IF : 4.547 | IC Value 80.26 VOLUME-6, ISSUE-7, JULY-2017 • ISSN No 2277 - 8160

Original Research Paper Gynecology

# "HIGH MATERNAL VIRAL LOAD KEY TO VERTICAL TRANSMISSION OF HEPATITIS B"

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**ABSTRACT** Hepatitis B virus continues to be a serious health problem globally. India contributes 10-15% of the total HBV carriers in the world. The leading cause of acquisition is vertical transmission from an infected mother to newborn. The aim of this article is to provide a concise review of risk factors in vertical transmission. Objectives: To assess the risk of perinatal hepatitis B virus (HBV) transmission by quantitative HBV DNA. Method: The present study was carried out at MLN Medical College Allahabad a total of 5000 pregnant patients were screened for HBV infection. HBsAg positive women were enrolled for the study. Infectivity status was determined by HBeAg positivity by Elisa and quantitative HBV DNA by RT PCR. At the time of delivery cord blood was taken to assess the perinatal transmission. Result: . Total vertical transmission was 37.5% .In all HBeAg positive mother 63.63% showed vertical transmission. In HBsAg positive women with High viral Load (>1x105 copies/ml)showed 90% vertical transmission. Thus vertical transmission depends upon the HBV viremia levels.

**KEYWORDS** : Diagnosis. HBeAg. HBsAg . HBV DNA . Hepatitis B. Perinatal transmission.

**Introduction -** Two billion people are infected with hepatitis B throughout the world. In the South-East Asia region, the estimated burden of chronic HBV infection is 100 million. 3.7% point prevalence means over 40 million HBV carriers. (1) India is considered to have an intermediate level of HBV prevalence. HBsAg positive in asymptomatic pregnant women out of which 1.1% is with high HBV DNA load.(2,3)The disease is endemic in Africa and Asia where the disease is transmitted from mother to new born or between close contacts in early childhood. Chronic hepatitis B infection causes cirrhosis, liver cancer and death. Perinatal transmission of HBV is most important cause of chronic HBV infection .Transmission is very high ie.80-90% if the mother suffers from hepatitis B infection during the third trimester or in peripartum period (4, 5). Low 10% transmission rate is noted if the infection is acquired during the first or second trimesters of pregnancy. Interruption of childhood HBV infection results in a significant decline in the prevalence of chronic HBV infection and its squelae.(6) Universal immunization is recommended as the best solution. The chronicity is caused by a very high viral load transmitted to the neonate. However in infant born to mother with high HBV DNA levels, despite immunoprophylaxis 8-32% of infant will develop perinatal infection (7, 8, 9, 10, 11, 12)

**Materials & methods:** The study was carried out at M L N Medical College, Allahabad during 2008 and 2009. Women in any trimester of pregnancy with or without jaundice attending the antenatal clinic were included. After a complete general, systemic and obstetrical examination, 5 ml blood was collected after consent. Sera were tested for HBsAg using ELISA. Women who tested HBsAg positive were enrolled in the follow up study after an informed consent. Personal history, history of risk factors, chronic hepatitis and obstetric history was obtained. At the time of admission for delivery, again a detailed history was taken, and general, systemic and obstetrical examination was done. Data on ultrasound findings, mode of delivery, indication for cesarean section if done, weight and maturity of babies at delivery, and results of neonatal physical and neurological examination were recorded. Cord blood was collected at the time of delivery and tested for HBsAg. The presence of HBsAg in cord blood was taken as evidence of vertical transmission.

Biochemical and serological tests- Serum was used for liver biochemical tests including bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (Autospan) and enzyme immunoassays for HBsAg and HBeAg. All HBsAg positive specimens were tested further for HBV DNA, anti-HBe antibody and anti-HBc IgM using Autospan Diagnostic kit as manufacturer instruction (Span Diagnostic Ltd, SuratIndia). Infants born to HBsAg positive mothers received HBV vaccine (10 µg) and hepatitis B immunoglobulin

(HBIG; 0.5 mL) were administered, one in each thigh, within 12 h of birth Detection of HBV DNA by PCR (qualitative HBV DNA) - All the HBsAg positive samples were done for the HBV DNA. Nucleic acids were extracted from 20µl serum that had been stored at -80C. High Pure Viral Nucleic acid Kit (Roche, Penzberg, Germany) was used. 5 µl of the extracted DNA was mixed with 45 µl of a PCR reaction mixture(Roche, Penzberg, Germany) containing 400 nM of the primer P1-1 (5'GCATGGAGACCACCGTGAA3':sense ) and P1-2(5' GGAAAGAAGTCAGAAGGCAA 3': antisense) and subjected to 35 cycle of 940C for 1 min,550C for2 min, 720C for 2 min. The PCR product was analyzed by agarose gel (2%) electrophoresis to see the HBV DNA bands under Trans gel illuminator.

HBV Real Time Quantitative PCR- It provides a direct and reliable estimate of the level of HBV replication After extraction of DNA real time PCR was done by use of Applied Bio system SYBR Green PCR Master Mix Method by adding 19 micro liters master mix to the necessary wells in the 96 well plate then add 1micro litre of DNA to appropriate wells in the plate .The plate was sealed and mixed and incubation was done at 50oC for 2 minutes, Taq activation was done at 950 C for 10 minutes. 40 cycles of denaturation at 95oC for 15 seconds and 40 cycles of annealing and extension at 72o C for one minute was done. The results were obtained in copies/ml.More than 105 copies were considered to be a high viral load. Less than 105 copies were considered to be a low viral load.

Statistical analysis- Inter-group comparisons were done using Student's t test and $\chi 2$ -test with; p values below 0.05 were taken as significant.

**Observation:** HBsAg prevalence rate in 5000 antenatal women screened for HBsAg by ELISA. Only 32 were found to be positive. This represented a seroprevalence of 0.64 %( 32/5000) in the present study

**Table 1 Quantitative HBV DNA level in All HBsAg positive women**

| Quantitative HBV | No. | High Viral load | Low viral load | |
|---|---|---|---|---|
| DNA level | | >1x105 copies/ml | <1x105 copies/ml | |

| | | | | |
|---|---|---|---|---|
| Total | | | | |
| | 32 | 10 | 22 | |

All HBsAg positive antenatal women were tested for quantitative HBV DNA by real time quantitative PCR assay then they are further categorized in high viral load(>1x 105 copies/ml )and low viral load groups(<1x105 copies/ml) Thus high viral load were present in 10 /32(31.2%)antenatal women.

**Vertical transmission rate and factors influencing it**

Out of 4 antenatal women with acute hepatitis, 3(75%) transmitted infection to their babies as shown by cord blood HBsAg positivity(p=.02) .9(32.14%) of 28 babies of asymptomatic mothers showed vertical transmission. Out of all 32 HBsAg positive antenatal women vertical transmission was seen in 12(37.50%) babies.

**Table 2 Maternal serological markers and vertical transmission**

| Serological markers | Antenatal women positive | Vertical transmission |
|---|---|---|
| HBsAg + HBeAg | 8 | 6 |
| +HBV DNA | | |
| HBsAg+HBeAg | 3 | 1 |
| HBsAg+AntiHBeAb | 9 | - |
| HBsAg +HBVDNA | 5 | 3 |
| HBsAg | 7 | 2 |
| Total | 32 | 12 |

Out of 8 HBeAg and qualitative HBV DNA positive mothers 6 babies (75%) showed vertical transmission. In HBeAg positive mothers 1 baby (33.33%) showed vertical transmission. In antiHBeAb positive group no one showed vertical transmission. In HBsAg and qualitative HBV DNA positive group Cord blood of 12 newborns were positive for HBsAg. Out of12, 7(58.33%) babies were delivered by HBeAg positive antenatal women while the 5(41.66%) were delivered by HBeAg negative antenatal women. Out of 20 babies who were negative for HBsAg 4(20%)were delivered by HBeAg positive antenatal women, the remaining 16(80%) were delivered by HBeAg negative antenatal women.7 of 11(63.63%) babies who developed vertical transmission were delivered by HBeAg positive mothers and 5 (23.80%) babies who developed vertical transmission were delivered by HBeAg negative mothers. (p=.02) which was significant

**Table 3 Correlation between maternal quantitative HBVDNA level and vertical transmission**

| Vertical transmission<br><br>Quantitative HBV DNA level | Cord blood HBsAg Positive | Blood HBsAg Negative | Total |
|---|---|---|---|
| 10( >1X105 copies/ml) | 9 | 1 | 10 |
| | | | |
| 22 (<1x10 copies/ml) | 3 | 19 | 22 |
| Total | 12 | 20 | 32 |

Out of 10 antenatal women who had high viral load 9 (90%) showed vertical transmission.(p=.001) In low viral load group OF 22 only 3 (15.78%) showed vertical transmission. Total vertical transmission was 37.5%.Women delivered with HBeAg positivitiy with high viremia 6(85.71%) babies had vertical transmission who were delivered by HBeAg positive mothers with high viremia and only 1(25%) baby showed vertical transmission in HBeAg positive mothers with low viremia.who were delivered by HBe Ag positive mother with low viremia.(p=.005).Out of 32 babies 24 women delivered vaginally and 11 (45.83%) had vertical transmission as compared to 8 babies delivered by LSCS only one(12.5%) showed vertical transmission.

**Discussion: T**he present study had seroprevalence of HBsAg in antenatal women was 32/5000 (0.64%).However higher prevalence seen in Sukone Pradutkanchana et al 2005(13) Khakhkhar Vipul et al (2012)(14) and Sayed et al (2013)(15).) . HBeAg positivity rate among HBsAg positive antenatal women have been shown to vary widely in different geographical regions around the world. HBeAg positivity was 34.37% in present study which is lower than by Vipul et al(14) and Wiseman et al(2009)(16).Total vertical transmission was 37.5% which is also lower than studies of Candoti et al (2007) i.e. 8.3%(17).HBeAg positive women showed 63.63% of vertical transmission as compared to 23.80% in HBeAg negative women which was statistically significant(x2-4.35,p<.05) that HBeAg positive mothers were having higher rate of vertical transmission . 31.2% had high viral load by quantitative HBV DNA PCR having 90% vertical transmission .HBe Ag positive women with high viremia showed 85.71% & HBe Ag positive women with low viremia showed 25% vertical transmission. Similar observation was seen in studies by Wiseman et al (2009)(16),Alison et al 2015(18).Ling et al 2014 (19) concluded that vertical transmission was higher in mother with high viremia (93.3%).Conclusion-Quantitative HBV DNA is better predictor of vertical transmission than HBeAg . Vertical transmission occurs despite postnatal active and passive immunization in infant of highly viremic mothers and risk correlates with maternal serum HBV DNA.

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