ORIGINAL ARTICLE

Prevalence and incidence of hepatitis B virus infection in STD clinic attendees in Pune, India

A Risbud, S Mehendale, S Basu, S Kulkarni, A Walimbe, V Arankalle, R Gangakhedkar, A Divekar, R Bollinger, D Gadkari, R Paranjape

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See end of article for authors' affiliations

Correspondence to: Dr Arun Risbud, MD, MPH, National AIDS Research Institute, G-73, MIDC, Bhosari, Post Box 1895, Pune 411 026, India; arunrisbud@yahoo.com or hivnet@vsnl.com

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Objectives: To estimate the prevalence and incidence of hepatitis B virus (HBV) infection among patients attending three STD clinics in Pune, India, and to identify associated risk factors.
Methods: Of the 2098 patients screened at STD clinics in Pune during 1996, 497, who returned for at least one follow up visit, were screened for various markers of HBV infection (HBsAg, anti-HBs, anti-HBc), HIV antibody, and VDRL.
Results: Of the 497 participants 3.6%, 26.5%, and 43.2% were positive for HBsAg, anti-HBs, and anti-HBc respectively. Tattooing (AOR 1.64, 95% CI 1.03 to 2.64) was found to be independently associated with presence of core antibody. Additionally, history of being in commercial sex work and history of a genital ulcer were independently associated with a positive anti-HBc antibody test (AOR 12.45, 95% CI 5.58 to 27.82 and AOR 1.70, 95% CI 1.09 to 2.66, respectively). 72 out of 497 (14.5%) participants were HIV positive at baseline. HIV-1 antibody positive patients were more likely to have a positive anti-HBc test (69.4% *v* 39.0%, p<0.001). 30 out of 282 participants, negative for anti-HBc antibody at enrolment, seroconverted subsequently, resulting in an incidence of 10.86 per 100 person years (95% CI 7.2%, 14.5%) (mean and accumulated follow up of 11.7 months and 276.17 person years, respectively).
Conclusions: A high prevalence and incidence of HBV infection, seen in STD clinic attendees underscore the need to provide HBV vaccine to commercial sex workers and their clients in India.

Hepatitis B virus (HBV) infection is an important health problem in developing countries, including India. According to World Health Report 2000, it is estimated that HBV infection accounts for about 600 000 disability adjusted life years (DALY) and about 31 000 deaths annually in South East Asia. About 4% of the population are estimated to be carriers of HBV, giving a total pool of approximately 36 million carriers in India.[1] HBV is reported to be responsible for 70% of cases of chronic hepatitis and 80% of cases of cirrhosis of the liver. About 80% of Indian patients with hepatocellular carcinoma have hepatitis virus associated liver disease.[2]

HBV infection is predominantly acquired at an early age in developing countries, which includes vertical transmission from mother to child, perinatal transmission, and horizontal transmission from child to child. However, HBV can also be transmitted sexually and sexual transmission—both heterosexual and homosexual, accounts for a majority of the transmission occurring in adult life.[3]

Numerous studies[4–7] have reported high prevalence of HBV markers in subjects practising risky sexual behaviour, like STD patients and commercial sex workers. A high prevalence of HBV infection has also been reported among individuals practising risky behaviours and HIV infected individuals in India.[8–10] However, a majority of such reports were based on the presence of HBV surface antigen only. Though spouses of HBV carriers were shown to have higher risk of HBV acquisition,[11] limited data are available with regard to the extent of sexual transmission of HBV. Data on incidence of HBV infection in STD patients and other population are not available from India.

HBV as well as HIV can be transmitted sexually. Dual infection of HIV and HBV can lead to reactivation of HBV infection and also increase in replication of HIV.[12] Currently, STD control programmes in India do not generally include administration of hepatitis B vaccine to STD patients. Therefore, this study was undertaken to assess the prevalence and incidence of hepatitis B infection, as well as the HBV risk determinants among patients attending three urban STD clinics in Pune.

MATERIAL AND METHODS

Study population and clinical methods

During the calendar year 1996, a total of 2098 patients visited three STD clinics of the National AIDS Research Institute in Pune. As a part of the ongoing study of HIV transmission among people with high risk behaviour, these patients were offered serological screening for HIV-1 and HIV-2 infection and STD diagnosis and treatment. People seronegative for HIV were invited to participate in a prospective study of HIV acquisition and instructed to return every 3 months for follow up. Seropositive patients were also encouraged to come for follow up. After informed consent, patients were administered a structured questionnaire, which included data on demographics, STD and medical history, sexual behaviour, and reproductive history in female patients. Presumptive diagnosis of sexually transmitted disease was based on detailed physical examination and clinic based laboratory tests. The diagnosis and treatment of STDs was administered in accordance with WHO guidelines.

Of the 2098 patients screened in the STD clinics, 497 (23.7%), who returned for at least one follow up visit till December 1998, were included in the present analyses.

HIV and syphilis serology

Baseline and follow up samples of participants were initially screened with a commercially available enzyme linked immunosorbent assay (ELISA) for detection of antibodies to HIV-1 and HIV-2 (Genetic Systems Corporation, USA). Specimens reactive in ELISA were evaluated by a rapid test for HIV-1 and HIV-2 (Recombigen HIV-1/HIV-2 Rapid test Device, Cambridge Biotech) and those found positive were confirmed by the respective HIV-1 or HIV-2 western blot (Diagnostic Biotechnology Ltd, Singapore). Syphilis antibodies were detected

Table 1 Univariate analysis of risk factors and prevalent anti-HBc antibody in STD patients

Characteristic	No of subjects	Prevalence of anti-HBC (%)	Odds ratio (95% CI)	p Value
Overall	497			
Risk group:				
Female/non-sex worker	32	8 (25.0)	1.0	
Female/sex worker	79	69 (87.3)	20.7 (7.3 to 58.5)	<0.001
Male/no exposure to sex worker	44	12 (27.3)	1.0	
Male/visited sex worker	342	126 (36.8)	1.6 (0.7 to 3.3)	0.215
Age (years):				
<20	68	17 (25)	1.0	
20–29	239	92 (38.5)	1.9 (1.0 to 3.4)	0.042
> 30	190	106 (55.8)	3.8 (2.0 to 7.0)	<0.001
Marital status:				
Never married	225	85 (37.8)	1.0	
Married	212	82 (38.7)	1.0 (0.7 to 1.5)	0.846
Widowed/divorced	60	48 (80.0)	6.6 (3.3 to 13.1)	<0.001
Formal education:				
Some	393	141 (35.9)	1.0	
None	104	74 (71.2)	4.4 (2.7 to 7.1)	<0.001
Lifetime number of sexual partners:				
1	67	14 (20.9)	1.0	
2–9	259	91 (35.1)	2.0 (1.1 to 3.9)	0.028
10–999	87	42 (48.3)	3.5 (1.7 to 7.3)	0.001
> 1000	84	68 (81.0)	16.1 (7.2 to 35.9)	<0.001
Condom use in past 3 months:				
No	247	92 (37.2)	1.0	
Yes	167	95 (56.9)	2.2 (1.5 to 3.3)	<0.001
Tattooed:				
No	303	104 (34.3)	1.0	
Yes	194	111 (57.2)	2.6 (1.7 to 3.7)	<0.001
History of genital ulcer:				
No	295	111 (37.6)	1.0	
Yes	202	104 (51.5)	1.7 (1.2 to 2.5)	0.002

Table 2 Logistic regression analysis of risk factors and prevalence of anti-HBc antibody in STD patients

Characteristic	AOR (95% CI)	p Value
Risk group		
Male/no exposure to sex worker	1.00	
Male/visited sex worker	1.21 (0.58 to 2.51)	0.609
Female/non-sex worker	0.64 (0.22 to 1.91)	0.426
Female/sex worker	11.21 (4.25 to 29.52)	<0.001
Age group		
<20 years	1.00	
20–29 years	1.71 (0.87 to 3.36)	0.117
>30 years	2.71 (1.36 to 5.40)	0.005
Tattooed		
No	1.00	
Yes	1.67 (1.09 to 2.55)	0.019
History of genital ulcer		
No	1.00	
Yes	1.70 (1.12 to 2.56)	0.012

by standard Venereal Disease Research Laboratory non-treponemal test (VDRL antigen, Span Diagnostic, Surat, India).

HBV serology

Baseline samples of all participants were tested for the following HBV markers by commercially available ELISA assays, HBsAg (Surase B-96, General Biologicals, Taiwan), anti-HBs (Anti Surase B 96, General Biologicals, Taiwan), and anti-HBc (Abbott, USA). Follow up samples of patients, who were negative for HBV markers at baseline, were tested similarly for respective markers. A subset of patients who, at enrolment, were positive for anti-HBc antibody only, were tested for the presence of anti-HBc IgM (Abbott, USA) to determine what proportion of them were recent infections.

Statistical analysis

Baseline characteristics of the subjects who were followed at the clinics were compared with those who never returned to the clinics for follow up. Univariate analysis was performed to assess the relation between various risk factors and anti-HBc antibody in contingency tables with χ^2 tests using the EPI-INFO 2000 package. Odds ratios were calculated. Variables found to be significantly associated with presence of anti-HBc antibody were included in multivariate analysis using SPSS 10.0 package.

HBV incidence rates were estimated by the ratio of the number of anti-HBc seroconversions to the person years of follow up expressed as percentage. The risk factors associated with anti-HBc conversion were analysed using EPI-INFO 2000. Variables found to be significantly or marginally associated with anti-HBc conversion were included in Cox proportional hazard analysis using S-PLUS 2000. For the variables related to sexual behaviours, Cox analysis was done separately for males and females.

RESULTS

Of the 2098 subjects screened during the year 1996, 497 participants, who completed at least one follow up by December 1998, were retrospectively enrolled in the study. These subjects were compared with the remaining 1601 subjects who attended clinics during the same period but only once. The two populations did not differ significantly by most socio-demographic and behavioural characteristics, including median age, sex, marital status, whether living alone (or not), and sexual behaviour such as visit to sex worker, being in the commercial sex work, number of sexual partners, condom use, STD history. However, people who reported for follow up and hence were included in the study were more likely to be literate (OR 1.55; 95% CI 1.21 to 1.99), non-smokers (OR 0.61; 95% CI 0.49 to 0.76) and more likely to have undergone surgery (OR 1.47; 95% CI 1.17 to 1.85).

Table 3 Univariate analysis of risk factors and incident anti-HBc antibody in STD patients

Characteristic	No of subjects	Incident HBV core antibody		
		No (%)	RR (CI)	p Value
Overall	282	30 (11)		
History of tuberculosis				
Yes	9	4 (44.4)	4.65 (2.05 to 10.52)	0.0091
No	272	26 (9.6)	1.00	
History of medical injections in past 6 months				
Yes	135	7 (5.2)	0.33 (0.15 to 0.75)	0.0079
No	147	23 (15.6)	1.00	
History of inguinal swelling				
Yes	41	8 (19.5)	2.13 (1.02 to 4.45)	0.0571
No	240	22 (9.2)	1.00	

Table 4 HBV marker profile at baseline and probable clinical staging

Clinical stage of HBV infection	HBV marker			No of subjects (%)
	HBsAg	Anti-HBs	Anti-HBc	
Early acute infection	Pos	Neg	Neg	9 (3.7)
Either acute or chronic Infection	Pos	Pos/Neg	Pos	9 (3.7)
Possibilities include: 1 HBV infection in remote past 2 "Low level" HBV carrier 3 "Window" between disappearance of HBsAg and appearance of anti-HBs 4 False positive reaction	Neg	Neg	Pos	95 (39.0)
Indicates previous HBV infection and immunity to HBV infection	Neg	Pos	Pos	111 (45.5)
Vaccine type response	Neg	Pos	Neg	20 (8.2)
Total				244

Of the 497 subjects included, 386 (77.7%) were males and 111 (22.3%) were females. Among women 79 (71.1%) were engaged in commercial sex work. Two hundred and thirty nine (48%) belonged to 20–29 years age group and 38% were aged 30 years or more. Nearly 45% were unmarried and a majority (79%) had some formal education.

The overall prevalence of HBV markers was seen to be high for HBsAg (3.6%; 95% CI 2.2 to 5.7), anti-HBs (26.5%; 95% CI 22.5 to 30.5%), and anti-HBc (43.2%; 95% CI 39.1 to 47.9%). A total of 244 (49.1%) participants revealed at least one of the HBV markers.

Table 1 summarises the results of univariate analysis of prevalence of HBV core antibody among these patients. Odds ratios in relation to appropriate baseline characteristics were calculated.

Determinants such as living with family, sexual orientation, history of anal sex in past 3 months, history of injections in past 6 months, history of blood transfusions, circumcision, current STDs (presence of genital ulcer, discharge, or warts), and VDRL reactivity were not associated with positive anti-HBc status.

Females practising sex work, and people who were older, uneducated, tattooed, widowed, or divorced and who had suffered from genital ulcer were more likely to possess anti-HBc antibodies. A dose-response relation was observed when anti-HBc prevalence was compared with number of lifetime partners. Individuals who had used condoms in the last 3 months were not protected from HBV exposure.

Logistic regression analysis (table 2) revealed that tattooing (AOR 1.67; 95% CI 1.09 to 2.55) and age more than 30 years (AOR 2.71; 95% CI 1.36 to 5.40) were independently associated with presence of anti-HBc antibody. Factors related to sexual behaviour such as being a commercial sex worker (AOR 12.45; 95% CI 5.58 to 27.82) and history of genital ulcer (AOR 1.7; 95% CI 1.09 to 2.66) were also independently associated with anti-HBc antibody.

Hepatitis B incidence

Two hundred and eighty two subjects who were negative for anti-HBc antibody at enrolment were followed for a mean of 11.7 months (range 0.5–34.7 months) accumulating 276.17 person years of follow up. Thirty of these individuals seroconverted during the follow up giving an incidence of 10.86 per 100 person years (95% CI 7.2 to 14.5 per 100 person years). Among those who seroconverted five were females and 25 were males.

Univariate analysis for risk determinants associated with incident HBV infections (table 3) revealed that history of tuberculosis (p value 0.0091) was found to be significantly associated with HBV incidence. History of injections in past 6 months (p value 0.0079) did not increase the risk of acquiring HBV infection. Additionally, a history of inguinal swelling also (p value 0.0571) revealed borderline significance. However, none of the sexual behaviour related variables were significantly associated with HBV seroconversion. A high relative risk (3.19, 95% CI (0.91 to 11.17)) was observed for men involved in anal sex with commercial sex worker. All the above variables were included in Cox proportional hazards analysis. However, because of a very small number of conversions during follow up, none of the variables except for males involved in anal sex with commercial sex worker (p value 0.012) showed any significance. This may possibly be the result of a very small number of conversions during follow up or due to the fact that none of these factors are independent risk factors for incident HBV infection in adult STD patients.

Association of HIV and HBV infection at baseline

Among the participants, HIV prevalence was 14.5% (72/497, 95% CI 11.7 to 18.1%) with 32.4% in females and 9.6% in males. Prevalence of HBV markers was higher in HIV positive subjects compared with HIV negative subjects. The differences were significant with respect to anti-HBc (69.4% *v* 38.8%; p value <0.001) and anti-HBs (36.1% *v* 24.9% p value 0.065). HIV infected individuals were four times (OR 3.95; 95% CI 2.19 to 7.18) more likely to have at least one positive marker of HBV infection.

Based on the presence of various combinations of HBV markers at baseline,[13] we classified our subjects into different stages of HBV infection. Of the 244 subjects who were found to harbour at least one serological marker of HBV, at least 3.7% subjects were more likely to be in early acute infection and 3.7% subjects may be in an acute or chronic infection state (table 4). Thirty nine per cent were positive for anti-HBc alone (n=95) at baseline. Anti-HBc alone positivity was significantly higher among HIV positives compared to HIV negatives (32.8% *v* 16.7%, p<0.01). Of these, 9.5% were found to be positive for anti-HBc IgM antibody indicating recent infection.

DISCUSSION

In countries where HBV infection is endemic, it is transmitted predominantly in childhood mostly through the parenteral route. However, it can also be transmitted through the sexual route. Predominant route of transmission also depends on the proportion of people indulging in the practice of "at-risk" behaviour. The HIV epidemic is reaching the general population and rural areas in India. The level of the HIV epidemic in the country is probably indicative of a high proportion of people practising at-risk behaviours. With this background it is possible that a significant transmission of HBV occurs through the sexual route. Though a high prevalence of HBV infection is documented earlier, the incidence of HBV infection among STD clinic attendees has not been previously reported from India. A high overall prevalence of all three HBV markers—namely, HBsAg, anti-HBs, anti-HBc, was seen in our study population. Similar high prevalence has also been reported among STD clinic attendees in some studies[4–10] and in spouses of HBV carriers.[11] Some of these studies reported comparatively low HBsAg carrier rate in the non-STD population—for example, 0.64% in voluntary blood donors.[10] Tattooing is known to be independently associated with prevalent anti-HBc antibody. Independent association of anti-HBc prevalence with sexual risk factors such as being a commercial sex worker and history of genital ulcer support that HBV transmission through the sexual route may be significant even in a country where HBV is an endemic infection.

A high incidence (10.86 per 100 person years) of HBV infection was detected among STD attendees in our study. This estimated incidence of HBV is higher than that reported in injecting drug users in Switzerland (2.1% per 100 person years),[14] and in Australia (1.8 per 100 person years).[15] HBV incidence observed in our study population was also higher than that reported in men having sex with men (MSM) in Brazil (4.44 per 100 person years).[16] Among the sexual behaviour variables, "anal sex in males with commercial sex workers" only was found to be associated with incident HBV infection. One important reason for lack of association of other sexual behaviour factors could be small number of incidence cases and the study setting where majority of the individuals have practised unsafe sex.

A history of tuberculosis was found to increase the likelihood of seroconversion for anti-HBc antibody. In this retrospective study, our study instruments did not seek additional information. It is therefore not possible to explain this association with the available data. Inguinal lymphadenitis can occur among patients having lymphogranuloma venereum, chancroid, herpes genitalis, syphilis, and granuloma inguinale. This association between inguinal swelling and incident HBV infection suggests that sexual transmission of HBV might be playing an important part among adults.

History of injections in the last 6 months did not increase the risk of acquiring HBV infection. Though, in fact, it appeared to protect the subjects from acquiring HBV infection, the association does not appear to be causal, as it does not have any pathophysiological basis.

Prevalence of HBV markers in HIV positive subjects was higher compared to that in HIV negative subjects. This difference was significant with respect to anti-HBc (69.4% *v* 38.8%) and anti-HBs (36.1% *v* 24.7%). Similar findings were noted in many other studies.[17–20]

HIV and HBV share the same routes of transmission. In Pune, the overall HIV incidence has been reported to be 10.2% per year,[21] which is more or less similar to that estimated for HBV infection in the same cohort. Interestingly, we have reported among other factors, old age, commercial sex work, lifetime number of sexual partners, current or previous genital ulcer/discharge/warts, and lack of formal education as the risk determinants of prevalent HIV infection[21]; some of these were found to be associated for HBV infection. The majority of study subjects had no other known risk factor, except at-risk sexual behaviour. Almost similar rates of incidence in both these infections that are transmitted sexually underscore the need for recognition of HBV as an important STD.

Among the subjects possessing any of the HBV markers, 23% were found to be HIV infected and 73.6% HIV infected subjects possessed at least one of the three HBV markers. This can be attributed to increased persistence and/or reactivation of HBV infection among HIV infected people. However, it may also be indicative of a greater efficiency of HBV transmission through the sexual route compared to that of HIV. This has important implications with regard to HIV transmission in couples, as reported previously. Suriyanan *et al* found HIV seropositivity in females to be significantly associated with HBsAg positivity in the male index in couple setting.[22] Coinfection with HIV can adversely affect HBV infection. Coinfection with HIV may lead to increased chances of persistence of HBV,[23 24] increased HBV viral load,[25 26] reduced response to HBV treatment,[27] and increased chances of reactivation.[28] This highlights the importance of prevention of HBV infection at all settings including STD clinic.

The study population comprised the subjects who attended follow up visits. The majority of the demographic characteristics and sexual behaviour factors did not differ significantly between study subjects and those who could not be enrolled since they did not come for follow up, thereby indicating that the study subjects represented STD clinic attendees in general. However, people who reported for follow up and hence were enrolled in the study were more likely to be literate (p=<0.001), non-smokers (p<0.001), and more likely to have undergone surgery (p<0.001). This suggests that our estimates of HBV prevalence could be an underestimate of the actual prevalence among STD patients. As against this, inclusion of HIV positive (14%) subjects in our study might have led to overestimation of prevalence and incidence of HBV infection.

We were interested to know what proportion of the subjects, positive for HBV markers at screening (244), were exposed in the recent past. Eighteen (7.3%) possessed surface antigen. However recency of infection in them cannot be confirmed in absence of additional data on anti-HBc IgM, HBeAg, or HBV DNA. Ninety five (39%) were positive for anti-HBc alone. This is not a typical HBV serology; however, we had similar results in our previous experience in the population in HBV endemic region. Additional tests undertaken then had ruled out the false positivity of anti-HBc in them. Additionally, anti-HBc alone positivity was significantly higher among HIV positives compared to HIV negatives (32.8% *v* 16.7%, p<0.01), which

Key messages

- High prevalence and incidence of HBV infection was observed in STD clinic attendees in Pune, India
- Extent of clinical interaction between HBV and HIV coinfected individuals may have to be studied
- Sexual transmission may have a significant contribution in spread of HBV
- Vaccination against HBV in sex workers and their clients needs consideration

may have been due to impaired immune status of HIV positives. Of the 95 subjects, nine were found to be positive for anti-HBc IgM, indicating probable recent infection.

To summarise, a high prevalence and incidence of hepatitis B virus infection was seen in STD clinic attendees. Some sexual risk factors were independently associated with prevalent anti-HBc antibody. These results suggest evidence of sexual transmission of HBV in India. HBV is a vaccine preventable infection and the results highlight the importance of HBV vaccination among commercial sex workers and their clients.

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CONTRIBUTORS

AR, SM, VA, RB, and DG were involved in conceptualising and designing the study; AR, RG, AD, SK, and RP were involved in implementation of clinical and laboratory procedures of the study. Statistical analysis was done by AR, SB, and AW; AR, RG, AD, VA, SB, SM, RP, DG, SK, AW, and RB contributed to writing and editing the manuscript.

Authors' affiliations

A Risbud, S Mehendale, S Kulkarni, R Gangakhedkar, A Divekar, D Gadkari, R Paranjape, National AIDS Research Institute, Pune, India
S Basu, Indian Statistical Institute, Calcutta, India
A Walimbe, V Arankalle, National Institute of Virology, Pune, India

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