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ORIGINAL ARTICLE

Seroprevalence and factors associated with surface antigen of Hepatitis B virus and anti Hepatitis C virus antibody among southern region of India, Tamil Nadu

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Abstract
Hepatitis B and C are emerging viral infections that are a leading cause of liver cirrhosis and hepatocellular carcinoma with particular relevance in liver transplantation around the world. Though several studies from different countries have estimated the prevalence of these viral infections, there are only a few studies regarding the Indian population. Our specific aim was to find out the prevalence and predicting factors of the HBV and HCV infections in urban, suburban, rural and tribal populations in southern region of India. This study was conducted from different areas of southern region and a questionnaire was collected from all participants along with blood for serological analysis of HBV and HCV. The prevalence of HBV infection in the population was 3.3%, and HCV infection was 0.3% and prevalence rate of HBV infection in urban area was 4.5%, suburban 1.6%, rural 3.2% and in tribal 0%. Incidentally, this is the first report from southern region of India in a large scale with clear area wise prevalence rate of infection. The multinomial logistic regression analysis showed that the risk of HBV infection was greater for males (OR- 0.589, 95% CI- 0.363 to 0.955, P = 0.032), place of living (OR- 0.702, 95% CI- 0.536 to 0.920 P = 0.010), marital status (OR- 2.783, 95% CI-1.130 - 6.853, P = 0.026) and occupation (OR- 0.789, 95% CI- 0.657 to 0.948, P = 0.011). Epidemiology of HBV and HCV infections in this southern region evidently elucidated a low prevalence for hepatitis C virus and slightly high prevalence for hepatitis B virus with higher rates in urban areas than other northern regions of India.

Keywords: Hepatitis B virus; Hepatitis C virus; Seroepidemiological studies; India.

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Introduction

Viral infection is most common cause for hepatitis, which may leads to cirrhosis or cancer in the liver. Viral hepatitis affects approximately 2 billion people around the world, nearly one in every three. There are five distinct types (A, B, C, D and E) of hepatitis viruses reported, these types are of greatest concern because of the burden of illness, life-threatening and the potential for outbreaks and epidemic spread. In particular, types B and C lead to chronic disease in millions of people throughout the world and together are the major cause of liver cirrhosis and cancer. According to WHO, more than 240 million people are affected with chronic hepatitis B liver infection. Of these, an estimated of 60 million people die annually due to the acute or chronic consequences of hepatitis B. Chronic hepatitis C infection affects 184 million people around the world and approximately 35 to 50 million people die annually from HCV-related liver diseases.[1] However, the epidemiology pattern and consequence of HBV and HCV infections varies greatly from one part of the world to another and changes in the time course.[2]

Majority of seroprevalence studies are restricted to hospital and cross sectional design; however population based studies are better representation of overall community. India is the second world's most populous country with estimated population of 1.25 billion people which covers approximately 17.4% of world population but data on the prevalence of HBV and HCV in the population is scarce especially in the southern region of India. Viral hepatitis has serious socioeconomic repercussion. It is the most important public healthcare issue and even a major political implication. A better knowledge of the burden of viral hepatitis and the risk factors associated with it are among the population's main concerns of any nation.[3,4] Reliable prevalence data on infection rates of viral hepatitis are necessary for devising national control strategy. Therefore, each country should adopt its own policy to combat viral hepatitis.

Countries are classified based on the endemicity of HBV infection, which varies into high (> 8% infection rate), intermediate (2% - 8%) or low (< 2%) occurrence areas. The prevalence of chronic HBV infection in India varied from 2% to 10% as shown by different epidemiological studies (WHO, 2000). According to a few population based studies conducted in India, West Bengal studies estimated 2.97% of prevalence for HBV,[5] other northern Indian population study predicted 2.1% for HBV and 19.5% for HCV[6] and a study in weaver's community of Tamil Nadu estimated the prevalence of HBV (2.34%), HCV (0.94%).[7] The true prevalence of HBV and HCV infection among the people in southern region is unknown.

The aim of this study was to determine the prevalence and predictors of HBV and HCV infection in a large population in urban, sub-urban and rural areas of southern region of India.

Materials and Methods

The study was conducted in southern region of India, Tamil Nadu, a high densely populated state is the seventh most populous state in the country. The study was conducted in randomly chosen locations of urban, rural and suburban regions of Tamil Nadu which includes Royapuram, Madhavaram, Porur, Poonamalle areas of Chennai District, Tambaram area of kanchipuram District, Uthukottai, Velliyur, Manavur, Periyapalayam, Palavedu areas of Tiruvallur District, Sholurmattam of Nilgris District and Vellore District.

Sample size was determined based on assuming the prevalence of 3% with 2% precision for each area and we estimated the sample size to be 3100. The study population was 3182 individual residents. All individuals residing in the locations were invited to participate in the study. Those who refused or were not willing to participate in the study were excluded (apart from those with mobility problems for whom samples were taken at their home). A week before commencement of the study, the venue and date of the study camp was announced to the public via press, loud speaker and local newspapers. The messages contained the nature of the infection with HBsAg, HCV and consequences of these infections. The need for screening and the benefits of early detection were also explained. Help was sought from local village health nurses (V.H.N) to spread the information regarding the screening camps venue among the local public. Posters were displayed in prominent places where people gather in large numbers i.e., bus station, ration shops, schools & markets. Local village Panchayat officials and district administrative official's

help were sought in mobilizing the people at the time of study. A register was maintained with details of personal information, address and mobile number for each person attending the study and it was rechecked with permanent resident census history in those areas included for the study.

The people were made aware of the importance of blood examination. The details of the blood examination were made clear to the participants. Informed consent was obtained from the volunteers with detailed questionnaire.

The research protocol was approved by institutional ethical committee. Each person who attended the study was evaluated clinically. Universal precautions were taken before obtaining blood for examination. Five millilitres of blood was collected from the individuals in sterile syringes. The blood samples were centrifuged on the spot and the serum was separated. The serum was transported to the Department lab within 6 hours in cold chain and serological test was done for hepatitis B and C.

Serology

A two stage testing was employed. In the first stage, samples were tested for HBsAg and Anti HCV using the commercially available rapid test kit (Reliable Pro-detect Biomedicals Pvt. Ltd., Shimla (HP), India). The procedures were executed as per the kit as follows: The samples were brought to room temperature before performing the analysis. 100µl of the serum was dispensed into the sample well of the card. As the sample flows, purple colour was seen moving across the result window in the centre of the test card. The results were interpreted in 15 minutes.

Interpretation was made as follows: the presence of two colour bands on "T" test line and "C" control line indicates a positive result. The weak intensity of test band indicates lower concentration of HBsAg in the specimen. The presence of only one band on "C" (Control) line indicates a negative result. The colour band on "T" test line is absent for such specimen. If the colour band does not appear in the "C" line within 15 minutes, with or without "T" test line the result is considered as invalid and it has to be re-tested.

In the second stage, all reactive samples were tested using the enzyme linked immunosorbent assay (ELISA). Samples showing repeat test activity on both methods were considered positive and were included for analysis.

Statistical Analysis

All data were entered on to an Excel spread sheet in a flat file and tested for consistence. Outliers were rechecked from case sheet, and where necessary with the individuals. All statistical analysis was done using SPSS version 15.0. The categorical variables were evaluated by Pearson's chi-square test or Fisher's exact test. Variables with P value <0.200 in the bivariate logistic regression analysis were incorporated in to the multinomial logistic regression analysis for odds ratio with 95% confidence intervals and statistical significance was assessed at level $p<0.01$ or $p<0.05$.

Results

A total of 3182 subjects were tested for HBsAg and HCV in different locations of southern region of India, Tamil Nadu. Among the population screened, 105 tested positive for HBsAg with a prevalence of 3.3% and 8 tested positive for Hepatitis C with a prevalence of 0.3%. Of subjects screened, 688 were from rural areas, 1607 from urban areas and 684 from sub urban areas and 203 from tribal areas. The area wise prevalence rate of HBV details are given in Figure 1. Higher prevalence rate of HBV was observed in the urban areas (4.5%), subsequently rural (3.2%), sub urban area (1.6%) and no infection was found in the screened population of tribal community in this study.

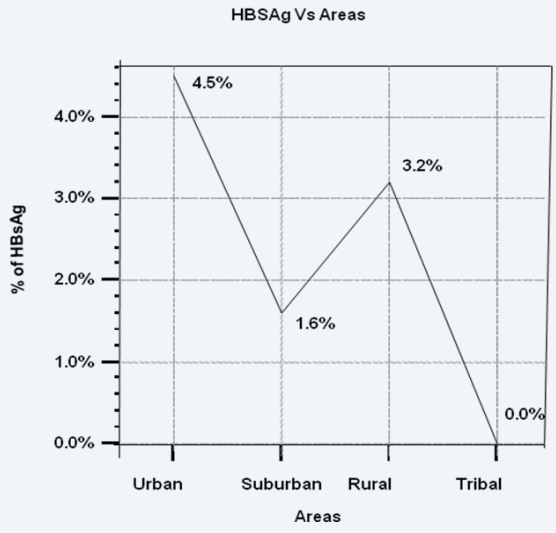

Figure 1. Prevalence rate of HBV in Urban, Suburban, Rural, Tribal Areas

Table I. Characteristic and bivariate logistic regression of factors associated with HBsAg status among population

Characteristic		HBsAg negative (N=3077) n (%)	HBsAg positive (N=105) n (%)	OR	95% CI	P-value
Age	<20	284 (9.2)	1 (1)	R	-	-
	20-29	731 (23.8)	23 (21.9)	8.936	1.201-66.477	0.032*
	30-39	596 (19.4)	23 (21.9)	10.960	1.473-81.559	0.019*
	40-49	634 (20.6)	27 (25.7)	12.095	1.635-89.442	0.015*
	50-59	541 (17.6)	17 (16.2)	8.924	1.182-67.401	0.034*
	>60	291 (9.5)	14 (13.3)	13.663	1.785-104.589	0.012*
Gender	Male	1451 (47.2)	68 (64.8)	R	-	-
	Female	1626 (52.8)	37 (35.2)	2.059	1.371-3.093	0.0001**
Place of living	Urban	1535 (49.9)	72 (68.6)	R	-	-
	Suburban	673 (21.9)	11 (10.5)	0.348	0.184-0.661	0.001**
	Rural	666 (21.6)	22 (21)	0.704	0.433-1.145	0.157
	Tribal	203 (6.6)	0 (0)	0.000	0.000	0.995
Education qualification	Un educated	544 (17.7)	20 (19.0)	R	-	-
	Upto school	2178 (70.8)	64 (61.0)	0.799	0.480-1.332	0.390
	Upto college	355 (11.5)	21 (20.0)	1.609	0.860-3.011	0.137
Marital status	Unmarried	408 (13.3)	6 (5.7)	R	-	-
	Married	2669 (86.7)	99 (94.3)	2.522	1.099-5.788	0.029*

Table I continued...

Characteristic			HBsAg negative (N=3077) n (%)	HBsAg positive (N=105) n (%)	OR	95% CI	P-value
Occupation	Un employed		837 (27.2)	29 (27.6)	R	-	-
	Goverment		484 (15.7)	26 (24.8)	1.550	0.903-2.663	0.112
	General		1147 (37.3)	39 (37.1)	0.981	0.602-1.600	0.940
	Business		330 (10.7)	7 (6.7)	0.612	0.266-1.411	0.250
	Education		279 (9.1)	4 (3.8)	0.414	0.144-1.187	0.101
Health issue / medication		No	2914 (94.7)	104 (99)	R		
		Yes	163 (5.3)	1 (1)	0.172	0.024-1.240	0.081
Hypertension / diabetes		No	2875 (93.4)	103 (98.1)	R	-	-
		Yes	202 (6.6)	2 (1.9)	0.276	0.068-1.128	0.073
Liver disease		No	2949 (95.8)	96 (91.4)	R	-	-
		Yes	128 (4.2)	9 (8.6)	2.160	1.066-4.375	0.032*
Alcohol		No	2579 (83.8)	75 (71.4)	R	-	-
		Yes	498 (16.2)	30 (28.6)	2.071	1.342-3.198	0.001**
Smoking		No	2955 (96.0)	98 (93.7)	R	-	-
		Yes	122 (4.0)	7 (6.7)	1.730	0.787-3.804	0.173
History of transfusion / surgery		No	3023 (98.2)	104 (99)	R	-	-
		Yes	54 (1.8)	1 (1)	0.538	0.074-3.929	0.541
History of jaundice		No	2932 (95.3)	100 (95.2)	R	-	-
		Yes	145 (4.7)	5 (4.8)	1.011	0.406-2.521	0.981

OR- odds ratio; CI- confidence interval, R- Referral
*p=0.05, **p=0.01, is given with the statistical significance

Out of total volunteers 1519 were male and 1663 were female with the male to female ratio of 47.7: 52.3. The age of the participants ranged from 7 months to 97 years with a mean age of male was 43.36 and female was 37.41 years. The age and gender wise infection were given in figure 2 and figure 3. The prevalence was higher in male (4.5%) when compared to female (2.2%) in HbsAg but about equal in HCV. Age wise prevalence rate showed that very low prevalence was reported in those less than 20 years (0.4%) group and higher prevalence in the above 60 years group (4.6%).

In the bivariate analysis, factors associated with evidence of HBV infection are shown in Table I. When living place is considered as risk for HBV, suburban (OR-0.348, CI-0.184-0.661, P =0.001) area are having lesser risk than urban. Age wise risk of HBV was observed as 20-29 years (OR-8.936, CI-1.201 to 66.477, P=0.032), 30-39 years (OR-10.960, CI-1.473 to 81.559, P=0.019*), 40-49 years (OR-12.095, CI-1.635 to 89.442, P=0.015), 50-59 years (OR-8.924, CI-1.182 to 67.401, P=0.034), >60 years (OR-13.663, CI-1.785 to 104. 589, P=0.012) than the less 20 years age. Males are 2 fold more likely to get infection than female gender (OR-2.059, CI-1.371 to 3.093, P=0.0001), Married people have 2.5 times higher risk than unmarried person (OR- 2.522, CI-1.099 to 5.788, P=0.029), history of past or present liver disease (OR-2.160, CI-1.066 to 4.375, P=0.032), alcohol consumption (OR-2.071, CI-1.342 to 3.198, P=0.001) were significantly associated with HBV infection. No significant differences were observed in the comparison of other variables. HCV infections were very minimally detected in all groups of volunteers evaluated in this study hence the risk factors association with HCV infection was not statistically significant (Table II).

Another important feature of this study is multivariate analysis of risk factors which has rarely been carried out in previous reports from India. Multivariate logistic regression analysis was performed using HBV infection (negative or positive) as the dependent variable. The following variables were included in the logistic regression model: age, gender, place of living, education qualification, marital status, occupation, health issue or medication, hypertension or diabetes, liver disease, alcohol consumption, smoking, history of transfusion or surgery, history of jaundice. In the

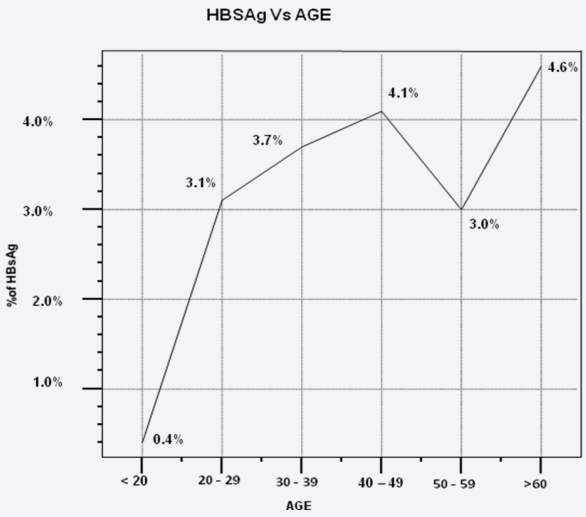

Figure 2. Prevalence rate of HBV in different age group

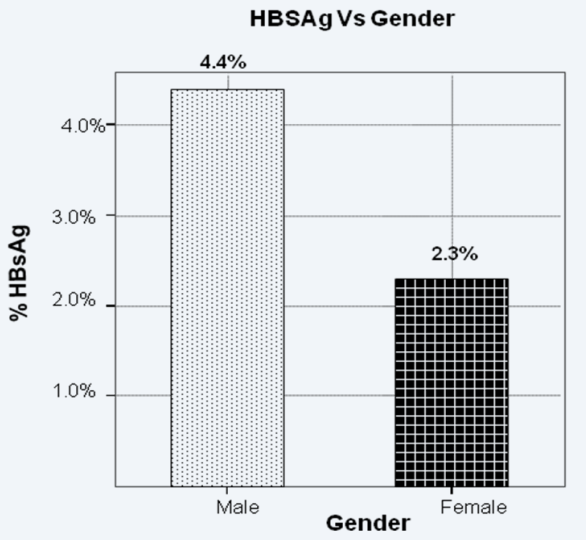

Figure 3. Gender wise prevalence of HBV

multivariate analysis (Table III), the variables that were independently associated with HBV infection were gender (OR- 0.589, 95% CI- 0.363 to 0.955, P = 0.032), place of living (OR- 0.702, 95% CI- 0.536 to 0.920 P = 0.010), marital status (OR- 2.783, 95% CI- 1.130 - 6.853, P = 0.026) and occupation (OR- 0.789, 95% CI- 0.657 to 0.948, P = 0.011).

Discussion

Hepatitis B virus (HBV) and Hepatitis C virus (HCV) infections are major health problems around the world. A significant proportion of infected patients will progress onto cirrhosis and hepatocellular

Table II. HCV positive status among the population

Characteristics		HCV positive	P-value
Age (years)	<20 (285)	2	0.579
	20-29 (756)	2	
	30-39 (619)	1	
	40-49 (661)	1	
	50-59 (558)	2	
	>60 (303)	0	
Gender	Male (1518)	4	0.898
	Female (1664)	4	
Place of living	Urban (1607)	5	0.334
	Suburban (684)	0	
	Rural (688)	3	
	Tribal (203)	0	
Education qualification	Un educated (567)	0	0.186
	Upto school (2249)	8	
	Upto college (366)	0	
Marital status	Unmarried (414)	3	0.039
	Married (2768)	5	
Occupation	Un employed (870)	2	0.557
	Government (503)	1	
	General (1189)	5	
	Business (337)	0	
	Education (283)	0	
Health issue / medication	No (3018)	7	0.347
	Yes (164)	1	
Hypertension / diabetes	No (2978)	8	0.459
	Yes (204)	0	
Liver disease	No (3045)	8	0.548
	Yes (137)	0	
Alcohol	No (2654)	5	0.112
	Yes (528)	3	
Smoking	No (3049)	7	0.225
	Yes (133)	1	
History of transfusion / surgery	No (3127)	8	0.707
	Yes (55)	0	
History of jaundice	No (3032)	7	0.298
	Yes (150)	1	

Table III. Multiple logistic regression of risk factors associated with HBsAg

Characteristic	AOR	95% CI	P-value
Age	1.016	0.864 - 1.195	0.846
Sex	0.589	0.363 - 0.955	0.032*
Place of living	0.702	0.536 - 0.920	0.010**
Education qualification	1.357	0.924 - 1.994	0.120
Marital status	2.783	1.130 - 6.853	0.026*
Occupation	0.789	0.657- 0.948	0.011*
Health issue/medication	0.195	0.027 - 1.428	0.108
Hypertension / diabetes	0.416	0.098 - 1.763	0.234
Liver disease	1.361	0.651 - 2.847	0.413
Alcohol	1.192	0.711 - 1.996	0.505
Smoking	2.305	0.904 - 5.872	0.080

*p=0.05, **p=0.01, is given with the statistical significance
AOR- adjusted odds ratio, CI- confidence interval.

carcinoma. There is an urgent need to undertake a study systematically on population and devise ways and means not only to cure but also to minimize its proliferation. We undertook the first prevalence study of the viral hepatitis infection among apparent healthy people in the southern region of India.

We have documented a 3.3% prevalence of HBV infection and low 0.3% prevalence of HCV infection in this large population based study. This study was conducted in different locations and prevalence rates of hepatitis B virus infection were compared across the populations of different areas of urban (4.5%), sub-urban (1.6%), rural (3.2%) and tribal (0%). There are several studies conducted on seroprevalence of HBV in India performed in the different population ranges in non-tribal group is 2.4% and in tribal population is 15.9%.[8] However, this divergence from our study suggested that no prevalence in this tribal group may reflect specific characteristics of tribal in southern region of India, it is therefore not surprising that the prevalence of HBV varied markedly from one region of India to another. We also noticed that urban areas includes Royapuram, Vellore had a higher likelihood of having hepatitis B and hepatitis C virus infection than the other sub-urban area. Compared with a northern India study among general population the prevalence rate (2.97%)[5] was 0.33% higher in this study. Prevalence of HCV infection was not similar to other studies in India. Thus the infection dynamics of HCV in our country appears to be similar to that observed in the other country like Spain where the difference between subtypes of 1a and 1b that might account for their different prevalence of various demographic region.[9] However, our study has not done the subtype of HCV, which was not the scope of our study.

Other than being population based prevalence study, there are some special characteristics from our study which should be noted. Our study was composed of more number of females than males, whereas many other previous studies have accounted almost exclusively on males, this is particularly true for blood donor studies where the female population has always been much lesser than the male donors. However, similarly to other previous hospital based study,[10,11] we

also have found a significantly higher rate of HBsAg in men than women but anti-HCV antibodies rates were similar in gender.

The factors associated with HBV infection have been identified in this study, very few studies only in India have studied these factors. We have demonstrated that there is a strong association with Gender, Place of living, Marital status, and occupation. Occupation is surprising since it was significantly correlated in multivariate logistic regression though it was not significant with any individual occupation in categorical bivariate logistic regression. Occupation is a known predisposing factor associated for HBsAg infection.[12] Compared with the general population, medical physicians and staff are exposed to a much higher risk of contracting acute viral hepatitis from HBV infected patients. In our study, we could not analyse such correlation since ther was not enough medical physicians and staff enrolled. Alcohol consumption was significantly associated with HBV infection in our study in the bivariate, but not in the multivariate logistic regression. Our results nearly justify the previous study on alcohol consumption and risk for the HBV infection, they have noticed that alcohol drinking practice were independent predictors of risk for carriers of inactive HBV to develop hepatocellular carcinoma (HCC).[13]

In this study, risk factors such as history of transfusion and/or surgery and history of jaundice were not significantly associated with HBV infection, which was associated in most of the hospital based studies. Habit of smoking was also not associated with HBV infection in our population study.

Strengths and limitations of our study

The unique strengths of this study are the large, population based with clear prevalence estimation in urban, rural, suburban than any other population study in southern region of India. We were also able to explore potential correlation between HBV and HCV infections and sociodemographic and medical conditions, as well as lifestyle.

The major limitation of the study is that, as a questionnaire survey, all data collection was limited to self-report and another important limitation is lacking correlation to sexual transmission of hepatitis infection. In the southern region of India, people are more traditional and religious and so did not accept to disclose such personal information, hence very poor response on these questions.

Conclusion

The accurate epidemiological data of HBsAg and anti-HCV among population in southern region of India suggests that our region has slightly high prevalence for hepatitis B with higher rates in urban areas and low prevalence for hepatitis C when compared to northern region of India. Strong associations between HBV infection were observed related to the gender, place of living, marital status, and occupation.

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