNA detected	Mean viral load	Standard deviation of viral load
Jaundice	33	14(42.42%)	789666.7	1724383
Non Jaundice	20	10(50%)	40463920	125669956.1

Table 7: HCV transmission.

Many have speculated that the jaundice may be associated with a more robust immune Th1 lymphocyte and cytokine response to the HCV. It is also thought that the cellular immune response contributes to control of the virus and potentially to liver inflammation in chronic disease [9-12].

No significant co-relation was noted between clinical findings such as hepatomegaly, arthritis, vasculitis etc with HCV viral load in contrast to other studies [13-17]. Patients who have received blood transfusions may have hepatomegaly or chronic hepatitis due to iron overload [13,14]. Presence of Splenomegaly may be explained by excessive destruction of RBCs [15].

The clinical spectrum of Hepatitis C infection includes both hepatic manifestations such as deranged liver functioning and cirrhosis and non - hepatic

with significant number of Hepatitis C virus PCR positive cases owing to higher number of Thalassemia patients in this age group. Older age groups who harboured the infection gave history of blood transfusion following surgery, in blood camps (needle prick / use of faulty needle), alcohol abuse and IV drug abuse [5-8].

HCV RNA could be detected in only 42.42% patients with jaundice and the mean viral load is 7,89,666 IU/ml in this group. In the non-jaundiced patients HCV RNA could be detected in 50% patients and the mean viral load is 4,04,63,920 IU/ml which is significantly higher than in the jaundiced patients. This may signify immune basis of jaundice which at least partially controls the infection and lowers viral load than those patients who cannot mount immune response with higher viral load.

manifestations such as arthritis, glomerulonephritis, HCV related systemic vasculitis features like kerato conjunctivitis sicca, lichen planus and essential mixed cryoglobulinemia. These are mainly due to HCV and Anti-HCV immune complex deposition [16-18].

Hepatitis C infectivity showed significant co-relation with liver parameters like AST, ALT, C. bilirubin and U. bilirubin. There was no co-relation with clotting time markers. Similar findings were also noted in other studies [19-22]. It should be noted that main source of Alkaline Phosphatase is the liver, but some amount of it is also secreted by the bones, intestines, pancreas and the kidneys. Hence P value was not calculated for ALP.

The following are the tables illustrating significant co-relation between few laboratory findings with HCV viral load.

The chi-square statistic is 6.3977. The p-value is .011427. The result is significant at p <.05.			
	PCR Positive	PCR Negative	Total
AST Elevated	22 (18.09)	10 (13.91)	32
AST Not Elevated	4 (7.91)	10 (6.09)	14
	26	20	46
The chi-square statistic is 8.4546. The p-value is .003641. The result is significant at p <.05.			
	PCR Positive	PCR Negative	Total
ALT Elevated	19 (14.13)	6 (10.87)	25

ALT Not Elevated	7 (11.87)	14 (9.13)	21
	26	20	46
The chi-square statistic is 6.075. The p-value is .013711. The result is significant at $p < .05$.			
	PCR positive	PCR negative	Totals
Thyroid profile not deranged	7 (10.00)	11(8.00)	18
Thyroid profile deranged	8 (5.00)	1 (4.00)	9
Total	15	12	27

Table 8: Significant co-relation between few laboratory findings with HCV viral load.

AST and ALT levels rise in the blood when hepatocytes are damaged or destroyed [20,21]. In acute hepatitis, elevated bilirubin levels indicate the severity of the acute illness [22-24].

Iron overload due to blood transfusions may result in endocrine abnormalities like hypothyroidism, hypogonadism etc. increased blood glucose levels [24-26]. Platelet counts decrease in chronic liver diseases due to decreased Thrombopoietin production and excessive destruction by the enlarged spleen [27]. Our study also co-related with other findings such as anaemia and hypothyroidism, however there was no evidence of increased blood glucose levels and decreased platelet counts.

Five patients have completed treatment with Sofosbuvir combination therapy. Among the treated patients, 3 patients reported genotype 3 and 2 patients reported genotype 1 and were treated accordingly.

Globally, genotype 1 and 3 are the most frequently reported [28-30]. In India, genotype 3 is more prevalent followed by genotype 1 [31,32]. The patients were treated as per the new treatment guidelines of Hepatitis C virus [33].

Conclusion

HCV PCR is extremely useful in establishing the diagnosis of acute HCV infection, since RNA is detectable as early as 1 week after exposure via needle-stick or blood transfusion and at least 4-6 weeks prior to seroconversion as demonstrated in a number of transmission settings. The diagnosis of HCV infection must be established with antibody screening followed by PCR for HCV RNA for confirmation as well as for follow-up of patients on treatment. Viral load assessment at baseline is very important for monitoring therapy.

Proper clinical evaluation to look for hepatic as well as non-hepatic manifestations as protocol must be

maintained by treating physicians so as to avoid catastrophic consequences. Significant co-relation exists between liver function tests and Hepatitis C infectivity and therefore regular monitoring of liver function tests along with complete blood count, renal function tests, blood glucose level and endocrine tests should be undertaken.

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