Epidemiol. Infect. (2002), **128**, 465–471. © 2002 Cambridge University Press
DOI: 10.1017/S095026880200691X Printed in the United Kingdom

Epidemiology of hepatitis B infection among the Nicobarese – a mongoloid tribe of the Andaman and Nicobar Islands, India

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(*Accepted 3 January 2002*)

SUMMARY

Andaman and Nicobar Islands, Union Territory of India, is home to six primitive tribes. Preliminary serological studies carried out earlier among the four accessible tribes revealed that hepatitis B virus (HBV) infection is hyper-endemic among them. The present study was carried out to understand important modes of transmission and to identify possible risk factors associated with HBV infection among the Nicobarese tribe. The epidemiology of HBV infection in these islands appears to be distinct with a high prevalence of the chronic carrier state (22·2%) associated with a comparable proportion of the population being anti-HBs positive (26·3%). More than half of the HBsAg and anti-HBs negative individuals have anti-HBc antibodies. Age, past history of hospital admission, intramuscular injections and number of carriers in the *tuhet* were found to be significantly associated with exposure to hepatitis B virus. Horizontal transmission through close contact with carriers and perinatal route appears to be an important mode of transmission of HBV in this community. Besides this, use of unsafe injections represents an independent risk factor for acquiring HBV infection in this population. Introducing HBV vaccine in the infant immunization programme and improving injection safety would help to control the infection in the tribal community of these islands.

INTRODUCTION

Hepatitis B virus (HBV) infection is one of the major diseases of mankind, with about 2 billion people infected globally and more than 350 million chronic carriers of the virus [1]. The disease is an important public health problem in India with an estimated 36 million HBV carriers. India comes in the intermediate zone of endemicity with a carrier rate of 4·71% [2].

The discovery of Australia Antigen (hepatitis B surface antigen, HBsAg) by Blumberg in the sera of an Australian aborigine [3] led to the identification of the infectious agent, and since then much information about the virus and the disease has been generated leading to the development of an effective vaccine. Following the discovery of Australia antigen, several studies have been carried out among the tribal populations worldwide and these studies generally have shown higher prevalence of HBV infection among these aborigines [4–9]. Association of some socio-cultural practices like bloodletting, scarification, tattooing and orally processed food are probable reasons for the observed higher prevalence [10].

In India, 427 scheduled tribal communities have been listed, including sub-tribes with varying population sizes. Studies carried out on some of the tribes revealed higher prevalence of hepatitis B than the

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national average [11, 12]. The Andaman and Nicobar Islands, Union Territory of India, situated in the Bay of Bengal, about 1200 km east of the Indian sub-continent is the home of six primitive tribes. Great Andamanese, Onges, Jarawas and Sentinelese belong to the Negrito race and Nicobarese and Shompens belong to the Mongoloid race. The population of these tribes is 26852 (1991 census), which is about 9% of the total population of these islands. The Jarawas and Sentinelese are still hostile to the external world. The population of these tribes, except the Nicobarese, has been declining continuously over the past several years.

The preliminary serological studies carried out earlier among the four accessible tribes of these islands revealed that HBV is hyper-endemic among them, with HBsAg carrier rates in the range 23·3–37·8% [13]. The carrier rates observed among these tribes are the highest reported rates from India. The present study was carried out to understand the epidemiology of HBV infection and to identify important modes of transmission of HBV infection among the Nicobarese tribe.

METHODS

Study area

The Nicobarese, with a total population of 26561, constitute more than 98% of the tribal population of Andaman and Nicobar islands. They are confined in Car Nicobar Island, Nancowry group of islands and in Harminder Bay of Little Andaman Island. Nicobarese living in Car Nicobar Island form the largest group of tribal population living in any single island of the territory. There are 15 villages in Car Nicobar Island with a total population of 19336 (1991 census). The medical care in this island is provided through one district hospital and six sub-centres. The present study was carried out in *Tamaloo* village of Car Nicobar Island which has a total population of 1347 residing in 27 *tuhets*. *Tuhet* denotes the principal homestead and large lineage group residing in their individual houses and huts. These are either built adjacent to the primary homestead or in their horticultural gardens and plantations. Thus, the word *tuhet* indicates an extension of a family and is the unit of all social life in Car Nicobar Island [14].

All the 887 permanent residents (65·9% of the total population of the village) of this village aged 45 years or less residing in 27 *tuhets* were included in the study. The number of people in the *tuhets* ranged from 11–79. Venous blood samples were collected from 841 persons after obtaining their informed consent. From the remaining 46 infants and small children, 3–4 drops of capillary blood were spotted on filter paper strips and blood was dried and stored at room temperature for 2 weeks, and then at −20 °C until further analysis. Blood samples were also collected from 73 pregnant women attending antenatal clinics in different sub-centres in this Island, 16 of whom were from this village.

Serology

Viral markers were tested employing commercially available ELISA kits. All the 887 samples were tested for HBsAg (Murex, UK). Forty six blood samples from the infants/young children on filter paper were tested for HBsAg only. HBsAg negative venous blood samples ($n = 651$) were tested for antibody to HBsAg (anti-HBs, Antisurase-B, General Biologicals, Taiwan). Individuals negative for both HBsAg and anti-HBs ($n = 480$) were tested for antibodies against hepatitis B core antigen (IgG-anti HBc, Anticorase-B, General Biologicals, Taiwan). All the HBsAg positive serum samples were tested for presence of hepatitis B e antigen (HBeAg, General Biologicals, Taiwan), antibodies to HBeAg (anti-HBe, General Biologicals, Taiwan) and IgM antibodies against hepatitis B core antigen (IgM-anti HBc, General Biologicals, Taiwan).

Samples positive only for anti-HBc (i.e. samples negative for HBsAg and anti-HBs and positive for IgG anti-HBc) were further tested for the presence of IgM-anti-HBc antibodies and anti-HBe antibodies. Repeat blood samples were collected 4 months following initial bleeding from anti-HBc alone positive individuals and retested for anti-HBc antibodies.

Risk factors

Information about different risk factors such as history of hospital admission, any form of parenteral treatment such as intra-muscular (IM) injections, intravenous (IV) medication, blood transfusion, etc. in the last 10 years, history of ear/nose piercing and past history of jaundice in the family was collected for each subject on a pre-designed questionnaire. The subjects' responses regarding past history of parenteral treatment and hospitalization were cross–checked with other family members. Responses given

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Table 1. *Age-related prevalence of HBsAg and anti-HBs among the Nicobarese tribe*

	HBsAg		Anti-HBs		Anti-HBc (IgG)		Overall exposure†	
Age group	No. positive/ No. tested	Prevalence (%)	No. positive/ No. tested	Prevalence (%)	No. positive/ No. tested	Prevalence (%)	No. positive/ No. tested	Prevalence (%)
< = 5	12/97*	12.4	2/46	4.3	4/44	9.1	11/51	21.6
6–14	48/241	20.2	34/193	17.6	48/159	30.2	130/241	53.9
15–24	50/211	23.7	45/161	28.0	82/116	70.7	177/211	83.9
25–34	52/190	27.4	47/138	34.1	71/91	78.0	170/190	89.5
35–45	35/148	23.6	43/113	38.1	54/70	77.1	132/148	89.2
Overall	197/887	22.2	171/651	26.3	259/480	54.0	620/841	73.7

* Includes 7/46 samples from children collected on filter papers and tested for HBsAg only.
† Includes those individuals whose venous samples were collected.

by these subjects were also verified from the records of hospitalization/outpatient treatment, available with the persons whenever possible. Reasons for hospitalization and IM injections were also noted. Detailed information about the last injection received by the person (person who prescribed and administered the injection, type of syringe and needle used and place where the injection was administered) were also collected. Persons positive for any of the three serological markers, i.e. HBsAg, anti-HBs or anti-HBc were considered as cases and those negative for all the three markers were considered as controls for logistic regression analysis.

Statistical analysis

The 95% confidence limits for the prevalence of HBsAg, anti-HBs and anti-HBc were estimated. The significance of difference in proportions was tested using χ^2 test. χ^2 for trend was calculated to see the linearity of association between age and serological markers of HBV infection. Logistic regression analysis was carried out to find out the association between possible risk factors with exposure to HBV. Statistical analysis was carried out using software EPI info version 6.1 and SPSS version 8.0.

RESULTS

HBsAg was detected in 197 of the 887 (22·2%) individuals (95% CI 19·5–25·1) (Table 1). All the *tuhets* had at least one individual positive for HBsAg. The mean numbers of carriers in the tuhet were $7{\cdot}3 \pm 4{\cdot}9$ (range 1–18). 12·4% of the under-five children and 5·3% (1/19) of the infants from this village were positive for HBsAg. The HBsAg positivity among the pregnant women was 20·5% (15/73). Of the 190 HBsAg positive persons whose serum was available, IgM anti-HBc was detected in only two (1%) individuals, indicating recent HBV infection. Thirty-five (18·4%) individuals were positive for HBeAg whereas 64 (33·7%) of the HBsAg positive individuals had antibodies against HBeAg. The HBeAg positivity was significantly higher in children aged 14 years or less than the older persons (19/53 vs. 16/137, $\chi^2 = 13{\cdot}29$, $P = 0{\cdot}0003$). Two (13·3%) of the 15 HBsAg positive pregnant women were HBeAg positive.

Of the 651 HBsAg negative individuals whose sera samples were available, antibodies against HBsAg were detected in 171 individuals with a prevalence of 26·3% (95% CI 23·0–29·9). Antibodies against hepatitis B core antigen were detected in 259 of the 480 (54·0%) subjects negative for both HBsAg and anti-HBs with a prevalence of (95% CI 49·5–58·4) (Table 1). None of these 259 persons was positive for IgM anti-HBc.

Thus, of the 841 persons from this village whose venous blood samples were collected, 620 individuals were positive for any of the three markers of HBV infection with an overall exposure of 73·7% (95% CI 70·7–76·6). The overall exposure to HBV, as defined by the presence of any of the three markers studied was found to linearly associated with age (χ^2 trend = 129·803, $P = 0{\cdot}000$).

The logistic regression analysis was carried out to assess the risk factors associated with HBV exposure with 620 cases (i.e. persons positive for any of the three serological markers HBsAg, anti-HBs or IgG anti-HBc) and 221 controls negative for all the three markers. The results of univariate and multivariate

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Table 2. *Univariate analysis of different risk factors associated with exposure to HBV*

Risk factor	Cases ($n = 620$)	Controls ($n = 221$)	Odds Ratio (95% CI)	P
Age (in years)				
0–5	11	40	1*	
6–14	130	111	4.26 (1.99–9.28)	0.000
15–24	177	34	18.93 (8.36–43.77)	0.000
25–34	170	20	30.91 (12.84–76.24)	0.000
34–45	132	16	30.00 (11.99–77.18)	0.000
Sex				
Female	300	104	1*	
Male	320	117	0.95 (0.70–1.29)	0.794
History of hospitalization				
No	351	153	1*	
Yes	269	68	1.72 (1.23–2.42)	0.001
History of IM injections				
No	265	105	1*	
Yes	355	116	1.21 (0.88–1.67)	0.251
History of IV medication				
No	467	173	1*	
Yes	153	48	1.18 (0.80–1.74)	0.428
History of blood transfusion				
No	604	213	1*	
Yes	16	8	0.71 (0.28–1.83)	0.574
History of ear/nose piercing				
No	419	150	1*	
Yes	201	71	1.01 (0.72–1.43)	0.996
No. of carriers in the *tuhet*				
1–2	26	39	1*	
3–5	210	76	4.14 (2.28–7.55)	0.000
6–10	273	80	5.12 (2.84–9.27)	0.000
>10	111	26	6.40 (3.17–13.04)	0.000
Family history of jaundice				
No	599	215	1*	
Yes	21	6	1.26 (0.48–3.86)	0.791

* Reference category.

logistic regression analysis are shown in Tables 2 and 3. Of the various risk factors studied, age, history of hospital admission, history of intra-muscular injections and the number of HBsAg carriers in the *tuhet* were found to be significantly associated with HBV exposure.

Fever was the most commonly reported reason for hospitalization (69·7%) and receiving intramuscular (IM) injections (66·4%). Other important reported reasons for hospitalization and receiving IM injections were respiratory tract infections (14·8% and 20·8%) and skin infections (6·2% and 4·9% respectively). Everyone who received injections reported that the injection was administered in hospital setting and was prescribed by a qualified doctor. In total, 89·2% of the individuals who received IM injections reported that a glass syringe was used for administering the injection and only 10·8% reported that a plastic disposable syringe was used. Everyone who received injections with glass syringe reported that the glass syringe and the needle were taken from a vessel containing water at the time of giving the injection.

DISCUSSION

The results of the present study document high endemicity of HBV infection in this village with about 74% of the population exposed to this infection. High prevalence of HBsAg observed in the present study is comparable to the earlier study carried out among the Nicobarese tribe settled in three areas namely Harminder Bay of Little Andaman, Car Nicobar Island and Nancowry group of islands [13]. Prevalence of anti-HBs is generally several folds higher than the

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Table 3. *Multivariate analysis of risk factors associated with HBV exposure*

Variable	Beta	Standard error	OR (95% CI)	*P*
Age (years)				0·000
0–5			1·00*	
6–14	1·45	0·38	4·28 (2·02–9·03)	0·000
15–24	2·94	0·41	18·91 (8·54–41·89)	0·000
25–34	3·39	0·43	29·63 (12·71–69·05)	0·000
35–45	3·24	0·45	25·63 (10·69–61·42)	0·000
Sex	0·1626	0·2444	1·18 (0·73–1·90)	0·506
No. of carriers in *tuhet*				0·000
1–2			1·00	
3–5	1·44	0·34	4·22 (2·17–8·20)	0·000
6–10	1·61	0·33	5·01(2·62–9·60)	0·000
>10	1·87	0·39	6·50 (3·04–13·91)	0·000
History of hospitalization	0·71	0·25	2·04 (1·26–3·30)	0·004
History of IM injections	0·56	0·24	1·75 (1·09–2·80)	0·021
History of IV medication	0·19	0·29	1·21 (0·68–2·14)	0·514
History of blood transfusion	−0·39	0·51	0·68 (0·25–1·84)	0·449
History of jaundice in family	0·23	0·56	1·26 (0·42–3·74)	0·681
History of ear/nose piercing	−0·27	0·27	0·76 (0·45–1·29)	0·309
Constant	−3·3101	0·5341		0·000

* Reference category.

prevalence of HBsAg [15]. However, epidemiology of HBV infection in these islands appears to be distinct with high prevalence of chronic carrier state associated with a comparable proportion of the population being anti-HBs positive. A similar picture was also observed in an earlier study carried out among the four accessible tribes of these islands [13]. Interestingly, almost half of the HBsAg and anti-HBs negative individuals were found to be positive for anti-HBc antibodies, suggesting the loss of anti-HBs antibodies. The possibility of these individuals representing anti-HBs response failures on account of tolerance developed following HBV exposure during infancy cannot be ruled out. It is noteworthy that none of the anti-HBc alone positives was positive for IgM anti-HBc, ruling out the recent infection [16]. The immediate question therefore was whether the high prevalence of anti-HBc alone positivity observed in the present study could possibly be due to the use of inefficient ELISAs, i.e. non-specific anti-HBc and/or insensitive anti-HBs ELISA. To address this issue, 25 HBsAg and anti-HBs negative, anti-HBc positive serum samples were retested for the presence of anti-HBs antibodies in Abbott ELISA and all were found to be non-reactive. Also, 58 of the 259 anti-HBc alone positive samples were tested for anti-HBe antibodies and 40 (68·9%) were found to be positive, indicating definite exposure to HBV. Follow-up samples collected 4 months after initial bleeding from 88 anti-HBc alone positive individuals were re-tested for anti-HBc antibodies. Continued presence of anti-HBc was documented in 86 (97·7%) persons. Anti-HBs testing of the follow-up samples was of no use in resolving this issue as all these individuals were vaccinated with hepatitis B vaccine on the basis of HBsAg and anti-HBs negativity. We therefore believe that the observed serological picture reflects true HBV activity in this population.

In the present study, 5·3% of the infants and 20·5% of the pregnant women were found to be HBsAg positive. Also, 13% of the HBsAg positive pregnant women were also HBeAg positive. Thus, vertical transmission seems to an important mode of transmission of HBV in this community. After the first year of age, the age-specific rates of HBV exposure were found to increase linearly from 21·6% for under-five children to 89·5% among persons aged 25–34 years emphasizing importance of other modes of transmission. As evident from Table 1, more than double increase in the overall HBV exposure was noted in children aged 6–14 years as compared to under-five children. These results strongly point towards continued horizontal transmission of HBV in these children.

All the 27 *tuhets* in this village had at least one chronic carrier with an average of 7·3 carriers per *tuhet*. Multiple logistic regression analysis showed the presence of HBsAg carriers in the *tuhet* to be

https://doi.org/10.1017/S095026880200691X Published online by Cambridge University Press

significantly associated with exposure to HBV, the odds of being exposed to HBV increased from 4·22 for *tuhets* having 3–4 carriers to 6·50 for *tuhets* having more than 10 carriers. The observed association of number of carriers in the family with the exposure to HBV and the linear increase of HBV exposure with age further support the importance of horizontal spread of the virus by close contact of the family members with HBsAg carrier. Though the exact mode of spread of the virus in horizontal transmission is uncertain, it is suggested that the infection is probably transmitted by percutaneous infection through saliva or traces of blood [17]. Horizontal transmission has been observed to be an important mode of transmission in many countries including Sub-Saharan Africa, China and Middle East and Singapore [17–20].

In order to ascertain the role of parenteral treatment in the spread of HBV infection in this island, information regarding the past history of hospitalization and parenteral treatment in last 10 years was collected. Multiple logistic regression analysis revealed that history of hospital admission and IM injections were significantly associated with exposure to HBV. HBV infection was not, however found to be associated with other form of parental treatment like intravenous medications, and blood transfusion, probably because these procedures are always carried out with disposable transfusion sets.

In the present study, 69·8% of the subjects reported that they were hospitalized for fever and 66·4% reported that they received injections for the treatment of fever. It is pertinent to mention that malaria is hyper-endemic in this area with an annual parasite index ranging between 60 and 140 [21] and it could be the probable reason for high hospitalization rates for fever observed in the study.

It is estimated that about 50% of all the injections in developing countries are unsafe and the population attributable risk of unsafe injections with respect to HBV infection, i.e. the proportion of infections attributable to unsafe injections was estimated to be in the range of 20–80% [22]. In India, several studies have found the use of unsafe injections to be an important risk factor for acquiring hepatitis B infection [23, 24]. A recent study from a single village of West Bengal, Eastern India with 76·1% participation rate [25], found use of glass syringe for injection to be the most significant risk factor (OR = 3·01). The findings of the present study indicate that history of hospitalization and IM injections were significantly associated with exposure to HBV. The use of disposable syringes and needles was also found to be low in Car Nicobar Island and majority of the injections were administered with glass syringes and needle. These findings suggest the possibility of transmission of the infection through unsafe injections in this community.

Cross-sectional analytical studies have their own limitations. In the present study, attempts were made to reduce the recall bias regarding the past history of parenteral treatment and hospitalization by cross-checking the responses with family members and also by verifying with hospital records available with the individuals whenever possible. Though the findings suggest the possibility of transmission of infection through unsafe injections, injection safety assessment survey, which would help identifying unsafe practices related to injection administration, sterilization, waste disposal, etc. is necessary in this island.

In conclusion, the findings of the present study document that hepatitis B infection is highly endemic among the Nicobarese tribe with both perinatal and horizontal mechanisms being operative. Horizontal transmission both in early childhood and later years of life, through close contact with HBsAg carriers appears to be significantly important mode of transmission. Besides, past history of injections also represents an independent risk factor for acquiring HBV infection in this community.

In India, hepatitis B vaccine has not yet been introduced in the National Immunization Programme probably because of high cost of the vaccine [26]. However, considering the high prevalence of HBV infection and small number of tribal population of these islands, it is necessary to introduce hepatitis B vaccine in the immunization programme for this community, especially for newborns. Improving injection safety by advocating the use of disposable syringes, and equipping the hospitals especially the sub-centres in peripheral areas with operational sterilization equipments could also help in reducing the transmission of HBV and other blood borne infections.

ACKNOWLEDGEMENTS

The study was carried out with financial assistance from Ministry of Health and Family Welfare, Government of India, New Delhi. The authors gratefully acknowledge the help and co-operation extended by Director of Health Services, Medical Superintendent, Civil Hospital, Car Nicobar and Director of Tribal

Welfare, Andaman and Nicobar Administration, Port Blair.

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