Hepat Mon. 2018 October; 18(10):e55064. doi: 10.5812/hepatmon.55064.

Published online 2018 April 30. **Research Article**

Influential Factors of Hepatitis B Virus cccDNA in Peripheral Blood Mononuclear Cells Among HBsAg-Positive Pregnant Females Neonates

Xiao-Hong Shi [1], Bo Wang [2], Shu-Ying Feng [2], Zhen Guo [1, 3], Jian Guo [1, 4], Xue-Fei Wang [1], Shu-Zhen Li [1], Yong-Liang Feng [1] and Su-Ping Wang [1, *]

[1] Department of Epidemiology, School of Public Health, Shanxi Medical University, Taiyuan, Shanxi Province, China
[2] Department of Obstetrics and Gynaecology, The Third People Hospital of Taiyuan City, Taiyuan, Shanxi Province, China
[3] Central laboratory, Henan Cancer Hospital, Affiliated Cancer Hospital of Zhengzhou University, Zhengzhou, Henan Province, China
[4] School of Public Health, Chinese Academy of Medical Sciences, Peking Union Medical College, Beijing, China

[*] *Corresponding author*: Su-Ping Wang, Professor, Department of Epidemiology, School of Public Health, Shanxi Medical University, 56 Xin Jian South Road, Taiyuan, 030001, Shanxi Province, China. Tel/Fax: +86-3514135103, E-mail: spwang88@163.com

Received 2017 June 16; **Revised** 2017 December 12; **Accepted** 2018 April 24.

Abstract

Background: Although many studies have measured HBV cccDNA molecules in Peripheral Blood Mononuclear Cells (PBMC) from patients with active chronic hepatitis B, the current pilot study found PBMC HBV cccDNA in PBMC among HBsAg-positive mothers and their neonates. However, the risk factor of HBV cccDNA in PBMC among HBsAg-positive pregnant female's neonates remains unclear.

Objectives: The aim of this study was to explore influential factors of HBV cccDNA in PBMC among HBsAg-positive pregnant female's neonates.

Methods: Peripheral blood samples and clinical data were collected from 151 pregnant females, who were positive for hepatitis B surface antigen (HBsAg) in the Third People Hospital of Taiyuan City. Blood samples from 152 neonates were collected before immune prophylaxes administration and tested for HBV markers, HBV DNA in serum, and HBV DNA in PBMC. Bayesian logistic regression with Cauchy prior were used to measure the association between maternal characteristics, neonatal characteristics, and HBV cccDNA in PBMC of neonates.

Results: Among neonates of HBsAg-positive mothers, the positive rate of cccDNA in PBMC was 4.61% (7/152). Maternal PBMC HBV cccDNA positivity (OR = 18.411, 95%CI: 3.025 - 66.022) and neonates PBMC rcDNA positivity (OR = 13.529, 95% CI: 1.948 - 93.690) were associated with HBV cccDNA in neonatal PBMC, respectively.

Conclusions: The study suggested that HBV cccDNA can be detected in PBMC of HBsAg-positive mother's neonates. Maternal PBMC HBV cccDNA positivity and neonatal PBMC rcDNA positivity are risk factors of HBV cccDNA in PBMC of neonates.

Keywords: PBMC, HBV, HBV cccDNA, Influential Factor

1. Background

Hepatitis B virus (HBV) infection, a major public health problem worldwide, increases the risk of terminal liver disease in more than 250 million people (1). China is in the intermediate prevalence region of HBV (2). Vertical transmission of hepatitis B virus is a major reason for the spread of HBV in areas where it is prevalent (3). Hepatitis B virus transmission from the mother to infant includes intrauterine transmission, intrapartum transmission, and puerperal transmission. Cellular transmission by peripheral blood mononuclear cells (PBMC) is regarded as a possible route for HBV intrauterine transmission. Some studies have shown that maternal HBV can traverse the placenta eventually by sera and PBMC and then may lead to HBV intrauterine transmission (4-7). Hepatitis B virus intrauterine transmission was defined as finding HBsAg and/or HBV DNA positivity in the peripheral blood of neonates within 24 hours of birth and before active or passive immune prophylaxis (8, 9). Previous researches showed that the sensitivity and accuracy of HBV covalently-closed circular DNA (cccDNA) was better than that of serum HBV DNA, which was widely regarded as the most specific biomarker of hepatitis B virus replication (10). Hepatitis B virus infections are maintained by the presence of a small and regulated number of episomal viral genome cccDNA in the nuclei of infected cells. Hepatitis B virus cccDNA is the template for the replication of HBV, which plays a key role in viral infection and persistence (11, 12).

Many studies have only measured HBV cccDNA molecules in PBMC from patients with active chronic

hepatitis B (13), while the current research group found HBV cccDNA in PBMC among HBsAg-positive mothers and their neonates (14). The risk factors of HBV cccDNA in PBMC among HBsAg-positive pregnant female's neonates remains unclear.

2. Objectives

The aim of this study was to explore the influential factors of HBV cccDNA in neonatal PBMC and to provide a theoretical basis for exploring potential etiology of HBV intrauterine transmission.

3. Methods

Eligible study subjects were HBsAg-positive mothers, who had given birth in the third people hospital of Taiyuan city between 1st of June 2001 and 31st of December 2002. A total of 151 pregnant females were eligible for the study. The basic information, including maternal demographics, history of disease, and HBV infection details before and during pregnancy, of the HBsAg-positive mothers and neonates were collected by well-trained interviewers utilizing standardized and unified questionnaires by face-to-face interviews or medical records. The research protocol was approved by the ethics committees of Shanxi Medical University (No:2016LL143), and all mothers signed a written informed consent.

3.1. Sample Collection

All participants donated 5 mL of peripheral blood before delivery, and 5 mL of femoral venous blood was collected from each infant within 24 hours after birth prior to inoculations of hepatitis B vaccine and HBIG. Blood samples were processed within 24 hours of being drawn. Peripheral blood mononuclear cells were isolated by Ficoll-Paque density gradient centrifugation. The plasma samples and PBMC were stored at -80°C for further experiments.

3.2. Serological Tests

HBsAg and HBeAg were measured by the enzyme-linked immunosorbent assay (ELISA) (Shanghai Kehua Biotechnology, Shanghai, China). All procedures were performed according to the manufacturers' instructions.

3.3. Molecular Tests

The total DNA from plasma or PBMC was extracted with hydroxybenzene-chloroform-isoamyl alcohol and the integrity of DNA was assessed by gel electrophoresis. The blood plasma or PBMC, which was positive for HBsAg, HBeAg, and HBV DNA was selected as the positive control, and the blood plasma or PBMC, which was negative for HBsAg, HBeAg, and HBV DNA was selected as the negative control. Additionally, sterile water was used as the blank control. All the three control specimens and case specimens were used for DNA isolation and PCR.

3.3.1. Hepatitis B Virus DNA Tests

Hepatitis B virus DNA was tested by nested Polymerase Chain Reaction (n-PCR) using nested primers (15) (Table 1). The first round of nested PCR was performed in 50-μL reaction system, which contained 25 μL of template, 5 μL 10 $\times$ Buffer, 4 μL $MgCL_2$ (25 mM each), 1 μL primer (50 pM/μL), 1 μL dNTP mixture (10 mM each), and 0.4 μL Taq DNA polymerase (5 units/uL). Thermal cycle parameters included pre-denaturation at 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 50 seconds, annealing at 55°C for 50 seconds, extension at 72°C for 5 minutes, and finally extension for 10 minutes at 72°C. The first round product of 5 μL was taken as a template for the second round of amplification with the same components and parameters as the first round of PCR. The PCR amplification products were electrophoresed on 2% agarose gels stained with ethidium bromide and examined under UV light.

3.3.2. Hepatitis B Virus cccDNA Tests

Hepatitis B virus rcDNA and cccDNA in PBMC were tested by selected polymerase chain reaction (s-PCR) using selected primers (16) (Table 1). The PCR was performed in a 20-μL reaction system, which contained 15 μL of template, 2 μL 10 $\times$ Buffer, 0.15 μL $MgCL_2$ (25 mM each), 0.2 μL primer (50 pM/μL), 0.4 μL dNTP mixture (10 mM each), and 0.2 μL Taq DNA polymerase (5 units/uL). Thermal cycle parameters, included pre-denaturation at 94°C for 5 minutes, followed by 30 cycles of denaturation at 94°C for 60 seconds, annealing and extension at 72°C for 3 minutes, and finally extension for 5 minutes at 72°C. The PCR amplification products were electrophoresed on 2% agarose gels stained with ethidium bromide and examined under UV light. Then, the products were sent for sequencing and blasted with HBV standard sequences.

3.4. Statistical Analysis

Neonates, for whom HBV cccDNA in PBMC was positive, were selected as cases, and those, for whom HBV cccDNA in PBMC was negative, were selected as controls. In univariate analyses, chi-square test was used for categorical data and Student's t-test was used for continuous variables. These analyses were performed using SPSS version 19.0 software. Bayesian logistic regression with Cauchy was used prior to estimation of odds ratio (OR) and 95% confidence interval (CI). The analyses were performed using R version 3.2.2 software. $P < 0.05$ was considered statistically significant.

Table 1. The Specific Primers Used for Amplifying Hepatitis B Virus DNA

Amplifying Fragment	Primer Name	Sequences (5' - 3')	Position, nt	Product Size, bp
HBV DNA, 1st round	Forward	CTGCTGGTGGCTCCAGTT	59 - 76	699
	Reverse	CAATACCACATCATCCA	758 - 741	
HBV DNA, 2nd round	Forward	CCTGCTCGTGTTACAGGC	189 - 206	500
	Reverse	GGCACTAGTAAACTGAGC	689 - 672	
HBV , rcDNA	Forward	CCGACCACGGGGCGCACCTCTCTTTACG	1515 - 1542	243
	Reverse	CTAATCTCCTCCCCCAGCTCCTCCCAGT	1758 - 1731	
HBV cccDNA	Forward	CCGACCACGGGGCGCACCTCTCTTTACG	1515 - 1542	373
	Reverse	CAAGGCACAGCTTGGAGGCTTGAACAGT	1888 - 1861	

Table 2. Distributions of Maternal and Neonatal Characteristics in Cases and Controls

Characteristics	Cases, (N = 7), No. (%)	Controls, (N = 145), No. (%)	P Value
Maternal characteristics			
Age, y, mean (SD)	28.30 (4.13)	29.00 (7.65)	0.679
Highest educational levels			0.699
< High school	5 (71.4)	80 (57.1)	
≥ High School	2 (28.6)	60 (42.9)	
Gestational weeks			0.582
< 37	0 (0.0)	12 (8.3)	
37 - 41	6 (85.7)	123 (84.8)	
≥ 41	1 (14.3)	10 (6.9)	
Neonates characteristics			
Gender			0.442
Girl	2 (28.6)	72 (51.1)	
Boy	5 (71.4)	69 (48.9)	
Deformity			1.000
Yes	0 (0.00)	2 (1.5)	
No	7 (100.00)	134 (98.5)	
Weight, mean (SD)	3207.1 (333.4)	3300.0 (449.1)	0.591
Height, mean (SD)	49.7 (1.49)	49.5 (1.64)	0.769

4. Results

4.1. Result of Molecular Tests

There were 151 HBsAg-positive mothers and 152 neonates, which included one pair of twins. In all of the neonates, ten neonates had positive results for HBsAg and 5 neonates had positive results for HBV DNA in serum. Two neonates were infected in the form of occult infection. Hepatitis B virus intrauterine transmission was defined as finding HBsAg and/or HBV DNA positivity in the peripheral blood of neonates within 24 hours of birth and before active or passive immune prophylaxis. The rate of HBV intrauterine transmission was 7.9% (12/151). In all of neonates, 7 neonates had positive results for HBV cccDNA and 35 neonates had positive results for HBV rcDNA in PBMC.

4.2. Associations Between Maternal Characteristics, Neonatal Characteristics, and Hepatitis B Virus cccDNA in Peripheral Blood Mononuclear Cells of Neonates

Neonates, for whom HBV cccDNA in PBMC was positive were selected as cases, and those, for whom HBV cccDNA in PBMC was negative, were selected as controls. The demographic characteristics were the same between cases and controls (Table 2). In univariate analyses (Table 3), maternal PBMC HBV rcDNA positivity (P = 0.009), maternal PBMC HBV cccDNA positivity (P < 0.001), neonatal HBsAg positivity (P = 0.007), and neonatal PBMC rcDNA positivity (P < 0.001) were significantly associated with HBV cccDNA in neonatal PBMC.

After adjusting for related covariates (Table 4), maternal PBMC HBV cccDNA positivity increased the risk of HBV replication in neonatal PBMC (OR = 18.411, 95% CI: 3.025 - 66.022). Neonatal PBMC rcDNA positivity was significantly associated with HBV replication in neonatal PBMC (OR = 13.529, 95% CI: 1.948 to 93.690).

5. Discussion

In this study, 7.9% of neonates born to HBsAg-positive mothers showed HBV intrauterine transmission, which was within the range of 5% to 40% in China (17, 18).

After uncoating, HBV was transported to the nucleus, in which the virus would be converted to a covalently

Table 3. Univariate Analyses of Associations Between Maternal Characteristics, Neonate's Characteristics, and HBV Replication in PBMC of Neonates[a]

Characteristics	Cases		Controls		P Value
	No	Yes	No	Yes	
Pregnancy					
History of hepatitis	6 (85.7)	1 (14.3)	117 (81.2)	27 (18.8)	1.000
Family history of HBV infection	4 (57.1)	3 (42.9)	93 (68.4)	43 (31.6)	0.681
History of blood transfusion	6 (85.7)	1 (14.3)	140 (97.9)	3 (2.1)	0.176
History of acupuncture	6 (100.0)	0 (0.0)	134 (93.1)	10 (6.9)	1.000
History of dental treatment	5 (83.3)	1 (16.7)	105 (72.9)	39 (27.1)	0.794
HBV vaccine injection	6 (100.0)	0 (0.0)	130 (90.9)	13 (9.1)	1.000
History of abortion or induced labor	7 (100.0)	0 (0.0)	93 (64.6)	51 (35.4)	0.096
History of labor	5 (71.4)	2 (28.6)	112 (77.8)	32 (22.2)	0.655
Pregnancy					
Medication use during pregnancy	6 (100.0)	0 (0.0)	108 (75.0)	36 (25.0)	0.336
Antepartum hemorrhage	6 (100.0)	0 (0.0)	130 (90.3)	14 (9.7)	1.000
Threatened premature labor	6 (100.0)	0 (0.0)	143 (99.3)	1 (0.7)	1.000
HBIG injection	4 (57.1)	2 (42.9)	44 (30.0)	98 (69.0)	0.087
Maternal HBeAg positive	3 (42.9)	4 (57.1)	90 (62.1)	55 (37.9)	0.431
Maternal serum HBV DNA positive	4 (57.1)	3 (42.9)	80 (55.2)	65 (44.8)	1.000
Maternal PBMC HBV rcDNA positive	1 (14.3)	6 (85.7)	96 (66.2)	49 (33.8)	0.009
Maternal PBMC HBV cccDNA positive	2 (28.6)	5 (71.4)	135 (93.1)	10 (6.9)	< 0.001
Delivery					
Caesarean section	3 (57.1)	4 (42.9)	83 (59.3)	57 (40.7)	1.000
After delivery					
Neonates HBsAg positive	4 (57.1)	3 (42.9)	133 (94.6)	7 (5.4)	0.007
Neonates anti-HBs positive	6 (100.0)	0 (0.0)	117 (97.3)	3 (2.7)	1.000
Neonates HBeAg positive	4 (57.1)	3 (42.9)	92 (66.7)	46 (33.3)	0.689
Neonates anti-HBe positive	4 (66.7)	2 (33.3)	71 (59.2)	49 (40.8)	1.000
Neonates HBcAg positive	6 (100.0)	0 (0.0)	119 (99.2)	1 (0.8)	1.000
Neonates anti-HBc positive	3 (50.0)	3 (50.0)	26 (21.7)	94 (78.3)	0.134
Neonates serum HBV DNA positive	6 (85.7)	1 (14.3)	129 (97.0)	4 (3.0)	0.229
Neonates PBMC HBV rcDNA positive	1 (14.3)	6 (85.7)	116 (80.0)	29 (20.0)	<0.001

[a]Values are expressed as No. (%).

Table 4. Multivariate Analysis of Associations Between Maternal Characteristics, Neonates Characteristics and Hepatitis B Virus Replication in Peripheral Blood Mononuclear Cells of Neonates

Variables	β	S.E.	Z	P Value	OR	OR 95% CI
Intercept	-5.754	1.205	-4.772	1.82E-06	-	-
Maternal PBMC HBV cccDNA	2.913	0.921	3.162	0.001	18.411	3.025 - 66.022
Neonates PBMC HBV rcDNA	2.604	0.988	2.635	0.008	13.529	1.948 - 93.690
Maternal PBMC HBV rcDNA	0.819	0.996	0.822	0.411	2.269	0.321 - 16.014
Neonates HBsAg	-0.005	0.241	-0.023	0.981	0.994	0.618 - 1.595

closed circular molecule (cccDNA), which would serve as the template for transcription (19). Although HBV is generally considered to be hepatotropic, HBV specific nucleic acids and relative antigen can be detected in many extra-

hepatic tissues (20, 21), and it has been proved that PBMC may be the extrahepatic place of HBV transcription and translation (22, 23). In the present study, 15 mothers and 7 neonates were positive for HBV cccDNA in PBMC, which suggested that HBV can exist and be replicated in PBMC.

Besides, it was observed that an increased risk of HBV cccDNA in PBMC of neonates was associated with maternal PBMC HBV cccDNA positivity. The positivity of HBV cccDNA is a sign of HBV replication (2, 24). Hepatitis B virus cccDNA in neonatal PBMC may suggest that there may be HBV replication in neonates. However, HBV cccDNA in neonatal PBMC may come from maternal PBMC. Furthermore, PBMCs are immigrant cells from mother to neonate's blood stream (25), which can carry cccDNA into neonates. Otherwise, they may be due to blood transfection of neonates with the mother during delivery. The source of neonatal PBMC HBV cccDNA remains to be explored further. However, the persistence of HBV cccDNA is the main source of failure to eliminate HBV, which can cause serious outcomes (26). Engineered site-specific nucleases and RNA interference therapeutics could clear or silence cccDNA (27). Thus, PBMC HBV cccDNA in HBsAg positive pregnant females can be detected during the gestation period, and clearance of HBV cccDNA by anti-viral treatment to decrease the risk of HBV replication in neonatal PBMC could be achieved.

Neonatal PBMC HBV rcDNA positivity may influence its PBMC HBV cccDNA. Hepatitis B virus is a small DNA virus that replicates by protein-primed reverse transcription (28). After infection, HBV rcDNA is converted to cccDNA, which serves as a viral persistence reservoir. This suggests that the development of HBV infection may be controlled by preventing transformation from rcDNA to cccDNA.

The researchers firstly detected HBV cccDNA in PBMC of HBsAg-positive pregnant females and their neonates. Furthermore, this study firstly focused on the risk factors of HBV cccDNA in PBMC among HBsAg-positive pregnant female's neonates. The current study has laid the foundation of subsequent research. However, the source of HBV cccDNA in neonatal PBMC is still unclear. The neonates, who were positive for HBV cccDNA in PBMC were not followed up and thus the persistence of HBV cccDNA in neonatal PBMC could not be understood. Future research will follow up neonate, who was positive for HBV cccDNA in PBMC and observe the nucleotide divergence of HBV cccDNA in maternal PBMC and neonatal PBMC and the variation of HBV sequences.

5.1. Conclusion

Hepatitis B virus cccDNA could be detected in PBMC among neonates born to HBsAg-positive mothers, and the positive rate of HBV cccDNA in PBMC was 4.61%. Maternal PBMC HBV cccDNA positivity and neonates PBMC rcDNA positivity may increase the risk of HBV cccDNA positivity in PBMC of neonates. This preliminary conclusion will provide the basis for future research of HBV intrauterine infection.

Acknowledgments

The authors are particularly grateful of the study subjects and their mothers. The authors also acknowledge the nursing staff of obstetrics and gynecology department, the third people hospital of Taiyuan city, Shanxi, P.R. China for their assistance. The authors gratefully acknowledge Dr. Weiwei wu, Dr. Hongfang Shao, and Dr. Yi Gao for critical review of this manuscript.

Footnotes

Conflict of Interest: None declared. Completed disclosure of interest form is available online as supporting information.

Funding/Support: This research was supported by the national natural science foundation of China, (Nos. 30070669 and 81573212), open project supported of state key laboratory of infectious disease prevention and control (No. 2017SKLID306).

References

1. Ott JJ, Stevens GA, Groeger J, Wiersma ST. Global epidemiology of hepatitis B virus infection: new estimates of age-specific HBsAg seroprevalence and endemicity. *Vaccine*. 2012;**30**(12):2212–9. doi: 10.1016/j.vaccine.2011.12.116. [PubMed: 22273662].
2. Chen Y, Wang L, Xu Y, Liu X, Li S, Qian Q, et al. Role of maternal viremia and placental infection in hepatitis B virus intrauterine transmission. *Microbes Infect*. 2013;**15**(5):409–15. doi: 10.1016/j.micinf.2013.02.008. [PubMed: 23500187].
3. Yu M, Jiang Q, Gu X, Ju L, Ji Y, Wu K, et al. Correlation between vertical transmission of hepatitis B virus and the expression of HBsAg in ovarian follicles and placenta. *PLoS One*. 2013;**8**(1). e54246. doi: 10.1371/journal.pone.0054246. [PubMed: 23382883].
4. Bai GQ, Li SH, Yue YF, Shi L. The study on role of peripheral blood mononuclear cell in HBV intrauterine infection. *Arch Gynecol Obstet*. 2011;**283**(2):317–21. doi: 10.1007/s00404-010-1366-8. [PubMed: 20107823].
5. Gatta A, Giannini C, Lampertico P, Pontisso P, Quarta S, Zignego AL, et al. Hepatotropic viruses: new insights in pathogenesis and treatment. *Clin Exp Rheumatol*. 2008;**26**(1 Suppl 48):S33–8. [PubMed: 18570752].
6. Xu YY, Liu HH, Zhong YW, Liu C, Wang Y, Jia LL, et al. Peripheral blood mononuclear cell traffic plays a crucial role in mother-to-infant transmission of hepatitis B virus. *Int J Biol Sci*. 2015;**11**(3):266–73. doi: 10.7150/ijbs.10813. [PubMed: 25678845].
7. Xue SL, Wei JN, Wang B, Shuang JY, Feng LP. Association of placental apoptosis and PBMC transfer with HBsAg of PBMC in neonates. *Chin J Public Health*. 2015;**31**:199–201. Chinese.
8. Li XM, Shi MF, Yang YB, Shi ZJ, Hou HY, Shen HM, et al. Effect of hepatitis B immunoglobulin on interruption of HBV intrauterine infection. *World J Gastroenterol*. 2004;**10**(21):3215–7. doi: 10.3748/wjg.v10.i21.3215. [PubMed: 15457579].

9. Zou H, Chen Y, Duan Z, Zhang H, Pan C. Virologic factors associated with failure to passive-active immunoprophylaxis in infants born to HBsAg-positive mothers. *J Viral Hepat*. 2012;**19**(2):e18–25. doi: 10.1111/j.1365-2893.2011.01492.x. [PubMed: 22239517].
10. Addison WR, Walters KA, Wong WW, Wilson JS, Madej D, Jewell LD, et al. Half-life of the duck hepatitis B virus covalently closed circular DNA pool in vivo following inhibition of viral replication. *J Virol*. 2002;**76**(12):6356–63. doi: 10.1128/JVI.76.12.6356-6363.2002. [PubMed: 12021368].
11. Singh M, Dicaire A, Wakil AE, Luscombe C, Sacks SL. Quantitation of hepatitis B virus (HBV) covalently closed circular DNA (cccDNA) in the liver of HBV-infected patients by LightCycler real-time PCR. *J Virol Methods*. 2004;**118**(2):159–67. doi: 10.1016/j.jviromet.2004.02.006. [PubMed: 15081611].
12. Torii N, Hasegawa K, Joh R, Hayashi N. Configuration and replication competence of hepatitis B virus DNA in peripheral blood mononuclear cells from chronic hepatitis B patients and patients who have recovered from acute self-limited hepatitis. *Hepatol Res*. 2003;**25**(3):234–43. doi: 10.1016/S1386-6346(02)00275-9. [PubMed: 12697244].
13. Cabrerizo M, Bartolome J, Caramelo C, Barril G, Carreno V. Molecular analysis of hepatitis B virus DNA in serum and peripheral blood mononuclear cells from hepatitis B surface antigen-negative cases. *Hepatology*. 2000;**32**(1):116–23. doi: 10.1053/jhep.2000.8541. [PubMed: 10869298].
14. Shi XH, Wang SP, Li SZ, Xie LJ, Feng LP, Shuang JY. Study on replication status of HBsAg positive pregnant women and newborns. *Chin J Public Health*. 2004;**20**(5):513–4. Chinese.
15. Fan JS, Zhuang H, Zhu XJ. Comparative study on the clinical and epidemiological characteristics of HBsAg negative and positive hepatitis B in 6 cities in China. *Chin J Prev Med*. 1996;**30**(Suppl):17–9. Chinese.
16. Kock J, Theilmann L, Galle P, Schlicht HJ. Hepatitis B virus nucleic acids associated with human peripheral blood mononuclear cells do not originate from replicating virus. *Hepatology*. 1996;**23**(3):405–13. doi: 10.1002/hep.510230303. [PubMed: 8617418].
17. Guo Z, Shi XH, Feng YL, Wang B, Feng LP, Wang SP, et al. Risk factors of HBV intrauterine transmission among HBsAg-positive pregnant women. *J Viral Hepat*. 2013;**20**(5):317–21. doi: 10.1111/jvh.12032. [PubMed: 23565613].
18. Shao ZJ, Zhang L, Xu JQ, Xu DZ, Men K, Zhang JX, et al. Mother-to-infant transmission of hepatitis B virus: a Chinese experience. *J Med Virol*. 2011;**83**(5):791–5. doi: 10.1002/jmv.22043. [PubMed: 21360547].
19. Chen Y, Sze J, He ML. HBV cccDNA in patients' sera as an indicator for HBV reactivation and an early signal of liver damage. *World J Gastroenterol*. 2004;**10**(1):82–5. Chinese. doi: 10.3748/wjg.v10.i1.82. [PubMed: 14695774].
20. Chen L, Wu C, Fan X, Gao J, Yin H, Wang T, et al. Replication and infectivity of hepatitis B virus in HBV-related glomerulonephritis. *Int J Infect Dis*. 2009;**13**(3):394–8. doi: 10.1016/j.ijid.2008.08.014. [PubMed: 19036623].
21. Yu MM, Gu XJ, Xia Y, Wang GJ, Kan NY, Wu KH. [Relationship between the expression of HBV DNA, HBV cccDNA in human ovary tissues and the HBV intrauterine infection]. *Zhonghua Liu Xing Bing Xue Za Zhi*. 2013;**34**(2):178–82. [PubMed: 23751477].
22. Coffin CS, Mulrooney-Cousins PM, van Marle G, Roberts JP, Michalak TI, Terrault NA. Hepatitis B virus quasispecies in hepatic and extrahepatic viral reservoirs in liver transplant recipients on prophylactic therapy. *Liver Transpl*. 2011;**17**(8):955–62. doi: 10.1002/lt.22312. [PubMed: 21462295].
23. Vakili Ghartavol Z, Alavian SM, Amini S, Vahabpour R, Bahramali G, Mostafavi E, et al. Prevalence of occult hepatitis B virus in plasma and peripheral blood mononuclear cell compartments of patients with chronic hepatitis C infection in tehran-iran. *Hepat Mon*. 2013;**13**(5). e10134. doi: 10.5812/hepatmon.10134. [PubMed: 23967017].
24. Caruntu FA, Molagic V. CccDNA persistence during natural evolution of chronic VHB infection. *Rom J Gastroenterol*. 2005;**14**(4):373–7. [PubMed: 16400354].
25. Badur S, Lazizi Y, Ugurlu M, Perk Y, Ilter O, Aydinli K, et al. Transplacental passage of hepatitis B virus DNA from hepatitis B e antigen-negative mothers and delayed immune response in newborns. *J Infect Dis*. 1994;**169**(3):704–6. doi: 10.1093/infdis/169.3.704. [PubMed: 8158060].
26. Abdelhamed AM, Kelley CM, Miller TG, Furman PA, Isom HC. Rebound of hepatitis B virus replication in HepG2 cells after cessation of antiviral treatment. *J Virol*. 2002;**76**(16):8148–60. [PubMed: 12134020].
27. Lin CL, Yang HC, Kao JH. Hepatitis B virus: new therapeutic perspectives. *Liver Int*. 2016;**36 Suppl 1**:85–92. doi: 10.1111/liv.13003. [PubMed: 26725903].
28. Koniger C, Wingert I, Marsmann M, Rosler C, Beck J, Nassal M. Involvement of the host DNA-repair enzyme TDP2 in formation of the covalently closed circular DNA persistence reservoir of hepatitis B viruses. *Proc Natl Acad Sci U S A*. 2014;**111**(40):E4244–53. doi: 10.1073/pnas.1409986111. [PubMed: 25201958].