Original Article

Seropositivity of Hepatitis B and Hepatitis C Virus and Its Coinfection: A Hospital Based Study

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Abstract

Background: The spectrum of Hepatitis B virus (HBV) and Hepatitis C virus (HCV) infection ranges from self-limiting to acute fulminant to chronic hepatitis. The Laboratory diagnosis of HBV is by detection of various sero-markers and HCV is by indirect and direct tests. HBV and HCV have same modes of transmission and can be co-transmitted simultaneously or by superinfection with significant clinical implications. Hence, this study is undertaken to estimate seroprevalence of HBV and HCV and its coinfection among general population attending hospital.
Methods: This retrospective observational study was carried out from Jan to Dec 2022 on OPD and IPD specimens tested for HBV and HCV screening by HBsAg ELISA and anti-HCV ELISA.
Statistical analysis: Seroprevalence was calculated as proportion of samples positive amongst tested and Chi Square test was used for statistical analysis.
Result: Out of 36873 samples tested for HBV ELISA, 747 were positive with 2.0% seroprevalence, and 36500 samples tested for HCV ELISA, 361 were reactive with 0.99% seroprevalence. Male to female ratio for HBV was 2:1, HCV 1.2:1 and for coinfection 4.6:1. The most affected age group was 18-40 years. There is a significant difference in sample seropositivity between OPD and IPD. In co-infection maximum positivity was from Nephrology, followed by GI medicine.
Conclusion: Emphasis on awareness about preventive measures, and curtailing high risk behaviours among young adults should be stressed. Effective HBV Vaccination coverage with more data on HBV and HCV coinfection needs to be obtained.

Keywords: serology; ELISA; hepatitis; coinfection

Introduction

Hepatitis B virus (HBV) is a DNA virus of family hepadenaviridae that primarily infect hepatocytes. The spectrum of disease ranges from self-limiting to acute fulminant to chronic hepatitis. Hepatitis C virus (HCV) is a small, enveloped, positive sense single stranded RNA virus belonging to flaviviridae family causes both acute (30%) who clears the virus within 6 months without treatment and (70%) chronic infections.[1] The Laboratory diagnosis of HBV infection is based on clinical symptoms along with detection of various serological markers, i.e, HBsAg, antiHBs, antiHBc, HBeAg, and antiHBe. HBsAg is the most prominent serological marker that appears in blood, even before biochemical markers and persists through acute phase of disease, hence used as most common marker for diagnosis among patients and carriers. HCV diagnosis is done by indirect serological tests which detect anti-HCV using rapid and ELISA test and direct tests that detects quantification of viral RNA particles.[2] HBV and HCV infections are major healthcare problem. WHO classified HBV prevalence into high endemic (>8%), intermediate endemic (2-7%) and low endemic (<2%), India falls in intermediate range with 40 million patients are projected to be chronically infected. The HCV seroprevalence has ranged from 0.09 – 2.02% with 10-20 million people with active HCV infection are living in India.[3] The co-infection of HBV and HCV is not

known in the present setup hence, this study is undertaken with the aim and objective to estimate seroprevalence of HBV and HCV infection and its coinfection among general population attending hospital.

Materials and Methods

This retrospective and observational study was carried out from Jan 2022 to Dec 2022 in a tertiary care teaching institute. Patients of both sexes and all age group who visited out-patient department (OPD) and indoor patient department (IPD) and were advised HBV and HCV screening on the basis of clinical findings or as a part of pre-operative or ANC (antenatal care) check-up were included.

HBsAg ELISA - This is direct, solid phase enzyme linked immunoassay. The microwells are coated with monoclonal anti-HBsAg antibodies. The assay was performed as per manufacturer's instructions; Blank = 0, Negative control (NC) <0.5, Positive control (PC) >1.0, Cut off = Mean of NC + 0.1, Grey zone = Cut off X 0.9. If found reactive with values above cut-off, confirmation done by repeating in duplicate with the same kit. The values lying in Grey zone were considered as indeterminate or equivocal, and retesting was done for them with the same kit (Merilisa HBsAg, Manufactured by Meril diagnostics, India).

Anti-HCV ELISA – This is a 3rd generation Sandwich format microplate enzyme linked immunoassay for the detection of antibodies of HCV. The microwells are coated with HCV specific recombinant proteins, i.e core, NS 3,4,5 derived from HCV RNA. The assay was performed as per manufacturer's instructions; Blank = 0, Negative control (NC) <0.5, Positive control (PC) >1.0, Cut off = Mean of NC + 0.2, Grey zone = Cut off X 0.9. If found reactive with values above cut-off, confirmation done by repeating in duplicate with the same kit. The values lying in Grey zone were considered as indeterminate or equivocal, and retesting was done for them with the same kit (Merilisa HCV, Manufactured by Meril diagnostics, India).

Statistical analysis- Seroprevalence was calculated as proportion of samples that tested positive for HBsAg and anti-HCV antibody from those that were tested. Chi Square test was used for statistical analysis. P<0.05 were considered statistically significant. This study was approved by the institutional ethics committee (IEC). The waiver of patient consent was granted by the IEC.

Results

Out of 36873 samples tested for HBV ELISA, 747 were positive with 2.0% seroprevalence, and out of 36500 samples tested for HCV ELISA, 361 were reactive with 0.99% seroprevalence. Gender distribution with male (M) to female (F) ratio for HBV was 2:1 and HCV 1.2:1. The most common affected age group for both HBV and HCV infection was 18-40 years [Table-1, Table-2]. There is a significant difference in sample seropositivity between OPD and IPD [Table-3]. For Coinfection, M:F ratio was 4.6:1 and commonest age group affected was 18-40 years [Table-4]. The highest positives for OPD were from Medicine, followed by GI Medicine and Obstetrics & Gynaecology, and for IPD were from Medicine, followed by Orthopaedics and Haematology. For co-infection maximum positivity was from Nephrology, where all nine patients were indoor, followed by GI medicine, where all four patients were from OPD [Figure-1, Figure-2].

Table 1: Distribution of OPD/IPD, age and gender in HBV ELISA positive samples (n=747)

HBV Age (yrs)	OPD M	OPD F	IPD M	IPD F	Total
<18	5	3	1	1	10
18-40	109	89	132	55	385
41-60	109	34	102	46	291
>61	21	5	21	14	61
Total	244	131	256	116	747

*p < 0.05; The p-value is 0.0000, statistically significant (Chi square test)

Table 2: Distribution of OPD/IPD, age and gender in HCV ELISA reactive samples (n=361)

HCV Age (yrs)	OPD M	OPD F	IPD M	IPD F	Total
<18	5	2	8	4	19
18-40	32	40	45	45	162
41-60	44	21	44	26	135
>61	7	8	15	15	45
Total	88	71	112	90	361

*p < 0.05; The p-value is 0.0001, statistically significant (Chi square test)

Table 3: Distribution of OPD and IPD in HBV and HCV ELISA samples

	Positive	Negative	Total
Total OPD samples	534	29335	29869
Total IPD samples	574	42930	43504
	1108	72265	73373

*$p < 0.05$; The p-value is 0.0000, statistically significant (Chi square test)

Table 4: HBV-HCV coinfection with OPD/IPD, age and gender distribution

Co-infection HBV HCV Age (yrs)	OPD M (N=534)	OPD F (N=534)	IPD M (N=574)	IPD F (N=574)	Total (N=1108)
<18	0	0	0	0	0
18-40	4	1	4	1	10
41-60	1	0	4	1	6
>61	0	0	1	0	1
Total	5	1	9	2	17

*The p-value is 0.0470, p value < 0.05 statistically significant (Chi square test)

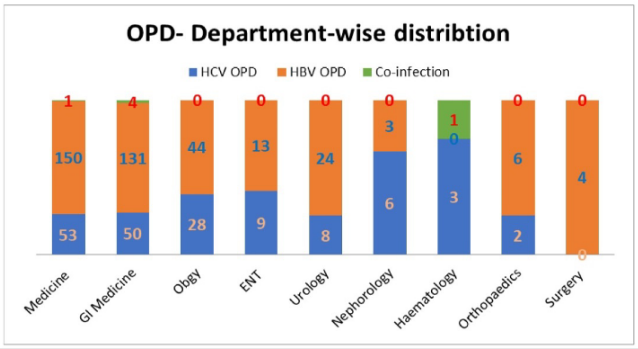

Figure 1: OPD- department wise distribution of ELISA positive samples (n=534).

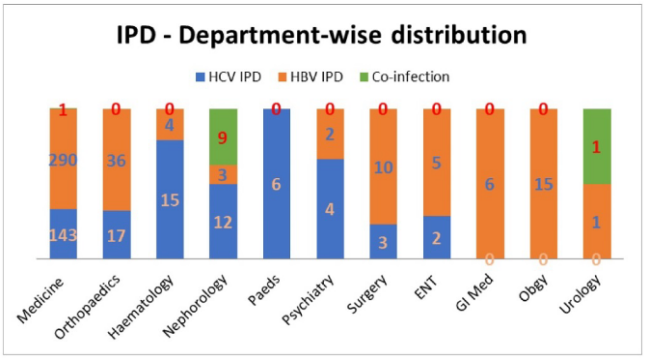

Figure 2: IPD- department wise distribution of ELISA positive samples (n=574).

Discussion

The seroprevalence of HBV and HCV in present study was 2% & 0.99% respectively. The HBV seroprevalence was similar as other studies by Singh et al,[4] Patil et al,[1] and Kulkarni et al[5] who reported it as 2.05%, 2.25%, and 2.34% respectively, while a lower seroprevalence was reported as 1.73%, 1.63% and 1.2% by Sood et al,[6] Quadri et al,[7] Naqshabandi et al[8] among hospital based general population. HCV seroprevalence of 0.99%, is similar to the meta-analysis by Gupta et al[9] where pooled prevalence of anti-HCV was 0.85% (0.00-3.98%), and Goel et al[10] reported 0.6% (0.20-1.20%). The varied HCV seroprevalence of HCV antibody was reported as 0.52% by Parimala TV in Tumkuru, Karnataka,[11] 1.06% by Rahaman J et al in Kolkata[12] and 2.3% by Kumar et al in Uttar Pradesh.[13] The variation in seroprevalence may be due to diverse geographical localities, difference in study population, study designs, sample size, data collection methods, diagnostic methods, age group, socio-economical levels, immunity variation, and behavioural practices. Mumbai being a cosmopolitan city with lot of migrants residing, socio-demographic factors affecting seroprevalence could be high

density living conditions, inadequate sanitation, and high-risk medical practices conducted by unauthorized personnel in slum areas, also high risk practices as sharing sharp objects like razors and blades by roadside barbers or unsafe tattooing and ear/skin piercing in community settings increases susceptibility. Resistance showed by certain communities and localities for vaccination despite the availability of HBV vaccines, may also be an important contributory factor.

In the current study, the male-female ratio for HBV was 2:1, with 67% males and 33% females and for HCV 1.2:1, with 55.4% male and 44.6% females. The gender based difference was not significant. The most common affected age group for both HBV and HCV infection was 18-40 years [Table-1, Table-2]; similar results were reported by Sigh et al,[4] and Kulkarni et al.[5] In contrast, Patil et al,[1] and Bhaumik et al[15] reported it in age groups of 51-60 years and 61-80 years. In this study, the reason for high positivity in young youth and middle aged group could be due to promiscuous behaviour as exposure to multiple sex partners, unsafe sexual practices and IV drug abuse.

In this study, there is a significant difference in sample seropositivity between OPD and IPD. Centers for Disease Control and Prevention (CDC) has recommended HBsAg and HCV testing for pregnant women, infants born to positive mothers, household contacts and sex partners of infected persons, people born in geographical regions with $\geq$2% HBsAg prevalence, injection drug abusers, homosexuals, and people who have been exposed to blood and body fluids such as needle stick injuries in healthcare workers and sexual assault.[16, 17] Hence patients visiting OPD for general check-up, for cataract removal or other elective surgeries had been screened for HBV and HCV. Pregnancy and delivery are physiological process for the women devoid of any signs or symptoms related to the infection are screened under ANC check-up protocol. Chronic nature of the disease and its accidental discovery during routine screening may be the reason for high seropositivity in OPD samples [Table-3]. The highest positive for OPD was from Medicine, followed by GI Medicine and Obstetrics & Gynaecology, and for IPD it was from Medicine, followed by Orthopaedics and Haematology.

In this study HBV and HCV co-infection was 1.5% (17/1108), meta analytical study by Desikan P et al reported prevalence of 1.89%[18] while Agarwal L et al reported 0.16%.[19] Saravanan et al reported 5.9% in chronic liver diseases[20] while Malhotra R et al reported 0.8% in hemodialysis patients.[21] In present study, maximum positivity for co-infection was from Nephrology, followed by GI medicine [Figure-1, Figure-2]. In these coinfected patients' disease was more crippling with 59% had chronic renal disease and were receiving multiple haemodialysis while 41% had hepatic involvement, in form of hepatosplenomegaly, liver failure or hepatic encephalopathy. M:F ratio was 4.6:1 with 18-40 years was the commonest age group affected [Table-4]. Higher male-to-female (4.6:1) ratio could be due to biological, hormonal, and behavioural factors. Testosterone increases HBV transcription and promotes liver cell damage, making males more susceptible to developing chronic, severe infections. Behavioural and environmental factors as higher alcohol consumption, substance abuse, incarceration and transactional sex may also be associated with male preponderance, while oestrogen in female protects liver cells.[22, 23]

HBV and HCV have same modes of transmission as sexual, IV drug use, blood transfusion and vertical transmission. Several studies all over the world showed HBV and HCV viruses can be co-transmitted simultaneously or by superinfection, i.e. one virus is acquired in a patient with pre-existing chronic infection by other virus. Occult HBV infection (OBI) is the presence of HBV DNA (often at low levels) in serum or liver tissue without detectable HBsAg mainly in patients with chronic HCV leading to rapid progression of liver fibrosis toward cirrhosis and development of hepatocellular carcinoma.[24] A critical concern is the reactivation of hidden HBV infection during or after starting treatment for HCV with directly acting antivirals (DAA), which may occur in 25%–87.5% of cases, sometimes leading to severe hepatitis.[25] Co-infection with HBV and HCV has significant clinical consequences hence, more studies needs to be conducted across the country in the routine laboratory set-ups and also among the specific settings such as blood donors, injecting drug users, pregnant women, patients with chronic liver disease, and patients on haemodialysis. This will help in knowing the actual data of coinfection in general and specific groups and will help to formulate prevention and management strategies.

Limitations of the study

Only using ELISA for diagnosis without confirmatory PCR for active infection is common concern in seroprevalence studies.

Conclusion

To conclude, in this study HBV seroprevalence was 2%, and HCV prevalence was 0.99%. Economically productive age group of 18-40 years was most affected, so emphasis on awareness about preventive measures for transmission, and curtailing high risk behaviours among young adults should be stressed. Effective HBV vaccination coverage with more data on HBV and HCV coinfection needs to be obtained across different geographic locations and specific settings.

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