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Research Article

Clinical Significance of Hepatitis B Virus-Genotypes and Correlation of HBV-DNA Viral Load with the Liver Enzyme in Pregnant Female

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Abstract

Objective: Hepatitis B virus (HBV) infection during prenatal period presents with unique management issues for both the mother and fetus. These include the effects of HBV on maternal and fetal health, the effects of pregnancy on the course of HBV infection, treatment of HBV during pregnancy, and prevention of mother-to-child transmission.

Methods: Study conducted on 770 in clinically suspected pregnant female patients and performed in central research station laboratory of Microbiology at Netaji Subhash Chandra Bose Subharti Medical College in Meerut, India between November 2017 and February 2020. Serum samples were tested for HBsAg, HBeAg using ELISA.DNA was isolated and Amplified by RT-PCR by using pre-S gene and correlation with ALT, AST. Amplified DNA was Sequenced and genotyped by sequencer and analysed it on NCBI.

Results: 68 were positive for HBsAg in Pregnant female. 47 were HBeAg positive. 13 sample were positive for PCR. ALT was significant ($P < 0.004$) when correlate with DNA viral load. Genotype-B and genotype-A were 61.5% (n = 8), and 38.5% (n = 5) respectively.

Conclusion: Genotype B was more prevalent in pregnancy. This study will improving current knowledge of genotype of hepatitis B in this region and will help clinicians to provide better therapy to the pregnant. For the differentiation of HBV infection level in diagnosis, HBsAg, HBeAg and ALT will be adequate in low income developing countries. Implementation of HBV-vaccine for both pre-exposure and post-exposure has been suggested to control HBV in Pregnant and new born baby. General population should get Vaccination from government hospitals to avoid this health problem.

Keywords: Hepatitis B Virus; Pregnancy; Genotypes; ALT; AST; RT-PCR

Introduction

Hepatitis B virus (HBV) infection is a serious public health issue around the world and a significant explanation for chronic hepatitis, cirrhosis, and hepatic cell carcinoma (HCC) with about 350 - 400 million human beings chronically infected globally and about 600,000 deaths yearly as a result of hepatitis B virus-associated liver diseases [1]. Transmission of HBV from carrier mothers to their babies can occur throughout the perinatal age and seems to be the foremost necessary think about decisive the prevalence of infection in high endemicity areas, significantly in India and Asian geographical area [2]. Before HBV immunizing agent was integrated into the routine immunization program, the proportion of babies that became HBV carriers were concerning 10 per cent to 30% for mothers which were HBsAg positive but HBeAg negative [3]. However, the frequency of perinatal infection was higher about 70% to 90%, once the mother was conjointly HBeAg positive [4-6]. In India, as an example, the prevalence of chronic hepatitis B virus disease in pregnant women is about 0.82%. During pregnancy, the risk of HBV transmission across the placenta will be increase (vertical transmission) [7].

Prevention of vertical transmission is a crucial part of world efforts to reduce the burden of chronic HBV since this transmission is to blame for about common fraction of chronic infections worldwide. The chance of developing chronic HBV infection is reciprocally proportional to the age at the time of exposure. The chance is as high as ninety percent in those exposed at birth while not vaccination [2], whereas the chance, is far lower (about twenty to thirty

percent) in those exposed during childhood [9]. Maternal screening programs and universal vaccination of infants have considerably reduced transmission rates.

Aim of the Study

This study aims to assess the seroprevalence of HBV and its genotypes in this place, in addition, to study the correlation of HBV-DNA with the liver enzymes in pregnant women.

Materials and Methods

Study background and subjects

This was conducted on 770 clinically suspected pregnant female patients. The blood sample was collected in a clean, sterile, small test tube from suspected HBV infections and its sequelae patients from Meerut and prosses in central research centre laboratory of Microbiology at Netaji Subhash Chandra Bose Subharti Medical College and Hospital Between November 2017 and February 2020.

Sample collection and processing

Ten-millilitre blood samples received from clinically suspected pregnant women in the Serology Section of the 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 and Co. Pvt. Ltd. New Delhi, India) and positive serum was stored in frozen (-20°C) until tested for the viral markers. The positive serum samples for HBsAg by Hepa Card were tested again for HBsAg using commercially available ELISA kit (ERBA Transasia Bio-medicals Ltd. Daman, India). Serum samples tested positive for HBsAg were tested for HBeAg (ELISA; Beijing Kewei Clinical Diagnostic Reagent Inc. Beijing, China).

DNA isolation from the serum samples was performed using the "QIAamp DNA Mini Kit" (Qiagen, Germany) following the manufacturer's recommendations [10]. The isolated DNA was amplified a portion of the HBsAg (s101-s237) by Real-Time PCR by using Artus HBV RG PCR Kit (Qiagen, Germany) following the manufacturer's recommendations [11] (Table 1).

SN	Primer	Nucleotide sequence (5' to 3')	Position (nt)	Polarity
	Pre-S gene			
1	PS1	GGGTCACCTTATTCTTGGGA	2814 - 2833	Forward
2	PS2	CCCCGCCTGTAACACGAGCA	208 - 189	Reverse
3	PS3	TTGGGAACAAGATCTACAGC	2828 - 2847	Forward
4	PS4	GTCCTGATGCGATGTTCTCC	176 - 157	Reverse
5	PS-B1	ATTCAAAGCCAACTCAGAAA	2946 - 2965	Forward
6	PS-B2	ACAGTATTCTGAG-CAGGGCTC	105 - 85	Reverse

Table 1: PCR primers for HBV-DNA Viral load and genotyping.

All PCR Positive Sample was analysed for ALT and AST using UV(IFCC) kinetic method by Randox Laboratories Ltd Kit (140 London Wall, London, UK) following the manufacturer's recommendations [12].

TRUGENE kit was for HBV Genotyping. The software was assigned for the viral genotype and polymorphisms present in Amplified HBV DNA [13]. The sequenced file generated by TRUGENE software was analysed on the NCBI website for HBV genotyping and also analysed by Bioedit software [14].

Statistical analysis

Obtained data were analyzed by using the SPSS software for windows version 18. The comparison of data in respect of age groups was performed by Z- test. Karl Pearson correlation test was used for correlation of HBV DNA viral load with ALT and AST. $P < 0.05$ was considered to be statistically significant.

Results

770 serum sample were taken from pregnant female which were suspected for HBV infection and screened for HBsAg, 68 (8.8.%) sample were positive for HBsAg, with the age range 21 - 50. In this study, it was observed that the highest positive case was found in the age group of 21- 30. Of the 68 HBsAg Positive case 47 were HBeAg positive and 21 female were negative for HBeAg, this was statistically significant ($P < 0.039$) by using the Z test (Table 2).

Age group (Years)	HBsAg -Ve	HBsAg +Ve (HBeAg +Ve)	Total
21 - 30	253	32 (27)	285 (37%)
31 - 40	229	25 (17)	254 (33%)
41 - 50	220	11 (3)	231 (30%)
Total	702	68 (47)	770

Table 2: Distribution of HBsAg and HBeAg among pregnant patients.

In RT-PCR, the results show that in 72% (n = 49) of patients were not detectable serum HBV DNA, 9% (n = 6) were threshold and 19% (n = 13) were PCR positive. Out of this 13 PCR positive patients 30.7% (n = 4) were between > 2000 IU/mL to 20000 IU/mL HBV DNA levels and 69.2% (n = 9) were > 20000 IU/mL HBV DNA levels (Table 3).

All the 13 PCR positive patients were analysed for AST and ALT to correlate with DNA viral load. ALT was significant (P-value < 0.004) when correlating with DNA viral load but the correlation of AST with HBV DNA viral Load was Non-significant (P-value < 0.330). The correlation between AST and ALT was also significant (P-value < 0.025) by using Karl Pearson correlation test (Table 4).

HBsAg +ve	HBeAg		Viral Load IU/ml			
	- ve	+ve	NTC Threshold	> Threshold (0.05)	≥ Cutoff (10)	Total
68	-	47	28	06	13	35
	21	-	21	-	-	21
68	21	47	49	06	13	68

Table 3: Distribution of HBV Viral DNA among HBsAg and HBeAg positive patients.

S.N.	Liver Enzyme (IU/L)		Viral Load (IU/ml)
	AST	ALT	
1	25	80	2.00313×10^6
2	25	54	2.75425×10^3
3	636	197	3.78339×10^5
4	59	78	2.86575×10^3
5	291	300	2.17394×10^5
6	198	243	7.0589×10^5
7	57	56	3.54325×10^3
8	> 750	204	4.200652×10^6
9	410	106	8.39205×10^5
10	> 750	220	1.08651075×10^6
11	647	139	5.576665×10^5
12	393	166	2.305925×10^4
13	107	123	6.958×10^3

AST Vs ALT	χ^2 value = 0.558 P value < 0.025 (significant)
ALT Vs Viral Load	χ^2 value = 0.674 P value < 0.004 (significant)
AST Vs Viral Load	χ^2 value = 0.261 P value <0.330 (Non-significant)

Table 4: Correlation of AST, ALT levels with HBV DNA viral load.

All 13 PCR positive sample were sequenced for HBV genotyping. The prevalence of HBV genotype-B and genotype-A were 61.5% (n = 8), and 38.5% (n = 5) respectively. Genotype-B was predominant (Table 5).

S.N.	Viral Load (IU/ml)	Genotype Target Regions-Pre-S gene(s101-s237)
		Genotypes with gene bank number
1	2.00313×10^6	B-D00329
2	2.75425×10^3	B-D00329
3	3.78339×10^5	A- AF 090842
4	2.86575×10^3	B-AB073846
5	2.17394×10^5	A- AF 090842
6	7.0589×10^5	B-D00329
7	3.54325×10^3	B-D00329
8	4.200652×10^6	B-D00329
9	8.39205×10^5	A- AF 090842
10	1.08651975×10^6	A- AF 090842
11	5.576665×10^5	B-D00329
12	2.305925×10^4	A- AF 090842
13	6.958×10^3	B-AB073846

Table 5: *HBV genotypes and their Viral load in pregnant women.*

Discussion

In high endemic areas, prenatal HBV screening is of paramount significance for health workers and program planners to develop prevention strategies in populations at threat for infection transmission. On this have a look at, we located that seroprevalence of HBsAg in clinically suspected pregnant ladies turned into 8.8%. This suggests a degree of endemicity of HBV infection qualifying as nearly excessive according to WHO guidelines [15].

The low occurrence was found of pregnant in pregnant ladies at national capital region of India which was 3.19%, 1.83% [16,17] and 1.5%, 1.6% and had been stated in Libya, Algeria and Nepal respectively [18] but, nearly similar 5%, 4.9%, 4.4%, 4.3% and 3.8% of prevalence which were reported from Salman Khan [19,20], Felege Hiwot [21], Arba Minch [22] and Bahir Dar city [23], respectively. Regarding HBeAg incidence, we discovered 6.1% HBeAg prevalence among HBsAg positive patients which become much like Kfutwah., *et al* [24]. But, Ott JJ., *et al*. suggested that the highest HBeAg occurrence (over 50%) changed into found in 0 to 9-year-old ladies. At the reproductive age, HBeAg occurrence becomes 20 - 50% [25].

The highest HBeAg positivity rate (3.5%) among HBsAg positive became determined inside the 21 - 30 age group. The feasible explanation for this finding might be that ladies in these age group are more sexually active and they may have a higher chance of multiple sexual partners. The history of abortion, nose piercing, surgery and records of multiple sexual partners had been massive predictors of HBV infection. Women with a record of abortion had a hazard of eleven times to develop HBV infection compared to their counterparts. This excessive incidence of infection may be attributed to poor practices of infection prevention manage during the abortion and associated activities. Furthermore, females with a history of multiple sexual companions have been 17 instances more likely to develop HBV infection compared with the ones having a single partner. Similar results suggested in Addis Ababa, Ethiopia [23] and in Nigeria [24]. This locating may be explained as because hepatitis B is blood born virus; blood, semen and other body fluids are not an unusual source of infection that sexual contacts function a method of transmission. Accordingly, sexually active women have a higher threat of having the infection, in particular, those who have a history of multiple sexual companions.

Genotype B was predominant in our isolate and the variable results were found in different studies conducted in different part of India; a take a look at carried out by Saket Chattopadhyay., *et al.* in new Delhi, handiest genotypes A and D were present and genotype D become dominant, genotype A became 16% and genotype D 84% and another studies conducted in national capital region of India was Genotype-B was more prevalent in comparison to Genotype-A [26-28]. A study carried out through Perumal Vivekanandan., et al. from Southern a part of India, genotype D detected in 57.3%, genotype A turned into detected in 18%, and genotype C turned into detected in 11.5% [29]. A look at performed through Swati S., et al. from the western part of India, detected the handiest genotype, Genotype D became the important (91.93%), genotype A became general in 8% [30].

Diverse observe conducted out aspect of India shown different prevalence, genotype C was important in Bangladesh [31]. Genotype D is predominant in Turkey [32]. The predominance of the HBV genotype E changed into detected in Niger [33]. Recent research cautioned that acute infection with HBV genotype A may additionally growth the risk of development to chronic infection. In Japan, the persistence of HBV infection after acute hepatitis B turned into higher in patients with genotype A (23%) than people with genotype B (11%) or C (7%) infections [34]. Of unique be aware, an increase of acute infections with HBV genotype A might result in a redistribution of HBV genotypes amongst patients with CHB in any country in which standard hepatitis B vaccination has now not yet been released. as an example, in a nation-wide survey, Matsuura., et al. located that the prevalence of HBV genotype A in chronic hepatitis B patients in Japan accelerated from 1.7% in 2000 to 3.5% in 2006 [35].

In our observation especially genotype A is associated with excessive viral load. In Northern India, genotype A became greater regularly associated with ALT elevation, HBeAg positivity, absence of anti-HBe and among elderly 25 years and above, cirrhosis of the liver, than genotype D [36]. HCC casualties with genotype C had an extra tumor recurrence rate after healing resection of HCC in comparison with genotype B [37]. A have a look at from India mentioned that genotype D was related to extra severe liver disease and HCC in younger patients than genotype A [38].

Different studies conducted in different countries by the different scientist showed the importance of HBV genotypes and their chronicity levels [39].

Recent research conducted by different researchers showed that the correlation of ALT and HBV-DNA found significant and these markers can be used to differentiate between HBeAg (-) active mutants patients from inactive patients and ALT can be use where PCR is not available specially in developing countries [40].

Various factors need to be assessed whilst figuring out the managing of pregnant ladies with chronic-HBV throughout being pregnant, along with the indicators for treatment, the expected period of therapy, and the ability damaging results to the fetus, the threat of growing drug resistance, and the accessibility and cost of the antiviral drugs. The fitness of the mother and fetus ought to be considered independently while selecting treatment. Pregnant girls with the chronic-HBV need to be controlled along with a hepatologist.

The study revealed that the problem of HBV was highest in pregnant women. The most predominant HBV seroprevalence age group was 21 - 30. Genotype B was more prevalent in comparison to genotype A. Our study also showed a strong correlation between ALT and HBV DNA viral load. Which can be used in low income countries for the differentiation and management of treatment of Hepatitis B virus inactive, active and chronic hepatitis B patients.

Conclusion

Genotype B was more prevalent in pregnancy. This study will improving current knowledge of genotype of hepatitis B in this region and will help clinicians to provide better therapy to the pregnant. For the differentiation of HBV infection level in diagnosis, HBsAg, HBeAg and ALT will be adequate in low income developing countries. Implementation of HBV-vaccine for both pre-exposure and post-exposure has been suggested to control HBV in Pregnant and new born baby. General population should get Vaccination from government hospitals to avoid this health problem.

Conflict of Interest

We declare that we have no conflict of interest.

Ethical Approval

Ethical approval for the study was taken from institutional research ethical committee.

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