Molecular epidemiology of hepatitis B virus in primitive tribes of Odisha, eastern India

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Background and objective: Among the indigenous population of India, Primitive Tribal Groups (PTGs) are vulnerable to various health related events and some of the PTGs are showing a decline in population associated with high mortality rates. The present study was undertaken to define the prevalence of Hepatitis B Virus (HBV) infection, its genetic characterization and possible risk factors for transmission in five PTGs in Odisha, India.

Methods: Cross-sectional observational studies were carried out in the Lodha, Saora, Khadia, Mankidia, and Juanga tribes residing in different parts of Odisha between 2006 and 2010.

Results: Hepatitis B surface antigen (HBsAg) prevalence was 0.8%, 0.9%, 0.9%, 3.7%, and 1.7% in Lodha, Saora, Khadia, Mankidia, and Juanga tribes, respectively. While 54.8% of seropositive (HBsAg) cases demonstrated HBV DNA, occult HBV infection was observed in 19.48% of cases. High viral load with detectable 'e' antigen was found in 29% of HBsAg-positive individuals. All HBV isolates ($n=17$) were genotype D without pre-core mutants. Only 15.6% of HBV positive individuals had symptoms of hepatic disease, though none had severe manifestations. Multivariate analysis of the prevailing risk factors indicated that shaving by the village barber was significantly associated with HBV transmission in males. Tattooing was found to be significantly associated with females.

Interpretation and conclusion: This is the first report on HBV infection in PTGs of Odisha that suggests a high potential for transmission of HBV infection in two PTGs (Mankidia and Juanga). It warrants early public health attention in tribal populations vulnerable to HBV infection.

Keywords: HBV, Primitive Tribal Groups, Risk factors, Odisha, India

Introduction

Primitive Tribal Groups (PTGs) are an especially vulnerable section of indigenous tribal population of India who are faced with either stagnation or decline in population growth. This may be attributed to various health related events, including acute and chronic infections that escape early detection and management. Chronic infections such as hepatitis B, C, and HIV which spread through parenteral routes can have a significant association with low levels of health awareness in the communities.¹ Hepatitis B Virus (HBV) infection has been shown to be the tenth most common cause of mortality in the world with India placed in the intermediate endemicity zone with an overall hepatitis B surface antigen (HBsAg) carrier rate varying from 2 to 7%.² A few studies undertaken in PTGs from Jharkhand, Arunachal Pradesh, and Andaman Nicobar islands in India revealed variable

HBsAg prevalence ranging from 2 to 65%.^{3–5} The circulating genotype, the routes of transmission and specific risk behavior in the community will add to the information available on tribal populations in the country to support development of specific prevention programmes/strategies for this vulnerable population.

There were no reports on HBV infection available from Odisha, eastern India, where >22% of the Indian tribal population, including 13 PTGs, live.¹ This study reports the prevalence of HBV infection in five PTGs in Odisha where there is potential for chronic morbidity with risk of mortality. The possible risk factors for transmission and the circulating genotypes are also reported that could be useful for public health planning in the region and other parts of the world with similar vulnerable populations.

Methodology

Study area and population

The study was undertaken between 2006 and 2010 covering five PTGs (Lodha, Juanga, Saora, Khadia,

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and Mankidia) from the State of Odisha, geographically located between 17°48' and 22°34' North and 81°24' and 87°29' East in the Eastern part of India on the coast of Bay of Bengal. Dwellings, located in isolated hilly/forest areas, were clustered in villages within different revenue blocks of six districts (Table 1). Representative samples were taken from all the clusters of dwellings of the above five tribes following multistage, proportionate to size, sampling procedures. A minimum sample size of 1286 was considered adequate, taking an approximate HBsAg prevalence rate of 5% at 90% confidence interval with 20% precision.⁷ Because of inherent difficulties in covering a larger population in the PTGs, the 90% confidence interval was considered for calculating sample size. However, it was planned to cover a larger proportion where total population of the tribe was low, i.e. less than 1000. The village was taken as the sample unit and subjects from all ages of either gender from all the households in the village were included as the study population.

Subject enrollment and sample collection

In the selected tribal inhabitations, repeated meetings and health awareness generation camps were organized in the villages before subject enrollment to help in rapport building and establishing communication with the PTGs. The assistance of village level health workers was sought to help the people understand in their respective tribal dialects. Through door-to-door visits, a population census was made and the individuals were informed about the study. Information on illness due to hepatitis infection, history of immunization, and risk factors for transmission were recorded in a pre-tested, structured questionnaire. Venous blood samples (4–5 ml) were collected aseptically and preserved in liquid nitrogen in the field. Samples were transported to the laboratory maintaining cold chain and stored at –80°C.

Human subject protection

The study was approved by the Human Ethical Committee of the Regional Medical Research Centre, Bhubaneswar. Permission from the Department of Tribal Welfare, Government of Odisha was also obtained because the study populations belong to scheduled tribes who are considered as especially vulnerable populations. Before enrollment in the

study, the individuals were informed in detail at group meetings as well as through individual interactions on the risks and benefits involved in the study. Village heads and ward members were also involved during the discussion. Communication was made in local tribal dialects understandable to them. Written informed consent was obtained from those who agreed to participate. Collaboration of medical officers of local primary health centers and officials of tribal development agencies in the respective districts were also obtained during the study. The individuals identified as HBsAg-positive were referred to the primary health-care system for management and follow-up. The results were also shared with the State Health Authority.

Laboratory investigation

All samples were tested for HBsAg, antibody to surface antigen (anti-HBs), and antibody to core antigen (anti-HBc). Only those samples which were positive to HBsAg, were tested for the 'e' antigen (HBeAg) and its antibody (anti-HBe), HBV DNA and antibody to Delta virus (HDV). ELISA kits from General Biologicals, Taiwan (HBsAg, anti-HBs, anti-HBc, HBeAg, and anti-HBe) and Diasorin, Italy (anti-HDV) were used.

DNA was extracted by using an extraction kit (Qiagen, Valencia, CA, USA). Nested PCR was performed for amplification of part of the S gene and pre-core mutation analysis according to previously described procedures.^{8,9} Quantitative viral load was determined by real-time PCR (Cobas Taqman 48; Roche).

A 363 bp fragment of S gene was sequenced by using the second-round primers and the ABI Big Dye terminator cycle sequencing ready reaction kit (DNA Sequencer, ABI, USA) and a phylogenetic tree was computed among genotypes of HBV (A to J) using Kimura 2 parameter matrix and neighbor joining method in Mega software, version 4.¹⁰ For pre-core mutants, a fragment of pre-core region was sequenced and analyzed.

Tests for liver function (ALT and AST) were done on all HBsAg-positive samples using diagnostic kits from Roche (Cobas Integra 400; Roche, USA). These tests were performed within 7 days of sample collection.

Quality control was assured by duplicate testing of all positive samples and 5% of the negative samples at

Table 1 Tribe wise population distribution and coverage under the study

Name of the PTGs	Name of the district with tribal population (no. of blocks)	Census population	Population covered
Lodha	Mayurbhanj (3)	2405	242
Saora	Mayurbhanj (2)	3740	212
Khadia	Mayurbhanj (3)	15 405	450
Mankidia	Mayurbhanj, Balasore, Deogarh Jajpur (6)	991	401
Juanga	Keonjhar, Dhenkanal (2)	31 823	460
5 PTGs	6 districts (10 blocks)	54 364	1765

National Institute of Virology, Pune, Maharashtra, India.

Assessment of risk factors for transmission of HBV infection

Information on the potential risk factors and routes for transmission of HBV prevailing in the community was obtained through individual and group questionnaires on the relevant risk behaviors, practices, and specific rituals which might contribute towards transmission. Risk factor questions put to individuals included: (1) history of multiple injection, i.e. more than two injection pricks received in preceding year; (2) whether shaving is done with village barber and razor shared with others in last 3 months; and (3) history of tattooing or body piercing in the past. Focus group discussions with young males and females and elderly people were held in separate settings, to derive qualitative and semi-quantitative information on the above.

Statistical analysis

Data were entered into Epi-info software and double checked for entry errors. Percentage prevalences were calculated and compared by Chi-square test in different age groups. Univariate and multiple logistic regression analyses were used to interpret the relative contribution of risk factors using SPSS version 17.0.

Results

During the study period, a total of 1765 individuals were enrolled. Age distribution of the subjects from different tribes is presented in Table 2. The study population included all age groups and both sexes; with male to female ratio of 1.07:1.

Clinical evaluation revealed presence of icterus in 19 (1.07%) and pallor in 186 (10.53%) individuals. Seven (0.39%) had hepatomegaly and 11 (0.62%) had splenomegaly. Ascites was not present in any case. However, features (periumbilical venous dilatation and upper GI tract bleeding) of portal hypertension were evident in one case. Overall 45 individuals (2.5%) had features of liver disease during the survey period. In addition, 140 (7.93%) individuals had past history of jaundice.

There was no evidence of jaundice outbreaks in the study villages. Reports of death due to liver failure were also infrequent. There was no ongoing HBV vaccination program for the community and none of the study population had received HBV vaccine.

Investigation showed HBsAg prevalence of 0.8–3.7% in the studied PTGs. Anti-HBs prevalence ranged from 0.9 to 9.5% of cases, whereas anti-HBc prevalence ranged between 1.4 and 26.2%. In total, 15.2% ($n=269$, male 148 and female 121) of the study population had recent or past exposure to HBV infection (Table 2).

Serological evidence of HBV infection in the different age classes is presented in Table 2. Evidence of HBV infection was observed in children ≤ 5 years in Khadia (4.4%), Mankidia (6.7%), and Juanga (8.3%) tribes.

Among 31 HBsAg-positive individuals, 17 (54.8%) had circulating HBV DNA, with HBeAg in 9 (29%) and antibody to HBeAg in 16 (51.6%) individuals.

Among individuals showing HBV DNA, nine had HBe antigen positivity along with higher HBV DNA load (1.62×10^2 – 2.62×10^8 IU/ml), the remaining eight were HBeAg-negative with low viral load (copy number <250). Occult HBV DNA was also detected by PCR assay in 15 (19.48%) of the 77 HBsAg-negative individuals having core antibody. Only one of the HBsAg-positive individuals had antibody to Delta virus (anti-HDV).

Sixteen of the 17 HBV DNA positive samples were sequenced. All belonged to genotype D (Fig. 1) and none had pre-core mutations. Among HBV-seropositive individuals, 53 (19.7%) had shown symptom and signs of hepatitis; however none had signs of hepatic failure. The individual co infected with HDV was also apparently healthy during the survey. All nine individuals with high viral loads were also apparently healthy on clinical evaluation. In all the HBsAg-positive individuals, mean ALT and AST levels were 29.7 and 33.18 IU/l, respectively.

The prevalences of possible risk factors for transmission of HBV, such as tattooing, sharing of razor, body piercing, history of multiple injections, and shaving by village barber, were obtained through questionnaire in the PTGs, and their frequency distribution along with univariate analysis results is shown in Table 3.

Multiple logistic regression showed that risk of getting HBV infections varied significantly among tribal groups (Chi-square = 34.1, Df = 16, $P = 0.01$). When risk factors were analyzed within individual tribes, no particular route was found to be relatively significant in Lodha, Saora, Khadia, and Mankidia tribe. In the Juanga tribe, shaving by village barber had a 2.3 (95% CI: 1.3–4.2) and 22.4 (95% CI: 2.9–169.7) times greater risk of acquiring HBV when compared to body piercing (OR = 1.0) and history of multiple injections (OR = 1.0). For all the tribes combined, persons using the service of a village barber has a 3.3 (95% CI: 2.0–5.5) times higher risk of acquiring HBV infection.

Focus group discussion results on the community behavior related to risk of transmission were expressed as common opinion on the practices in the community. It was recorded that tattooing was made mostly in females as adolescents or young adults. Sharing of needles during tattoo was reported

by 70% of respondents. History of multiple injections for common ailments such as fever and body pain was reported as a practice, but use of disposal syringes could not be ascertained. Shaving by the village barber was recorded as a response by 65% of males. Sharing of razors among peers and family members was reported by two-third of the males.

Discussion

This investigation provides the first data on HBV infection in the primitive tribes of Odisha in eastern part of India together with its molecular epidemiology. Investigation showed an overall HBV infection rate of 15.2%. The HBsAg prevalence (0.8–3.7%) was similar to those reported from Jharkhand (Paharia

Table 2 Age wise distribution of Sero markers of HBV infections

	HBsAg (+ve) (%)	Anti-HBS (+ve) (%)	Anti-HBC (+ve) (%)	Any marker (+ve) (%)
Lodha				
<1 Yr (n=0)	0	0	0	0
2–5 Yr (n = 21)	0	0	0	0
6–14 Yr (n = 57)	0	1 (1.8)	0	1 (1.8)
15–30 Yr (n = 65)	0	0	2 (3.1)	2 (3.1)
31–45 Yr (n = 38)	1 (2.6)	1 (2.6)	4 (10.5)	4 (10.5)
>45 Yr (n = 61)	1 (1.6)	2 (3.3)	5 (8.2)	6 (9.8)
All age groups (n = 242)	2 (0.8)	4 (1.6)	11 (4.5)	18 (7.4)
				95% CI: 4.76–11.45
Juanga				
<1 Yr (n = 1)	0	0	0	0
2–5 Yr (n = 23)	0	1 (4.3)	1 (4.3)	2 (8.7)
6–14 Yr (n = 210)	3 (1.4)	15 (7.1)	17 (8.1)	21 (10)
15–30 Yr (n = 87)	2 (2.3)	10 (11.5)	19 (21.8)	19 (21.8)
31–45 Yr (n = 77)	1 (1.3)	9 (11.7)	20 (26)	21 (27.3)
>45 Yr (n = 62)	2 (3.2)	9 (14.8)	17 (27.4)	20 (32.3)
All age groups (n = 460)	8 (1.7)	44 (9.5)	74 (16.1)	83 (18)
				95% CI: 14.7–21.7
Khadia				
<1 Yr (n = 0)	0	0	0	0
2–5 Yr (n = 46)	0	0	2 (4.4)	2 (4.4)
6–14 Yr (n = 102)	0	0	0	0
15–30 Yr (n = 136)	2 (1.5)	9 (6.6)	13 (9.6)	17 (12.5)
31–45 Yr (n = 86)	0	12 (14.1)	14 (16.3)	18 (20.9)
>45 Yr (n = 80)	2 (2.5)	7 (8.8)	13 (16.3)	18 (22.5)
All age groups (n = 450)	4 (0.9)	28 (6.2)	42 (9.4)	55 (12.2)
				95% CI: 9.5–15.5
Mankidia				
<1 Yr (n = 5)	0	0	1 (20)	1 (20)
2–5 Yr (n = 40)	0	1 (2.5)	2 (5.0)	2 (5)
6–14 Yr (n = 106)	4 (3.8)	9 (8.5)	18 (17.0)	19 (17.9)
15–30 Yr (n = 86)	5 (5.9)	8 (9.4)	24 (28.2)	28 (33.3)
31–45 Yr (n = 85)	2 (2.4)	7 (8.2)	24 (28.2)	26 (30.6)
>45 Yr (n = 79)	4 (5.1)	12 (15.4)	36 (46.2)	37 (47.4)
All age groups (n = 401)	15 (3.8)	37 (9.3)	105 (26.3)	113 (28.1)
				95% CI: 24.0–32.7
Saora				
<1 Yr (n = 1)	0	0	0	0
2–5 Yr (n = 24)	0	0	0	0
6–14 Yr (n = 55)	0	0	0	0
15–30 Yr (n = 46)	1 (2.2)	0	0	1 (2.2)
31–45 Yr (n = 43)	0	0	0	0
>45 Yr (n = 43)	1 (2.4)	2 (4.8)	3 (7.1)	4 (9.5)
All age groups (n = 212)	2 (0.9)	2 (0.9)	3 (1.4)	5 (2.3)
				95% CI: 1.0–5.4
All tribes				
<1 Yr (n = 7)	0	0	1 (14.3)	1 (14.3)
2–5 Yr (n = 154)	0	2 (1.3)	5 (3.3)	6 (3.9)
6–14 Yr (n = 530)	7 (1.3)	25 (4.7)	35 (6.6)	41 (7.7)
15–30 Yr (n = 420)	10 (2.4)	27 (6.5)	58 (13.9)	67 (16.1)
31–45 Yr (n = 329)	4 (1.2)	29 (8.9)	62 (18.9)	69 (21)
>45 Yr (n = 325)	10 (3.1)	32 (9.9)	74 (22.9)	85 (26.3)
Total (1765)	31 (1.7)	115 (6.5)	235 (13.3)	269 (15.2)
				95% CI: 13.64–16.9

Note: Chi-square test on inter age group differences in prevalence of HBV infection (any marker-positive) are as under:

For Lodha tribe, $P = 0.103$ and Chi-square value = 7.71.

For Juanga tribe, $P = 0.001$ and Chi-square = 24.52.

For Khadia tribe, $P = 0.001$ and Chi-square = 30.62.

For Mankidia tribe, $P = 0.001$ and Chi-square = 31.77.

For Saora tribe, $P = 0.034$ and Chi-square = 12.06.

Figure 1 Phylogenetic tree of hepatitis B virus based on the partial S gene sequences of the isolates ($n = 16$) (numbers represent the percentages of bootstrap replicates for the node. The capital letters A to H designate HBV genotypes. Reference sequences are denoted by their accession number and the country from which the strains were isolated).

tribe: 2%)⁶ and Madhya Pradesh (Saharia tribe: 5% and Baiga tribe: 4.4%);^{11,12} both are neighboring states of Odisha. However, studies on primitive tribes from Andaman and Nicobar islands, away from mainland India, have shown higher HBsAg prevalences (11.6–65%).^{4,5,12} One report from northeastern India has shown a 21.2% HBsAg prevalence in IduMishmi tribe of Arunchal Pradesh.³

In the PTGs studied here, anti-HBc positivity was observed to be high in Mankidia (26.2%) and Juanga

(16.1%) tribes, which was similar to Baiga tribe (33%) from Madhya Pradesh.¹² Tribes from Andaman and Nicobar islands also show high prevalence of Anti-HBc (27–68.8%).^{13,14} These reports of high HBsAg and anti-HBc indicate that high levels of transmission occur within the PTGs from different parts of the country, including some tribes (Mankidia and Juanga) residing in Odisha.

In the present study, 29% of HBsAg-positive individuals in the study had envelope antigen with

Table 3 Distribution of risk factors for HBV transmission in five PTGs with univariate analysis

Associated risk factors		HBV (+ve)	HBV (–ve)	OR (95% CI)	P
Tattooing	Present ($n = 300$)	66 (22.0%)	234 (78%)	0.57 (0.48–0.77)	0.000
	Absent ($n = 1465$)	203 (13.9%)	1262 (86.1%)		
Sharing of razor	Present ($n = 350$)	82 (23.4%)	268 (76.6%)	0.49 (0.37–0.66)	0.000
	Absent ($n = 1415$)	187 (13.2%)	1228 (86.8%)		
Body piercing	Present ($n = 712$)	122 (17.1%)	590 (82.9%)	0.78 (0.60–1.01)	0.079
	Absent ($n = 1053$)	147 (14%)	906 (86%)		
Shaving by village barber	Present ($n = 455$)	89 (19.6%)	366 (80.4%)	0.65 (0.49–0.86)	0.004
	Absent ($n = 1310$)	180 (13.7%)	1130 (86.3%)		
History of multiple injection	Present ($n = 253$)	21 (8.3%)	232 (91.7%)	2.16 (1.35–3.45)	0.001
	Absent ($n = 1312$)	248 (16.4%)	1264 (83.6%)		

high viral load (1.62×10^2 – 2.62×10^8 IU/ml), indicating a high potential to transmit the virus. Circulating HBV DNA was seen in 54.8% of HBsAg-positive individuals.

Genotype D was shown to be circulating within the studied PTGs and a recent hospital-based study in Odisha has also shown genotype D to be prevalent in general population.¹⁵ This could indicate that introduction of HBV infection to the PTGs has occurred from the general population residing in vicinity of the tribal inhabitations. The states of Jharkhand and Madhya Pradesh bordering on Odisha have also reported the same genotype, suggesting active transmission in the region of eastern and central India where majority of Indian tribal populations reside. This raises public health concern and requires specific awareness and health-care programs in the PTGs and other isolated tribes within India. Age-wise prevalence analysis indicated that both vertical and horizontal transmission contribute towards HBV infection within the PTGs. High endemicity of HBV infection in tribal populations has been suggested to be due to their association with a number of socio-cultural practices, such as endogamy, bloodletting, scarification, and tattooing in India⁴ as well as in other countries.^{16,17} This study reported prevailing risk factors, such as body piercing, tattooing, sharing of razor, shaving by village barber, and history of multiple injections. Among them, shaving by village barber in Mankidia and Juanga was shown to have statistically significant association with HBV infection. However, being a cross-sectional study, the risk factors were analyzed at one point of time; hence, it could not determine exact age of infection or the contribution of each risk factor.

In this report, two of these PTGs, Mankidia and Juanga, showed a higher prevalence of HBV infection, although all five PTGs studied share many similarities in respect to socio-cultural development, economy, geographical location, and relative isolation from the general population.

The higher rate of HBV infection observed in Mankidia tribe (28.1%) may be explained by their life style and behavior. The population typically lives a nomadic life style, wandering from hilly to plain areas for livelihood. The nomadic behavior gives a greater chance of entry of the virus from the general population and the risk factors prevailing in the community would have helped transmission within the tribe. The other tribe (Juanga) who had high prevalence of HBV had adopted an agricultural economy to certain extent alongside their traditional forest gathering practices. This has resulted in social contact with the general population residing in nearby villages and shaving by village barber was seen as significant route for HBV infection in this tribe.

The low rate of liver disease presentation may either be due to genotype D circulating in the general community,¹⁸ recent entry of the infection into these ethnic groups and thus, a short mean interval following infection or alternatively an unexplained host protection mechanism to disease development following exposure.

Owing to remoteness of the study area and primitive tribal populations, optimal sampling techniques could not be used and the study did not provide tribe-wise prevalence and risk factor data since it was not designed to do this. The cross-sectional nature of the study also did not allow prospective follow-up of the population to conclusively determine the age of entry of infection and the attributable route of transmission.

Conclusion

This study has shown high rates of HBV infection in some of the primitive tribes of Odisha in eastern India with genotype D in circulation. This is of concern for these PTGs who are showing low population growth or decline because of low longevity. Shaving by village barbers was shown as the most important risk factor. Although horizontal transmission contributed more, vertical transmission was also apparent. It may be recommended that immunization during infancy is provided along with health awareness campaigns to prevent sharing of blades during shaving by community barber. The report shows a need for further study of PTGs living in relative isolation who were conventionally thought to be protected from parenterally transmitted infections because of social isolation.

Disclaimer Statements

Contributors BD: designing study protocol, field investigation, result interpretation, manuscript preparation; JS: laboratory investigation, quality control, result interpretation, manuscript preparation; LMH: clinical examination, field investigation, result interpretation; SPS: protocol design and result interpretation, manuscript correction; PS: laboratory investigation, and quality control; RA, protocol design and review; SKK: protocol design, guidance and supervision, result interpretation, manuscript correction.

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