Detection of hepatitis B virus in clinically suspected infectious hepatitis pregnant and non-pregnant women

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ABSTRACT

Background: Vertical transmission of HBV from infected mothers to their offspring, either in peripartum or in utero, remains a major source of perpetuating reservoir of chronically infected individuals globally.

Objectives: To determine the prevalence of HBsAg & HBeAg in clinically suspected infectious hepatitis in pregnant women at a tertiary care hospital in Ghaziabad.

Material and Method: This prospective study was conducted on 188 patients between age ranges 18–65 years in Department of Microbiology at Santosh Medical College, Ghaziabad between December 2014 to June 2016. 5 ml blood samples were taken from each patient suspected of acute infectious hepatitis were analyzed. The sera were separated and screened for HBsAg by immune-chromatographic card then positive serum samples for HBsAg were again tested for HBSAg using ELISA kit. The positive sample for HBsAg in pregnant was tested for hepatitis B e antigen (HBeAg) by ELISA test.

Results: HBsAg were identified in 11(5.85%) female samples. Among them, 7 (3.72%) were from 21-35 years age groups, this was statistically significant (P<0.05), compared to the other age groups. From 11 HBsAg positive female, six were pregnant, and the prevalence of HBsAg-positive pregnant female is 3.19%. 4 female were positive for HBeAg and prevalence of HBeAg-positive pregnant woman is 2.13%. 75%(n=3) HBeAg-positive case was found in the age group of 21-35, this was statistically significant (P<0.05), compared to the other age groups.

Conclusions: Study showed that HBV is prevalent in pregnant women. Screening & vaccination should be considered by health policy makers to prevent transmission of HBV.

Keywords: Prevalence; Pregnant Women; Hepatitis B virus; Hepatitis B Surface Antigen; hepatitis B e antigen; Vertical transmission

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INTRODUCTION

The human hepatitis B virus (HBV) is a small-enveloped DNA virus causing acute and chronic hepatitis. Although a safe and effective vaccine is available, HBV infection still represents a major global health problem. Due to HBV-associated liver pathologies, about 240 million people chronically infected worldwide and approximately 600,000 deaths per year.1

In India, nearly 3-4% of the population are infected by HBV infection, and constituting > 50% chronic hepatitis cases. The reason for such phenomena are due to a large population of India and absence of a national immunization program would spell off a projected increasing burden of infection and liver diseases due to HBV. In this contrast, the HBV epidemiology in India has become relevant because of the possibility that India may soon have the largest HBV infection pool in the world. The prevalence of chronic HBV infection in pregnant women is 0.82 % in India and presents the risk of mother-to-child (vertical) transmission during pregnancy.2

Chronic HBV infection represents the primary risk factor for the development of hepatocellular carcinoma. The rate for chronicity is approximately 5% in adult infections, but it reaches 90% in neonatal infections. Although under the normal infection conditions, HBV does not induce direct cytopathic effects of liver damage (fibrosis, cirrhosis, and eventually hepatocellular carcinoma) and is believed to be caused by the ongoing immune response and a consistent inflammation of the liver.3

HBV is present in blood, saliva, semen, vaginal secretions and menstrual blood of the infected individuals and could be transmitted easily through contact with these infected body fluids. The most common mode of transmission is perinatal vertical transmission.4 In households of a chronically infected individual, HBV transmission can occur both via person-to-person and non-sexual contact.5

The neonates born to mothers infected with chronic hepatitis B have 90 percent risk of acquiring chronic HBV infection and have high chance to be chronic.4 In contrast, when HBV is acquired during adulthood, only 5-10 percent of adults develop persistent chronic HBV infection.6 Most of the developed countries screen all pregnant women for HBV infection. However, in the developing countries,
it depends on the risk factors. In India, there is no consistent policy of screening the pregnant women across the country. A meta-analysis of the prevalence of hepatitis B in India showed a 2.4 percent prevalence in general population. However, the prevalence rate of HBsAg positivity in pregnant women varied from 1-9 percent in different parts of the country, and e antigen (HBeAg) positivity rates among them varied from 4.8 - 68.7 percent.6

A large single center study conducted on 20,104 pregnant women in north India showed that the prevalence of chronic HBsAg positivity rate was 1.1 percent.7 Most of the pregnant women with viral hepatitis B are considered as chronic hepatitis B, but a few may develop acute hepatitis in the third trimester of pregnancy resulting in 1 percent of fulminant hepatitis.8 During pregnancy, acute viral hepatitis involves a particular risk both for the mother and the baby.

Of the two secretory proteins of HBV (HBsAg and HBeAg), HBsAg does not usually cross the placenta. However, small sized HBeAg might passes through the placental barrier even with low maternal viral load titer.9 In newborn, transplacental HBeAg can be detected at one month of age, but it would disappear before the fourth months. However, in several infected infants with HBV viral titers, persistent detection of HBeAg for more than four months strongly suggested an HBV chronicity.10

HBeAg spillage through placenta induces HBV specific T cell tolerance in utero, and intrauterine infection could be the main reason for the failure of immunoprophylaxis.11-13 However, there are several pieces of evidence to show that the incidence of intrauterine transmission is rare and only happens in the case of placental leakage.14

Infants born to HBeAg positive mothers are likely to be infected and progress to chronicity, however, infants born to HBsAg-positive mothers develop acute hepatitis and less likely to progress to chronicity.15 In North India, HBeAg positivity was 7.8 percent, and risk of perinatal transmission was 18.6 percent from HBsAg-positive mothers vs. 3 percent among infants of HBsAg-negative mothers.15,16

Thus, the aim of this study was to assess sero-prevalence of HBV in this area, and the presence of HBsAg and HBeAg in Pregnant and Non-Pregnant Women as the HBV is increasing in India.

MATERIAL AND METHODS

2.1. Study background and subjects
A prospective study was conducted on 188 female patients with clinically suspected acute infectious hepatitis, attending out-patients and inpatients departments of Santosh Medical College and Teaching Hospital, Ghaziabad, India, from December 2014 to June 2016. The inclusion criteria were: age between 18 – 65 years old, suspected to had HBV infections and its sequelae, pregnant women patients, able to provide written informed consent indicating awareness of the investigational nature of this study. Those who received any Immunization for HBV, co-infection with HBV – HCV, HBV- HIV, or HBV – HDV, Liver disease due to other viruses, alcoholism, or had diabetes mellitus were excluded.

2.2. Sample collection and processing
5 ml of blood samples received from pregnant women in the serology section of Department of Microbiology from patients suspected of acute infectious hepatitis were analyzed. The sera were separated and screened for HBsAg by Hepa Card (J. Mitra & Co. Pvt. Ltd. New Delhi),18 and the positive serums were stored at −20°C until tested for the viral markers. The positive serum samples for HBsAg by HepaCard were tested again for HBsAg using commercially available enzyme-linked immunosorbent assay kit (ELISA; ERBA Transasia Biomedicals Ltd. Ringawada, Daman India).19 Serum samples tested positive for HBsAg in pregnant were tested for hepatitis B e antigen (HBeAg) (ELISA; Beijing kewei clinical diagnostic reagent inc. Gucheng Xi Rd, Shi Jing Shan District, Beijing, China).20

2.3. Statistical analysis
Data obtained were analyzed using the SPSS software for Windows version 18. Comparison of data in respect of age-groups was performed by Chi-square. P values of <0.05 were considered to be statistically significant

RESULTS

From the 188 serum samples that were screened for HBsAg, 11 (5.85%) were positive (Table 1). The age ranged from 18 – 65 years old, and the majority were from 21 - 35 years age groups (n=7; 63.63%). The difference in the age distribution was statistically significant.

Of the 11 HBsAg Positive female, six were pregnant which indicate that the prevalence of HBsAg-positive pregnant woman was 3.19% (Table 2). Four women were positive for HBeAg while two were negative, which represent prevalence rate of 2.13% for HBeAg. 75% (n=3) HBeAg-positive case was found in the age group of 21-35 years which was statistically significant (P<0.05), compared to the
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DISCUSSION

Hepatitis B occurs throughout the world with no seasonal variation. The incidence is higher in urban than in rural areas and lowers in children than in adults in developed countries. However, the transmission through close personal contact or mother to baby is quite common in infants and children in Africa and the Far East. Most individuals are infected perinatally, by vertical transmission, or in early childhood in endemic areas, where carrier rates are >5%. The carrier rate is lower in the temperate regions than in the tropical, but in this region, its incidence is higher in males than in females.

In this study, 188 female were tested for HBsAg with age ranged from 18 – 65. The present study shows a 5.85% seroprevalence of HBsAg among clinically suspected infectious hepatitis pregnant patients which is similar to studies conducted by Monika Rajani et al. (5%). The frequency of seropositivity was found to be higher than that reported in other studies carried out by P. A. Bart et al. (9.5%). Furthermore, the seroprevalence of HBsAg was found to be lower than that reported in other studies conducted by B.R. Tiwari et al. (1.2%). Variable results are found between 1.09% to 1.55% in deferent studies in India. Those studies found were higher seroprevalence (3.72%) in 21-35 age group which was in accordance with Monika Rajani et al. that was also observed higher seroprevalence (7.6%) in 20-30 age group.

The seroprevalence of HBsAg in pregnant females was 3.19% which was roughly similar to studies conducted by Vandana Bansal et al. (2.37%). Meanwhile, the seroprevalence of HBsAg in pregnant women was found to be higher according to MA Adegbesan-Ofilabu et al. report (7.3%), and the seropositivity of HBeAg (66.67%) in pregnant females was different with their report (36.4%).

The present study revealed a trend of HBV sero-positive according to samples age. The positivity for HBsAg in pregnant women was also higher. A higher prevalence of HBsAg detected in the present study is presuming due to lack of HBV immunization in India. The higher levels are also related to the existence of un-educated population and unlicensed practitioners in developing countries like India who, for several decades, had taken advantage of a lack of consumer information in the medical services market and urged the patient

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Table 1: Age distribution among patients with clinically suspected infectious hepatitis female and HBsAg positive female patients

| Age group (in years) | Non- Pregnant 5(2.66%) | Pregnant 6(3.19%) | Total 11(5.85%) |
|---------------------|-------------------------|------------------|-----------------|
| < 20                | 20                      | 3                | 23(12.23%)      |
| 21 - 35             | 117                     | 7                | 124(65.96%)     |
| 36 - 50             | 33                      | 1                | 34(18.09%)      |
| 51 - 65             | 7                       | -                | 7(3.72%)        |

Table 2: Distribution of HBsAg positive in pregnant and Non-Pregnant women

| Age group (in years) | Non- Pregnant | Pregnant | Total |
|---------------------|---------------|----------|-------|
| < 20                | 1             | 2        | 3(27.27%) |
| 21 - 35             | 3             | 4        | 7(63.64%) |
| 36 - 50             | 1             | -        | 1 (9.09%) |
| 51 - 65             | -             | -        | -     |

Table 3: Distribution of HBeAg in pregnant women

| Age group (in years) | Positive 4 (2.13%) | Negative 2(1.06%) | Total 6(3.19%) |
|---------------------|---------------------|-------------------|---------------|
| < 20                | 1                   | 1                 | 2(33.33%)     |
| 21 - 35             | 3                   | 1                 | 4(66.67%)     |
| 36 - 50             | -                   | -                 | -             |
| 51 - 65             | -                   | -                 | -             |

Figure 1: Comparative seroprevalence of HBsAg & HBeAg in pregnant women
to take unnecessary injections which may lead to iatrogenic transmission of the disease.

CONCLUSION
The study showed that hepatitis B was significantly more prevalent in pregnant than non-pregnant women. Therefore, it is imperative for nations to formulate and implement consistent population-based screening and universal vaccination programs to address the global health burden that chronic HBV infection imposes.

CONFLICT OF INTEREST STATEMENT
We declare that we have no conflict of interest regarding this article.

ETHICAL APPROVAL & FUNDING
Ethical approval for the study was taken from institutional research ethic committee.

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