



SEROPREVALENCE OF ANTI-HCV ANTIBODIES & COEXISTENCE OF HBV IN ANTI -HCV ANTIBODY POSITIVE PATIENTS AT SMS AND ALLIED HOSPITALS

Dr. Bhupendra Kumar Mandawat

Associate Professor, Department of Microbiology, Government Medical College, Kota (Rajasthan).

ABSTRACT Within a few years of Hepatitis C virus discovery, it has been recognized as a major pathogen causing significant morbidity and mortality throughout the world including India. More than 200 million carriers of HCV exist in the world and constitute the reservoir of this infection. The carrier rate of HCV infects ranges from 10-20%. **BACKGROUND:** Since year 1991 screening of blood for HCV antibody in blood banks has been made mandatory in many parts of the world. In India screening of blood for HCV antibodies became mandatory from 1st July, 1997. HCV is the commonest cause of post transfusion hepatitis accounting for nearly 80-90% of cases. 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. **OBJECTIVES:** The aims of this study are: To find out the seroprevalence of anti HCV antibodies in different categories of individuals from different OPDs, wards and ICUs of SMS Hospital and Allied Hospital Jaipur and also to evaluate the coexistence of HBV infection in anti HCV antibody positive patients. **METHODOLOGY:** The present study was conducted in the clinical microbiology laboratory of the S.M.S. Hospital, Jaipur from period of 1st January, 2007 to 13th November 2007 to evaluate the prevalence of anti HCV antibody in symptomatic and asymptomatic individuals of various categories. Categories were identified based on clinical evaluations & various investigations. **RAPID TEST and ELISA TEST** was done at clinical microbiology laboratory S.M.S. Medical College & Hospital, Jaipur. **RESULT:** In our present study a total no. of 1857 samples were tested out of which 34 samples (1.83%) were positive for antibody to HCV. Out of 1318 samples of males, 32 (2.42%) were positive (2.42%) whereas 539 samples of females, 2 (0.37%) were positive. This clearly shows HCV seroprevalence was more in males than females. Highest prevalence of anti HCV antibodies was observed in the age group of 31-40 years and lowest prevalence in 0-20 years of age. There is a scarcity of information on HCV prevalence particularly in developing countries like India, hence present study was conducted for early detection & prevention of HCV infections. Out of 34 HCV positive samples, there were only 3 (8.82%) samples reactive for HBsAg. Male and female ratio were 2:1.

KEYWORDS

HCV, HBV, COEXISTENCE RAPID TEST, ELISA TEST.

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*Corresponding Author Dr. Bhupendra Kumar Mandawat, Associate Professor, Department of Microbiology, Government Medical College, Kota (Rajasthan). mandawatbhupendra744@gmail.com

INTRODUCTION:

Hepatitis C virus is a pathogen causing significant mortality & morbidity throughout the world including India. HCV is a flavivirus. More than 200 million carriers of HCV exist in the world and constitute the reservoir of this infection. The carrier rate of HCV infects ranges from 10-20% [12]. HCV is the commonest cause of post transfusion hepatitis accounting for nearly 80-90% of cases. Most of the HCV infections are sub clinical, and in more than 50% of cases, HCV infections lead to chronic persistent & active infections accompanied by complications of liver cirrhosis, autoimmune diseases, cryoglobulinemia and hepatocellular carcinoma (HCC) which may develop after about 25-35 yrs [12]. 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 [2]. In India approx 1.8-2.5% of the population is presently infected by HCV [8] and 20 million are suffering from HCV infection & its complications [15]. 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% of cases of HCV infections will progress to cirrhosis over 20-50 yrs interval and others to hepatitis and hepatic carcinoma [17]. 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. The routes of transmission of Hepatitis B & Hepatitis C virus are similar thus the virus can co-infect the same individual simultaneously resulting in an aggravated disease process [2-8]. Prevalence of co-infection has been

reported to vary from 2.0% to 29.0% [14]. There is a higher degree of epidemiological similarity between Hepatitis C virus and Hepatitis B virus. Since the route of transmission is same, the preventive measures to control the transmission will be similar.

AIMS & OBJECTIVE: The aims of this study are to find out the seroprevalence of anti HCV antibodies in different categories of individuals from different OPDs, wards and ICUs of SMS Hospital and Allied hospitals and also to evaluate the coexistence of HBV infection in anti HCV antibody positive patients. This study is conducted to aid in early detection and treatment and its prevention in community.

MATERIAL AND METHODS: The present study was conducted in the clinical microbiology laboratory of the S.M.S. Hospital, Jaipur from period of 1st January, 2007 to 13th November 2007 to evaluate the prevalence of anti HCV antibody in symptomatic and asymptomatic individuals of following categories: Liver disease patients, Healthy voluntary blood donors, Renal failure /Dialysis patients/following Renal transplant patients, Thalassemia/Haemophilic patients, Intra venous drug abusers, Health care workers. Various categories were identified based on clinical evaluations & various investigations. A total of 1857 blood samples were collected from the outdoor and indoor patients of S.M.S. and its allied hospitals. A total of 500 blood samples were collected from healthy voluntary blood donors from the S.M.S. Hospital blood bank and health care workers of S.M.S. Hospital. The collected blood was allowed to clot & serum was separated. The sample was stored at 2-8°C & tested within 7 days of collection. Patients' serum samples were subjected to following tests for detection of Anti-HCV antibodies.

A-Rapid test: - DOT immunoassay for detection of Anti-HCV antibodies.[13,26] B- ELISA test :- For Detection of Anti-HCV antibodies[11,27]

HCV MICROELISA TEST: The 3rd generation HCV Microlisa is an in vitro qualitative enzyme linked immunosorbent assay for the detection of antibodies against HCV (anti-HCVs) in human serum or plasma. This kit is manufactured by J. Mitra & co. Pvt. Ltd. New Delhi, India.

Principle:-The 3rd generation HCV Microlisa is based on a highly sensitive technique, Enzyme Linked Immunosorbent Assay which detects antibodies against HCV in human serum and plasma. The 3rd generation HCV Microlisa utilizes a combination of antigen with the sequence of both HCV structural and non-structural antigen i.e. CORE, E1, E2, NS3, NS4 and NS5. The results were read on Microplate spectrophotometer at 450 nm. Cut off value was calculated as per the manufacturer's guidance and the results were interpreted accordingly. Cut off value = $0.1 \times PCx + 0.1$, $PCx = \text{Mean absorbance of positive control}$ Interpretation :- According to their absorbance values, samples were interpreted as either reactive for HCV antibody (HCV positive) or non reactive for HCV antibody (HCV negative) if test specimens with absorbance value within 10% below the cutoff should be considered suspect for the presence of antibodies and should be retested in duplicate. Sample found to be reactive initially by HCV Microlisa test were again tested by visual rapid test which is HCV TRI-DOT test.

HCV TRI-DOT:-The 4th Generation HCV TRI-DOT is a rapid, visual, sensitive and qualitative in vitro diagnostic test for the detection of antibodies to Hepatitis C Virus in human serum or plasma. They are for the putative core (structural), protease/helicase NS3 (non-structural) NS4 (non-structural) and replicase NS5 (non-structural), regions of the virus in the form of two test dots "T1" & "T2" to provide a highly sensitive and specific diagnostic test. This Kit is manufactured by J. Mitra & Co. Pvt. Ltd. New Delhi, India.

Principle:-4th generation HCV TRI-DOT has been developed and designed using modified HCV antigens representing the immunodominant regions of HCV antigen. HCV antigens are immobilized on a porous immunofiltration membrane. Interpretation: - Results are noted as per manufacturer's guidelines and results were interpreted accordingly. If test dots T1, & T2, either both dark and light in colour (pink), result should be considered reactive for antibody to HCV. If only control dot appear it indicates that the sample is non-reactive for anti-body to HCV. Sample found to be positive for HCV antibodies by both HCV microlisa test & HCV TRI-DOT method would be further tested for hepatitis B Surface antigen by ELISA test.

HEPALISA TEST:- This is a microwell ELISA test for the detection of hepatitis B surface antigen (HBsAg) in Human Serum / Plasma. HBsAg has been accepted as a universal and the most reliable seromarker in case of acute HBV infection[12,14]. This Kit is manufactured by J. Mitra & Co. Pvt. Ltd. New Delhi, India.

Principle :-HEPALISA is a solid phase enzyme linked immunosorbent assay (ELISA) based on the "Direct Sandwich" principle. The microwells are coated with Monoclonal antibodies with high reactivity for HBsAg. The samples are added in the wells, followed by standard procedure. The intensity of developed blue colour is proportional to the concentration of HBsAg in sample. To limit the enzyme-substrate reaction, stop solution is added and a yellow colour develops which is finally read at 450 nm spectrophotometrically. Test procedure & results were interpreted as per the manufacturer's guidelines. Sample were interpreted as reactive for HBsAg (HBsAg positive) or non reactive for HBsAg (HBsAg negative).

Bio-Safety:-All standard precautions, bio-safety measures & biomedical waste managements in our study according to Biological waste managements Rules 1998 were observed.

RESULT: In the present study a total no. of 1857 samples were tested out of which 34 samples (1.83%) were positive for antibody to HCV. Out of 500 of samples tested from the patients with liver diseases 21(4.2%) samples, 3 positive samples in (0.6%) Renal diseases patients, 7 positive samples in (1.4%) in Healthy voluntary blood donors, 2 positive samples in (0.8%) thalassaemia patient and 1 sample positive

out of 7 tested sample in Intravenous drug abuser (14.2%) were positive for antibody to HCV. There is no positive sample in Health care workers (0%). Out of 34 HCV positive samples, there were only (8.82%) samples reactive for HBsAg. Comparison of studies conducted by other researchers showed slight variations in prevalence of HCV infection.

TABLE – 1 HCV SERO PREVALENCE AMONG VARIOUS RISK GROUPS

Various Risk Group	Total No. of sample tested	Total No. of HCV (+) cases
Liver disease patients (AVH, FHF, SAFHF, CLD, LC, HCC)	500	21 (4.2%)
Renal disease patients (RF, RF with dialysis RF without dialysis, Following Renal transplant)	500	3 (0.6%)
Healthy voluntary Blood donors	500	7 (1.4%)
Thalassaemia /Haemophilic patients	250	2 (0.8%)
Intravenous drug abusers	7	1 (14.2%)
Health Care workers	100	0 (0%)
Total Number of Cases	1857	34 (1.83%)

* AVH – Acute viral hepatitis, FHF – Fulminant hepatic failure, SAFHF – Sub acute fulminant hepatic failure, CLD – Chronic liver diseases, LC – Liver cirrhosis, HCC – Hepatocellular Carcinoma, RF – Renal failure.

This table shows that Total no. of 1857 samples were tested out of which 34 samples (1.83%) were positive for antibody to HCV. Out of 500 of samples tested from the patients with liver diseases 21(4.2%) samples were positive for antibody to HCV. Table also shows 3 positive samples in (0.6%) Renal diseases patients, 7 positive samples in (1.4%) in Healthy voluntary blood donors, 2 positive samples in (0.8%) thalassaemia patients, 1 sample positive out of 7 tested sample in Intravenous drug abuser (14.2%). There is no positive sample in Health care workers (0%)

TABLE - 2 SEXWISE DISTRIBUTION OF ANTI HCV ANTIBODIES AMONG VARIOUS RISK GROUPS

S. No.	Various Risk groups	Total No. of Sample tested	Male		Female		Total no. of HCV positive cases
			Total sample tested	HCV positive cases	Total sample	HCV positive cases	
1.	Liver disease patients (AVH, FHF, SAFHF, CLD, LC HCC)	500	311	19 (6.10%)	189	2 (1.05%)	21/500 (4.2%)
2.	Renal diseases patient (Renal failure RF with dialysis & RF without dialysis. Following Renal transplant)	500	356	3 (0.84%)	144	0 (0%)	3/500 (0.6%)
3.	Healthy Voluntary Blood donor	500	409	7 (1.71%)	91	0 (0%)	7/500 (1.4%)
4.	Thalassaemia/Haemophilic	250	168	2 (1.19%)	82	0 (0%)	2/250 (0.81%)
5.	Intravenous drug abusers	7	7	1 (14.2%)	0	0 (0%)	1/7 (14.2%)
6.	Health Care workers	100	67	0 (0%)	33	0 (0%)	0/100 (0%)
	Total	1857	1318	32 (2.42%)	539	2 (0.37%)	34 (1.83%)

Age Group	Total Sample Tested		Liver Disease patients		Renal Disease patients		Healthy Voluntary Blood Donors		Thalassaemia/Haemophilic patients		Intra Venous Drug Abusers		Health Care Workers		Total No. of HCV Positive (%)
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	
0-10yr	117	79	0	0	0	0	0	0	1	0	0	0	0	0	1/34 (2.94%)
11-20yr	126	53	0	0	0	0	1	0	0	0	0	0	0	0	1/34 (2.94%)
21-30yr	169	68	0	0	1	0	1	0	1	0	0	0	0	0	3/34 (8.82%)
31-40yr	354	163	9	1	0	0	5	0	0	0	1	0	0	0	16/34 (47.05%)
41-50yr	263	104	3	0	1	0	0	0	0	0	0	0	0	0	4/34 (11.76%)
>51yr	289	72	7	1	1	0	0	0	0	0	0	0	0	0	9/34 (26.47%)
Total No. of Cases	1318	539	19	2	3	0	7	0	2	0	1	0	0	0	34/1857 (1.83%)

This table clearly shows that out of 1318 samples of males, 32 (2.42%) were positive (2.42%) whereas 539 sample of females, 2 (0.37%) were positive. This clearly shows HCV seroprevalence were more in males than females.

TABLE – 3 AGE WISE DISTRIBUTION OF ANTI HCV ANTIBODIES

Table shows highest prevalence of anti HCV antibodies was observed in the age group of 31–40 years and lowest prevalence in 0–20 years of age.

TABLE - 04 HBsAg CO-INFECTION IN HCV ANTIBODY POSITIVE PATIENTS

Total No. of HCV positive case tested	HBsAg. Positive Cases		Total number of HBsAg positive cases
	Male	Female	
34	2 (5.88%)	1 (2.94%)	

Out of 34 HCV positive samples, there were only (8.82%) samples reactive for HBsAg and Out of 3 HBsAg positive sample, 2 samples are of males (5.88%) and 1 sample is of female(2.94%) patients.

TABLE – 05 AGE WISE,SEX WISE & RISK GROUP WISE CO-EXISTENCE OF HBV – INFECTION IN HCV POSITIVE PATIENTS

Age group	Liver disease patients		Renal disease patients		Healthy Voluntary Donor		Thalassaemia/ Haemophilic		Intravenous Drug Abuser		Health Care Workers		Total No. of HBsAg +ve cases
	HCV +ve cases	HBsAg Positive cases	HCV +ve cases	HBsAg Positive cases	HCV +ve cases	HBsAg Positive cases	HCV +ve cases	HBsAg Positive cases	HCV +ve cases	HBsAg Positive cases	HCV +ve cases	HBsAg Positive cases	
	M	F	M	F	M	F	M	F	M	F	M	F	
0-10 year	0	0	0	0	0	0	0	0	0	0	0	0	0
11-20 year	0	0	0	0	0	0	1	0	0	0	0	0	0
21-30 year	0	0	0	1	0	0	1	0	0	0	0	0	0
31-40 year	10	2	1	0	0	0	3	0	0	0	1	0	3/10 (30%)
41-50 year	3	0	0	1	0	0	0	0	0	0	0	0	0
> 51 years	8	0	0	1	0	0	0	0	0	0	0	0	0
Total	21	2	1	3	0	0	7	0	0	0	1	0	3/34 (8.82%)

Table shows that HCV and HBV co-infection in 31 – 40 years (30%) age groups only. Table shows that HCV and HBV co-infection only in liver diseases patients (30%), of which 2 HBsAg positive cases were of males and 1 HBsAg positive case of females. 3 (1.42%) were reactive for HBsAg out of 21 HCV positive patients and all the three were of Liver disease patients.

DISCUSSION: 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. In India, screening for Hepatitis C virus has been made mandatory for all blood banks from 1st July 1997, due to which the transmission of Hepatitis C virus has greatly

reduced. In the year 2000, WHO chose a theme to promote health for all on 'World Health Day' i.e. 7th April as-"Safe blood start with me – blood save lives". The life force in all Human beings is blood which can be transfused to save lives. A healthy person has healthy blood can and does save life[23].

In our study a total of 1857 cases comprising of 1318 males and 539 females. Out of 1857 samples, 34(1.83%) samples were positive for anti HCV antibody and Hepatitis C virus and Hepatitis B virus co infection were positive in 8.82%. Our finding is supported by various other studies done in other parts of the world and India.

TABLE-6 COMPARATIVE STUDY ON HCV SEROPREVALANCE FROM DIFFERENT PARTS OF THE WORLD & INDIA

S.No.	Authors & Year	Place	Prevalence of Anti HCV
1	Kuog, et al 1989[16]	USA	0.59%
2	Choo QL, et al 1990[9]	Japan	1.2%
3	Yapi, et al 1991[30]	Singapore	1.7%
4	Pramoolsinap, et al 1992[20]	Thailand	1.42%
5	Abdel-Waheb, et al 1994[1]	Egypt	1.52%
6	V.A. Arankalle et al 1995[28]	Westren India	1.52%
7	Jaiswal et al 1995[29]	Central India	1.63%
8	M.S. Gosavi et al 1997[18]	Mumbai	16.9%
9	Harjeet Kaur et al 2000[13]	Ludhiana	1%
10	S.A.Ganju et al 2001[24]	Shimla	0.06%
11	Anima Xess et al 2001[6]	Patna	5.4%
12	Pratima Gupta et al 2002[21]	Uttranchal	0.91%
13	S. Mishra et al 2002[25]	Orrisa	1.57%
14	Commun. Dis. Intell 2004[10]	Australia	2.3%
15	Present Study 2007	Jaipur	1.83%

Our findings are in accordance with various authors as above. Our observations are in total agreement with the reported literature

• Our finding regarding HCV seroprevalence are supported by various authors like 0.59% by Kuog et al (1989), 1.2% by Choo QL, et al (1990), 1.7% by Yapi, et al (1991), 1.42% Poovarwan et al (1991), 1.52% by Ranger et al (1993), 1.5% by V.A. Arankalle et al (1995), 1.63% by Jaiswal et al (1995), 16.9% by M.S. Gosavi et al (1997), 1% by Harjeet Kaur et al (2000), 0.06% by S.A. Ganju et al (2001), 5.4% by Anima Xess et al (2001), 0.9% by Pratima Gupta et al (2002), 1.57% by S. Mishra et al (2002), 2.3% in Australia (Commun. Dis. Intell 2004). In our study, out of 1318 males patients 32 male (2.42%) were positive and out of 539 females patients, 2 female (0.37%) were positive. The male female ratio among HCV positive patients are 16 : 1 which are comparatively higher than other studies, but all studies says male were affected more as compared to females. Our study findings are supported by Ashish Kumar et al (2002) 2.5: 1, in Australia, (Commun. Dis. Intell 2004) 1.8: 1 male female ratio. In our study, higher prevalence of HCV positivity was seen in 31 – 40 year age group (47.05%) followed by > 51 years age group (26.47%) and 41-50 year age group (11.76%). These finding are supported by authors as 16 – 25 year age group (Arankalle et al 1995), > 51 year age group (Pratima Gupta et al 2002), 30-50 year age group (Ashish Kumar et al 2004) and 20 – 24 year age group (Australia, Commun. Dis. Intell 2004).

TABLE-7 Comparison of studies done for prevalence of HCV and HBV co-infection

S.No.	Study (Author)	Percentage HCV and HBV co-infection
1	O.khawa et al 1997[19]	15%
2	Sinha et al [6,14]	7.4%
3	Jamb et al [6,14]	2%
4	Gangwal et al [6,14]	29%
5	Ranga et al 1998[22]	10.3%
6.	Anil Gupta et al 1999[5]	2%

7	Anima Xess et al 2001[6]	3%
8	Ashish Kumar et al 2004[7]	28.5%
9	AK Tripathi et al 2006[3]	0.16%
10	Present Study 2007	8.82%

Our findings are in accordance with various authors as above.

The prevalence of HCV and HBV co-infection has shown wide variation in different studies which is shown in above table in present study it was 8.82%. The prevalence of HCV and HBV co-infection varies from 2.0 to 29%. In our study HCV and HBV co-infection mainly seen in liver disease patients, Male and female ratio were 2:1. & mainly in 31-40 year age groups our finding regarding supported by authors like 15% in male & 7.7 % in female by Anil Gupta et al (1999), mainly in male by Anima Xess et al (2001), 40.9% by berry et al (1998), 41-50 year age group (54.56%) & 31-40 years age group (18.18%) by Ashish Kumar et al 2004.

SUMMARY AND CONCLUSION: The present study was conducted in the Department of Microbiology & Immunology, SMS Medical College, Jaipur The object was assessing the seroprevalence of anti HCV antibodies in SMS and Allied Hospital and evaluates the coexistence of HBV infection in anti HCV antibody positive patients.

In all, 1857 patients were screened. The observations were made with reference to age sex, constitutional symptoms, various risk groups and investigations.

- The seroprevalence of HCV has declined since the screening of blood for donation in blood banks for anti HCV antibodies became mandatory in 1991 in some parts of the world and in India since 1997.
- HCV infection prevalence varies with geographical distribution and social characteristic of population groups.
- We observed 1.83% seroprevalence of anti HCV antibodies and 8.82% HBsAg seropositivity among HCV positive patients.
- Our study is a step ahead in this direction with the purpose of providing authentic scientific data based on the affected population attending our hospital.
- HCV infection is the most important cause of chronic hepatitis in several countries of the world. But at present no vaccine is available for it. Because of the increasing prevalence rate, this is necessary that medical personnel and health care workers must be educated and trained about the danger and consequences of HCV infection. All anti HCV antibody positive patients must be considered highly infectious and must be prohibited from donating blood, organ, tissues or semen. Therefore, routine screening of all the blood donors should be done in Blood Bank.
- We conclude that HCV directly affects epidemiology, morbidity, mortality, socioeconomic and preventive aspects. It is very important that the priority for HCV control is concentrated on early detection and effective treatment of both HCV and HBV of which may offer the greater chance of prolonging the life of those suffering from HCV infection. It is suggested that education of public at large to increase the general awareness towards the transfusion transmitted diseases and how to prevent them. The prevalence of HBV and HCV co-infection is definitely present in the general population as is shown by the present study, the extent of which may vary from region to region and the study group screened. Both viruses contribute to the development of a chronic liver disease entity complementing each other during the progressing pathology. Reusage of unsterilised, contaminated needles and syringes were perhaps the main reason determined for the spread of HBV and HCV or both. This calls for stringent screening measures for blood borne viruses at departmental laboratories and blood banks for all sera/blood processed.

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