INTERNATIONAL JOURNAL OF INNOVATIVE RESEARCH & DEVELOPMENT

ISSN 2278 – 0211 (Online)

Seroprevalence of Hepatitis B Surface Antigen and Antibodies to Hepatitis C Virus at an Indian State Bordering Myanmar: A Hospital-Based Study

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Abstract:
In Manipur, one of the six high HIV-prevalent states of India, information is limited regarding Hepatitis B and C infections, which share a similar parenteral mode of transmission. The aims of this study are to investigate the seroprevalence of Hepatitis B surface antigen (HBsAg) and Hepatitis C antibodies (HCVAb) and to identify the seropositivity rates at different age-groups. A retrospective cross-sectional study was conducted at the Microbiology department of Jawaharlal Nehru institute of Medical Sciences, Manipur, on existing data from 2010- 13. A total of 21358 serum samples were screened for HBsAg and HCVAb using rapid immunochromatography tests and 3rd generation enzyme-linked immunosorbant assays (ELISA). 75(1.57%) and 259(5.4%) of 4790 males were positive for HBsAg and HCVAb respectively, and 132(0.8%) and 78(0.5%) of 16568 females were positive for HBsAg and HCVAb respectively. 21(0.44%) males and 5(0.03%) females were positive for both infections. For HBsAg the yearly seroprevalence ranged from 1.55 to 2.38% among the males and from 0.54 to 1% among the females. For HCVAb, it ranged from 3.91-8.17% among the males and from 0.30-0.64% among the females. Difference in seroprevalence between males and females was found to be statistically significant at P-value < 0.05. Seropositivity rates were maximum at 41-50 years for both infections among positive males, and at 21-30 years for HBsAg and 31-40 years for HCVAb among positive females. The rising seroprevalence rates of both hepatitis infections, especially those of HCV infection among the males, need urgent attention.

Keywords*: ELISA, hepatitis B, hepatitis C, immunochromatography, seroprevalence*

1. Introduction

Manipur is one of the six high prevalence states of India regarding human immunodeficiency virus (HIV) infection. HIV, hepatitis B virus (HBV) and hepatitis C virus (HCV) all share a common route of parental transmission. Hepatitis B and C affect the liver chronically and hence is a cause of high morbidity and mortality. It was against the above backdrop that the present study was undertaken to estimate the seroprevalence of hepatitis B and hepatitis C infections among individuals attending our medical institute in the capital of this state and to identify the seropositivity rates at different age-groups of infected persons.

2. Materials and Methods

The study was carried out in the Serology and Immunology Section of the Department of Microbiology, Jawaharlal Nehru Institute of Medical Sciences, Imphal, Manipur. It was a retrospective cross-sectional data review, and the identity of the patients had been delinked from the data. Hence, Institutional Ethical Board clearance was not sought. Data evaluated included the findings of blood samples from all individuals who registered consecutively at the Out Patient Department (OPD) or were admitted to the In Patient Department (IPD), from January 2010 to December 2013 and who were advised to undergo screening for Hepatitis B surface antigen (HBsAg) and Hepatitis C Virus antibodies (HCVAb). About five ml of venous blood was received from each individual and the blood was allowed to clot for 30 minutes at room temperature. The serum sample was separated after centrifugation at low speed.

The serum was tested for HBsAg using a rapid card method (Hepacard, manufactured by Biomed Industries/ Diagnostic Enterprises, India). Reactive samples were re-confirmed by another rapid test (Hepaview, manufactured by Qualpro Diagnostics, India) or by an Enzyme Linked Immunosorbant assay (ELISA) (Hepalisa, manufactured by J. Mitra & Co. Pvt Ltd., India).

Antibodies to HCV were determined using rapid test device (Flavicheck-HCV WB, manufactured by Qualpro Diagnostics, India). Samples reactive by this test were re-confirmed using another rapid test (HCV Tridot, manufactured by Diagnostic Enterprises, India) or by a 3rd generation ELISA (SD HCV ELISA 3.0 , manufactured by Standard Diagnostics, Inc., Korea).

All the tests were performed in accordance with the manufacturer's instructions using adequate controls. A patient is considered positive for any one or both of these infections if at least two of three tests used for each type of infection were reactive.

The prevalence of both hepatitis B and C infections were analysed by using Student's t-test at probability level of 5%. A *P*- value of less than or equal to 0.05 was considered statistically significant.

3. Results

Of 21417 consecutive blood samples received over a period of four years, five failed to produce adequate amount of serum for the tests, and 54 were considered not fit for the tests as they were haemolysed. A total of 21358 serum samples were included in the study. Table 1 shows the year-wise distribution of individuals screened for HBsAg and HCVAb in terms of gender and religion. Females (77.57%) outnumbered males (22.43%). More than half of them were Hindu by religion (64.56%). Table 2 shows the year-wise seropositivity rates of HBsAg and HCVAb. Over a period of four years from 2010 and 2013, not only can we see a steady increase in the number of individuals screened, but an overall increase in the percentage of positivity rates for both sexes. Out of 4790 males screened 2% (96=14+17+28+37) were HBsAg positive and 5.8% (280= 34+43+76+127) were HCVAb positive. Out of 16568 females screened, 0.8% (137=22+23+40+52) was HBsAg positive and 0.5% (83= 10+14+26+33) were HCVAb positive. The difference in the seroprevalence of HBsAg between the male and the female patients was found to be statistically significant for all four years of the study at *P*-values of 0.0019, 0.0063, 0.0003, and 0.0024 consecutively. The corresponding *P*-values for HCVAb were all statistically significant at zero.

Year	Total	OPD	IPD	Male	Female	Hindu	Muslim	Christian	Others
2010	4084	3069	1015	767	3317	2750	482	429	423
2011	4560	3536	1024	1100	3460	2892	554	634	480
2012	5978	4556	1422	1368	4610	3686	702	978	612
2013	6736	5079	1657	1555	5181	4461	747	844	684
Total	21358	16240	5118	4790	16568	13789	2485	2885	2199

Table 1: Year-wise distribution of individuals screened for HBsAg and HCVAb.

		2010		2011		2012		2013	
		Pos / Total tested	% Pos	Pos / Total tested	% Pos	Pos / Total tested	% Pos	Pos / Total tested	% Pos
HBsAg	M	14/ 767	1.83	17/ 1100	1.55	28/ 1368	2.05	37/ 1555	2.38
	F	22/ 3317	0.66	23/ 3460	0.66	40/ 4610	0.87	52/ 5181	1.00
	T	**36/ 4084**	**0.88**	**40/ 4560**	**0.88**	**68/ 5978**	**1.14**	**89/ 6736**	**1.32**
HCVAb	M	34/ 767	4.43	43/ 1100	3.91	76/ 1368	5.56	127/ 1555	8.17
	F	10/ 3317	0.30	14/ 3460	0.40	26/ 4610	0.56	33/ 5181	0.64
	T	**44/ 4084**	**1.08**	**57/ 4560**	**1.25**	**102/ 5978**	**1.71**	**160/ 6736**	**2.38**

Table 2: Year-wise seroprevalence rates of HBsAg and HCVAb
M = Males, F = Females, T = Total, Pos = Positive

Table 3 shows the seropositivity rates of positive patients in different age groups for both infections. Among the males the highest seroprevalence for both HBsAg and HCVAb were found to occur in the 41-50 years age group. Among the females highest seroprevalence for HBsAg and HCVAb were found in the 21-30 and 31-40 years age groups respectively.

Age Group	HBsAg Positive		HCV antibody Positive		HBV-HCV Positive	
	Male N=75	Female N=132	Male N=259	Female N=78	Male N=21	Female N=5
0 - 10	0	2 (1.52%)	0	0	0	0
11 - 20	7 (9.33%)	16 (12.12%)	0	2 (2.56%)	0	0
21 - 30	9(12%)	55 (41.67%)	11 (4.25%)	13 (16.67%)	3 (14.29%)	2(40%)
31 - 40	21 (28%)	34 (25.76%)	101 (39%)	25 (32.05%)	7(33.33%)	2(40%)
41 - 50	27 (36%)	4 (3.03%)	122 (47.10%)	16 (20.51%)	9(42.86%)	1(20%)
51 - 60	8 (10.67%)	4 (3.03%)	25 (9.65%)	9 (11.54%)	2 (9.52%)	0
61-70	3 (4%)	2 (1.52%)	0	9 (11.54%)	0	0
71 & above	0	0	0	4 (5.13%)	0	0

Table 3: Seropositivity rates of HBsAg and HCVAb in different age-groups

4. Discussion

Out of 21358 serum samples studied a majority of 16240 (76.04%) were from patients registered at the OPD and the rest were from those admitted in the wards.(Table 1) A majority of 16659 (78%) samples were from asymptomatic individuals and 4699 (22%) had complaints related to viral hepatitis such as icterus with or without fever, anorexia, nausea, vomiting, right upper quadrant abdominal pain and hepatomegaly. Among the 16568 female patients, most of them 13420 (81%) were females undergoing antenatal screening for Hepatitis B and C infections. A much lesser 149 (0.9%) of them were undergoing pre-operative screening and none of them revealed a history of being HIV positive. Of the 4790 males 204 (4.3%) were known HIV positive patients and 575 (12%) were screened pre-operatively.

Out of 4790 males screened it has been found that 75 (1.57%) of them were only HBsAg positive whereas a much higher 259 (5.4%) tested positive for only HCVAb. Out of 16568 females studied, 132 (0.8 %) and 78 (0.5 %) of them were only HBsAg and only HCVAb positive respectively. 21 (0.44%) males and 5 (0.03%) females were positive for both infections and all were found to be symptomatic for viral hepatitis. Though it could not be determined whether these were co-infections or super-infections, it is certainly known that both lead to high morbidity and mortality. [1]HBsAg in serum is the first seromarker to indicate active HBV infection, either acute or chronic. Based on the prevalence of chronic hepatitis B, countries have been variably classified as high (≥8%), intermediate (2-7%), and low prevalence (≤2%) areas.[2] India has been placed in the intermediate zone of prevalence by WHO.[3] In this study, the yearly seroprevalences among the males ranged from 1.55 to 2.38% whereas those among the females ranged from 0.66 to 1%. (Table 2) Comparing the HBsAg seropositivity rates in different age groups, we can observe that they are higher among the females upto 30 years of age. Thereafter the seropositivity rates start to reverse and the percentage of positive males predominate. (Table 3)

Among the 96 HBsAg positive males 28 (29.17%) were in the 31-40 years age-group and 36 (37.5%) were in the 41-50 years age-group. Considering the fact that all of the known HIV positive males belonged to these two age-groups, and that most HIV positives in this state are or were intravenous drug users (IVDUs) it is easy to understand why more number of males were positive in these two age-groups.[4] Beyond these two age-groups, the numbers of HBsAg positive males are much lower at 12(12.5%) and 10(10.42%) in the 21-30 and 51-60 years age-groups respectively.

Comparing the HBsAg seropositivity rates in different age-groups of 137 HBsAg positive females, we observed that a maximum of 93 (67.88%) are in the 21-30 and 31-40 years age-groups put together. Taking into consideration the high percentage (81%) of pregnant females among the total number of females screened this high rate of seropositivity in the child bearing age is expected to translate into high perinatal transmission rates. Perinatal transmission is the most common mode of HBV transmission worldwide. It occurs at or near the time of birth, because neonatal vaccination prevents new born infection in about 80% -95% of cases.[5] Centers for Disease Control and Prevention (CDC) guidelines include mandatory screening of all women for hepatitis B during the first prenatal visit because this virus is highly contagious, and the risk that the newborn infant will develop hepatitis B is 10 -20% if the mother is positive for HBsAg.[6] CDC also noted that risk factor-based screening did not identify 35%-65% of all HBsAg positive mothers. Thus screening the expectant mother could go a long way in prevention of prenatal HBV transmission. In India, unlike the policy for HIV infection screening among pregnant women, there is no policy for HB infection screening for them. Perinatal transmission of HBV infection has declined steadily in the United States, consistent with the successful implementation of universal screening of pregnant women and vaccination policies.[7] If the mother is HBsAg positive, appropriate active and passive immunoprophylaxis should be given in the form of hepatitis B immunoglobulin and hepatitis B vaccine.

The presence of anti-HCV Ab indicates previous exposure to hepatitis C virus. This antibody is present in only 40% of acute infections but in more than 95% of chronic infections.[8] Hepatitis C virus (HCV) infection establishes a state of chronic infection in as many as 85% of acutely infected patients, whereas about 15% of acutely infected patients spontaneously clear the infection.[9] In India, anti-HCV Abs are present in approximately 15 million people with a prevalence rate of 1.2 – 1.8%. [10,11,12]. In this study, the yearly overall seroprevalence ranged from 1.08% to 2.38%. Those among the females continued to be under 1%. In contrast, those among the males were well above the national average and ranged from 3.91 to 8.17%. (Table 2) This high seroprevalence of HCV infection among the males could be due to the fact that this institute also runs an Anti-retroviral Therapy ART) centre under the aegis of the National AIDS Control Organisation (NACO). In Manipur, HIV-HCV co infection among people living with HIV (PLHIV) is 79.1% in 2008.[4] In India, HCV is the most infectious disease among IVDUs. A recent study conducted in another medical institute in the same city reported the seroprevalence of HCVAb as 0.40% among the voluntary blood donors and 1.11% among the replacement/relative blood donors.[13] Evidently more lives could be lost due to HCV than did HIV among people who have or had high risk behaviour such as sharing of needles and syringes, and also of other injecting-related equipment, because the cost of treatment is very high and there is no Government policy to screen these patients as in the case of HIV/AIDS.

On the other hand, universal screening for hepatitis C in pregnancy is not recommended. Firstly, the efficiency of hepatitis C virus (HCV) transmission by sexual activity remains controversial. [14] Secondly, an effective hepatitis C vaccine has not been developed and thirdly, the drugs used most commonly to treat hepatitis C in both children and adults, interferon and ribavirin are not recommended for use in pregnancy. [15,16] HCV-infected children may be born to mothers who were anti-HCVAb positive. Intrapartum infection is more common than in-utero infection. [17] Elective CS is not recommended; as even non-viremic women cannot be assured they have no chance of infecting their neonates, although the risk appears to be very low. [17,18] Yet, in this study, a large number of pregnant females were screened for HCVAb as clinicians have a tendency to test both HBV and HCV infections together.

In this study, 575 (12%) of 4790 males and 149 (4.7%) of 3148 non-pregnant females underwent pre-operative screening for these two infections. Out of 575 males only 5 (0.87%) and 1 (0.17%) were positive for HCV Ab and HBsAg respectively. Only 1 (0.67%) of the 149 females were reactive for HBsAg. It has been suggested that a minimum prevalence of 1 in 1000 (0.1%) in the general population justifies screening of an infection. [19] Universal pre-operative testing for HBV and HCV may be done if a protocol for management of infected patients, including testing of family members of confirmed cases, is available as non-sexual intra-familial modes of transmission of HBV and HCV have been reported.[20,21,22] Patient and institutional resources may be saved by elective screening dependent on a carefully worked out clinical risk assessment plan.

5. Conclusion

This study shows that the ever rising seroprevalence rates of hepatitis B and C infections, especially that of HCV infection among the males, is a cause of alarm in this state of NE India bordering Myanmar. Taking into consideration that there is no vaccine or immunoglobin prophylaxis for hepatitis C infection, and that screening is justified in terms of the seroprevalence rates of both infections for four consecutive years, a large study among the general population could be conducted to make the consideration for national policies for both hepatitis more conclusive.

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