厚生労働科学研究費補助金（肝炎等克服政策研究事業）
令和４年度　研究報告書
全国規模の肝炎ウイルス感染状況の把握及びウイルス性肝炎 elimination に向けた
方策の確立に資する疫学研究

カンボジアにおける 1,565 例の妊婦及びその新生児を対象とした HBV 母子感染状況に関する前向き血清疫学研究

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研究要旨

日本では、1986 年に開始した B 型肝炎母子感染防止事業（Hep B ワクチン＋HBIG）やその後の HBV 母子感染予防対策により、垂直感染による HBV 感染はほぼ制圧可能と考えられる[1]。

一方、HBV 高浸淫地域として知られているアジア・アフリカ地域の 1 国であるカンボジアでは、WHO が推奨するユニバーサルワクチネーション（Hep B ワクチンを出生 24 時間以内に初回投与、その後 6 週、10 週、14 週後に追加投与）を 2005 年から全土で導入している。なお、カンボジアでは、HBIG 投与は、母子感染予防のためにその投与を希望し個人負担によるケースはあるが、一般化されてはいない。

WHO は、妊婦の血中ウイルス量が高値（>200,000IU/ml, あるいは >10^6 copies/mL）である場合、妊婦への抗ウイルス剤投与による母子感染予防を推奨しているが、日本、カンボジアでは一般には行われていない。

本研究では、HBV 母子感染のリスクと有効な予防策を検討するため、カンボジア保健省国立母子保健センターと共同で、カンボジアの妊婦および新生児を対象とした HBV 母子感染状況に関する前向き血清疫学調査研究を実施した。

本研究は、2020 年 2 月～2021 年 12 月の期間にシェムリアップの 3 産科医療機関で妊婦検診を受けた全妊婦のうち同意を得た 1,565 人（平均年齢 28.3±5.7 歳）を対象とした【study 1】と、出生した児を対象とした【study 2】からなっている。

study-1 では、妊婦の HBsAg 検査を行った。study-2 では、HBsAg 陽性の母親全員と HBsAg 陰性の母親の 4 分の 1 から生まれた児を対象にフォローアップを行い、出産時と 6 ヵ月後に、血清または DBS (HemaSpot™) を採取した。

本研究は、広島大学疫学倫理審査委員会およびカンボジア保健省倫理審査委員会の承認を得て実施した(approval number: E-1693, 223-NECHR)。

その結果、以下の結果を得た。

1. 1,565 人の妊婦のうち、4.3%（67/1565 人）が HBsAg 陽性であった。
2. 67 人（平均年齢 29.4±5.4 歳）の HBsAg 陽性者のうち HBeAg 陽性者は 41.8%であり、HBeAg 陽性例では HBeAg 陰性例と比較して有意に HBV DNA 量が高値であった（各中央値：1.1×10^8 copies/mL、4.0×10^3 copies/mL）。
3. ウイルス量高値群（HBV DNA>200,000 IU/mL あるいは $>10^6$ copies/mL）は HBsAg 陽性妊婦の 28.4%を占めた。
4. HBV 遺伝子配列の SP 領域と S 領域の nested PCR により Genotype の判定は 61 例で可能であり、主要な Genotype は C（68.9%）と B（31.1%）であった。
5. 116 人の妊婦（HBsAg 陽性 35 人、HBsAg 陰性 81 人）とその児が、生後 6 か月のフォローアップを完遂した。
6. HBsAg 陽性の母親から生まれた 35 人の児は全員、出生 24 時間以内に Hep B ワクチンを投与され、その後追加 3 回の Hep B ワクチンを投与された。個人負担により出生時に HBIG を投与されたのは 35 人中 3 人であった。3 人のうち 1 人は、母親の妊娠中血中 HBV ウイルス量が WHO 基準よりも多く（1.2×10^9copies /mL）、2 人は同基準よりも低かった（8.6×10^3copies /mL, 2.0×10^2 copies /mL）。
7. 出産後 6 か月のフォローアップを完遂した HBsAg 陽性妊婦 35 人のうち、17 人については出産時に臍帯血を採取できた。17 人のうち、HBe 抗原陽性妊婦 8 人中 5 人、HBe 抗原陰性妊婦 9 人中 1 人、合計 6 人において、臍帯血中の HBsAg が陽性であった。ただし、そのうち 3 人は臍帯血中に HBV DNA が検出されず、検体採取時に母体血がコンタミネーションした可能性が考えられた。臍帯血中に HBV DNA が検出された 3 人のうち 2 人については、生後 6 か月時点において児血中の HBsAg は陰性で、HBV DNA は検出されなかったことから、出産時に HBV 曝露はあったものの Hep B ワクチンにより感染成立を阻止できたものと考えられた。一方、臍帯血中に HBV DNA が検出された別の 1 人については、生後 6 か月時点においても児血中 HBsAg は陽性であり、HBV DNA が検出され（1.2×10^9copies /mL）、胎内感染であった可能性が示唆された。同ケースの母親は HBeAg 陽性で、HBV ウイルス量は WHO 基準よりも多く（1.2×10^9copies /mL）、児は Hep B ワクチン（出生時＋3 回）に加えて HBIG も投与されたケースであった。HBIG については製品の品質が確保されているかどうかなど、詳細は不明である。
8. HBsAg 陽性の母親から生まれた 35 人の児のうち、生後 6 カ月に HBsAg 陽性が確認されたのは上記の 1 人のみであった（母子感染成立率：1/35, 2.9%）。
   Hep B ワクチン（出生時＋3 回）により、35 人中 34 人（97.1%）は母子感染を予防できた。
9. なお、HBsAg 陰性の母親からの児（81 人）に、生後 6 か月時点での HBV 感染例はなかった。

以上より、

妊婦 1,565 人を対象としたカンボジアとの国際共同研究により、カンボジアでは妊婦の HBsAg 陽性率が 4.3%と依然高いこと、HBsAg 陽性妊婦のうち 28.4%が HBV 母子感染の高リスク妊婦（高ウイルス量）であることを提示した。Hep B ワクチン（出生時＋3 回）により、HBsAg 陽性の母親から生まれた 35 人中 34 人（97.1%）は生後 6 ヶ月の段階では HBV 母子感染を予防したと考えられた。生後 6 ヶ月に HBV 感染が確認された 1 人については、妊婦血中ウイルス量が多く（1.2×10^9copies /mL）、臍帯血中にも HBV DNA が検出されたことから、胎内 HBV 感染であった可能性が示唆された。

1 Sugiyama A, Tanaka J et al. Epidemiological assessment of interventions to eliminate mother-to-child transmission of hepatitis B virus in Japan. *GastroHep.* 2021;3:72-79

A. 研究目的

Japan has been doing well in preventing mother-to-child transmission (MTCT) of hepatitis B virus (HBV) through a nationwide project that started in 1986 and universal vaccination against HBV since 2016. Since then, the incidence of HBV infection has significantly decreased. However, Japan has yet to implement antiviral prophylaxis treatment for pregnant women at risk of MTCT, which is recommended by the World Health Organization (WHO). The WHO recommends introducing antiviral prophylaxis for pregnant women with high viral load (>200 000 IU/mL, which equal to >106 copies/mL) or HBeAg positive, in settings where HBV DNA measurement is limited (WHO, 2020). It is essential for Japan to gather evidence on the risk and effectiveness of various interventions for preventing HBV MTCT, not only within the country but also from outside sources to tailor more effective program against HBV.

This study was developed based on the results of a nationwide study conducted previously in Cambodia in 2017 (PI: Prof. Junko Tanaka), which indicated that the prevalence of HBsAg among 5–7-year-old children was 0.56%, while that among their mothers was 4.39%, highlighting concerns for mother-to-child transmission (MTCT) of HBV (1).

The research group led by Prof. Junko Tanaka (Hiroshima University), collaborated with the National Maternal and Child Health Center (NMCHC), Ministry of Health of Cambodia, decided to conduct a study on the prevalence of HBsAg among pregnant women and the rate of HBV MTCT in Cambodia where hepatitis B vaccine (HepB) at birth and three follow up doses have been the only interventions to reduce the MTCT of HBV. As Hepatitis B Immune Globulin (HBIG) is an optional if available and affordable. The results of this study will provide insight into the risk of HBV MTCT in the setting without antiviral prophylaxis and will contribute to establishment of HBV screening system linkage to care for pregnant women in Cambodia.

B. 研究方法

1. The study aimed to investigate the mother-to-child transmission of hepatitis B virus (HBV) which required a minimum sample size of 31 HBsAg-positive mothers. Therefore, we estimated to screen 1,500 pregnant women.
2. The study consisted of two parts. In Study-1, all pregnant women who visited one of three designated hospitals in Siem Reap Province, Cambodia, were tested for HBsAg (rapid test, Abbott Determine™, USA) and collected serum samples and socio-demographic information. In Study-2, all HBsAg-positive pregnant women and one-fourth of HBsAg-negative pregnant women and their babies were invited for follow-up at delivery and 6 months after delivery. Cord blood and dried blood spot (DBS, HemaSpot™) samples were collected.
3. All collected samples were stored at -25°C until analysis. HBV serological markers were detected using chemiluminescent enzyme immunoassay (CLEIA, Lumi-pules, Fujirebio Inc., Japan).
4. Viral load was quantified using real-time polymerase chain reaction (Real Time-PCR, Thermo Fisher Scientific), and sequencing was also performed using Sanger method.
5. Univariable and multivariable logistic regression analysis was employed to identify potential risk factors for HBsAg positivity. A p-value of less than 0.05 was considered statistically significant.
6. Ethical Considerations
   This study was approved by the Epidemiological research Ethic Committee of Hiroshima University (No. E-1693) and the Cambodian National Ethic Committee for Health Research (No. 223-NECHR).

C. 研究結果

In Study-1, 1565 pregnant women were screened for HBsAg, and the prevalence of HBsAg was found to be 4.28% (67/1,565). The prevalence of anti-HBs, anti-HBc, and anti-HCV among pregnant women was 38.5%, 23.1%, and 0.51%, respectively (Fig. 1 and Fig. 2). Multivariable analysis of HBsAg positivity indicated that pregnant women who ever received the HepB vaccine had significantly decreased odds of being positive for HBsAg (AOR: 0.22, p=0.011) (Table 1).

Out of 67 HBsAg positive samples from pregnant women, HBeAg was detected in 41.8% (28/67) and had significantly high viral load than HBeAg-negative samples (p<0.0001) (Fig. 3). Genotypes were identified in 61 samples. HBV genotype C was predominant (68.9%), followed by B (31.1%) (Fig. 4).

In Study-2, 116 six-month-old infants completed the follow-up until six-month-old, 35 of whom were born to HBsAg-positive mothers. 100% and 98.3% of the follow up babies received HepB vaccine at birth and three follow up doses, respectively. Of the 35 babies born to HBsAg positive mothers, only 3 babies received HBIG.

One of these 35 infants tested positive for HBsAg, resulting in a MTCT rate of 2.86% (Fig. 5). The infected mother-child pair belonged to genotype B4, and the phylogenetic analysis revealed a 100% homology sequence in the whole genome.

The infected infant was born to a 30-year-old mother who was HBeAg positive with a high HBV viral load (1.2×10^9 copies/mL), which exceeded the cut-off recommended by the WHO for antiviral prophylaxis. The delivery occurred at 39 weeks of gestation by on-demand cesarian section. HBsAg was detected in her cord blood. The infant received HepB vaccine birth dose

and HBIG within 24 hours and completed a three-dose pentavalent vaccine. The mother of the infected child did not receive antiviral prophylaxis during her pregnancy.

D. 考察

The study found that 4.28% of pregnant women had HBsAg, which is consistent with previous findings(1). However, this number is higher than that in Japan, where the prevalence of HBsAg among pregnant women is 0.52% (2).

Our study found that a baby infected with HBV at 6 months old despite receiving timely HepB vaccination and HBIG. The mother of the infected baby had a high viral load and HBeAg positive. Furthermore, HBsAg and HBV DNA were detected in the cord blood likely acquired the infection from the mother during pregnancy which could not be prevented by vaccination. Antiviral prophylaxis treatment during pregnancy might have prevented the transmission of HBV from mother to her baby.

Therefore, although HepB vaccination and HBIG are an essential intervention for preventing HBV transmission, it is crucial to recognize that it may not be enough. This highlights the need for additional interventions, antiviral prophylaxis, to further reduce the risk of MTCT, for pregnant women have high viral loads.

E. 結論

In settings where HepB vaccination and HBIG are the only interventions, a residual risk of HBV MTCT has been observed (2.9%, 1/35). The infected baby, who had received a complete series of HepB vaccine and HBIG at birth, was born to a mother with a high viral load. Additionally, HBsAg was detected in the cord blood, indicating that the onset of infection most likely occurred during pregnancy and could not be prevented by the vaccine or HBIG. This suggests that these interventions may not provide complete protection against HBV MTCT. Therefore, in addition to HepB vaccine and HBIG, we should consider administering antiviral prophylaxis during pregnancy for pregnant women with high viral load (>10^6 copies/mL) to further reduce the risk of transmission.

F. 健康危険情報

特になし。

G. 研究発表

1．論文発表

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https://doi.org/10.1186/s12879-023-08249-1

2．学会発表

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H. 知的財産権の出願・登録状況（予定を含む。）

特になし。

Reference

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2. Tanaka J, Akita T, Ko K, Miura Y, Satake M. Countermeasures against viral hepatitis B and C in Japan: An epidemiological point of view. Hepatol Res. 2019;49(9):990-1002.

Figure 1: Study Flow Chart

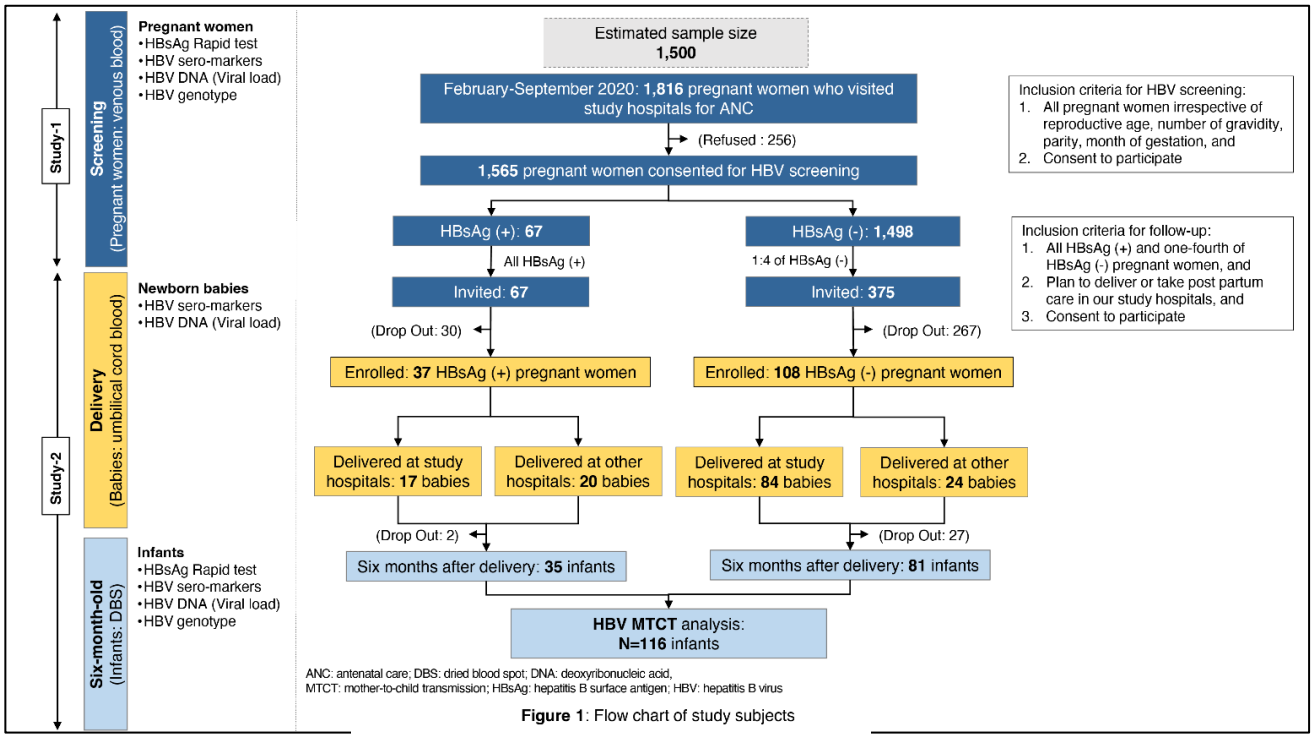

Figure 1: Flow chart of study subjects

Figure 2: Distribution of HBV and HCV sero-markers

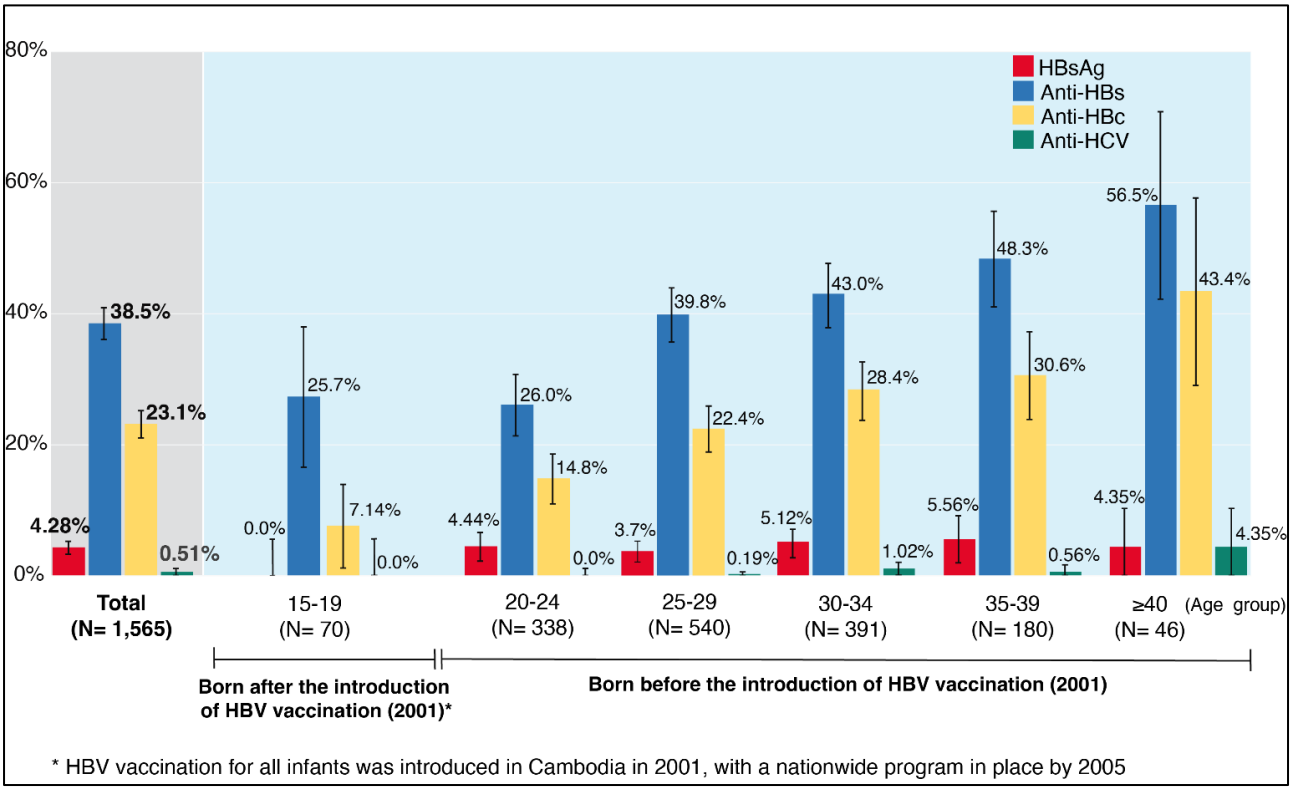

Figure 3: Distribution of HBV viral load by HBeAg

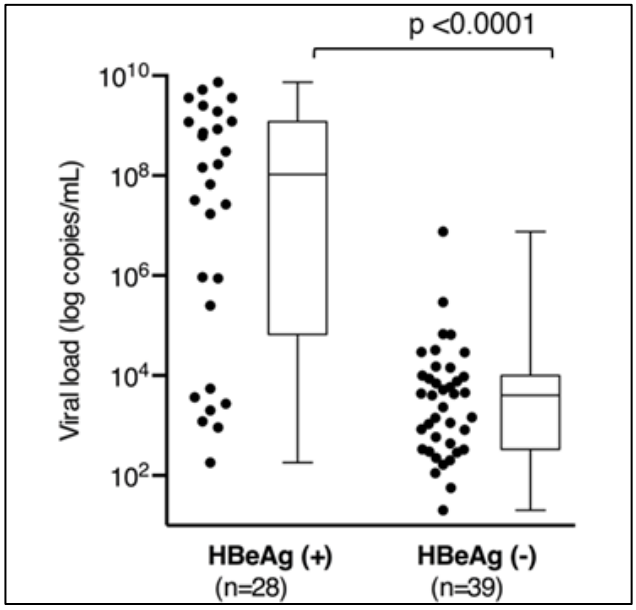

Figure 4: HBV Genotype Distribution

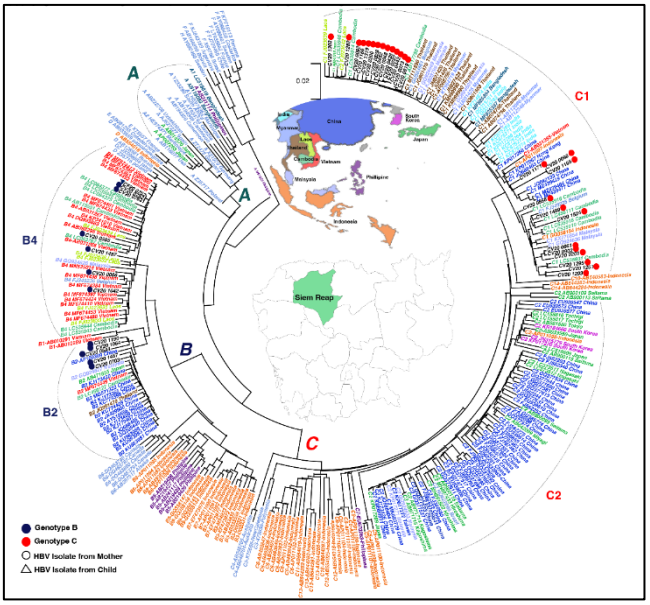

Figure 5: HBsAg status of infants at six-month-old

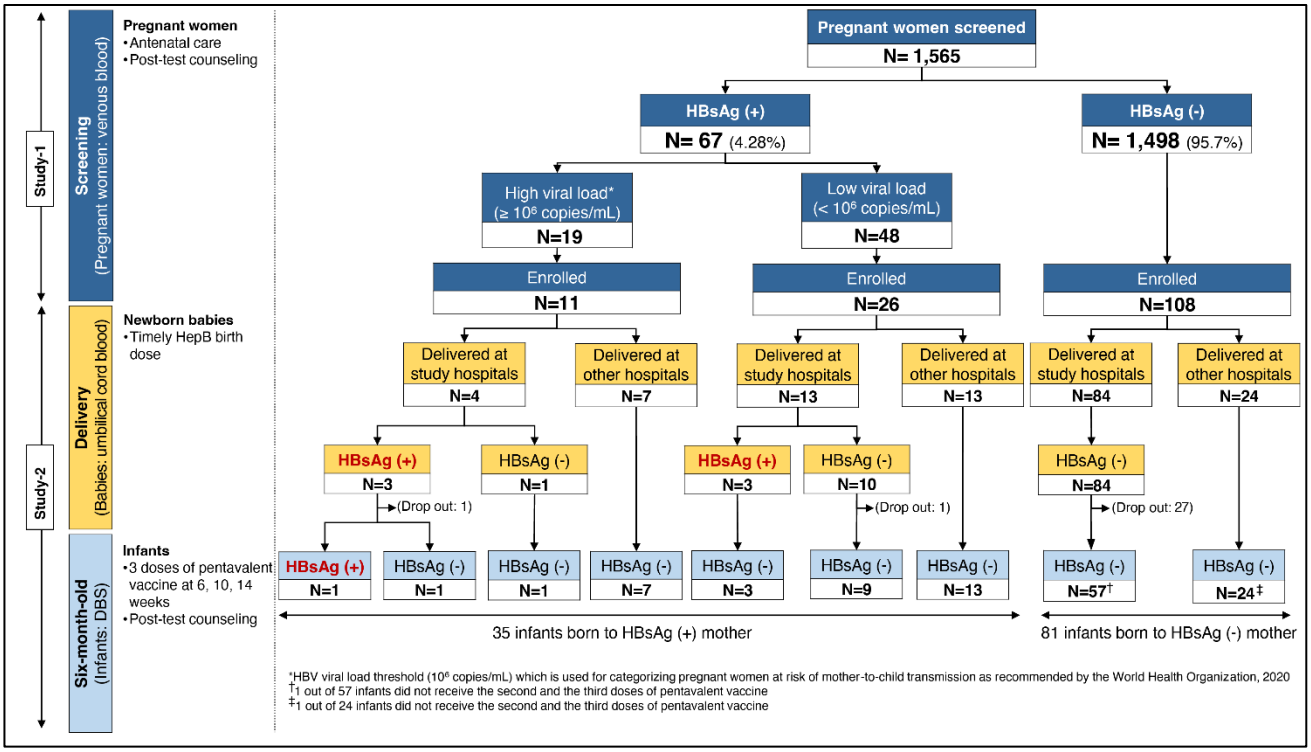

Table 1: Background characteristics and Factors associated with HBsAg positivity among pregnant women

Variables	Total (N=1565)	HBsAg (+)	Univariable analysis (N=1565)			Multivariable analysis (N=1506)		
		n (%)	OR	[95% CI]	p-value	AOR	[95% CI]	p-value
Age (mean=28.3 ± 5.7)								
15-19	70	0 (0)	0	-	-	0	-	-
20-24	338	15 (4.44)	1	(Reference)		1	(Reference)	
25-29	540	20 (3.70)	0.83	[0.42-1.64]	0.589	0.79	[0.39-1.58]	0.501
30-34	391	20 (5.12)	1.16	[0.58-2.30]	0.670	1.14	[0.57-2.28]	0.711
35-39	180	10 (5.56)	1.27	[0.55-2.88]	0.573	1.36	[0.59-3.19]	0.468
≥40	46	2 (4.35)	0.98	[0.22-4.42]	0.978	1.19	[0.25-5.61]	0.818
Education level								
≤Primary School	324	13 (4.01)	1	(Reference)	-	1	(Reference)	
High School	857	35 (4.08)	1.02	[0.53-1.95]	0.956	0.99	[0.49-1.98]	0.984
University	384	19 (4.95)	1.25	[0.61-2.56]	0.551	1.37	[0.61-3.03]	0.441
Occupation								
Farmer/Fishery/Laborer	255	6 (2.35)	1	(Reference)		1	(Reference)	
Public Officer	217	10 (4.61)	2.00	[0.72-5.61]	0.185	2.18	[0.73-6.40]	0.158
Privat Company Employee	495	26 (5.25)	2.30	[0.93-5.66]	0.070	2.37	[0.92-6.11]	0.074
Self-Employed	598	25 (4.18)	1.81	[0.73-4.47]	0.198	1.92	[0.75-4.91]	0.171
Number of children								
1-3	1469	63 (4.29)	1	(Reference)		-	-	-
≥4	96	4 (4.17)	0.97	[0.35-2.72]	0.954	-	-	-
Blood transfusion history								
No	1527	65 (4.26)	1	(Reference)		-	-	-
Yes	38	2 (5.29)	1.25	[0.29-5.30]	0.763	-	-	-
Surgical history								
No	1361	62 (4.56)	1	(Reference)		1	(Reference)	
Yes	204	5 (2.45)	0.53	[0.21-1.33]	0.173	0.5	[0.19-1.28]	0.152
Ever received HepB								
Yes	236	3 (1.27)	0.25	[0.08-0.81]	0.020	0.22	[0.06-0.72]	0.011
No	1313	64 (4.87)	1	(Reference)		1	(Reference)	
Don’t know	16	0 (0)	0	-	-	-	-	-