Section: Microbiology

Original Research Article

A STUDY ON THE SEROPREVALENCE OF HBV AND HCV COINFECTION IN HIV INFECTED INDIVIDUALS ATTENDING JNIMS HOSPITAL, MANIPUR

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Abstract

Background: In the context of effective antiretroviral therapy (ART), liver disease has emerged as a major cause of morbidity and mortality in HIV-infected persons. Further, co-infection with viral hepatitis may complicate the delivery of ART by increasing the risk of drug-related hepatotoxicity and impacting the selection of specific agents. Therefore, this study was conducted to determine the seroprevalence of HBV and HCV coinfection amongst the newly detected treatment naïve HIV infected individuals. Aim: 1.To study the seroprevalence of HBV and HCV coinfection in HIV infected individuals. 2. To study the common routes of transmission of HBV and HCV for developing strong preventive measures. **Materials and Methods:** A cross-sectional study carried out in the Dept. of Microbiology, JNIMS, Imphal from 1st September 2015 to 31st August 2017 amongst the 170 newly diagnosed (treatment naïve) HIV infected clients attending (ICTC), JNIMS. HIV detection was done according to Strategy III as per NACO guidelines 2015 by two rapid tests and one ELISA i.e SD bioline HIV1/2 3.0, HIV 1/2 Trispot test Kit & Qualisa HIV1+2. For HBV & HCV detection, screening was done with using rapid diagnostic kit (SD Bioline HBsAg, SD bioline HCVAb) and reactive tests were tested with ELISA (Hepalisa HBV, Qualisa HCV). Quantitative PCR (CobasTaqman HBV test) & (COBAS TaqMan HCV Test) were performed for the positive samples as per manufacturer's instructions. Statistical test: Fischer's Exact test & t test were done to see the association between the different variables and coinfection. **Result:** The seroprevalence of HBV-HIV coinfection was 5.9%. The seroprevalence of HCV-HIV coinfection was 14.1%. The seroprevalence of HIV-HBV-HCV was 2.9%. 22.3% of the HCV co-infected participants were male and only 1(1.5%) was female and difference in gender was statistically significant. Though the difference in gender was statistically insignificant for HBV coinfection. 30.4% of the HCV co-infected participants were either IVDUs or IVDUs who also had multiple sexual partners, which was statistically significant. But statistically insignificant for HBV coinfection. Amongst the 24 HCV coinfected participants, 31.6% were unmarried, 8.6% were married and 22.2% were widow/widower. The difference in the marital status was statistically significant for HCV coinfection, though statistically insignificant for HBV coinfection. **Conclusion:** In this study, HBV/HCV coinfection was seen more commonly in IVDUs and also those IVDUs who also had multiple sex partners. Therefore, this study emphasises on the need to conduct large scale studies to understand the burden better.

INTRODUCTION

Manipur is one of the six high prevalence states of India and Manipur is among the states which have shown high levels of HIV prevalence among injecting drug users (IDUs), MSM and FSW.[1,2] In the context of effective antiretroviral therapy (ART), liver disease has emerged as a major cause of

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morbidity and mortality in HIV-infected persons. Further, co-infection with viral hepatitis may complicate the delivery of ART by increasing the risk of drug-related hepatotoxicity and impacting the selection of specific agents.[3]

Aims and Objects

1. To study the seroprevalence of HBV and HCV coinfection in HIV infected individuals.
2. To study the common routes of transmission of HBV and HCV for developing strong preventive measures.

MATERIALS AND METHODS

Study Design: The study was a cross-sectional study.

Study Area

The study was carried out at the Department of Microbiology, Jawaharlal Nehru Institute of Medical Sciences Hospital, Porompat, Imphal –East Manipur.

Study Population

The study population included newly diagnosed HIV infected clients attending Integrated Counselling and Testing Centre (ICTC), JNIMS (referred from the Out Patient Department, In Patient Department, and non-governmental organisations and voluntarily testing).

Study Period

From 1st September 2015 to 31st August 2017

Sample Size:

Formula: $n=\frac{z^2pq}{d^2}$, n = sample size, p = 11.1% (prevalence from previous study in Manipur, India) q = 100-11.1=88.9, z = 1.96 at 95% Confidence Interval, d (allowable error) = 5%

$n=\frac{1.96^2\times11.1\times88.9}{5^2}$=151, Assumming 10% non-response rate, n =170

Sample Design: consecutive non-duplicate

Operational definition:

1. A case of HIV and HBV coinfection: HIV positive as per strategy III NACO guideline and HBV positive with two different tests of different principles with or without quantitative detection by PCR.
2. A case of HIV and HCV coinfection: HIV positive as per strategy III NACO guideline and HCV positive with two different tests of different principles with or without quantitative detection by PCR.
3. A case of HIV coinfection with both HBV and HCV: HIV positive as per strategy III NACO guideline and HBV & HCV positive with two different tests with different principles respectively with or without quantitative detection by PCR.

Tools and Procedures

Sample Collection

Blood Collection was done as per NACO guidelines.[4]

5 ml of blood was collected aseptically after cleaning the venepuncture site with 70 % alcohol and immediately transferring into a sterile red top evacuated tube which was labelled with atleast two patient identifier (patient name/ID/Laboratory number). Separation of Sera was done as per NACO guidelines.[4] Storage of sera was done as per NACO guidelines.[4] Tests done for Detection for HIV detection was done according to Strategy III as per NACO guidelines 2015 by two rapid tests and one ELISA i.e SD bioline HIV1/2 3.0, HIV 1/2 Trispot test Kit & Qualisa HIV1+2. For HBV & HCV detection, screening was done with using rapid diagnostic kit (SD Bioline HBsAg, SD bioline HCVAb) and reactive tests were tested with ELISA (Hepalisa HBV, Qualisa HCV). Quantitative PCR (CobasTaqman HBV test) & (COBAS TaqMan HCV Test) were performed for the positive samples as per manufacturer's instructions.

Consent

Consent in the form of written was taken from all the participants.

Analysis of Data

Prevalence data were calculated. Chi-square test and Fisher's exact test (categorical variables) were used to compare variables and to evaluate the association between the presence of HIV-HCV coinfection and associated factors and HIV-HBV coinfection and associated factors. Differences were considered statistically significant, when p value was < 0.05.Statistical evaluations were performed using SPSS (version 11.0; SPSS inc., Chicago, USA, 1999).

RESULTS

Table 1. Shows background socio-demographic profile of the participants

Variable	Categories	Number (%)
Age groups (yr)	≤25	12 (7.1)
	26-35	44(25.9)
	36-45	79(46.5)
	46-55	26(15.3)
	56& above	9(5.3)
Gender	Female	67(39.4)
	Male	103(60.6)
Religion	Hindu	114(67.1)
	Muslim	15(8.8)
	Christian	39(22.9)
	Others	2(1.2)

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Marital status	Married	116(68.2)
	Unmarried	38(22.4)
	Widow/widower	9(5.3)
	Divorce/separated	7(4.1)

Table 2. Showing risk factors of the participants for HIV infection

Risk factors	**IVDU**	**67(39.4)**
	IVDU & multiple sex partners	2(1.2)
	MSM	4(2.4)
	FSW	1(0.6)
	Spouse positive for HIV	43(25.3)
	Spouse positive & multiple sex partner	4(2.4)
	Multiple sex partner	34(20.0)
	Bood & blood products	1(0.6)
	Others	5(2.9)
	No risk revealed	9(5.3)

IVDU- intravenous drug users, MSM- men having sex with men, FSW-female sex worker.

Table 3. t test showing comparison of mean age by HCV status

HCV status	**Age (yr) Mean(SD)**	**Mean difference**	**t-value**	**p-value**
Positive (24)	38.5(8.24)	-0.430	-0.215	0.830
Negative (146)	39.0(9.23)			

Table 4. Showing association between HCV status and gender

Gender	**HCV status**		**p-value**
	Positive N (%)	Negative N (%)	
Female	1(1.5)	66(98.5)	0.0001
Male	23(22.3)	80(77.7)	

*Fischer's exact test.
The difference in gender was found to be statistically significant.

Table 5. Showing Association between HCV status and marital status

Marital status	**HCV status**		**p-value**
	Positive N (%)	Negative N (%)	
Married	10(8.6)	106(91.4)	0.004*
Unmarried	12(31.6)	26(68.4)	
Widow/widower	2(22.2)	7(77.8)	
Divorce/separated	0(0.0)	7(100)	

*Fischer's exact test
The difference in the marital status was found to be statistically significant.

Table 6. Association between HCV status and reason for testing

Reason for testing	**HCV status**		**p-value**
	Positive N (%)	**Negative N (%)**	
NGO	11(33.3)	22(66.7)	**0.011***
Voluntary	9(9.8)	83(90.2)	
OPD	1(5.6)	17(94.4)	
IPD	3(11.1)	24(88.9)	

*Fischer's exact test
The correlation between HCV status and reason for testing was found to be statistically significant.

Table 7. Showing prevalence of HBV & HCV by different test

Variable	**Test**	**Positive N (%)**	**Negative N (%)**
HBV	ICT	10(5.9)	160(94.1)
	ELISA	10(5.9)	160(94.1)
	PCR	10(5.9)	160(94.1)
HCV	ICT	24(14.1)	146(85.9)
	ELISA	24(14.1)	146(85.9)
	PCR	22(12.9)	148(87.1)
Both HBV & HCV		5(2.9)	165(97.1)

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Table 8. Showing PCR results for HCV & HBV in HIV co-infected participants

Laboratory number (HCV)	PCR value (IU/ml)
1	$2.41X10^3$
2	$7.23X10^2$
3	$2.76X10^5$
4	$3.42X10^3$
22	$5.79X10^1$
23	$4.54X10^5$
24	Not detected
33	$3.74X10^2$
36	$9.32X10^3$
49	$9.21X10^2$
50	$7.91X10^7$
51	$6.23X10^4$
52	$4.12X10^5$
54	$7.27X10^3$
55	$2.75X10^8$
57	$3.95X10^2$
59	$4.12X10^5$
60	$5.83X10^6$
61	$2.28X10^3$
84	$6.47X10^6$
94	Not detected
100	$5.93X10^4$
107	$4.47X10^4$
110	$4.19X10^3$
Laboratory number (HBV)	PCR value(IU/ml)
19	$1.11X10^5$
23	$3.31X10^3$
33	$4.27X10^3$
36	$5.78X10^7$
38	$6.31X10^3$
39	$5.29X10^5$
51	$3.37X10^4$
57	$1.76X10^4$
123	$1.32X10^3$
128	$2.49X10^3$

Table 9. Showing association between HBV status and gender

Gender	HBV status		p-value
	Positive N (%)	Negative N (%)	
Female	2(3.0)	65(97.0)	0.195
Male	8(7.8)	95(92.2)	

Table 10. Test showing comparison of mean age by HBV status

HBV status	Age (yr) Mean(SD)	Mean difference	t-value	p-value
Positive (10)	39.8(8.13)	0.90	0.303	0.762
Negative (160)	38.9(9.15)			

DISCUSSION

In the present study, majority of the participants were in the age group 36-45 years. Rodriguez A M et al also found the same.5 In the present study, it was found that the most common risk factor for HIV infection was IVDU which comprised of 39.4% .Though the association was statistically insignificant. It has clearly been stated that people who inject drugs are 24 times more likely to acquire HIV than adults in the general population.6 Gregory M et al also observed that participants in their study reported high rates of needle/syringe sharing.7 A study in Manipur in 2014 by Armstrong G et al found that compared to PWID (people who inject drugs) aged 35 years or older, PWID aged 18 to 24 years were more likely share needles/syringes.[8] In this study, the seroprevalence of HIV and HCV co-infection was found to be 14.1%. Ruan Y H et al observed that HCV/HIVcoinfection was 11.3% and Dienstag J L et al also found that HCV infection rate in HIV positive individuals 9.2%, which are comparable with the present study.[9,10] In studies done in Manipur in 2017 by Roy Arup et al the seroprevalence of HCV was as high as 64.7% among PWID individuals and in 2019, Antara R et al found that HCV coinfection with HIV was 21.47%.[11,12] Though Kushwaha J.S et al found that HCV co-infection was 7 % in HIV seropositive patients.13 On the other hand the seroprevalence of HCV coinfection was found to be only 2 % by Kalyani CS et al and 2.43% by Gupta S et al which are lower as compared to the finding of this study.[14,15] But it is worth mentioning that their study population of their study comprised essentially of heterosexually transmitted HIV infection. Barua P et al found in their study that although acquisition of HCV by sexual route may

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not be as efficient as parenteral route, yet sexual transmissibility of HCV among FSWs poses high risk to the community.[16] And heterosexuality was found to be the predominant sexual behaviour followed by bisexuality and homosexuality by Jindal N et al.[17] In our study, HCV co-infection was most commonly seen in participants who had the risk factor of being an IVDU at present or in the past and also those IVDUs who also had multiple sex partners. And the association was found to be statistically significant, which is in concordants with other studies by Panda S et al and Basu D et al.[18,19] A study by Javadi A et al also concluded that all HIV infected IDUs were infected with HCV as well.[20] In a study conducted by Devi KhS et al among IDUs from de addiction centre in Co-infection of HIV and HCV was as high as 52.4% and IDUs were in sexually active age group with a risk of infection to their sexual partner.[21] Ruan Y H et al , Hajiani E et al and Degenhardt L et al also reported the same findings.[19,22,23] In 2016 in Manipur, Kermode M et al also concluded that among the 31 per cent of HIV positive PWID, 95 per cent were co-infected with HCV.[24] In the present study, mean age for HCV co-infection was found to be 38.5±8.24 years. Out of the 24, 23(22.3%) were male and only 1(1.5%) was female. The difference in gender was found to be statistically significant, which is similar to the finding of Antara R et al.[15] The difference in the marital status was found to be statistically significant (31.6% unmarried). In this study, most of the participants who were found to be HCV positive had come for testing for HIV on recommendation by non-governmental organisations (NGOs). The correlation between HCV status and reason for testing was found to be statistically significant. This may be because of the fact that the NGOs brought those cases who were identified to be exposed to the risk factors .As was found by Kermode M et al.[24] In the present study of all the 24 cases of HCV coinfection that were positive by both immunochromatography and ELISA tests, only 22 (12.9%) were detectable by quantitative PCR. The discrepancy seemed to be due to low concentrations of viral RNA as was the case in a study conducted by Netski D M et al.[25] In our study the seroprevalence of HIV and HBV co-infection was found to be 5.9%, which is similar to the findings of Gupta et al (5.3%), Kumarasamy et al (4.8%) and Nagamani P et al (4.5%) but lower than that reported by Kushwaha J.S et al (11%) and Kalyani CS et al (12 %) .[15,26,27,11,14] Though, in a study done in Manipur in 2017 by Roy A et al also found that seroprevalence of HBV among the PWID was 17.6%.[12] In the present study the mean age for HBV co-infection was 39.8±8.[13] years which was similar to the findings of Baveja U K et al and Rodriguez A M et al.[28,5] In this study, HBV co-infection was most commonly seen in participants who had the risk factor of being an IVDU at present or in the past and also those IVDUs who also had multiple sex partners which was in concordance with the findings of other studies done by Saha M K et al in Manipur and Baveja U K et al in Tamil Nadu as well as in Manipur.[29,28] In a study conducted by Price H et al HBeAg was detected among 37% of HBsAg-positive patients. Also in HBsAg-positive patients, HBV DNA was undetectable in 21%. A total of 96% of HBeAg-positive and 70% of HBeAg-negative patients had detectable HBV DNA. Persistent isolated anti-HBc may also be due to occult HBV infection (with low-level detectable HBV DNA viral load) or loss of anti-HBs with time or immunosuppression in patients who have resolved infection. Immunosuppression associated with HIV coinfection can result in reactivation of HBV infection, with reappearance of HBsAg or HBeAg, or a reduction in the rate of loss of either marker over time.[30] On the other hand, Nelson P et al and Saha K et al showed that about 95% of adults with acute HBV infection will clear the virus, lose HBsAg and develop anti-HBc and hepatitis B surface antibodies (anti-HBs) and the presence of anti-HBc indicates previous exposure and it is also a more durable marker than HBsAg.[31,32] And, Gerlich W Het al found that the best available marker for the presence and number of infectious HBV particles is the number of HBV DNA molecules. However, the detection of HBV DNA in serum does not always correspond to infectivity or to the number of HBV progeny viruses released from hepatocytes.[33] Indeed, in the majority of viral infections, the number of physical virus particles is much larger than the number of fully infectious virions. On the other hand, Hollinger FB et al found that there also appears to be a correlation between levels of HBV DNA and serological status among patients with OBI (occult HBV infection). HBV DNA levels are lowest in seronegative patients, intermediate in anti- HBc negative and anti-HBs positive patients, and highest in subjects who are anti HBc-positive but anti-HBs negative.[34] Fabbri G et al highlighted the risk of HBV reactivation with interferon-free directly acting antivirals (DAA) treatment in HIV/ HCV co-infected patients previously exposed to HBV and who have contraindications for treatment with nucleoside/nucleotide reverse transcriptase Inhibitors because of comorbid conditions. [35] But in the study it was hard to classify if the HBV coinfections were acute, chronic or reactivation due to immunosupression as all the other serological markers were not tested and CD4 count association was also not studied. In the present study, all of which HBV coinfected cases which were positive by immunochromatography and ELISA test, also were detectable in Quantitative PCR. Geretti A M et al showed similar findings.[36] In the our study, 2.9% showed the triple infection of HIV, HBV and HCV. Solomon S S et al also found that the overall 2.74% were positive for HIV, anti-HCV and were chronically infected with HBV.[37] Though in 2005

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when Devi KhS et al conducted a study among IDUs from de addiction centre in Manipur, the prevalence of HIV, HBV and HCV was 6%.[21]

Conflict of interest: No conflict of interest associated with this work.

Ethical issues: Ethical clearance was sought from the institutional ethics committee.

CONCLUSION

In this study, HBV/HCV coinfection was seen more commonly in IVDUs and also those IVDUs who also had multiple sex partners. In the present study it was hard to classify if the HBV coinfections were acute, chronic or reactivation due to immunosupression as all the other serological markers were not tested and CD4 count association was also not studied. All samples reactive by ELISA for HBsAg could be further tested for the presence of hepatitis "e" antigen (HBeAg) and IgM antibody to hepatitis B core antigen (HBcIgM) and anti HCV IgG and IgM for better understanding of the coinfection status. In this study, we also did not have the data on the Hepatitis B vaccination status. The overall prevalence of occult HBV infection reactivation in HIV and the frequency of the specific triggers of reactivation needs to be assessed in prospective longitudinal studies.

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