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Original Research Article

Seroprevalence of hepatitis B virus surface antigen among pregnant females attending antenatal clinic at a tertiary care hospital in North India

Noor Jahan[1], Anil Kumar[2]*, Asma Nigar[3], Siraj Ahmad[4], Razia Khatoon[5], Nitin Chaudhary[6]

[1]Department of Microbiology, Integral Institute of Medical Sciences and Research, Integral University, Lucknow, Uttar Pradesh, India
[2]Department of Medical Microbiology, Vananchal Dental College and Hospital, Garhwa, Jharkhand, India
[3]Department of Obstetrics and Gynaecology, [4]Department of Community Medicine, Integral Institute of Medical Sciences and Research, Integral University, Lucknow, Uttar Pradesh, India
[5]Department of Microbiology, Hind Institute of Medical Sciences, Mau, Ataria, Sitapur, Uttar Pradesh, India
[6]Department of Medical Microbiology, Saaii College of Medical Science and Technology, Kanpur Nagar, Uttar Pradesh, India

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***Correspondence:**
Anil Kumar,
E-mail: anilchaudhary1019510@gmail.com

ABSTRACT

Background: Hepatitis B infection is one of the most common public health problems worldwide. Hepatitis B virus Surface Antigen (HBsAg) positive mothers may transmit the virus vertically to neonates transplacentally during pregnancy, perinatally during delivery or postnatally through breast milk. Such neonates being carriers of virus are at a very high risk of developing chronic liver diseases at a younger age and also, they act as reservoirs of infection in the community. Objective of the study is to evaluate the seroprevalence of HBsAg in pregnant females attending Antenatal Clinic.
Methods: A hospital based cross-sectional study was done from January to June 2019. A total of 840 pregnant females were included in the study who attended Antenatal Clinic for routine checkup whose blood samples were sent to Microbiology Laboratory for screening of HBsAg by Enzyme Linked Immunosorbent Assay (ELISA).
Results: Out of 840 pregnant females included in the study, 8 were reactive to HBsAg, hence, prevalence was found to be 0.95%. The seroprevalence of HBsAg was found to be more (1.40%) in 26-35 year females. Maximum seropositivity was seen in females from urban areas (1.0%), those attending OPD of ANC (1.03%) and those who belonged to lower socio-economic class (1.02%).
Conclusions: Routine free screening for HBV infection should be offered to all antenatal females to reduce the risk of vertical transmission to the neonates born to infected mothers, thereby, preventing them from becoming carriers and developing chronic hepatitis and hepatocellular carcinoma later in life.

Keywords: Antenatal clinic, Enzyme linked immunosorbent assay, Hepatitis B virus surface antigen, pregnant females, Seroprevalence

INTRODUCTION

Hepatitis B infection is one of the most common public health problems worldwide.[1,2] Hepatitis B virus belongs to the family "Hepadnaviridae". It is an enveloped DNA virus and infects the liver and causes liver inflammation and hepatocellular carcinoma.[3,4] Hepatitis B virus infections are 100 times more contagious than HIV.[5,6] According to the World Health Organization (WHO), 240 million individuals are having chronic hepatitis B

infection and due to this infection 6.5 lac individual deaths occur annually in the whole world.[4]

Globally, the prevalence and routes of transmission of the infection HBV vary in different areas. According to the WHO, on basis of prevalence of HBsAg carrier rate in the general population, sub-saharan, African, east Asian and Alaskan population are having high HBV endemicity (HBsAg carriage >8%), but population of southern parts of Eastern and central Europe, the Amazon basin, the middle east and the Indian subcontinent are having intermediate HBV endemicity (HBsAg carriage 2-7%), and low endemicity of population are western and northern Europe, Northern America, and Australia (HBsAg carriage <2%).[7]

In India number of carrier of HBV infection is 40 million. WHO reported that 15-40% of the hepatitis infected patients suffers from liver cirrhosis, liver failure and hepatocellular carcinoma**.**[8]

According to the previous study, prevalence level of hepatitis B surface antigen in pregnant females of India was 2.3 to 6.3%.[9-11]

A study from Northern India documented that the prevalence level of HBsAg in pregnant females was 3.7%, positive rate of hepatitis B viral protein (HBeAg) was 7.8% and rate of vertical transmission was 18.6%.[12]

When carrier mother is HBsAg positive, but HBeAg negative only 10-30% of the babies become infected to the HBV, whereas, when carrier mothers are positive from both HBsAg and HBeAg around 70-90% of infants that become carrier.[13]

Most of the studies, have recommended that before delivery it is important to know that mother is HBsAg positive or negative, if mother is positive to the HBsAg, after the delivery, babies should be given immunoprophylaxis in the form of active immunization and passive immunization with Hepatitis B immunoglobulin and 3 doses of Hepatitis B recombinant vaccine respectively, thereby, preventing them from being chronically infected by the virus.[14]

The transmission of hepatitis B virus is due to the transfusion of infected blood and its products, urine, semen, sweat, saliva, tears, breast milk, vaginal secretions, pathological effusions and virus can be also transmitted by mother to the newborn.[15,16] Most common mode of transmission is perinatal transmission and responsible for approximately one-third of chronic hepatitis B virus infections.[17,18]

Keeping the above facts in mind the present study was done to evaluate the seroprevalence of HBsAg among pregnant females attending Antenatal Clinic of our hospital.

METHODS

A hospital based cross sectional study was done over a period of 6 months from January to June 2019, among pregnant females attending Antenatal Clinic of Integral Institute of Medical Sciences and Research, Lucknow, to determine the prevalence of HBsAg among them. The study was approved by Institutional Ethical Committee. An informed consent was taken from pregnant female patients included in the study prior to sample collection. A pre-designed questionnaire was used to get the information regarding socio-demographic profile of the enrolled patients.

Inclusion criteria

- Apparently healthy pregnant females from both Out-Patient Department (OPD) and Indoor-Patient Department (IPD) who attended Antenatal Clinic of our hospital for routine check-up and were advised to undergo screening for HBsAg, and gave their consent were included in the study.

Exclusion criteria

- Patients whose blood sample was not requested for screening for presence of HBsAg as well as patients who refused to give consent were excluded from the study.

A total of 840 subjects were included in the present study whose blood samples were screened for presence of HBsAg. From each pregnant female, 3 ml venous blood withdrawn aseptically in a well labeled plain vacutainer tube was received in the Immunology section of Microbiology Laboratory. The tubes were allowed to stand for 30 mins followed by centrifugation at 3500 rpm for 15 min to separate serum. The sera were then screened for HBsAg by using HEPALISA, a 3rd generation Enzyme Linked Immunosorbent Assay (ELISA) method (J. Mitra and Company Private Limited, India). The HEPALISA is a solid phase ELISA based on direct sandwich principle. The microwells are coated with monoclonal antibodies with high reactivity for HBsAg. According to manufacturer's instruction 100 µl negative controls (wells A1 and B1), 100 µl positive controls (wells C1 and D1) and 100 µl samples (well E1 onwards) were added in the respective wells followed by addition of 50 µl working enzyme conjugate and then the plate was covered and incubated at 37ºC±1ºC for 60 minutes. The plate was then washed with working wash buffer 6 times followed by the addition of 100 µl working substrate solution in all the wells and the plate was covered and incubated at room temperature (20-25ºC) for 30 minutes in dark. Finally, 100 µl stop solution was added to each well and the absorbance was read at 450 nm in an ELISA reader within 30 minutes. The Cut-off value was calculated by formula: mean absorbance of Negative control (NC)+0.1. Samples with absorbance value less than the cut-off value were considered as non-

reactive, whereas, those with absorbance value equal to or greater than the cut-off value were considered as reactive by the criteria of HEPALISA.

Statistical analysis

The collected data was analyzed using SPSS Data Editor Software version 20. Chi-square test was performed for categorical variables and p value ≤0.05 were considered statistically significant.

RESULTS

Out of 840 apparently healthy pregnant females included in this study who attended Antenatal Clinic for routine check-up, whose blood samples were screened for presence of HBsAg by ELISA, 8 females were found to be reactive and 832 were found non-reactive to HBsAg, thus the prevalence was found to be 0.95% (Figure 1).

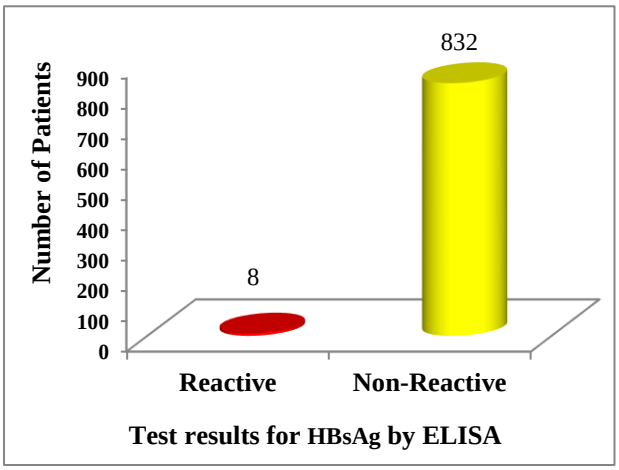

Figure 1: Distribution of pregnant females on the basis of their reactivity to HBsAg.

Female patients enrolled in this study varied from 15 to 45 years, with maximum number of patients 46.90% (394/840) belonging to age group of 15-25 years. However, maximum HBsAg seropositivity 1.40%(5/357) was seen in patients with age group of 26-35 years (Table 1).

Table 1: Distribution of pregnant female patients according to their age groups and their relation with HBsAg reactivity (N=840).

Age group (in years)	HBsAg		Total
	Reactive N (%)	Non-reactive N (%)	
15-25	3(0.76%)	391(99.24%)	394(100%)
26-35	5(1.40%)	352(98.60%)	357(100%)
36-45	0(0.00%)	89(100%)	89(100%)
Total	8(0.95%)	832(99.05%)	840(100%)

N = Number of patients.

Although majority of patients belonged to rural areas, 76.07% (639/840), maximum HBsAg seropositivity was found among patients belonging to urban areas, 1.00%. However, this difference of reactivity among urban and rural patients was not found to be statistically significant (p=0.943) as shown in (Table 2). It was found that majority of subjects enrolled in the study were indoor patients 65.47% (550/840), while maximum seropositivity to HBsAg was found among outdoor patients, 1.03% as compared to indoor patients 0.91%. As depicted in Table 3, this finding was found to be statistically insignificant (p=0.858). Table 4 shows that majority of enrolled pregnant females belonged to lower socio-economic class 70.23% (590/840), and least patients belonged to upper class 2.02% (17/840). Maximum HBsAg seropositivity was found among patients belonging to lower class (1.02%), followed by patients belonging to middle class (0.86%), while none of the patients belonging to upper class were found to be HBsAg reactive.

Table 2: Distribution of pregnant female patients according to their residence and HBsAg reactivity (N=840).

Residence	HBsAg		Total	Chi-Square (χ^2) and *p value
	Reactive N (%)	Non-reactive N (%)		
Urban	2 (1.00%)	199 (99.00%)	201 (100%)	$\chi^2 = 0.005$
Rural	6 (0.94%)	633 (99.06%)	639 (100%)	df = 1
Total	8 (0.95%)	832 (99.05%)	840 (100%)	p = 0.943

* p<0.05 was considered as statistically significant. df = degree of freedom. N = Number of patients.

Table 3: Distribution of pregnant female patients according to their registration status and HBsAg reactivity (N=840).

Registration status	HBsAg		Total	Chi-Square (χ^2) and *p value
	Reactive N (%)	Non-reactive N (%)		
OPD	3 (1.03%)	287 (98.97%)	290 (100%)	$\chi^2 = 0.032$
IPD	5 (0.91%)	545 (99.09%)	550 (100%)	df = 1
Total	8 (0.95%)	832 (99.05%)	840 (100%)	p = 0.858

* p<0.05 was considered as statistically significant. df = degree of freedom. N = Number of patients. OPD = Outdoor patient department. IPD = Indoor patient department.

Table 4: Distribution of pregnant female patients according to their socio-economic status and HBsAg reactivity (N=840).

Socio economic status	HBsAg		Total
	Reactive N (%)	Non-reactive N (%)	
Upper class	0(00%)	17(100%)	17(100%)
Middle class	2(0.86%)	231(99.14%)	233(100%)
Lower class	6(1.02%)	584(98.98%)	590(100%)
Total	8(0.95%)	832(99.05%)	840(100%)

DISCUSSION

The prevalence of hepatitis B infection is found to be different in various part of the world with variations from country to country, one region to another region and one group to another group of the country.[19-21] Global prevalence of hepatitis B in pregnant females is 1.5 to 2.5%. In India prevalence rate of hepatitis B infection in pregnant females varies from 0.2 to 7.7%.[22] Previously done studies has shown that prevalence rate of HBsAg positivity varies between 2.3 and 6.3% in female who are pregnant.[9]

Out of 840 pregnant female patients enrolled in this study for the detection of hepatitis B surface antigen we found that 8 pregnant females were positive to HBsAg by ELISA test. The prevalence of HBsAg infection in pregnant females was found to be 0.95%. This finding is concordance with another study done by Dwivedi and Mishra, 2011 who reported prevalence rate of 0.9%.[12] Various other studies done in the past depicted the prevalence of HBsAg in pregnant woman as 0.83%**,** 0.61%, 0.82%**,** 0.76% respectively.[22-25] Table 5 shows the chronological distribution of prevalence rates of HBsAg positivity depicted in previous studies as compared to this study.[26-31]

Table 5: Chronological order distribution of prevalence rates of HBsAg positivity among pregnant females as depicted in various researches done in India as well as abroad as compared to this study.

Study	Year	Location	Prevalence of HBsAg positivity
This study	2019	Lucknow	0.95%
Sujatha A et al,[25]	2019	Telangana	0.76%
Cetin S et al,[26]	2018	Turkey	2.1%
Mishra S et al,[27]	2017	Madhya Pradesh	1.09%
Garg R et al,[28]	2017	Agra	2.04%
Rajendiran S et al,[29]	2017	Tamil Nadu	1.01%
Afzali H et al,[30]	2015	Iran	1.56%
Mehta K et al,[23]	2014	Jamnagar	0.83%
Khakhkhar VM et al,[31]	2012	Gujarat	3.07%
Dwivedi and Mishra[12]	2011	Allahabad	0.9%

The study depicted highest prevalence of HBsAg positivity in middle age group 26-35-year pregnant females 1.04% and least in 36-45 years 0.00% (0 out of 8) and rest among 15-25 years 0.76%. This finding is in agreement to another study done by Rajendiran S et al, who reported that the prevalence of HBV infection was highest in the age group 26-30(46.1%).[29] However, another study done by Chernet A et al, reported highest positivity in 35-44 years (7.6%) age group followed by positivity seen in 25-34 years (4.7%) age group.[32]

The study showed that majority of enrolled subjects were indoor patients 65.47% as compared to outdoor patients 34.53%, whereas, highest seropositivity to HBsAg was found among outdoor patients, 1.03% as compared to indoor patients 0.91%. This finding is in agreement to another study done by Mishra S et al, who reported the prevalence rate of HBsAg in indoor pregnant females' patients to be 1.09%.[27] In this study authors found that majority of patients belonged to rural areas (76.07%) as compared to those from urban areas (23.93%), whereas, maximum HBsAg seropositivity was found among patients belonging to urban areas, 1.00% followed patients from rural areas 0.94%.

This finding is in concordance with another study done by Sibia P et al, who reported higher seroprevalence of HBsAg among patients from urban areas (1.23%) as compared to those from rural areas (1.07%).[24]

On evaluating the socio economic status of the pregnant female patients enrolled in this study, authors found that majority belonged to lower class (70.24%), followed by those belonging to middle class (24.74%) and upper class (2.02%), with highest seropositivity of HBsAg seen in lower class (1.02%), as compared to middle class (0.86%) and upper class (0.00%)**.**

CONCLUSION

Keeping in view the dangerous outcome of vertical transmission HBV from infected pregnant females to

their new-born, it is strongly recommended that routine free screening of all pregnant mothers for HBV infection should be done. The babies born to HBsAg reactive mothers should immediately receive post exposure prophylaxis consisting of simultaneous administration of vaccine in one arm and immunoglobulin in another arm. This would prevent infection in this age group, thereby, help in decreasing the overall carrier rate and development of chronic liver disease and hepatocellular carcinoma later in life.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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