

Seroprevalence of hepatitis B surface antigen and antibodies to hepatitis C in patients attending tertiary care hospital

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Abstract

Background: Hepatitis B and hepatitis C viruses are the two blood borne viruses that have been significantly related to the complications of hepatitis like cirrhosis and hepatocellular carcinoma and mortality in people worldwide. The seroprevalence of Hepatitis B surface antigen and anti HCV antibodies is useful in estimating the magnitude of HBV and HCV infection in general population which can aid in framing preventive measures. For detection of HBV and HCV, rapid diagnostic tests based on immunochromatographic principles and ELISA are widely used in most developing countries, including India. The study aims at estimating the seroprevalence of HBsAg and anti HCV antibodies among general population attending OPD and IPD at a tertiary care hospital and in turn, assessing the burden of Hepatitis B and Hepatitis C among them using ELISA.

Material and Methods: This hospital-based, prospective cross-sectional study was conducted in Jhalawar Medical College and Hospital Jhalawar for a period of 1 year on the patients who registered at the OPDs and those

admitted to the IPDs with clinical history of hepatitis, jaundice for evaluation and alcoholic hepatitis.

Result : Seropositivity of HBsAg was found to be 3.8% (19/500) and seropositivity of anti HCV antibodies was found to be 1.2% (6/500) with the HBV and HCV coinfection seroprevalence being 0.2% (1/500). Males were found to have higher overall seropositivity of both HBsAg (12/302; 3.9%) and anti-HCV antibodies (4/302; 1.32) compared to females. Highest seropositivity was found in 31-40 years age group for both HBV and HCV.

Conclusion: Seroprevalence of HBV and HCV was found higher in males in the sexually active age group. Efforts should be made to target the reproductive age group to control the spread of the virus. Attempts should be made to prevent HBV and HCV infection by focusing on associated risk factors and promoting early diagnosis and screening for HBV and HCV infections.

Keywords: HBsAg, anti-HCV antibodies, seroprevalence.

Introduction

HBV causes a spectrum of disease from self-limited hepatitis to acute fulminant and chronic hepatitis which

may result in complications like liver cirrhosis and hepatocellular carcinoma.³ WHO estimates that nearly 2 billion people are infected worldwide, and more than 296 million people were suffering from chronic HBV infection in the year 2019. 1.5 million new cases are added every year.¹ Hepatitis B virus (HBV) is highly infectious and can be transmitted covertly by percutaneous routes and frankly by blood transfusion. The incubation period varies from 30–180 days. If HBsAg (Hepatitis B surface antigen) remains positive for more than 6 months it is called chronic hepatitis B virus infection. A proportion of patients may also become chronic carriers and transmit the disease to others.⁴

The diagnosis of HBV infection is accomplished by testing for a series of serological markers of HBV.⁴ The hepatitis B surface antigen (HBsAg) in serum is the first sero-marker to indicate active HBV infection, either acute or chronic.⁵ In the absence of other tests, presence of HBsAg is usually taken as a marker for chronic HBV infection. HBsAg ELISA is used for the qualitative determination of hepatitis B surface antigen (HBsAg) in human serum or plasma. This test is indicated for the screening of blood and blood products to be used for transfusion and an aid for the diagnosis of existing or previous hepatitis B infection.⁴

Hepatitis C virus (HCV) is a positive stranded RNA virus belonging to genus Hepacivirus in the family Flaviviridae.⁷ HCV is the major cause of chronic liver disease leading to cirrhosis of liver and hepatocellular carcinoma. Hepatitis C virus (HCV) is especially dangerous in that its morbidity rate is high as it establishes a state of chronic infection in as many as 85% of acutely infected patients, whereas about 15% of acutely infected patients spontaneously clear the

infection.²⁴ The major channels of HCV transmission are all related to exposure to blood and blood products. A significant percentage of individuals suffering from HCV infection are asymptomatic and are detected only on random check-ups for various purposes. The presence of anti-hepatitis C virus antibody (anti-HCV Ab) indicates earlier exposure to hepatitis C virus but does not indicate whether the infection is acute, chronic or resolved. This antibody is present in only 40% of acute infections but in more than 95% of chronic infections.²⁴ In India, antibodies against HCV are present in approximately 15 million people with a prevalence rate of 2%.⁹ According to WHO estimates 180 million people are affected worldwide. Anti-Hepatitis C virus (HCV) antibody prevalence in the general population is estimated to be between 0.09-15%. Based on some regional level studies, it is estimated that there are 6-12 million people with Hepatitis C in India.¹ HCV is detected by detecting anti-HCV antibodies in the serum through rapid immunochromatographic test as well as ELISA.

EIAs (Enzyme immunoassay) and RIAs (radioactive immunoassay) are the most sensitive methods. EIA methods are generally used by reference laboratories and blood banks because of its accuracy, low cost and safety in comparison with RIA methods. For detection of HBsAg, rapid diagnostic tests based on immunochromatographic principles are widely used in most developing countries, including India. In 2016, the WHO Global Health Sector Strategy obligated for hepatitis elimination by 2030 through scaled-up prevention, testing, and treatment.¹ In India, the same strategy is being followed titled “National Viral Hepatitis Control Program” which is running as a national program that aims at elimination of hepatitis as a public health threat and was defined as a 90% reduction in incidence

and a 65% reduction in mortality, compared with the 2015 baseline.¹⁷

Thus HBV and HCV are posing a great challenge in the health sector and need for their elimination or containment is eminent, especially in the developing countries. The study aims at estimating the seroprevalence of HBsAg and anti HCV antibodies in this region using rapid card tests and ELISA for HBV and HCV, in turn, promoting the diagnostic techniques that are highly sensitive, cheap, safe and less time consuming.

Material methods

This hospital-based, prospective study was conducted for a period of 1 year (October 2021 to October 2022) at the Department of Microbiology, Jhalawar Medical College and Hospital Jhalawar (a tertiary care teaching hospital) Rajasthan on the suspected patients of hepatitis B and hepatitis C infections attending the OPDs & IPDs in the hospital regardless of their age, sex, occupation, religion and ethnicity in tertiary-care hospital in Jhalawar, Rajasthan. The study included patients who registered at the OPDs and those admitted to the IPDs with clinical history of viral hepatitis, jaundice for evaluation and alcoholic hepatitis and excluded the patients who were unable or unwilling to give a written consent. High risk groups such as immunocompromised, Intravenous drug users were also excluded from the study. Informed, verbal and written consent (Annexure I) was taken from patients/guardians who were allowed to take part in the study. The patients/guardians were assured of confidentiality of the information. Study was conducted after taking ethical approval from institutional ethical committee.

Sample collection /transport /storage

3-5ml of venous blood was drawn aseptically from all eligible patient using a sterile needle and syringe in a

vacutainer after taking informed oral consent. The blood samples were collected at the central laboratory SRG hospital, Jhalawar and were allowed to clot for 45 min at room temperature and transported to VRDL lab of Jhalawar Medical College. The blood samples were centrifuged at 1,300 x g for 10 minutes at 4°C. A sterile, graduated, disposable transfer pipette was used to transfer serum into sterile screw-capped cryo tubes for serology. Rapid tests for screening for HbsAg and antibodies to HCV was performed on the same day and the samples were stored at 2-8°C for ELISA.

Specimen processing

Hepatitis B surface antigen was screened by Hepacard Rapid Immunochromatographic test. Screening for Hepatitis C antibodies was done by HCV tridot rapid test. The Serum samples for HBsAg or anti HCV antibody screened by rapid tests were further confirmed for Hepatitis B surface antigen and antibodies to Hepatitis C by ELISA. Hepatitis B surface antigen was investigated by enzyme immunoassay (EIA) using ELISA kit HEPALISA manufactured by J. MITRA & Co. Pvt. Ltd, NEW DELHI. This kit is a solid phase enzyme linked immunosorbent assay for the qualitative detection of HBsAg in human serum. Unlike HBV, the levels of HCV proteins in the serum are not sufficient enough to be detected by ELISA. Thus host generated antibodies to these viral proteins are used for immune-diagnosis of HCV. The 3rd generation HCV Microlisa manufactured by J. Mitra & Co. Pvt. Ltd., is a highly sensitive in -vitro enzyme linked immunosorbent assay used for the qualitative determination of antibodies to Hepatitis C virus in human serum.

Results

During the study period from October 2021 to October 2022, 22400 samples were received out of which 500 samples were selected for HBV and HCV seropositivity

testing. The Immunochromatographic tests for HBsAg and anti HCV antibodies were performed at the Central laboratory , SRG hospital, Jhalawar. The same samples were tested by ELISA as well and those tested positive by ELISA were used to calculate the seroprevalence of HBV and HCV. In all, 500 samples were tested as per the inclusion criteria for HBV and HCV screening by immunochromatographic rapid test. The samples were confirmed by ELISA for HBsAg and anti HCV antibody at Viral Research and Diagnostic Laboratory, Department of Microbiology, Jhalawar Medical College Jhalawar. 19

samples were found positive by ELISA for HBsAg while 6 samples were found positive by ELISA for anti HCV antibodies. Overall seroprevalence as well as age wise seroprevalence for HBV and HCV were calculated. In present study, the overall seroprevalence of HBsAg was 3.8% and that of anti HCV antibodies was 1.2%. Systematic representation of the observations with age wise and gender wise distribution of HBsAg and anti HCV antibody positives in the present study have been shown in the following tables.

Table 1: Age and Gender wise seroprevalence of HBsAg

Age (years)	No. of males tested	Positive	Prevalence %	No. of females tested	Positive	Prevalence %	Age wise prevalence (%)
<=20	10	0	0	16	1	6.2	3.8
21-30	80	4	5.0	101	3	3.0	3.9
31-40	66	4	6.1	38	2	5.3	5.8
41-50	71	2	2.8	29	1	3.4	3.0
51-60	24	1	4.2	11	0	0	2.9
61-70	36	1	2.8	1	0	0	2.7
71 & above	15	0	0	2	0	0	0
Total	302	12	3.9 %	198	7	3.5 %	3.8

Table 1. shows the age and gender distribution of the total number of cases with HBsAg seroprevalence. The overall seroprevalence of HBV was found to be 3.8%. The gender wise seroprevalence of HBV among males was found to be 3.9% and among females was 3.5%. Seroprevalence of HBV in males was higher in the age group 31-40 years 4 (6.1%), followed by the age group 31-40 4 (5.0 %). Seroprevalence of HBsAg in females was higher in the age group <20 years (6.25 %), followed by the age group 31-40 4 (5.3 %). Combined (male + female) seroprevalence of HBsAg was highest in the age group 31-40 years.

Figure 1: Age and gender wise Seroprevalence of HBsAg

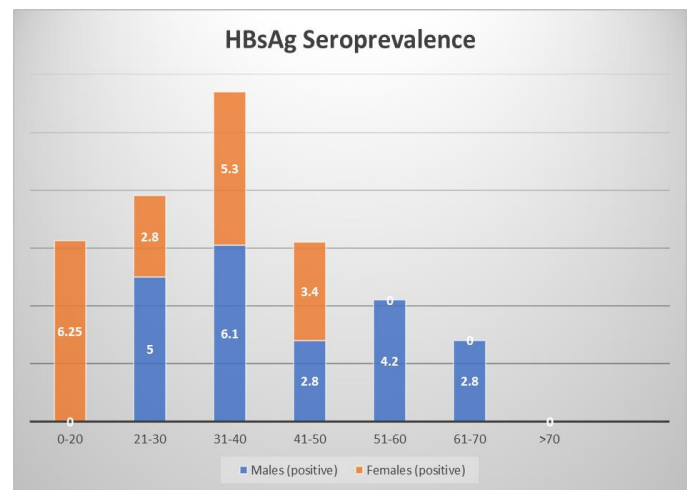
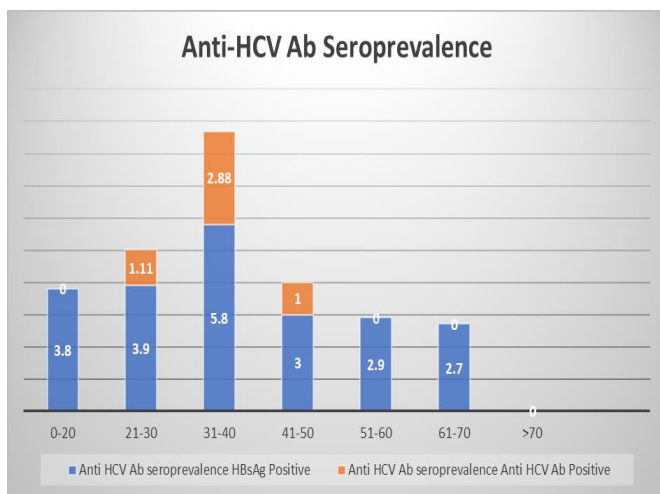


Table 2: Age and gender wise anti-HCV Antibody Seroprevalence

Age (years)	No. of males tested	Positive (males)	% (males)	No. of females tested	Positive (females)	% (females)	Total (%)
<=20	10	0	0	16	0	0	0
21-30	80	1	1.25	101	1	0.99	1.11
31-40	66	2	3.03	38	1	0.2.63	2.88
41-50	71	1	1.41	29	0	0	1.0
51-60	24	0	0	11	0	0	0
61-70	36	0	0	1	0	0	0
71 & above	15	0	0	2	0	0	0
Total	302	4	1.32	198	2	1.01	1.2

Table 2. shows the age and gender wise distribution of the total number of cases with HCV seroprevalence. The seroprevalence of HCV was found to be 1.2 %. The seroprevalence of HCV among males was found to be 1.32 % and among females was 1.01%. Seroprevalence of HCV in males was higher in the age group 31-40 years, 2/66 (3.03%), followed by the age group 41-50 years, 1/71 (1.41 %). Seroprevalence of HCV in females was higher in the age group 31-40 years, 1/38(2.63 %), followed by the age group 21-30 years, 1/101 (0.99 %). Combined (male + female) seroprevalence of HCV was highest in the age group 31-40 years, 3/104 (2.88%).

Figure 2: Age and gender wise anti-HCV Ab Seroprevalence



Discussion

Hepatitis B is a viral infection that attacks the liver and can cause both acute and chronic disease. The virus is most commonly transmitted from mother to child during birth and delivery, as well as through contact with blood or other body fluids during sex with an infected partner, unsafe injections or exposures to sharp instruments. Hepatitis B can be prevented by vaccines that are safe, available and effective. A safe and effective vaccine that offers 98% to 100% protection against hepatitis B is available. Hepatitis B infection acquired in adulthood leads to chronic hepatitis in less than 5% of cases, whereas infection in infancy and early childhood leads to chronic hepatitis in about 95% of cases. This is the basis for strengthening and prioritizing infant and childhood vaccination. WHO recommends that all infants receive the hepatitis B vaccine as soon as possible after birth, preferably within 24 hours. In a study regarding vaccination status among health care workers, it was observed that none of the vaccinated staff had hepatitis B infection while it was there in some of the unvaccinated staff.¹² Preventing hepatitis B infection averts the development of complications including chronic disease and liver cancer. Hepatitis B and C infections pose global public health problem. WHO estimates that 296 million

people were living with chronic hepatitis B infection in 2019, with 1.5 million new infections each year and 350 million are chronic carriers(WHO). In 2019, hepatitis B resulted in an estimated 820 000 deaths, mostly from cirrhosis and hepatocellular carcinoma (primary liver cancer).(WHO). The burden of hepatitis B infection is 18 million in the WHO South-East Asia Region. Based on the prevalence of HBV it is classified into high prevalence (>8), intermediate prevalence (2-7%), and low prevalence (<2%)¹

Prevalence of Hepatitis B

Table 6. shows the seroprevalence of HBV by ELISA. In the present study, the prevalence of hepatitis B in patients attending SRG hospital, Jhalawar was found to be 3.8% This is close to the prevalence rates of hepatitis B in developing countries. This prevalence rate is similar to the prevalence rate 2.99 % of the study by Poonam Sagar and Nand Kishor during 2021 in Rajasmand, Rajasthan.⁴ In 2020, Malhotra et al. in his study estimated the seroprevalence of hepatitis B to be 5.23% which is higher than the seroprevalence observed in the current study.¹¹ The seroprevalence was found lower in the studies done by Tripathi & Chakraverti (1.69%) in the year 2015, 0.87% in the study done by Smita Sood in the year 2010 and 0.56% in the study by Trupti B Naik.^{6,5,16}

Prevalence of Hepatitis C

Table 2. shows that the prevalence of hepatitis C in present study is 1.2 %. This seroprevalence of 1.2 % is almost similar (1.5%) to that found in the study conducted by Pratima Gupta in 2020.¹⁰ In India seroprevalence of HCV varies from 1.5% from Cuttack to 4.8% from Pondicherry. Study conducted by Malhotra et al. in 2020 showed the seroprevalence for Hepatitis C as 5.18%, which is quite high as opposed to that in the present study.¹¹ On the other hand, studies conducted by Tripathi (2015) and Smita Sood (2010) have shown low

seroprevalence of HCV infection of 0.4% and 0.28% respectively as compared to that of the present study (1.2%).^{6,5}

Prevalence of HBV and HCV

Both HBV and HCV were found predominantly in the age group 31-40 that corresponds to the reproductive age group.

Age and Gender wise seroprevalence of HBV

Table 9. in the present study, the highest seroprevalence among males (6.1%) was observed in the age group 31-40 years. In males the second highest seroprevalence was observed in the age group 21-30 years (5.0%). In females, the highest seroprevalence of HBV was observed in the age group of <20 years, which might be due to maternal transmission of HBV, whereas second highest seroprevalence was observed in the age group 31-40 years. Good amount of HBV seroprevalence was also found in the age groups 41-50 & 21-30 years which corresponds with the reproductive age group thus demonstrating sexual transmission as major route of transmission of the disease. A study done in 2016 by Kumar S. et al. supports the findings in the present study in which seroprevalence of hepatitis B is 2.37% among women of child bearing age and it correlates well with the high seroprevalence of 3-5% in the women of child bearing age in the present study, as a result of high sexual activity, which is a major cause of HBV transmission.¹⁴

Age and Gender wise seroprevalence of HCV

Table 15. shows age wise and gender wise seroprevalence of HCV. In the present study, HCV seroprevalence was highest in the age group 31-40 years in both males (3.03%) and females (2.63%). In males, it was followed by the older age group, that is 41-50 years, which goes well with the fact that HCV infection has a long incubation period. In females, another age group in which HCV was found prevalent in this study was 21-30

years. The females in age group 21-40 years are sexually active and so sexual transmission seems to be the major route of HCV transmission among females.

HBV and HCV coinfection

The present study found coinfection of HBV and HCV in 0.2% of the population. Rajeshwari Pillai (2020) in her study found that coinfection with HBV and HCV was seen in 0.10% of the individuals visiting the hospital, which is quite comparable to that found in this study (0.2%).¹⁶ The estimated worldwide prevalence of HBV/HCV coinfection is 1-15%.¹ Parenteral viral transmission is more likely to cause HBV /HCV coinfection. Most clinical studies have shown that progression of disease is faster in HBV-HCV coinfecting patients compared to those with mono-infection.²² In such patients, the risk of developing liver cirrhosis and hepatocellular carcinoma is higher than those with mono infection of either virus. HBV/HCV coinfection is preferably treated by or direct acting antiviral (DAA) based therapy, which was earlier treated by peginterferon (pegIFN). However, these drugs are more effective in HCV mono-infection clearance. Studies have suggested that HBV activity is suppressed when HCV is actively multiplying and the risk of HBV reactivation is higher and onset is earlier when HCV is cleared from the blood with antivirals.²³ So, viral replication in coinfecting cells is characterized by a dominance of HCV over HBV replication.²⁴

Conclusion

Hepatitis B virus is very contagious disease in the world and requires serious medical management as well as vaccination for proper control and prevention of the virus. Efforts should be made to educate more and more people, especially in the sexually active age group, and create awareness among them about the transmission of HBV and HCV viral hepatitis. Males show a higher

seroprevalence of HBsAg and anti HCV antibodies, probably due to more outgoing activities. They should be educated to take necessary precautions to avoid viral transmission. People should be motivated to adapt behavioural practices that minimize the risk of transmission of viral hepatitis. Efforts should be made to promote hepatitis B vaccination program among general population through awareness campaigns as this will reduce the chances of HBV transmission at an early age.

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