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Original Article

Obstetrics and Gynaecology Section

Hepatitis B Viral Load Patterns in HBsAg-Positive Antenatal Women and their Correlation with Stages of Pregnancy: A Cross-sectional Study

PIRBOX RAFIQUL HUSSAIN[1], PINKEE PHUKON[2], BARBI GOGOI[3], ANGSUREKHA DAS[4], PURNIMA BARUA[5]

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ABSTRACT

Introduction: Screening for Hepatitis B Surface Antigen (HBsAg) during pregnancy is essential, as maternal viremia is directly proportional to perinatal transmission. However, there is insufficient literature available on the viral load pattern of Hepatitis B Virus (HBV) in different trimesters of pregnancy.

Aim: The aim of this study was to investigate the seroprevalence and estimate the viral load of HBsAg among antenatal women in different trimesters of pregnancy.

Materials and Methods: A hospital-based cross-sectional study was conducted in the Department of Microbiology at Jorhat Medical College and Hospital in Jorhat, Assam, India, from June 2017 to May 2018. A total of 7833 samples were collected from antenatal cases attending the Outpatient Department of Obstetrics and Gynaecology. HBsAg positive cases were screened using a rapid diagnostic kit and Enzyme-linked Immunosorbent Assay (ELISA). Viral load was estimated using Reverse Transcription Polymerase Chain Reaction (RT-PCR) after obtaining written consent. Positive patients were followed-up, and viral load estimation was repeated in each trimester. Statistical analysis was performed using Epi Info software and the Mann-Whitney U test.

Results: The mean age of the HBsAg positive antenatal women who participated in the study was 25.97±3.72 years. Out of the 7833 patients screened, 32 antenatal patients were found to be positive for HBsAg. The highest viral load was found to be 1 $\log^{10}$ (25%) and 6 $\log^{10}$ (25%) IU/mL. The median viral load was highest in the second trimester at 1.434×10^6 IU/mL, followed by the third trimester at 7.003×10^5 IU/mL, and the first trimester at 43.6482 IU/mL.

Conclusion: The pattern of HBV viral load indicates that patients in the first trimester either had borderline or low levels compared to those in the second and third trimesters. Since the viral load pattern varies in different trimesters of pregnancy, viral load estimation is crucial for the treatment of positive patients and to reduce HBV vertical transmissions.

Keywords: Hepatitis B surface antigen, Maternal viraemia, Perinatal transmission, Trimester

INTRODUCTION

The HBV is a blood-borne enveloped Deoxyribonucleic Acid (DNA) virus with a partly double-stranded, relaxed circular genome, belonging to the Hepadnaviridae family [1]. Although there are effective vaccines and treatment strategies against Hepatitis B (HB), it is still a significant health concern worldwide which can present in different forms and result in high morbidity and death. HBV is transmitted predominantly through percutaneous or mucosal exposure to infected blood and body fluids. Mother-to-child transmission, also known as perinatal transmission, is the major route of HBV transmission in many parts of the world [2]. India accounts for 10-15% of the entire pool of HBV carriers worldwide [3]. The prevalence of chronic HBV infection varies widely, with rates ranging from 0.1% to 20% in different parts of the world [3]. Countries are categorised into high, intermediate, and low endemic regions based on the prevalence rate of HBsAg [4]. Although India has been placed in the intermediate zone (2-7% prevalence) for HBsAg prevalence, the positivity rate varies in different regions of the country [5]. The northeastern region of India, including Assam, is home to several tribal communities, and the prevalence rate is high among isolated tribal groups such as Naga, Mising, Deuri, etc. [6]. Jorhat Medical College and Hospital (JMCH) serves several neighboring districts of Assam that belong to numerous tribal communities, indicating a potential risk of high prevalence of HBV infection. Therefore, the present study aimed to determine the seroprevalence and viral load of HBsAg among antenatal women in different trimesters of pregnancy, as well as the socio-demographic profile of HBsAg-positive antenatal women attending JMCH.

MATERIALS AND METHODS

A hospital-based cross-sectional study was conducted in the Department of Microbiology at Jorhat Medical College and Hospital (JMCH) in Jorhat, Assam, India, from June 2017 to May 2018. Ethical clearance was obtained from the Institutional Ethics Committee (Human) of JMCH, Jorhat, Assam prior to the commencement of the study (No.-SMEJ/JMCH/MEU/841/Pt-1/2011/6631). Written informed consent was obtained from the subjects. The samples were collected from antenatal cases attending the Outpatient Department of Obstetrics and Gynaecology at JMCH.

Inclusion criteria: Every consecutive antenatal patient attending the Obstetrics and Gynaecology Department of JMCH was included in the study.

Exclusion criteria: Cases with negative pregnancy tests, diagnosed molar pregnancy, women who did not provide written consent, and HBsAg-positive cases with a recent history of HBV vaccination were excluded from the study. HBsAg-negative cases were not further evaluated for ELISA.

Study Procedure

Sample collection: Blood samples were aseptically collected from each antenatal woman by venipuncture, and serum was separated by centrifuging at 3000 rpm for three minutes [7], then transferred to -20°C for further analysis. Clinical and socio-demographic data were collected using a pre-designed proforma.

Screening of HBsAg by rapid diagnostic kit: Serum samples from antenatal cases were screened for HBsAg using a rapid diagnostic kit (Alere Truline Rapid Test Kit) following the manufacturer's instructions.

Hepatitis B Surface Antigen (HBsAg) detection by ELISA: Serum samples that tested positive by the rapid kit were confirmed for HBsAg using an ELISA kit following the manufacturer's instructions (HEPALISA ULTRA).

HBV viral load estimation with real-time PCR: DNA extraction was performed using the QIAamp DNA Mini Kit (QIAGEN), and quantification was done using the 7500 fast RT-PCR system (Applied Biosystems) with the PCR kit supplied by artus® HBV RG/TM PCR Kit v1.

The seropositivity rate of HBsAg among antenatal women and the number of positive cases were determined in different age groups. The hepatitis B viral load pattern was estimated in different trimesters of pregnancy, and the correlation of viral load was analysed among the positive follow-up cases in each trimester.

STATISTICAL ANALYSIS

Statistical analysis was carried out by using Epi Info software version 7.1.4.0 (CDC, Atlanta) and Microsoft Office Excel 2010. The Mann-Whitney U test was used to determine statistical significance, with a p-value <0.05.

RESULTS

A total of 7833 non-duplicate consecutive antenatal patients in their three trimesters who attended JMCH were screened, and 32 patients were found to be positive for HBsAg. All the positive patients showed 100% concordance with the rapid diagnostic HBsAg ELISA test kit, and there was no ambiguity between the results of the two tests. The mean age of the HBsAg-positive antenatal women in the current study was 25.97±3.72 years.

Socio-demographic profile of the HBsAg-positive antenatal cases: The highest frequency (53.13%) of cases was found in the age group of 26-30 years [Table/Fig-1]. Out of the 32 patients, 26 cases (81.25%) belonged to the rural population, while only 6 (18.75%) cases were from urban areas. Among the 32 HBsAg-positive antenatal cases, a major proportion (n=19, 59.38%) were from tribal populations, and n=13 (40.62%) were from non-tribal populations.

Age group (years)	Number of HBsAg positive (n=32), n	Percentage (%)
20-25	13	40.63
26-30	17	53.13
>30	2	6.25

[Table/Fig-1]: Age distribution of HBsAg positive antenatal cases.

Frequency of risk factors among HBsAg-positive antenatal cases: The highest frequency of risk factor was observed in positive antenatal cases (43.75%) with a history of abortion/Medical Termination of Pregnancy (MTP) [Table/Fig-2]. Out of the 32 HBsAg-positive antenatal cases, 14 (43.75%) cases were primigravidae, and 18 (56.25%) cases were multigravidae; 7 (21.88%) cases were presented in the first trimester, 11 (34.38%) cases in the second trimester, and 14 (43.75%) cases presented in the third trimester of pregnancy.

Viral load estimation of HBsAg-positive antenatal cases: The viral load was analysed for the 32 HBsAg-positive antenatal cases using RT-PCR and was found to be in the range of 7.412 IU/mL to 1.702×108 IU/mL. Among the positive cases, HBV DNA was detectable in 28 (87.5%) cases. The highest number of viral loads was found in the 1 log^{10} and 6 log^{10} IU/mL categories (each 25%, n=8) of positive antenatal cases. Moreover, viral load was not detected in 4 (12.5%) cases, and lower viral loads were found in the 3 log^{10} and 8 log^{10} IU/mL categories (each 3.13%, n=1) of positive antenatal cases [Table/Fig-3].

Risk factors	Frequency (n)	Percentage (%) (95% Confidence interval)
H/O Abortion/MTP		
No	18	56.25 (37.66%-73.64%)
Yes	14	43.75 (26.36%-62.34%)
H/O general surgery		
No	23	71.88 (53.25%-86.25%)
Yes	9	28.13 (13.75%-46.75%)
Body tattoo		
Absent	27	84.38 (67.21%-94.72%)
Present	5	15.63 (5.28%-32.79%)
H/O blood transfusion		
No	28	87.50 (71.01-96.49%)
Yes	4	12.50 (3.51%-28.99%)
H/O dental procedures		
No	28	87.50 (71.01%-96.49%)
Yes	4	12.50 (3.51%-28.99%)

[Table/Fig-2]: Frequency of risk factors in HBsAg positive antenatal cases.

Viral load category (IU/mL)	Number of cases (n=32), n	Percentage (%)
Not Detectable (ND)	4	12.5
1 log_{10}	8	25
2 log_{10}	3	9.38
3 log_{10}	1	3.13
4 log_{10}	3	9.38
5 log_{10}	2	6.25
6 log_{10}	8	25
7 log_{10}	2	6.25
8 log_{10}	1	3.13

[Table/Fig-3]: Distribution of cases according to viral load log IU/mL.

Viral load pattern in different trimesters of pregnancy: The viral load pattern varies in different trimesters of pregnancy, and the median viral load was found to be highest in the second trimester, followed by the third and first trimesters [Table/Fig-4]. The viral load was found to be highest (>10^7 IU/mL) in patients in the second and third trimesters, at 9.09% and 14.29%, respectively [Table/Fig-5]. It was also observed that the majority of patients in the first trimester, n=6 (85.71%), had a viral load less than 100 IU/mL compared to the second and third trimester cases, which was found to be statistically significant (Chi-square value=8.8869 with 1 df, p-value=0.0029).

Trimester	Number of HBsAg positive, n	Median (IU/mL)	IQR (IU/mL)
First	7	43.6482	23.85-131.50
Second	11	1.434×10^6	4.99×10^4 -4.92×10^6
Third	14	7.003×10^5	130.06-5.9×10^6

[Table/Fig-4]: Viral load levels in different trimesters.
IQR: Interquartile range

Viral load category (IU/mL)	First trimester n (%)	Second trimester n (%)	Third trimester n (%)	Total n
>10^7	0	1 (9.09)	2 (14.29)	3
10^6-10^7	0	4 (36.36)	4 (28.57)	8
10^2-10^6	1 (14)	4 (36.36)	4 (28.57)	9
<10^2	6 (85.71)	2 (18.18)	4 (28.57)	12
Total, n	7	11	14	32

[Table/Fig-5]: Frequency of positive cases in different viral load categories according to trimesters.

Viral load pattern in follow-up patients: A total of 12 patients out of the 32 included in the present study could be followed-up. The median viral load was 72.334 IU/mL [Interquartile Range (IQR):

26.67-224.57 IU/mL] in the first trimester and 151.12 IU/mL (IQR: 20.31-347.63 IU/mL) in the second trimester [Table/Fig-6].

Trimester	Median VL (IU/mL)	IQR (IU/mL)	Mann-Whitney U test	
			U value	p-value
First	72.334	26.67-224.57	12.5	0.920
Second	151.12	20.31-347.63		

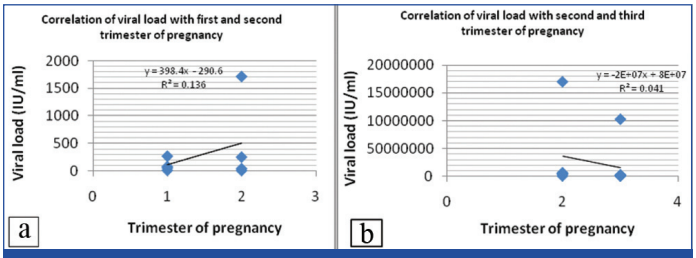
[Table/Fig-6]: Viral Load (VL) pattern in first and second trimester of follow-up cases.

Seven patients in the second trimester were followed-up, and the viral load was estimated in their third trimesters. The median viral load of the follow-up patients was 3.89×106 IU/mL (IQR: 1.69×106-8.81×10^7 IU/mL) in the second trimester and 1.2×106 IU/mL (IQR: 1.45×103-1.98×106 IU/mL) in the third trimester [Table/Fig-7]. However, in two patients, the viral load was not detectable in the second trimester; it became detectable in their third trimesters {Viral Load (VL) 1.692×102 and 1.451×103 IU/mL}. A total of 71.43% (n=5) of second trimester follow-up cases showed a decrease in the level of viral load, while 28.57% (n=2) of cases showed an increase in viral load in the third trimester.

Trimester	Median VL (IU/mL)	IQR (IU/mL)	Mann-Whitney U test	
			U value	p-value
Second	3.89×10^6	1.69×10^6-8.81×10^7	7	0.105
Third	1.2×10^6	1.45×10^3-1.98×10^6		

[Table/Fig-7]: Viral load pattern in second and third trimester of follow-up cases.

Correlation of viral load with pregnancy trimesters in the follow-up cases: Five patients in the first trimester were followed-up, and the viral load was estimated in their second trimesters. A positive correlation was seen in the viral load between the first and second trimesters (r-value=0.36934, p-value=0.920) [Table/Fig-8a]. Therefore, there is a probability of an increase in HBV viral load with progression from the first to the second trimester of pregnancy. Seven patients in the second trimester were followed-up, and the viral load was estimated in their third trimesters. A negative correlation was seen in the viral load between the second and third trimesters (r-value=-0.2026, p-value=0.105) [Table/Fig-8b]. Therefore, there is a probability of a decrease in HBV viral load with progression from the second to the third trimester of pregnancy.



[Table/Fig-8]: Correlation of viral load with (a) first and second trimester and (b) Second and third trimester of pregnancy.

DISCUSSION

The seropositivity rate of HBsAg in antenatal cases was found to be 0.41%. This rate was relatively lower than the prevalence rates reported by Biswas SC et al., (2.3%) and Gupta I et al., (2.5%) [8,9]. Since this study is a hospital-based study including only one center, it may not reflect the true prevalence of Hepatitis B in the state. The mean age of the HBsAg-positive antenatal women in this study was 25.97±3.72 years. This was in accordance with Thakkarwad S and Mundlod S (26.9 years) and Garg R et al., (26.9 years) in India, as well as Vazquez-Martinez JL et al., (26 years) in Mexico [10-12]. The highest frequency (53.13%) of cases was found in the age group of 26-30 years. Bose M et al., (2018) also found the highest number (50%) of HBsAg-positive antenatal cases in the age group of 26-30 years [13]. The highest rate of infection in these age groups could be due to their greater exposure and interaction in society compared to younger and older ages [14].

The majority (81.25%) of HBsAg-positive antenatal women belong to rural areas, while 18.75% are from urban areas. The tertiary care hospital where the present study was conducted caters to a large rural population, which explains the higher positivity of cases from rural areas. The HBsAg positivity was found to be highest in the tribal population, with n=19 (59.38%) and the majority, n=17 (53.13%) of the HBsAg-positive antenatal women, belonging to the Mishing community. Similarly, in Tripura, a higher seroprevalence (5.3%) of Hepatitis B was found in the tribal community, with a predominance of cases in the Chakma community (11.41%) [15]. Biswas D et al., in 2007 reported an even higher prevalence of HBsAg in the Idu Mishmi tribe of Arunachal Pradesh (21.2%) [6]. Although the Northeastern (NE) region is home to many tribes, limited literature is available on the seroprevalence of HBsAg in the different tribes of NE India. Therefore, the current study reports the frequency of HBsAg positivity among antenatal women in the Mishing community of Assam for the first time. In tribal communities in India, Murhekar MV et al., found that hepatitis B infection was highly endemic, with over 60% of people testing positive for HBsAg [16]. The high endemicity of HBV infection in tribal populations has been attributed to several factors by different researchers [17,18]. However, the causal association of these factors was not analysed in the present study.

The majority (n=18, 56.25%) of the HBsAg-positive antenatal women of the present study were multigravida, and 43.75% (n=14) were primigravidae. Garg R et al., reported a higher positivity of HBsAg in multigravida in their study [11]. The highest number (n=14, 43.75%) of HBsAg-positive antenatal cases were presented in the third trimester, followed by the second and first trimesters. According to Lennox Josiah A et al., the prevalence of HBsAg was highest (5.88%) among women in their second trimester of pregnancy, when the fetus is going through key developmental phases that carry a substantial danger to the growing fetus [19]. Similarly, Khakhkhar VM et al., reported that the highest incidence, n=37 (3.56%), was found among HBsAg-positive mothers during the third trimester of pregnancy, followed by the second and first trimesters of pregnancy [20].

The HBV DNA was detected in 87.5% of HBsAg-positive antenatal cases by RT-PCR. The highest number of cases had viral loads of 1 $\log^{10}$ and 6 $\log^{10}$ IU/mL (each 25%, n=8), followed by 2 $\log^{10}$ and 4 $\log^{10}$ (each 9.38%). The distribution of viral load >10^7 IU/mL was found in 9.37% of cases, which is similar to the results studied in the United Kingdom (UK) in 2013 for HBsAg-positive antenatal cases [21]. Several studies from Italy, the United States of America (USA), and India have revealed that maternal HBV viral load above 6 $\log^{10}$ copies/mL (equivalent to >1.72×105 IU/mL) at delivery seems to be the most important predictor for mother-to-child transmission [22,23]. Moreover, different studies have also reported that maternal pre-delivery HBV DNA levels above 6.0 $\log^{10}$ IU/mL are associated with HBV immunoprophylaxis failure in newborns [24]. The American Association for the Study of Liver Diseases and the American College of Obstetricians and Gynecologists propose viral load assessment before 28 weeks of gestation, as the likelihood of HBsAg transmission may damage newborns from positive mothers [25]. This helps to decrease the viral load in the pre-delivery period, thereby reducing the chances of mother-to-child transmission or HBV immunoprophylaxis failure in newborns [26].

Seven patients in the second trimester were followed-up, and their viral load was re-estimated in their third trimesters. The majority, n=5 (71.43%) of these cases, showed a decrease in viral load levels in the third trimester compared to the second trimester. However, this decrease was not found to be statistically significant. Matejko H and Matvisiv M in 2017 also reported an increase in viral load by 1-2 log in the second trimester, which decreased to a boundary level in the third trimester [27]. Viral load was found to be negatively correlated with the second and third trimesters, reflecting the probability of a decrease in HBV viral load with progression from the second to

the third trimester of pregnancy. A similar study was reported by Pereverten LY et al., which indicated a subsequent decrease in the level of viremia before childbirth [28]. During pregnancy, the maternal immune system undergoes several immunological modifications that may be responsible for the typical viral load pattern observed in patients during the three trimesters of pregnancy [29]. In the present study, the HBV viral load pattern revealed that patients in the first trimester either had a low level of viral load compared to second and third trimester patients. The followed-up cases revealed that viral load levels are positively correlated with the first and second trimesters, implying that the HBV viral load tends to increase as pregnancy progresses from the first to the second trimester. The reverse was noticed in the case of the second and third trimesters of pregnancy.

Limitation(s)

Fewer cases were detected in the first trimester, and not all cases could be followed-up in the subsequent trimesters of pregnancy.

CONCLUSION(S)

The frequency of HBsAg positivity in the present study was highest in the tribal Mishing community compared to non-tribal communities. Therefore, it is necessary to conduct an in-depth study on HBV infection in the Mishing community and other populations in Assam. Since the viral load level tends to increase during the first to second trimester of pregnancy, antiviral medication is necessary for HBsAg-positive prenatal women with viral loads (>2 lakh IU/mL). Therefore, it is necessary to determine the viral load in the second and third trimesters of pregnancy, as well as to ensure passive-active immunisation of their neonates.

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PARTICULARS OF CONTRIBUTORS:
1. Demonstrator, Department of Microbiology, Jorhat Medical College and Hospital, Jorhat, Assam, India.
2. Scientist, Department of Microbiology, Jorhat Medical College and Hospital, Jorhat, Assam, India.
3. Scientist, Department of Microbiology, Jorhat Medical College and Hospital, Jorhat, Assam, India.
4. Associate Professor, Department of Microbiology, Jorhat Medical College and Hospital, Jorhat, Assam, India.
5. Professor, Department of Microbiology, Jorhat Medical College and Hospital, Jorhat, Assam, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Purnima Barua,
Professor, Department of Microbiology, Jorhat Medical College and Hospital,
Jorhat-785001, Assam, India.
E-mail: vrdljmch2016@gmail.com

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