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HIV, HBV, HCV, and syphilis co-infections among patients attending the STD clinics of district hospitals in Northern India

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Summary
Objective: The objective of the study was to assess the risk of co-infections with human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV), and syphilis among patients attending sexually transmitted disease (STD) clinics, antenatal clinics (ANC) and Ob-Gyn outpatients department (OPD) clinics which were part of the sentinel surveillance program.
Methods: A serological screening was carried out during the period August—November 2002 to assess the risk of infection with HIV-1/2, and co-infection with HBV, HCV, and syphilis among the outpatients attending STD clinics, Ob-Gyn OPD clinics, and ANC of three district hospitals (Agra, Etawah, and Farrukhabad) of Uttar Pradesh state in Northern India. Unlinked and coded serum samples received from 863 patients (635 females and 228 males) were screened by laboratory tests commonly used for laboratory diagnosis of HIV, HBV, HCV, and syphilis.
Results: Among the 863 samples serological reactivity was detected for HIV-1/2 in 21 (2.4%), HBV in 25 (2.9%), HCV in nine (1.0%), and syphilis in 47 (5.4%). The incidence of HBV was higher among males than females, i.e. 10/228 (4.4%) versus 15/635 (2.4%). Co-infection was observed for HIV—HBV in two (0.2%), HBV—HCV in one (0.1%), and HIV—syphilis in one (0.1%). None were found to have co-infection with HIV—HCV, HBV—syphilis, and HCV—syphilis. Age, sex, literacy level, occupation, locality, migration, and presence of different sexually transmitted infections did not significantly influence the rate of HIV positives.
Conclusion: A substantial percentage of the outpatients seen in the clinics of the district hospital in Uttar Pradesh harbor HIV and viral hepatitis infections, which otherwise would remain undiagnosed without serological screening.

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Introduction

Among the blood-borne viruses transmissible through the parenteral route, by blood transfusion, as well as by sexual intercourse, human immunodeficiency viruses (HIV-1/2), hepatitis B virus (HBV) and hepatitis C virus (HCV) are important and have several implications. Not only do they establish asymptomatic persistent infections with occasional sequelae, but they also cause significant morbidity and mortality when transmitted through transfusion of blood and blood products.[1] Chronic infections with HIV, HBV, and HCV are major public health problems. Many risk behaviors as well as the routes of transmission for HBV and HCV infection are identical to those for HIV and other sexually transmitted diseases (STDs). Early diagnosis and effective treatment of STDs, especially those that cause ulcers and blood-borne infections, are an important strategy for the prevention of HIV transmission.

Globally, the HIV sentinel surveillance system has been recognized as an optimal mechanism to monitor trends of HIV infection in specific high-risk groups as well as low-risk groups. In India, high-risk populations include patients attending STD clinics, men who have sex with men (MSM) clinics, and drug addiction centers, while mothers attending antenatal clinics are regarded as a proxy for the general population.

This study was carried out at the Voluntary Confidential, Counselling & Testing Centre (VCCTC) at the Central JALMA Institute for Leprosy & Other Mycobacterial Diseases, Agra, India. The objective of the study was to assess the risk of co-infections with HIV, HBV, HCV, and syphilis among patients attending the STD, ANC and Ob-Gyn OPD clinics who were part of the sentinel surveillance program.

Screening for HIV, HBV, HCV, and syphilis was carried out in order to determine the prevalence levels of these infections, as biological markers of risk, modes, and time functions of their transmission.

Study design

The study design, developed according to the guidelines and operational procedures for sentinel surveillance, and the method of testing, i.e., unlinked anonymous testing, were formulated by the National AIDS Control Organization (NACO) of the Government of India. The study was carried out over a period of 4 months, between August and November 2002. The sample size and sampling period were decided at the beginning of sentinel surveillance.

The sentinel sites were the outpatient department (OPD) of STD clinics, Ob-Gyn OPD clinics, antenatal clinics (ANC), and intervention clinics of different district hospitals (Agra, Etawah, and Farrukhabad) of Uttar Pradesh, India. The rationale for choosing sentinel sites in these clinics was that the samples from the respective risk groups attending the clinic-based settings could be collected at regular intervals. Sites with 75% coverage of desired sample size were included for analysis. The rationale for the choice of sample size for the various groups (STD clinic and Ob-Gyn OPD attendees, are considered to be high-risk groups whereas ANC clinic attendees are a low-risk group) was that during this 4-month period, approximately 900 samples could be collected conveniently. The target was to test 900 samples but due to operational difficulties, this was not achieved by the end of the sampling period.

The inclusion criterion for ANC cases was all antenatal women attending the ANC clinic for the first time during the sampling period. Criteria for STD patients were male patients presenting with genital ulcer/warts/urethral discharge and female patients presenting with genital ulcer/warts/cervical discharge as confirmed by per speculum examination. Criteria for Ob-Gyn OPD patients were new STD patients attending Ob-Gyn OPD during the sampling period and patients attending the intervention clinics, with or without symptoms.[2]

The whole procedure was unlinked and anonymous. Thus, unlinked anonymous coded sera samples received from 863 patients were screened simultaneously for co-existing infections with HIV, HBV, HCV, and syphilis.

Materials and methods

Sampling technique

The sampling of STD/ANC clinic and Ob-Gyn OPD and intervention clinic attendees was commenced at the beginning of the sampling period, i.e., 1 August 2002, and continued until the end of November 2002. Blood samples (5 mL) were drawn at the respective clinic sites to avoid dropouts. Consecutive blood samples were collected aseptically from each survey participant, i.e., 250 new STD patients (150 from the STD clinics and 100 from the Ob-Gyn OPD of Agra), 400 antenatal cases attending the ANC clinic of Etawah, and 250 clinic attendees for syphilis and HIV testing (150 from the STD clinics and 100 from the Ob-Gyn OPD of Farrukhabad). The procedure was to continue consecutive sampling until the pre-determined sample size was reached or the pre-determined sampling period was over. Due to operational difficulties the pre-determined sample size was not reached, however this study was completed using the number of samples actually collected even though less than anticipated. The serum samples, collected after centrifugation at 2500 *g*, were delivered to our laboratory under strict cold chain precautions. These samples were stored at $-20\ ^{\circ}C$ until assays were performed.

Method of testing

Unlinked anonymous testing means that the sample was originally collected for another purpose, so, for example, a sample collected for syphilis diagnosis is tested for HIV after all information that could identify the source of the blood sample has been eliminated from the sample.

ELISA was carried out for HIV detection using Genedia HIV-1/2 EIA kit (Greencross, Korea). Those found positive were confirmed by rapid (HIV capillus latex aggregation assay, Trinity Biotech plc, Ireland) and rapid spot assays (Genelabs Diagnostics Pte. Ltd, Singapore).

The samples were further tested by HBV kit-HEPALISA (Microwell ELISA test for the detection of hepatitis B surface antigen (HBsAg) in human serum/plasma, J. Mitra & Co. Ltd,

New Delhi, India), HCV kit-LG HCD 3.0 plus enzyme immunoassay for the detection of hepatitis C virus (anti-HCV) in human serum/plasma (LG Chemical Ltd, Seoul, Korea), and VDRL-CARBOGEN rapid plasma reagin (RPR) card test/carbon antigen for syphilis testing (Tulip Diagnostics (P) Ltd, Goa, India). Serum samples were diluted and tested according to the kit's specifications.

Statistical analysis

Data were analyzed to determine the statistical significance of the association between HIV status and sex, age, locality, literacy, occupation, period of stay, and history of STDs of the attendees using Pearson's Chi-square test. The significance of the difference in the proportion of HIV positives among the different categories was also examined with *Z* statistics at 5% level of significance.

Results

The results of 863 samples received from different sentinel sites of district hospitals of Uttar Pradesh, which were screened for HIV and other co-infections are shown in Tables 1—3.

Table 1 shows the socio-demographic characteristics, HIV status, and history of STDs among patients attending the STD clinics. Of the 863 patients, 16 (2.5%) females and five (2.2%) males were infected with HIV. Among the outpatient clinics, 13 (2.6%) patients in the 21—29 years age group and seven (2.6%) in the 30—44 years age group were HIV positive. Their marital status was not defined. The difference in HIV positive status was not significant ($p = 0.321$) between urban and rural areas (12 (3.0%) in urban and nine (1.9%) in rural areas). Ten of the 358 (2.8%) illiterate patients were HIV positive. Further, among the 125 (14.5%) clinic attendees who were graduates, four (3.2%) were HIV positive.

Laborers, mainly migrants, were those most frequently infected with HIV and had the maximum number of STDs. Among the 863 patients were agricultural and unskilled workers, housewives, business and commercial workers, service staff, students, industrial workers, unemployed, drivers and hotel staff. Among these groups, the percentage of HIV positives was highest in unemployed males (4.2%). The difference in percentages of HIV positivity in the various occupational groups of females was found not to be statistically significant.

Those who had stayed in one place for more than 6 months were regarded as non-migrants. Among 814 non-migrants, 20 (2.5%) were HIV positive. Among migrants 1/49 (2.0%) was

Table 1 The socio-demographic characteristics, HIV status and history of STDs among patients attending the outpatient clinics

STD patients ($n = 863$)		Male ($n = 228$)		Female ($n = 635$)		Total ($n = 863$)		Statistics
Parameters	Categories	No.	HIV pos	No.	HIV pos	No.	HIV pos	p value
Age group (years)	<20	32	1	32	0	64	1	$p = 0.818$
	21—29	109	2	391	11	500	13	
	30—44	69	2	204	5	273	7	
	>45	18	0	8	0	26	0	
Locality	Urban	126	3	275	9	401	12	$p = 0.321$
	Rural	102	2	360	7	462	9	
Literacy status	Illiterate	53	2	305	8	358	10	
	Literate and to 5th grade	32	0	102	1	134	1	$p = 0.550$
	To 12th grade	83	3	163	3	246	6	
	Graduate and above	60	0	65	4	125	4	
Occupation	Agriculture/unskilled worker	83	3	204	8	287	11	
	Truck/taxi/auto drivers/Cleaners	11	0	0	0	11	0	$p = 0.692$
	Industrial workers	5	0	22	0	27	0	
	Hotel staff	7	0	1	0	8	0	
	Service staff	43	0	58	1	101	1	
	Business and commerce	40	0	80	2	120	2	
	Unemployed	9	1	15	0	24	1	
	Student	30	1	47	1	77	2	
	Housewife	0	0	208	4	208	4	
Migration	Migrant	14	0	35	1	49	1	$p = 0.623$
	Non-migrant	214	10	600	10	814	20	
STD	Genital ulcers	90	2	35	2	125	4	
	Urethral discharge	105	3	0	0	105	3	
	Genital ulcers and urethral/ cervical discharge	15	0	10	0	25	0	$p = 0.617$
	Cervical discharge	0	0	187	2	187	2	
	Genital warts	18	0	403	12	421	12	

Table 2 HIV, HBV, HCV, and syphilis status of patients attending the STD, ANC, Ob-Gyn OPD Clinics

STD patients		Male (n = 228)	Female (n = 635)	Total number of patients screened (n = 863)
HIV status	Reactive	5 (2.2%)	16 (2.5%)	21 (2.4%)
	Non-reactive	222 (97.4%)	619 (97.5%)	842 (97.6%)
HBV status	Reactive	10 (4.4%)	15 (2.4%)	25 (2.9%)
	Non-reactive	218 (95.6%)	620 (97.6%)	838 (97.1%)
HCV status	Reactive	2 (0.9%)	7 (1.1%)	9 (1.0%)
	Non-reactive	226 (99.1%)	628 (99.0%)	854 (98.9%)
Syphilis status	Reactive	10 (4.4%)	37 (5.8%)	47 (5.4%)
	Non-reactive	218 (95.6%)	598 (94.2%)	816 (94.6%)

Table 3 HIV, HBV, HCV, and syphilis status of the STD, Ob-Gyn OPD and ANC clinic attendees

Sentinel sites	Samples screened	HIV positive	HBV positive	HCV positive	Syphilis positive
STD clinic, Agra	150	5 (3.3%)	6 (4%)	2 (1.3%)	8 (5.3%)
Ob-Gyn OPD, Agra	100	—	1 (1%)	2 (2%)	3 (3%)
STD clinic, Farrukhabad	135	3 (2.2%)	6 (4.4%)	2 (1.5%)	6 (4.4%)
Ob-Gyn OPD, Farrukhabad	78	1 (1.3%)	—	—	4 (5.1%)
ANC clinic, Etawah	400	12 (3%)	12 (3%)	3 (0.8%)	26 (6.5%)
Total	863	21 (2.4%)	25 (2.9%)	9 (1.0%)	47 (5.4%)

HIV positive of which none were male; 1/35 (2.9%) of migrant females was HIV positive.

The chief presenting symptoms among the 863 patients at the time of visit to the clinic were genital warts, genital ulcers, urethral discharge and cervical discharge. HIV positivity occurred in 4/125 (3.2%) clients with genital ulcers, 3/105 (2.9%) with urethral discharge, 2/125 (1.6%) with genital ulcers and urethral/cervical discharge, 4/287 (1.4%) with cervical discharge and 8/221 with genital warts. Genital ulcers and warts are very important since the risk of acquiring HIV infection associated with STDs increases in their presence.

Of 863 patients, 21 (2.4%) were HIV positive and 47 (5.4%) were syphilis reactive. This suggests that STDs affect a large percentage of clinic patients.

The results revealed that none of the variables — sex, age, literacy status, occupation, locality, migration, and even the presence of different STDs — were found to significantly influence HIV positivity.

Table 2 shows that of the 863 patients, 21 (2.4%) STD, ANC and Ob-Gyn OPD clinic attendees were HIV positive, 25 (2.9%) were positive for hepatitis B virus by both rapid assays and ELISA, nine (1.0%) were positive for antibodies to the hepatitis C virus by ELISA, and 47 (5.4%) had antibodies to syphilis (reactive for syphilis by the simple RPR card test). More males (10 (4.4%)) than females (15 (2.4%)) had HBV.

Table 3 shows the HIV, HBV, HCV, and syphilis status of the STD, Ob-Gyn OPD and ANC clinic attendees. HIV positivity ranged from 1 to 3% among the clinic attendees, 1 to 4% for HBV, 0.8 to 2% for HCV and 3 to 6% for syphilis.

No HIV positivity was reported from the Ob-Gyn OPD clinic in Agra. No positivity for HBV or HCV infection was reported from the Ob-Gyn OPD clinic in Farrukhabad. Attendees of the ANC clinic in Etawah had positivity at 3% each for HIV and HBV, 0.8% for HCV and 6.5% for syphilis reactivity. This is of serious concern as ANC cases were considered to be the low-risk group.

Discussion

Sexually transmitted diseases (STDs) remain a public health problem of major significance in most parts of the world.[3–5] The incidence of acute STDs is high in many countries, including India although the precise magnitude of the problem is still not clear.[6–8] Failure to diagnose and treat STDs at an early stage results in serious complications and sequelae, including infertility, fetal wastage, ectopic pregnancy, cancer, and death.[9] The explanation for the increase in STDs is multifactorial, heterosexual promiscuity being one of them.[10]

Analysis of our results revealed that none of the variables — sex, age, literacy status, occupation, locality, migration, and even the presence of different STDs — significantly influenced the rate of HIV positivity among the attendees of the STD clinics. More males, i.e., 10 (4.4%) had HBV than females, 15 (2.4%). With regard to HCV (7/635) and syphilis (37/635), females were more affected than males but this could be due to the larger number of females, 635 as compared to 228 males screened during the surveillance. There were co-infections: two (0.2%) with HIV—HBV, one (0.1%) HBV—HCV, and nine (1.0%) with HIV—syphilis infections. Although the percentage of patients with co-infections is lower, the combination of viral infections such as HIV and HBV or HBV and HCV is a dangerous co-existence[11–13] and may have a detrimental effect on the patient and the treatment outcome. None were found to be co-infected with HIV—HCV, HBV—syphilis, or HCV—syphilis.

The results reveal that patients attending the STD, Ob-Gyn OPD, and ANC clinics harbor blood-borne viral infections like HIV, HBV, and HCV, which would otherwise remain undiagnosed in the absence of screening. Further, they are unaware of the underlying co-infection because this was an unlinked anonymous testing of coded samples.

Many studies showing the varying rates of HIV and HCV infections among STD patients have been reported by several authors in India and abroad. Kaur and Marshalla screened 233 serum samples for HIV, HBV, HCV, and syphilis and found that 21% were positive for syphilis, 3% for HBsAg and HIV-1 and 0.8% for HCV, and no correlation was observed in the transmission of two or more pathogens.[14] Garg et al. evaluated 46 957 donors for HIV, HBV, HCV, and syphilis. Of these, 90.1% were replacement donors and 9.0% were voluntary donors and the incidence of HIV was 0.44%, HBV was 3.44%, HCV was 0.29%, and VDRL was 0.22%.[15] Nanu et al. screened 132 093 voluntary and replacement donors and reported that HBsAg rates remained below 2.5%, HIV 0.55%, syphilis 0.52%, and HCV 1.49% among donors, and those with multiple infections were uncommon.[16] Patel screened 60 780 donors in Mumbai over a 6-year period, from 1994 to 1999, and found that 0.78% had antibodies to HCV, 0.26% had antibodies to HIV, and 1.7% had antibodies to HbsAg.[17] Gupta et al. screened 44 064 blood units in Ludhiana, during the period 2001—2003 and reported 0.08% had antibodies to HIV, 0.60% had antibodies to HBsAg, 0.11% had antibodies to anti-HBc, 1.09% were HCV positive, and 0.85% had antibodies to syphilis.[18] Ruan et al. reported 71.0% of the 379 intravenous drug abusers (IDAs) in China had antibodies to HCV and 11.3% had antibodies to HIV. HCV—HIV co-infection among IDAs was 11.3%.[19] Taketa et al. screened 98 IDAs, 100 commercial sex workers (CSWs) and 50 males with STDs in Thailand and reported that HCV is transmitted primarily by blood contact, HIV primarily by blood contact and secondarily by sexual contact, but HBV by both blood and sexual contact.[20] Galvin and Cohen reported that one in every 1000 episodes of sexual intercourse leads to HIV infection[21] thereby suggesting slow efficiency of HIV spread. Gordin et al. screened 616 patients in the USA and found that 23 (3.7%) were HIV positive and 2.0% (12/612) were positive for HbsAg.[22] Segurado et al. screened HIV-infected individuals in Brazil[23] and reported that exposure to blood and sexual partnership with IDUs constitute the main risk factors for HCV acquisition among HIV-positive patients. Choy et al. screened for HCV infection in STD-infected patients[24] in New Jersey, USA and reported that inner-city obstetric patients are at high risk for HCV infection when compared with the general population. Increasing age and HIV positive status are the risk factors that are significantly associated with HCV infection. In STD clinics, integrating risk-based screening into routine clinic services is an efficient way to identify HIV-infected persons.[25—27] The increased risk of HBV, HCV, and HIV infection among STD patients warrants specific preventive action.[28—30] HIV, HCV, and HBV may promote each other and be related to different cultures and living habits[31] though this does not appear to be the case in our study population.

Screening the high-risk population for these viral infections would aid early detection of co-infections and hence early treatment, which, if initiated, would help to decrease the further spread of these blood-borne infections. There is a need, therefore, to support an approach of targeted screening of all these viral infections, integrating viral hepatitis testing, counseling and referral services into the existing STD prevention and treatment services.

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Conflict of interest: No conflict of interest to declare.

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