

SEROPREVALENCE OF HEPATITIS C VIRUS IN HEPATIC DISORDERSSyeda Amtul Moqueeth¹, J. K. Surekha², Syeda Amtul Moheeth³, S. Vaisakhi⁴, Mohd. Abdul Moiz⁵**HOW TO CITE THIS ARTICLE:**

Syeda Amtul Moqueeth, J. K. Surekha, Syeda Amtul Moheeth, S. Vaisakhi, Mohd. Abdul Moiz. "Seroprevalence of Hepatitis C Virus in Hepatic Disorders". Journal of Evolution of Medical and Dental Sciences 2015; Vol. 4, Issue 23, March 19; Page: 4016-4023, DOI: 10.14260/jemds/2015/577

ABSTRACT: Seroprevalence of Hepatitis C virus among hepatic disorder patients. Various hepatic disorders like Hepatitis, cirrhosis & HCC cause multi organ failure leading to death. The most important viral cause of hepatic disorders after HBV is HCV. Hence this study is conducted to aid in early detection & treatment & its prevention in community. The study is conducted on 100 confirmed hepatic disorder patients from Gastro intestinal ward of Osmania General Hospital, Hyderabad. ELISA was done at Osmania General Hospital & Osmania Medical College, Hyderabad PCR was done at Deccan College of Medical Sciences, Hyderabad. Third generation ELISA containing an antigen derived from the Non-structural NS5 region which encodes as RNA polymerase was used. Principle of the test: Sandwich principle-Enzyme immune assay. Guanidine Isothiocyanate Method (GITC) of PCRELISA: Total No: of anti HCV antibody positive cases in our study were 5% & HBV +HCV cases were 1%. All positive cases were above the age of 40 yrs. HCV positivity ratio among Male: Female was 1:1.45. HCV was detected to be maximum in HCC (20%), followed by Hepatitis (9%) & then Cirrhosis (7%). **PCR:** All the 5 anti HCV antibody positive cases detected by ELISA were also detected to be positive for HCV-RNA by RT-PCR in all samples. The sample positive for HBV+HCV by ELISA also showed positive for HBV DNA by PCR and HCV RNA by RT-PCR. Various hepatic disorders like CAH, Cirrhosis and HCC caused by HBV and HCV have become a major public health problem throughout the world. HCC is one of the ten most common cancers in the world and is the only cancer which can be prevented by vaccination. HCV infection can be transmitted by blood transmission, injectable drugs, perinatally, improperly sterilized dialysis equipment, unprotected sex with infected partner specially MSM group and with other STDs & HIV. Hence this study is conducted to aid in early detection and treatment and its prevention in community. Comparison of studies conducted by other researchers showed slight variations in prevalence of HCV infection. 100 patients suffering from hepatic disorders were screened for HCV and HBV, which included 70 males and 30 females with mean age of 30 years. Prevalence of HCV was 5% and combination of HCV+HBV was 1% by both PCR and ELISA. The HCV positivity was detected to be highest in HCC followed by hepatitis and cirrhosis. There is a scarcity of information on HCV prevalence particularly in developing countries like India, hence present study was conducted for early detection

KEYWORDS: HCV, HBV, HIV, PCR, ELISA, HCC.

INTRODUCTION: Since time immemorial mankind is suffering from various hepatic disorders like hepatitis, cirrhosis and hepatic carcinoma causing multi organ failure leading to death.

The causes of hepatic disorders are broadly classified into: bacterial, chemical, parasitic, viral and drug induced.

Various types of viruses cause liver infection or hepatitis. Among hepatic viruses A, B, C, D, E and G are responsible.

ORIGINAL ARTICLE

Both hepatitis B virus and hepatitis C virus share common modes of transmission i.e., by blood and blood products mainly and also noticed in drug addicts.

These viruses are highly infectious (About hundred times more than HIV virus).

Globally, HCV has infected more than 170 million people and thus represents a viral pandemic 7 times more widespread than HIV infection.^[1]

In India approx 1.8-2.5% of the population is presently infected by HCV ^[2] and 20 million are suffering from HCV infection & its complications ^[3]

Previously blood transfusion was a major mode of HCV transmission but now that donor blood is thoroughly screened, majority of cases are injectable drug users.

HCV is also transmitted perinatally, by improperly sterilized dialysis equipment (68% of cases) and by unprotected sex with infected partners specially MSM group and with other STDs and even patients with HIV.^[4]

An estimated 20%cases of HCV infections will progress to cirrhosis ^[5] over 20-50yrs interval and others to hepatitis and hepatic carcinoma.

AIMS & OBJECTIVE: Seroprevalence of hepatitis C virus among hepatic disorders. The epidemic proportion of HCV infection, the limited efficacy and expensive nature of approved therapeutics, the high cost of liver transplants and huge burden on health care system all point out to the need for extensive search for seroprevalance and prophylactic vaccine development and need new therapies to treat the disease and prevent its complications.

Hence a study has been conducted to detect the seroprevalence of HCV among patients with hepatic disorders to aid in early detection, treatment and prevention in the community.

The study was conducted on 100 confirmed hepatic disorders patients from Gastroenterology department of Osmania General Hospital, Hyderabad.

ELISA done at Osmania General Hospital and Osmania Medical College, Hyderabad. PCR done at Deccan College of Medical Sciences, Hyderabad.

MATERIAL AND METHODS: Third generation ELISA containing an antigen derived from the non-structural NS5 region which encodes as RNA polymerase was used.

PRINCIPLE OF THE TEST: SP-NAN BASE C-96, 3.0 adopts the second antibody “sandwich principle” as the basis for the assay to detect antibodies to HCV. It is an enzyme immunoassay kit which employs synthetic HCV peptide (core and NS4 antigen) and recombinant HCV antigens (NS3 & NS5) for the detection of antibodies to HCV in human serum or plasma.

PROCEDURE: All reagents & specimens were brought to RT and the reagents were gently mixed well before use:

- 10 micro liters of controls (2xNC, 3xPC) and 10 micro liter per specimen was added into well of sample dilution plate 200 micro liters of specimen diluents was dispensed into wells.
- 100 micro liters of diluted conjugate added to each well except 2 blanks.
- The plate was incubated at 37degrees for 30 min.
- The plate was washed.
- 50ml of TMB substrate solution A was added to well and then.

ORIGINAL ARTICLE

- 50ml of TMB substrate solution B was added and mixed well gently.
- Incubated at RT for 30min.
- 100 microliters of 2N H₂SO₄ was added into each well.
- Absorbance at 450/650nm was determined.

PCR:

SAMPLE: Blood.

PROCEDURE: Guanidine Isothiocyanate method (GITC method).

For PCR Mixture:

1. The blood was collected from patients using EDTA as anti-coagulant (0.5ml/5ml blood).
2. 200 micro liters of serum sample was taken in a new eppendorf to this 500 micro liters of solution D. Sodium Acetate 50 micro liters, water saturated phenol 500 micro liters and chloroform 200 micro liters were added.
3. The entire mixture was vortexed for proper mixing and immediately kept in ice for about 20 mins.
4. After incubation in ice, the sample was centrifuged at 10000 rpm for 15mins at 4°C and the supernatant was collected in a new eppendorf, then 800 micro liters of chloroform, isoamyl alcohol (24:1) were added and centrifuged at 10000 rpm for 15 mins at 4°C.
5. To the supernatant equal volume of chilled isopropanol (100 micro liters) was added and kept for overnight incubation at -20°C.
6. The sample was centrifuged at 10000 rpm/15/4°C. To the pellet 800 micro liters of 80% alcohol was added and again centrifuged at 10000 rpm/15/4°C.
7. To the pellet 20 micro liters of autoclaved distilled water was added.

PROCEDURE FOR HCV-RNA ISOLATION: Template RNA and Reverse primer DNTPS and reverse transcriptase+Enzyme buffer were subjected to heating at 42°C for 1 hour to yield cDNA which was taken and treated as DNA template.

- 10 micro liters of DNA template was taken in PCR tube and 25 micro liters of PCR mixture was added.
- Kept in PCR machine automated to run 35cycles for approx 3 hours.
- Denaturation done at 94°C for 30secs.
- Annealing done at 56°C for 30secs.
- Extension done at 72°C for 60secs.
- Repeated for 35cycles and final extension done at 72°C for 5mins.
- Gel electrophoresis with agarose gel and ethidium bromide was prepared and set for about 20mins.
- Drops of bromophenol blue stain was added to the sample PCR mixture and mixed, pipetted and placed in the gel wells and electrophoresis run.
- Visualized in gel documentation system (Biorad) the fluorescent line, keeping positive and negative controls.

ORIGINAL ARTICLE

ETHICS: The procedures followed were in accordance with the ethical standards of the ethical committee on human experimentation

RESULTS: TOTAL NUMBER OF ANTI HCV ANTI BODY CASES IN MY STUDIES WERE:

Total No. of liver disease patients	HCV positive patients	HBV + HCV positive patients
100	5	1

Table 1

THE OVERALL AGE WISE PREVALENCE OF HCV INFECTION IN LIVER DISORDER PATIENTS WAS AS FOLLOWS:

Sl. No	Age Range	Total No. of Cases Tested	Total No. of Positive Cases	Percentage
1	Upto 10yrs	1	-	-
2	11-20 yrs	3	-	-
3	21-30 yrs	23	-	-
4	31-40 yrs	22	-	-
5	41-50 yrs	17	1	6%
6	51-60 yrs	18	1	6%
7	61-70 yrs	10	2	20%
8	71-80 yrs	1	1	100%

Table 2

Total No of HCV positive cases detected were 5 % (5/100 is No positive/No Tested):

- Age wise prevalence of HCV was 20% in age group 61-70 yrs.
- Age wise prevalence of HCV was 6% in age group 51-60 yrs and 41-50 yrs.
- All positive cases were above the age of 40yrs.

Sl. No.	Male			Female			Total		
	No. of Cases Tested	No. of Positive cases	Percentage	No. of Cases Tested	No. of Positive cases	Percentage	No. of Cases Tested	No. of Positive cases	Percentage
1	70	3	4%	30	2	7%	100	5	5%

Table 3: SEX WISE PREVALENCE OF HCV INFECTION

Total No of HCV positive cases detected were 5 % (5/100 is No positive/No Tested):

- Male HCV positive cases were 4% (3/70).
- Female HCV positive cases were 7% (2/30).

ORIGINAL ARTICLE

- HCV positive ratio among Male and female is M: F 1:1. 45.
- Showing slightly higher prevalence among females

Sl. No.	Hepatic Disease	Total No. of Cases Tested	No. of HCV Positive Cases Detected	Percentage
1	Cirrhosis	43	3	7%
2	Cirrhosis with ALD	19	-	-
3	Acute/Chronic viral hepatitis	11	1	9%
4	Jaundice complicating pregnancy	4	-	-
5	Primary carcinoma liver	5	1	20%
6	Secondary, s in liver	3	-	-
7	Amoebic liver abscess	6	-	-
8	Others	9	-	-

Table 4: PREVALENCE OF HCV VIRUS IN VARIOUS HEPATIC DISORDERS

HCV was detected to be maximum in:

- HCC cases 20% (1/5 No Positive/No Tested).
- Hepatitis cases 9% (1/11).
- Cirrhosis cases 7% (3/43).

Sl. No.	Total No. of anti-HCV antibodies positive samples tested	No. of samples positive for HCV RNA	Percentage
1	5	5	100%

Table 5: RESULTS OF PCR FOR HCV-RNA

Out of five anti HCV antibody positive samples detected by ELISA, HCV RNA was detected by RT-PCR in all samples.

Sl. No.	Total No. of HBsAg and anti-HCV antibodies positive samples tested	No. of samples positive for HBV DNA	No. of samples positive for HCV RNA	Percentage
1	1	1	1	100%

Table 6: RESULTS OF PCR FOR BOTH HBV-DNA + HCV-RNA

The sample positive for HBV + HCV by ELISA also showed positive for HBV-DNA by PCR and HCV-RNA by RT-PCR.

DISCUSSION: Hepatic disorders like chronic active hepatitis, cirrhosis & carcinoma caused by HBV & HCV have become a major public health problem throughout the world affecting millions of people. It is the cause of considerable morbidity & mortality in humans both from acute infections & its chronic sequelae.

ORIGINAL ARTICLE

HCC is one of the ten most common cancers worldwide & is the only cancer which can be prevented by vaccination.

Mortality due to HCV infection is increasing at alarming rate, therefore detection of seroprevalence of HCV in hepatic disorders is very essential.

All individuals infected with HIV should be screened for the presence of HIV infection.^[6]

Spontaneous recovery with viral clearance is likely to occur with HIV positive patients some, particularly those with higher CD4 cell counts, do spontaneously clear HCV infection.^[7]

Co infection with HIV has resulted in chronic liver damage in HIV patients, and more than 15% have developed severe liver damage or cirrhosis.^[8]

The present study conducted at OSMANIA GENERAL HOSPITAL, Hyderabad shows seroprevalence of HCV as 5% among patients suffering from various hepatic disorders.

Seroprevalence of HCV in hepatic disorders in our study was 5% almost similar to results of R. K. Jain [Gandhi Medical College, Bhopal IJG,2001 vol. 20 suppl 2Nov] prospective study of etiology & clinical presentation of CLD who reported 8% HCV prevalence.

Whereas study from North India –seroprevalance of HCV & HBV viral markers in patients with CLD Indian Journal of Med Microbiology oct 2005, shows infection as 25.7% in patients with CLD.

The prevalence of HCV infection is not uniform throughout India. Our findings of 5% HCV prevalence are comparable with some studies like that of chatterjee C Mithra Hazrasc, Banerjee Gubask-prevalence of HCV infection among patients of CAH & Cirrhosis of liver diseases-Indian Journal of Med Micro 2001.

The HCV prevalence rate in liver disorders in our study was as follows-cirrhosis-7%, viral hepatitis-9% HCC-20%.

According to Neeti Agarwal, Sita Naik et-al HCV as a cause of liver cirrhosis, frequency of genotype distribution, Indian Journal of Gastroenterology 2001.vol 20, suppl 2 Nov 83, shows 8-12% HCV infection in cases of cirrhosis.

A study conducted in 1991 at department of medical research reported 35% HCV among HCC cases. According to Devi. K. S. Singh et al (seroprevalence of HBV and HCV virus among hepatic disorders and injecting drug users in Manipur-Indian journal med micro 2004 vol 22 issue page 136-137) HCV prevalence is 30% in viral hepatitis, 27% in alcoholic hepatitis and 32% in cirrhosis of liver.

HCV positivity among hepatic disorders was found to be maximum in age group above 40yrs and these results are similar to the reports of a study by Devi.K.S.Singh which shows maximum HCV prevalence. In age group above 33yrs, Other similar studies also show that HCV prevalence is higher in older age groups only.

HCV prevalence was slightly more in females than males in present study with M: F ratio 13:22. These findings are supported by studies by khinpyone Kyi (Prevalence of HCV in healthy population and patients with liver ailments in Myanmar-Regional Health forum, WHO south-east Asia region vol-6 No. 1) which shows M:F ratio as 22.3 :29.9

HCV co infection with HIV in India has been reported infrequently. A study by Bhattacharya et al ^[9] has shown a 6% prevalence of HCV infection and a 21% seroprevalence of HCV among HIV infected individuals.

ORIGINAL ARTICLE

Our study also indicates 5% prevalence of HCV and 20% prevalence of co infection with HIV. However another study by Baveja et al ^[10] has shown a prevalence rate of 9.64%.

SUMMARY AND CONCLUSION:

- 100 patients suffering from established liver disease were screened for hepatitis B and C viruses.
- Study included 70 males and 30 females with mean age of 30yrs (Range 20-75).
- Prevalence of HCV in 100 liver disorder cases was 5%.
- All HCV positive cases were above the age of 40 yrs with highest prevalence in age group 61-70yrs (20%).
- The anti HCV anti body was detected to be highest in cases of HCC (20%) followed by hepatitis (9%) and cirrhosis (7%).
- All HCV positive cases were subjected to RT_PCR which showed 100% positivity.
- Infection with HCV is a growing health problem assuming epidemic proportion specially effecting young adults causing morbidity and mortality resulting in loss of man power thus badly affecting the economy of countries.
- There is a scarcity of information on HCV prevalence particularly in developing countries like India, the present study and other similar studies by early detection of viral prevalence go a long way in assessment of disease burden in community, in controlling the complications of viral infections like CAH, cirrhosis and malignancies and for effective implication of preventing and curative strategies.
- Routinely Ag-Ab detection tests and viral assays are done.
- PCR is a recent and confirmatory investigation capable of early detection and even when minute quantities of viruses are present.
- Innovative diagnostic techniques that are more efficient, rapid and cost effective should be made available for both rural and urban population to identify cases as early as possible & to prevent and control the spread of this deadly virus.

REFERENCES:

1. Abraham P, John J, Hepatitis C- a review with particular reference to the Indian scenario, *Indian J Med micro* 1995; 14; 5-14.
2. Chandra M, Khaja MN Farees N, Poduri CD, Hussain MM, Aejaz H, et al, prevalence, risk factors and genotype distribution of HCV & HBV infection in tribal population. A community based study in south India. *Trop Gastroentrol* 2003; 24: 193-5.
3. Khaja MN Madhavi C, Thippavazzula R, Nafeesa F, Habib AM, Habibullah CM, et al High Prevalence of HCV infection and genotype distribution among general population, blood donors and risk groups *Infect Genet Evol* 2006;6:198-204.
4. Alter MJ prevention of spread of hepatitis C-hepatology 2002; 36; 37-38.
5. Lauer GM, Walker BD, Hepatitis C Virus infection. *N England J Med* 2001; 345: 41-52.
6. Benson CA Kaplan JE Masur H Pau A. Holmes KK; CDC, et al Treating opport unistic infections among HIV-infected adultd and adolescents. Recommendations from CDC, the National institute of Health and HIV Association/Infectious Diseases Society of America. *MMWR Recomm Rep* 2004; 37: 1357-64.

ORIGINAL ARTICLE

7. Augenbraun M, Goedert JJ, Thomas, Feldman J, Seaberg EC, French AL et al ,Incident HCV in women with HIV infection. Clin Infect Dis 2003; 74: 1357-64.
8. Mayor AM, Gomez MA, Fernandez. DM, Rios-Olivares E, Thomas JC, Hunter RF, Morbidity and mortality profile of HIV infected patients with and without HCV Co-infection. Am J Trop Med Hyg 2006; 74: 239-45).
9. Bhattacharya S, Badrinath S, Hamide A, Sujatha S. Co-infection with HCV And HIV among patients with STD in Pondicherry, South India. Indian J Pathol Microbiol 2003; 46: 495-7.
10. Baveja UK, Chattopadhyaya D, Khera R, Joshi P. A cross sectional serological study of co-infection of HBV, HCV and HIV amongst a cohort of IDUS at Delhi. India J Med Microbiol 2003; 21: 280-3.

AUTHORS:

1. Syeda Amtul Moqueeth
2. J. K. Surekha
3. Syeda Amtul Moheeth
4. S. Vaisakhi
5. Mohd. Abdul Moiz

PARTICULARS OF CONTRIBUTORS:

1. Assistant Professor, Department of Microbiology, Gandhi Medical College.
2. Assistant Professor, Department of Microbiology, Gandhi Medical College.
3. Sonologist.

FINANCIAL OR OTHER

COMPETING INTERESTS: None

4. Microbiologist.
5. Anaesthesiologist.

NAME ADDRESS EMAIL ID OF THE CORRESPONDING AUTHOR:

Dr. Syeda Amtul Moqueeth,
16-1-24/b/c/10,
Farha Colony Saidabad,
Hyderabad-500059,
Telangana State.
E-mail: syedaamtul6556@gmail.com

Date of Submission: 09/02/2015.
Date of Peer Review: 10/02/2015.
Date of Acceptance: 09/03/2015.
Date of Publishing: 18/03/2015.