

Seroepidemiological Study of Hepatitis B and C Viral Infection in Acute and Chronic Hepatitis Patients in a Hospital Setup in Orissa

Prajyoti Sahu¹, S.K. Kar¹, A. Mohapatra¹, B. Dwibedi¹ and S.P. Singh²

¹Clinical Epidemiology Division, Regional Medical Research Centre,
Chandrasekharpur, Bhubaneswar- 751 023, India.

²Department of Gastroenterology, SCB Medical, Cuttack, India.

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The present study determines the distribution of seroepidemiologic markers of HBV and HCV virus infection among acute and chronic hepatitis patients attending the tertiary care hospital in Orissa. 529 blood samples were collected from patient attending the tertiary care hospital during the time period 2006 to 2008. Among them males were more frequently infected as compared to females. The overall HBV positivity rate reveals decreasing trends from the lower age group to the higher age groups. The overall HBV positivity was 33% and HCV positivity was only 0.76%. The risk factor analysis was carried out by using multivariate and univariate analysis which shows that shaving at barbour shop and sexual route of transmission ($P < 0.05$) were significantly associated with HBsAg positivity. The present study conclude that hepatitis B viral infection was responsible for 25% of cases of acute viral hepatitis and HCV infection was not a cause of acute viral hepatitis in coastal Orissa. Besides, HBV infection was responsible for 35% of chronic liver disease. Further, HBV infection was also responsible for 88% (15/17) of cases of primary liver cancer in our study. Besides, 15% of cases of chronic liver disease were positive for anti-HBc alone in the absence of HBsAg.

Key words: Hepatitis, Viral Infection, Acute, Chronic, Hospital and Orissa.

Hepatitis virus infection is a public health problem worldwide, causing high morbidity and mortality in the form of acute and chronic hepatitis, liver failure and hepatocellular carcinoma. According to WHO, a third of the world's population (2 billion people) has been infected with

hepatitis B virus and about 5% are chronically infected (WHO, 1996). 82% of world's 530,000 cases of liver cancer per year are caused by viral hepatitis infection, out of which 316,000 cases are associated with hepatitis B and 118,000 with hepatitis C virus (WHO, 1996). HBsAg seroprevalence in India is approximately 5 percent (Kurien *et al.*, 2005). Around 25% HBV carriers progress to chronic hepatitis, which in the long run may lead to cirrhosis or primary carcinoma of liver. Both these irreversible changes may lead to death of the subject (Serag *et al.*, 1999). India, with a carrier rate of 3% contributes to nearly 10% of the HBV carriers in the world. Without any organized HBV prevention programme and with 25 million live births in a year, nearly 1 million HBV infections are added to the HBV pool in India

* To whom all correspondence should be addressed.
Tel.: +91-674- 2303424, +91-9437258680;
Fax: +91-674- 2301351; E-mail: Kukun45@sify.com

(Choudhury *et al.*, 2005). HCV is a major causative agent for parenterally transmitted non-A, non-B hepatitis (Choo *et al.*, 1990). Epidemiological studies for the detection of antibodies to HCV have been carried out all over the world (Choo *et al.*, 1990). One of the distinctive features of HCV infection is that the risk of chronicity varies greatly with the age, at which the infection is acquired (Deuffic *et al.*, 1999).

There is very scanty data on Hepatitis B and C viral infection from Orissa. A study from coastal Orissa studied the prevalence of anti-HCV in blood donors and found the lowest national figure of only 0.01%. Another study by the same workers based on blood bank donor screening during the period 2002–2006 revealed an overall anti-HCV prevalence of 0.06%. Besides, the study also revealed an increasing trend in anti-HCV prevalence rate from 0.02% in 2002-03 to 0.14% in 2005-06. However, another report from the state AIDS cell (2003) revealed prevalence rates of 0.59% for HBV and 0.16% for HCV infection amongst the blood samples screened. The present study was conducted to determine the distribution of seroepidemiologic markers of HBV and HCV virus infection among symptomatic acute and chronic hepatitis patients attending the tertiary care hospitals in Orissa.

MATERIAL AND METHODS

Study area and Subject selection

The subjects were enrolled from patients attending the medical outpatient department [OPD] at MKCG Medical College Hospital (OPD) Berhampur and the gastroenterology OPD at SCB Medical College Hospital at Cuttack, which are the two major referral centres in the eastern state of Orissa. These two medical colleges act as the referral centers for most parts of Orissa including northern, coastal and south Orissa. The study subjects were selected from patients with clinical symptoms of acute/chronic hepatitis, either from the OPD or the medical/gastroenterology wards during the period March 2006 to December 2008. The study includes clinical diagnosis of acute hepatitis, chronic hepatitis (liver cirrhosis, hepatocellular carcinoma). Patient willing for giving blood sera and for liver biopsy if necessary. Written informed consent was obtained from the

patients to be enrolled for the study.

Diagnostic criteria

The diagnostic parameters were clearly outlined as per the standard procedures and guidelines. Care has been taken to avoid any bias opinion or definition as per the literature. The details of the definition followed are as follows:

Acute hepatitis

Acute Hepatitis HBV: Acute hepatitis is diagnosed by characteristic clinical feature with ALT elevation of at least 5xULN and acute HBV infection is defined if IgM anti HBc is positive in patient sera.

Chronic hepatitis

Symptomatic biochemical or serologic evidence of continuing inflammatory hepatic disease for more than 6 months without steady improvement is called chronic hepatitis (Tyagarajan *et al.*, 1996).

Serological screening for hepatitis markers by ELISA (Enzyme-Linked Immuno Sorbent Assays)

Three to four ml of intravenous blood was collected from 529 symptomatic patients, using standard procedures. Demographic data, case history and clinical examination findings were noted in a pre designed Performa. All the subjects were questioned about history of jaundice, blood transfusion, intravenous injection use, tattooing, multiple sexual partner behaviour for sexual route of transmission and other related history with probable risk of exposure to hepatitis B. The samples were stored and transported to RMRC laboratory with maintained cold chain. In the laboratory the sera are separated and assayed for presence of serological markers including HBsAg, AntiHBc, Anti-HBs, HBeAg and HCV (BioELISA, Ranbaxy), as per manufacturer's standard procedure. Positive samples were retested for confirmation by a second ELISA.

RESULTS AND DISCUSSION

Out of the total 529 subjects, 350 were acute (AH), 43 were chronic (CH), 90 were cirrhosis of liver, 17 were HCC and 25 were asymptomatic carrier. The mean age of the 529 patients i.e. acute hepatitis (AH), chronic hepatitis (CH), liver cirrhosis and HCC case were (34.13±11.0), (46±13.43), (47±11.32) and

(45±9.66) respectively. Among them, 87.2% were males while 12.7% were females; thus males were found to be more frequently infected as compared to females. The probable reason for this may be that the men are more exposed to the risk factors; the males are more educated and employed and work outside their homes or in agricultural lands, while women are mostly involved in household activities (Muhammad *et al.*, 2007). Males are commonly involved in the practices leading to the transmission of infections like blood transfusion and visiting barber's shop and so are more prone to get the infection at a much frequent rate as compared to their female counterparts in society. The overall HBV positivity rates reveal a decreasing trend from the lower age group (15-30) to the higher age group (>60). Seventy HBsAg positive subjects belonged to the age group 15-30 years, followed by 60 subjects in the age group 31-45 years and 34 subjects in the age group 46-60 years. Only 8 HBsAg positive subjects belonged to the age group of over 60 years (Table 1). Comparison of the different HBsAg positive age groups by chi-square test revealed that there was a highly significant trend ($P<0.0001$) in difference in the positivity rates. The analysis further indicated that HBV positivity has a predilection for the lower age groups in comparison to the higher age groups. The HBV positivity rate decreases from the lower age groups to the higher age groups because the positivity is proportional to exposure, and since the lower age group is more exposed to the probable risk factors, the positivity also is more among them which gradually stabilizes with age. The patients' resolving HBV infection correlates with their age and the strength of the initial immune response to HBV. Approximately

90% of adults resolve the HBV infection, whereas 30 to 90% of young children fail to resolve the infection and thus develop chronic HBV infection. The weak immune response generated by young children acutely infected with HBV is responsible for their failure in killing the HBV infected hepatocytes and clearing the infection (Ganem *et al.*, 2004).

The overall HBsAg positivity among the 529 subjects was 32.7% and the IgG anti-HBc positivity in cases of chronic liver disease was 53%. Out of 350 acute cases 25% cases were found positive for HBsAg, and 15% were positive for anti HBs. In early course of HBV infection, antiHBs antibodies bind to the envelop antigen on the intact hepatitis B virion and to the subviral lipoprotein particles. As the immune system clears the viral particles and antigens, free unbound anti-HBs become detectable in blood (www.hepwebstudy.org). In chronic hepatitis cases (43), HBsAg was positive in 51%, IgG anti-HBc in 53%, HBeAg in 7% and anti HBs was positive in 2%. In cirrhosis of liver cases, HBsAg was positive in 27%, IgG Anti-HBc in 37%, anti-HBs in 3% and HBeAg was positive in 2%. However, in HCC cases, HBsAg was positive in 88%, IgG anti-HBc in 71%; anti-HBs were not detected in any case, but HBeAg positivity was seen in 12%. In asymptomatic HBV carriers, HBsAg positivity was found obviously in 100% cases, but IgG anti-HBc was positive in 72% only. Besides in 20 of 133 (15.0%) cases of chronic liver disease (chronic hepatitis and liver cirrhosis), IgG AntiHBc alone was present despite HBsAg being negative. In individuals with chronic infection, HBsAg and antiHBc generally persist for life and HBV DNA may be detected by nucleic acid amplification

Table 1. Age and genderwise distribution of patients positive for hepatitis B virus

Age Groups	Number of subjects [n]	HBV +ve		HBV +ve Total
		Males	Females	
15-30	187	60 (85.7%)	10 (14.2%)	70(40.6%)* @
31-45	205	53 (88.3%)	7 (11.6%)	60(34.8%)*##
46-60	111	31 (91.1%)	3 (8.8%)	34(19.76%)* **^
>60	26	6 (75%)	2 (25%)	8(4.6%)@ #^
Total	529	150 (87.2%)	22 (12.7%)	172 (33%)

*P= 0.001, **P= 0.005, @P< 0.0001, #P<0.0001, ^P<0.001

method. The continued presence of HBeAg reflects higher HBV DNA and greater infectiousness (www.hepwebstudy.org). In this present study, HBsAg was as expected universally positive in all cases of HBV infection—both acute and chronic, while IgG-AntiHbc was present in patients with chronic HBV infection only.

Among the HBsAg positive patients, the percentage of anti Hbc positivity was highest in the older age group (>60yr) and lowest in lower age group (15-30 year). This is in contrast to the HBsAg positivity reverse trend, i.e., this is highest in the 15-30 year age group and gradually decreases with increasing age group (Fig. 1). From this study it is clear that Anti Hbc positivity increases steadily with increasing age. These findings are similar to the findings reported by Wolfgang *et al.*, 2001.

The overall prevalence of HCV infection among all cases was found to be 0.76%. This low

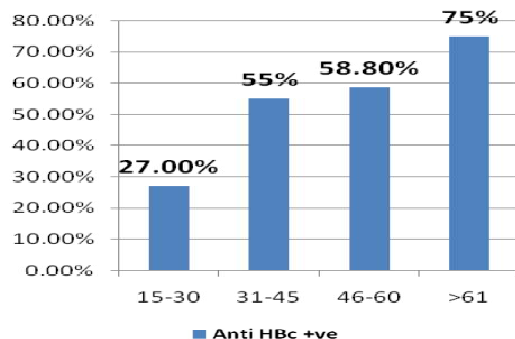


Fig 1. Percentage of anti Hbc among HBsAg positive individual

incidence of HCV infection is in keeping the low prevalence of HCV infection in Orissa in comparison to other states (Singh *et al.*, 2001 & 2009). The studies conducted by Singh *et al.* (2001 & 2009) for the seroprevalence of antibodies to HCV in coastal Orissa found to be 0.014-%. However, another study conducted by Misra *et al.*, (2002) for the seroprevalence of anti HCV antibody in and around Cuttack district in Orissa found a higher prevalence of 1.57%. The risk factor analysis was carried out by multivariate logistic regression using SPSS package (version 10). It may be noted that in the risk factor analysis, multiple

responses were possible and were taken into consideration for analysis. A person can be exposed to more than one route of transmission; hence multiple responses are considered for this analysis purpose.

In this analysis a univariate analysis was carried out using all possible routes of transmission for each type of case in which shaving at barber's shop (PdH0.000) and sexual route of transmission (PdH0.05) were significantly associated with HBsAg positivity. A multivariate analysis for logistic regression using all types of cases and possible route of transmission together revealed that injury/bleeding during shaving at barber's shop was significantly associated with HBsAg positivity (pdH0.05).

CONCLUSION

The present study revealed that Hepatitis B viral infection was responsible for 25% of cases of acute viral hepatitis. Hepatitis C viral infection was not a cause of acute viral hepatitis in coastal Orissa. Besides, HBV infection was responsible for 35% of chronic liver disease (chronic hepatitis and cirrhosis of liver). Further, HBV infection was also responsible for 88% (15/17) of cases of primary liver cancer in our study. Besides, 15% of cases of chronic liver disease were positive for anti-HBc alone in the absence of HBsAg. Thus addition of Anti-HBc to HBsAg testing resulted in the identification of HBV as the etiology in 15% more cases of chronic liver disease. This could have major implications in screening of blood of donors for transfusion.

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