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Original Research Article

EPIDEMIOLOGICAL INSIGHTS INTO HIV, HBV, AND HCV CO-INFECTIONS IN A TERTIARY CARE CENTRE: A CROSS-SECTIONAL STUDY

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Abstract

Background: Hepatitis B virus (HBV) and Hepatitis C virus (HCV) co-infection is prevalent among individuals living with Human Immunodeficiency Virus (HIV) due to shared transmission routes, including intravenous drug use, sexual contact, and perinatal transmission. This study investigates the prevalence of HBV and HCV infections among HIV-infected patients, focusing on transmission routes and analyzing risk factors for co-infections, with a particular emphasis on CD4 count and HBV/HCV viral load. **Materials and Methods:** This descriptive cross-sectional study was conducted at the Department of Microbiology, Government Medical College and Hospital, Thiruvallur during April to October 2023. A total of 2198 People living with HIV (PLWH) on highly active antiretroviral therapy (HAART) enrolled at the ART Centre were analysed for HBV and HCV co-infection. **Result:** Out of the 2198 HIV positive patients on HAART screened for co-infection 65 (2.9%), were identified as co-infected with hepatotropic viruses. Among these, 59 individuals (2.6%) were seropositive for HBV, and 6 individuals (0.2%) were seropositive for HCV. No patients with triple infection of HBV, HCV, and HIV were observed. Among the 65 co-infected patients, 34 were males, 30 were females, and 1 was transgender, resulting in a male-to-female ratio of 1.13:1. Males were more commonly affected with co-infection (52.3%). The age group between 25 to 40 years showed a significant association with HBV and HCV infection (40%). Heterosexual contact (89.2%) was the primary mode of acquiring HBV and HCV infection, followed by mother-to-child transmission (3.07%), MSM (1.5%), blood transfusion (1.5%). CD4+ T-lymphocyte count less than 250 was observed in 10 (15.3%) out of 65 co-infected patients. Plasma detection of HBV DNA was achieved in 31 individuals out of 59 HBsAg-positive samplesin our cohort and HCV RNA was detected and quantified in 3 out of 6 Anti-HCV-positive samples. **Conclusion:** This study underscores the high risk of HBV and HCV co-infections among HIV patients, necessitating routine screening and monitoring.

INTRODUCTION

Hepatitis B virus (HBV) and Hepatitis C virus (HCV) co-infection is prevalent among individuals living with Human Immunodeficiency Virus (HIV) due to shared transmission routes, including intravenous drug use, sexual contact, and perinatal transmission.[1,2]The impairment of cell-mediated immunity by HIV intensifies the replication of hepatotropic viruses. Although co-infection accelerates the clinical course of HIV, individuals

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co-infected with either HCV or HBV exhibit a more aggressive progression of liver disease.[3]

HIV-induced immunosuppression significantly impacts the natural history of HBV infection, leading to higher HBV DNA levels, increased reactivation rates, and reduced spontaneous clearance of HBsAg. Concomitant HIV infection elevates the risk of morbidity and mortality from HBV-related complications such as cirrhosis, end-stage liver disease, and hepatocellular carcinoma.[4]

HIV-infected individuals are less likely to naturally clear HCV infection, leading to more severe liver disease progression.In patients with HIV/HCV co-infection, end-stage liver disease remains a major cause of death. HIV infection increases the levels of HCV viremia by 2–8-fold. HIVco-infection also worsens the histological course of HCV infection by increasing and accelerating the risk of cirrhosis or leading to rare but lethal fibrosing cholestatic hepatitis.[5]

A systematic review and meta-analysis highlights the global burden of HBV infection in People Living With HIV (PLWH), emphasizing disparities according to region, development level, and country HIV prevalence.[6] Most studies on HIV/HBV and HIV/HCV co-infection have been reported from Western countries and understanding the co-infections is crucial in Asian countries due to the higher background prevalence of HBV and HCV.[7-9]

On this background this study investigates the prevalence of HBV and HCV infections among HIV-infected patients, focusing on transmission routes and analyzing risk factors for co-infections, with a particular emphasis on CD4 count and HBV/HCV viral load.

MATERIALS AND METHODS

Study Design: This is a descriptive cross-sectional study aims to explore the regional variations, risk behaviors, and demographic associations of HIV with HBV and HCV co-infection.

Sample Size: A total of 2198 participants were included in the study.

Study Site: The study was conducted at the Department of Microbiology, Government Medical College and Hospital, Thiruvallur during April to October 2023.

Ethical Approval: The study received approval from the Institutional Ethics Committee. Informed consent was obtained from the participants assuring confidentiality and anonymity.

Study Population:

Inclusion Criteria

People living with HIV (PLWH) on highly active antiretroviral therapy (HAART) enrolled at the ART Centre.

Exclusion Criteria

Participants with pre-existing hepatitis B and/or hepatitis C before the diagnosis of HIV.

Data Collection: Data collection involved obtaining detailed information from the study participants, including demographics (name, age, gender, residential area), and a comprehensive history of sexual activities, blood transfusion, intravenous drug abuse.

Sample Collection: Venous blood (2 ml) was collected in a plain red vacutainer from all the study participants under aseptic precaution. Serum was separated by centrifugation 1500rpm for 5 minutes and tested for HBsAg and anti-HCV antibodies using immunochromatography rapid detection method and confirmation by enzyme-linked immunosorbent assay (Erba Lisa, India)following manufacturers' instructions.

From patients tested positive for the co-infections, 2 ml of blood was collected into a K2EDTA vacutainer for HBV-DNA and HCV-RNA testing by quantitative polymerase chain reaction (qPCR).

About 1 ml of venous blood was tested for CD4+ T cells count using FACS Calibur (BD, USA).

Laboratory Testing: HBV quantification was assessed by testing 200ul of plasma sample with qPCR(Mylab Discovery Solutions Patho Detect TMHBV quantitative PCR kit), with high specificity and accuracy targeting conserved target sequence of S gene and detection by target specific probe for HBV DNA.

HBV DNA analytical detection threshold of this test is 5 IU/mL.Whole nucleic acid isolation process done with steps of sample lysis, DNA binding to the silica columns, washing and elution. The purified DNA obtained was subjected to the detection and quantification. Detection was carried out using an automated Real-time PCR system (QIAGEN).

Interpretation: The quantification range of this assay is 10 to 108 IU/mL.An "Undetected" result indicates that HBV DNA was not detected in the specimen.

A result of <10 IU/mL indicates that HBV DNA is detected, but the HBV DNA level present cannot be quantified accurately below this lower limit of quantification of this assay. Upper limit of quantificationis> 108IU/mL.

HCV-RNA testing (Mylab Discovery Solutions Patho Detect TM HCV quantitative PCR test) involved specimen preparation, utilizing reverse-transcriptase reaction to convert RNA into cDNA, polymerase chain reaction for the amplification of specific target sequences (5' UTR region) and target specific probes for the detection.The analytical detection limit of the kit is 40 IU/mL and the range of detection is from 40 IU/mL to 7*107 IU/mL.

CD4 T cells counting was done by adding 20ul of whole blood to the monoclonal antibody reagent,incubated for 15mins at room temperature in dark place ,the flourochrome labelled antibodies in the reagent bind specifically to leucocyte surface antigens. The stained samples are then treated with BD FACS(USA) lysing solution,which lyses erythrocytes under gentle hypotonic conditions while preserving the leucocytes and the CD4 T cells

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are acquired on the flow cytometer and the stained cells are analysed.

Statistical Analysis: Continual variables are presented as minimum and maximum values and as median together with quartile IQR. Categorical variables are presented as the number and percentage of patients in whom a given variable was found.

RESULTS

Out of the 2198 HIV positive patients on HAART(Tenofovir, Lamivudine and Dolutegravir) screened for co-infection 65 (2.9%), were identified as co-infected with hepatotropic viruses. Among these, 59 individuals (2.6%) were seropositive for HBV, and 6 individuals (0.2%) were seropositive for HCV. No patients with triple infection of HBV, HCV, and HIV was observed.

[Table 1] provides details on the demographics and risk behaviors of HIV patients with HBV and HCV co-infections.Among the 65 co-infected patients, 34 were males, 30 were females, and 1 was transgender, resulting in a male-to-female ratio of 1.13:1. Males were more commonly affected with co-infection (52.3%). The age group between 25 to 40 years showed a significant association with HBV and HCV infection (40%). Heterosexual contact (89.2%) was the primary mode of acquiring HBV and HCV infection, followed by mother-to-child transmission (3.07%), MSM (1.5%), blood transfusion (1.5%). In 3 (4.6%) of them no clue of the route of transmission was obtained even after thorough probing and counselling.

CD4+ T-lymphocyte count less than 250 was observed in 10 (15.3%)out of 65 co-infected patients. However, CD4 count was not significantly reduced in patients with HIV/HBV and HCV co-infection, as detailed in [Table 2].

Plasma detection of HBV DNA was achieved in 31 individuals out of 59 HBsAg-positive samplesin our cohort, and in 18 HBV viral load was less than 500IU/ml and in 7 it was >100,000 IU/mlas shown in [Table 3]. HCV RNA was detected and quantified in 3 out of 6 Anti-HCV-positive samples. By qPCR2 person were quantified to be<100,000IU/mland one was>100,000IU/ml.

Table 1: Demographic and risk behaviours of HIV patients with HBV and HCV Co-infection (n=65)

Characteristics				**Numbers (%)**
Age	**Males (n=34)**	**Females (n=30)**	**Transgender(n=1)**	
25-40	17	22	1	40(61.5)
41-60	15	8		23(35.3)
>60	2	-		2 (3.07)
Mode of transmission				
Heterosexual contact	33	24	1	58(89.2)
Mother to child transmission	2	-		2(3.07)
MSM	1	-		1(1.5)
Blood transfusion	-	1		1(1.5)
Unknown	1	2		3(4.6)

Table 2: Prevalence of HIV HBV and HCV Co-infection in the study population and CD4 count status.

Characteristics	**HIV/HBV Coinfection(n=59)**	**HIV/HCV Coinfection(n=6)**	**Total (n=65)**
Prevalence	59/2198(2.6%)	6/2198(0.2%)	
Gender			
Male	30(50.8%)	4(66.6%)	34(52.3%)
Female	28(47.4%)	2(33.3%)	30(46.1%)
Transgender	1(1.6%)	-	1(1.6%)
CD4 Count(cells/mm3)			
<50	-	-	-
50-100	1(1.6%)	-	1(1.5%)
100-250	6(10.1%)	3(50%)	9(13.8%)
250-500	25((42.3%)	2(33.3%)	27(41.5%)
>500	27(45.7%)	1(16.6%)	28(43.07%)

Table 3: Quantification of HBV DNA

HBV DNA(59)	**<250IU/mL**	**250 -500IU/mL**	**500- 1000IU/mL**	**1000-100,000 IU/mL**	**>100,000IU/mL**
Detected and quantified(31)	9	9	4	2	7
Detected not quantified(28)	-	-	-	-	-

DISCUSSION

This cross-sectional study delves into the prevalence and associated factors of Hepatitis B (HBV) and Hepatitis C (HCV) co-infections among HIV-infected individuals. As per Joint United Nations Programme on HIV/AIDS (UNAIDS) estimates, in 2016, with an adult HIV prevalence of 0.8% and

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considerable variation between countries, 36.7 million (30.8–42.9 million) people were estimated to be living with HIV globally. India has a low HIV prevalence of 0.22%.[5] The country's endemic is concentrated among high-risk groups and is heterogeneously distributed with wide geographic variations in the vulnerabilities that drive the epidemic.[9] Even with this low prevalence, in terms of absolute numbers, India has the third highest burden of HIV in the world with an estimated 2.14 million people living with HIV, 87,000 estimated new infections, and 69,000 AIDS related deaths annually.[10]Co-infections of HIV patients with HBV and HCV are major public health problems, contributing to the emerging burden of HIV associated hepatic mortality. Chronic viral hepatitis has emerged as an important cause of morbidity and mortality among HIV-positive patients resulting in an increase in inpatient healthcare utilization and an evolving discussion on the use of liver transplantation in these patients.[11]

The prevalence of HBV and HCV co-infections in HIV has been variably reported in different studies and geographical location is one of the major determinants of prevalence. The prevalence of HBV co-infection varies from 5 to 7% in low endemic areas. In intermediate and high endemic areas, it varies from 6 to 20%.The prevalence of HCV co-infection varies from 9 to 16%. This is much more common in certain groups, such as people who inject themselves with drugs.[11]

We found HBV co-infection in 2.6% of HIV-positive individuals which is consistent with the 2.3% of co-infection in Chennai reported by Saravanan et al. The HBV co-infection was higher in our study compared to 1.6% reported in Lucknow.[12,13]

The HCV co-infection in the present study was 0.2%. In a study conducted in the central part of India, HIV/HCV co-infection was found to be 0.84%.[14] The difference may be due the difference in riskbehavior.Co-infection of HBV and HCV with HIV (triple infection) was found to be nil which is similar to the study by Kalyani CS et al.[15]

The age group between 25 to 40 years showed a significant association with HBV and HCV infection (40%) in this study which is the common age group for HIV positivity in India and similar to a study by Naval Chandra et al which investigated co- infection in HIV infected patients.[16] This study showed male predominance (52.3%) amongst HIV infected patients which correlated with studies of Kalyani CS et al,[15] (58%) Sanjiv Ahuja et al. (62%).[17]

In this study, the route of transmission of HIV in most of our patients was heterosexual contact which is similar to the study by Gupta S et al.[18]

In a study by Matthews GV et al, analyzing the factors significantly associated with detectable HBV DNA included CD4 count <200 cells/mm3 (OR, 2.21 [95% CI, 1.30–3.77]),[19] The depleting CD4+ T cells count is a marker of immune dysfunction and HIV progression and indicators of acquiring multiple opportunistic infections and co-infections.[20]In this study CD4+ T-lymphocyte count less than 250 was observed in 10(15.3%) out of 65 co-infected patients. CD4+T lymphocyte count <250 cells/µL was seen in 07/59 (12%), 03/6 (50%), of HBV co-infected, and in HCV co-infected patients, respectively. The same proportions were found to be 26.6%, and 100% in a study by Riddhi Pradhan et al.[14]

In this study viremia was detected in 31out of 59 (52.5%) HIV/HBV co-infected patients on HAART and in 18 HBV viral load was less than 500IU/ml and in 7 it was >100,000 IU/mland HCV RNA was detected and quantified in 3 out of 6 HIV/HCV co-infected patients. In contrast to this only 16/56 (28.6%), of the co-infected patients on treatment had detectable level of plasma HBV DNA as per a Brazilian study on Hepatitis B viremia in HIV-coinfected individuals under antiretroviral therapy.

A quantitative result expressed in IU/mL indicates the degree of active viral replication in the patient. High level of HBV DNA is an independent risk factor for development of cirrhosis and HCC.As DNA levels have a fluctuating nature, monitoring HBV DNA levels over time is important for assessing disease progression or monitoring a patient's response to therapy.

This study highlights the complex landscape of HIV, HBV, and HCV co-infections in India by uncovering the regional variations, risk behaviors, and demographic associations.

Future plan- A subsequent longitudinal follow up study will be undertaken to evaluate the impact of HAART on HBV and HCV co-infections in HIV and found the proportion of patients with HIV who developed chronic HBV.

CONCLUSION

This study underscores the high risk of HBV and HCV co-infections among HIV patients, necessitating routine screening and monitoring. Targeted interventions and region-specific strategies are essential to address the heterogeneity of the epidemic. The study calls for a longitudinal follow-up to assess the impact of Highly Active Antiretroviral Therapy (HAART) on co-infections and emphasizes the importance of early diagnosis and treatment for improved clinical outcomes.

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