CHAPTER 3

Results

3.1 Characteristics of HBV/HIV co-infected pregnant women

Among 245 HBV/HIV-infected pregnant women enrolled in the previous study [24], 131 women had HBV DNA levels equal to or greater than 3.5 $\log_{10}$ IU/mL. The study population was selected as described below (Figure 3.1).

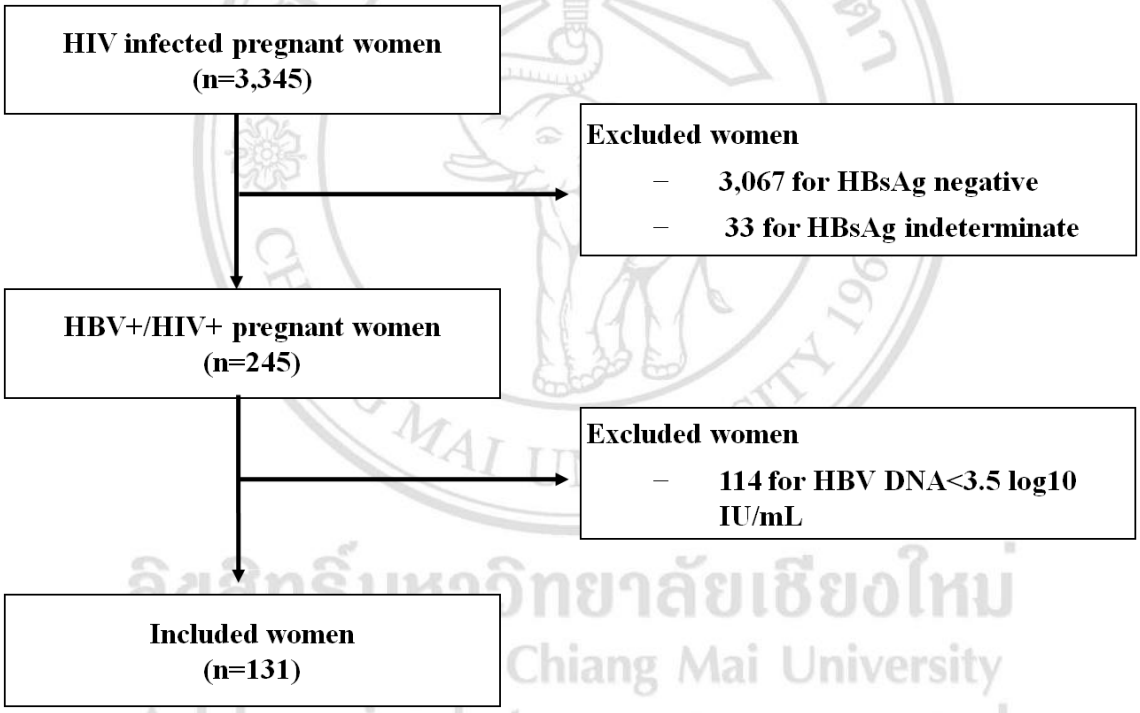

Figure 3.1 Overall study population

Baseline characteristics of 131 HBV/ HIV co-infected pregnant women with HBV DNA levels ≥3.5 log_{10} IU/ mL, are shown in table 3.1. Median age was 25 (Interquartile range; IQR: 21-28) years old. Median CD4+ and CD8+ T-cell count were 335 (IQR: 220-440) and 880 (IQR: 658-1,178) cells/ mm^3, respectively. Median serum alanine transaminase enzyme (ALT) was 19 (IQR: 13-28) IU/L. Two percent of women had antibodies against hepatitis C virus (anti-HCV). Median HIV RNA level was 4.09 (IQR: 3.35-4.66) log_{10} copies/mL and median HBV DNA level was 7.55 (IQR: 6.64-7.88) log_{10} IU/mL. A total of 114 pregnant women were positive and 17 were negative for HBeAg. (Table 3.1)

Table 3.1 Baseline demographic and clinical characteristics of HBV/HIV co-infected pregnant women

Baseline characteristics	**HBV- HIV co-infected pregnant women**		**High HBV DNA ≥3.5 log_{10} IU/mL**	
	N	**Median (IQR) or N (%)**	**N**	**Median (IQR) or N (%)**
Age at enrollment (years)	245	25 (22-29)	131	25 (21-28)
Body weight (kgs)	120	56.3 (51.5-62.0)	131	56.5 (51.3-63.0)
White blood cell count (cells/mm^3)	242	8,380 (7,000-10,000)	129	8,300 (7,100-10,000)
Absolute CD4+ T-cell (cells/mm^3)	242	342 (219-462)	129	335 (220-440)
Absolute CD8+ T-cell (cells/mm^3)	220	855 (626-1,189)	119	880 (658-1,178)
Serum ALT (IU/L)	240	17 (12-26)	129	19 (13-28)
Serum creatinine (mg/dL)	237	0.6 (0.5-0.7)	127	0.6 (0.5-0.7)
Anti-HCV antibody positive	244	4 (2)	131	2 (2)
HIV RNA load (log_{10} copies/mL)	243	3.96 (3.36-4.59)	130	4.09 (3.35-4.66)
Anti-syphilis antibody positive	240	7 (3)	128	6 (5)
HBV DNA (log_{10} IU/mL)	240	4.37 (1.83-7.63)	131	7.55 (6.64-7.88)
HBeAg positive	245	125 (51)	131	114 (87)

Note: IQR: Interquartile range; ALT: Alanine transaminase; N: Number of women

3.2 Analysis of HBV Genotypes

3.2.1 Characteristics of the study population

A total of 56 HBV/HIV co-infected pregnant women were included in the 2 two sub-studies. Baseline characteristics of these women are shown in table 3.2. Median age was 25 (IQR: 22-30) years old. Median CD4+ and CD8+ T-cell count were 354 (IQR: 228-462) and 840 (IQR: 658-1,170) cells/ mm^3, respectively. Median serum ALT was 17 (IQR: 13-26) IU/L. Two percent of women had antibodies against hepatitis C virus (anti-HCV). Median HIV RNA level was 4.09 (IQR: 3.51-4.66) log_{10} copies/mL and median HBV DNA level was 7.48 (IQR: 4.50-7.85) log_{10} IU/mL. A total of 39 pregnant women were positive and 17 were negative for HBeAg. (Table 3.2)

Table 3.2 Baseline demographic and clinical characteristics of the study population

Baseline characteristics	Study population	
	N	Median (IQR) or n (%)
Age at enrollment (years)	56	25 (22-30)
Body weight (kgs)	55	55.7 (51.0-61.2)
White blood cell count (cells/mm^3)	55	8,700 (6,900-10,2000)
Absolute CD4+ T-cell (cells/mm^3)	55	354 (228-462)
Absolute CD8+ T-cell (cells/mm^3)	51	840 (658-1,170)
Serum ALT (IU/L)	55	17 (13-26)
Serum creatinine (mg/dL)	54	0.7 (0.5-0.8)
Anti-HCV antibody positive	56	1 (2)
HIV RNA load (log_{10} copies/mL)	54	4.09 (3.51-4.66)
Anti-syphilis antibody positive	55	2 (4)
HBV DNA (log_{10} IU/mL)	56	7.48 (4.50-7.85)
HBeAg positive	56	39 (70)

Note: IQR: Interquartile range; ALT: Alanine transaminase; N: Number of women

3.2.2 HBV Genotyping

Of 56 samples, 44 had full-length genome successfully amplified and sequenced. For 5 samples (patient ID 0324, 0523, 0900, 1032 and 1278), only the surface region sequences were obtained. The full-length HBV genome or surface region cannot be amplified in 7 samples (patient ID 0270, 0470, 0729, 1448, 3804, 3853, and 3974) by the PCR method used in this study.

The neighbor-joining phylogenetic tree analysis was constructed based on full-length HBV reference sequences (genotype A–J) along with 44 samples (Figure 3.2). Reference sequences were retrieved from GenBank database. For sample with only the *surface* (*S*) region available, the phylogenetic tree was constructed based on *S* region of HBV reference sequences (genotype A–J) along with 49 samples (Figure 3.3). The full-length HBV genome and *S* region reproduce the same phylogenetic analysis results HBV genotypes C (n=44, 90%), B (n=4, 8%) and G (n=1, 2%).

In addition, HBV genotype was further confirmed by two web-based tools: 1) the HIV-GRADE HBV tool and 2) the NCBI Genotyping tool. The web-based HBV genotyping confirmed the genotypic result obtained from the phylogenetic tree analysis of the full-length HBV genome/*S* region. Interestingly, phylogenetic tree analysis and the HIV-GRADE HBV tool identified HBV ID 0725 as a genotype G whereas NCBI blast demonstrated that this patient had a recombination of genotype G and C (Figure 3.4).

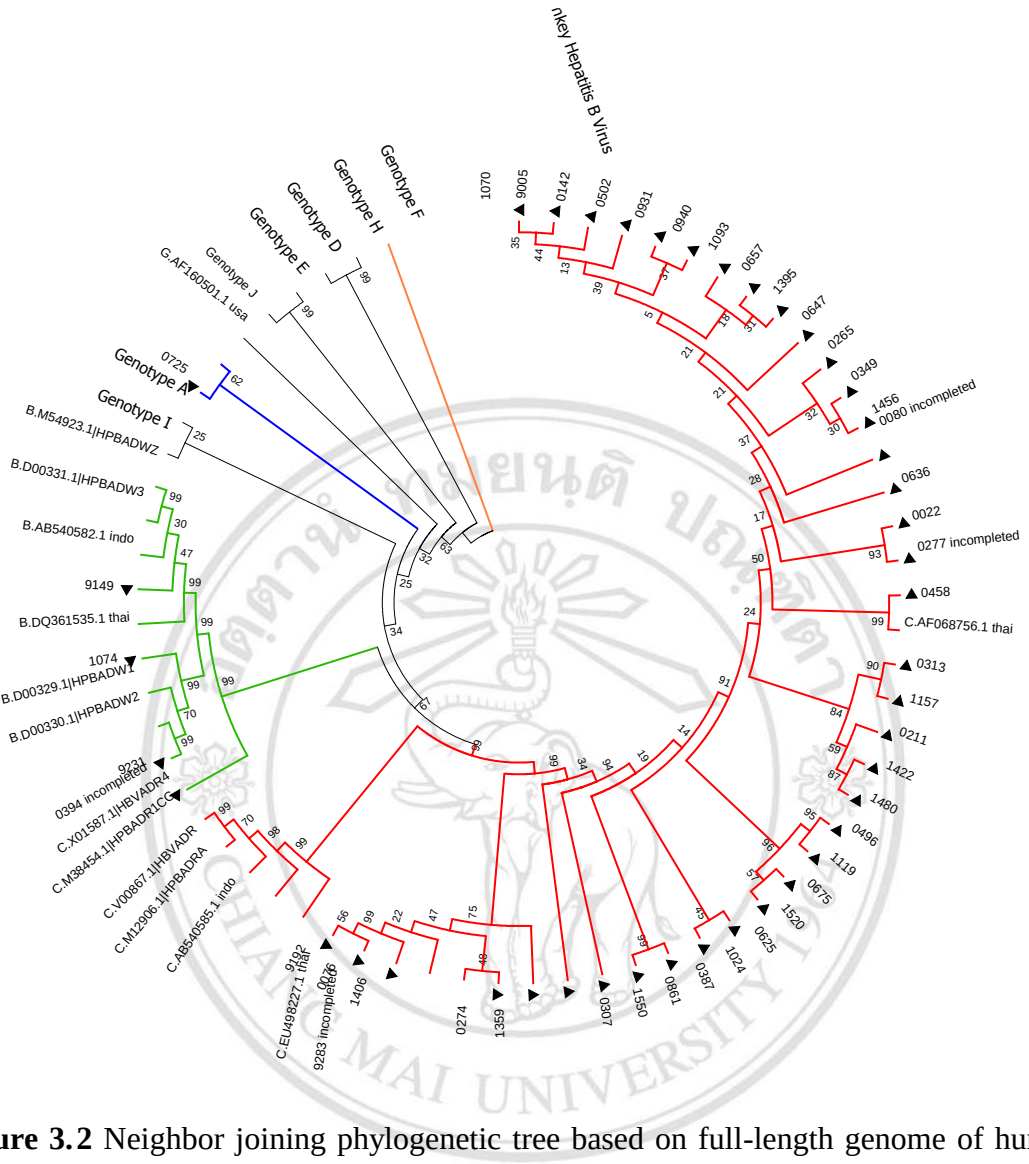

Figure 3.2 Neighbor joining phylogenetic tree based on full-length genome of human HBV with 37 reference sequences derived from GenBank and 44 HBV isolates from HIV-1 co-infected pregnant women. The sample sequences are identified with the symbol▲, followed by the sample number. Green line branch indicated genotype B. Red line branch indicated C. Blue line branch indicated G. The reference sequences are identified by genotype followed, by the accession numbers. Woolly monkey Hepatitis B virus (WMHBV) was utilized as an out-group (orange line). Bootstrap values (%) are shown at the nodes.

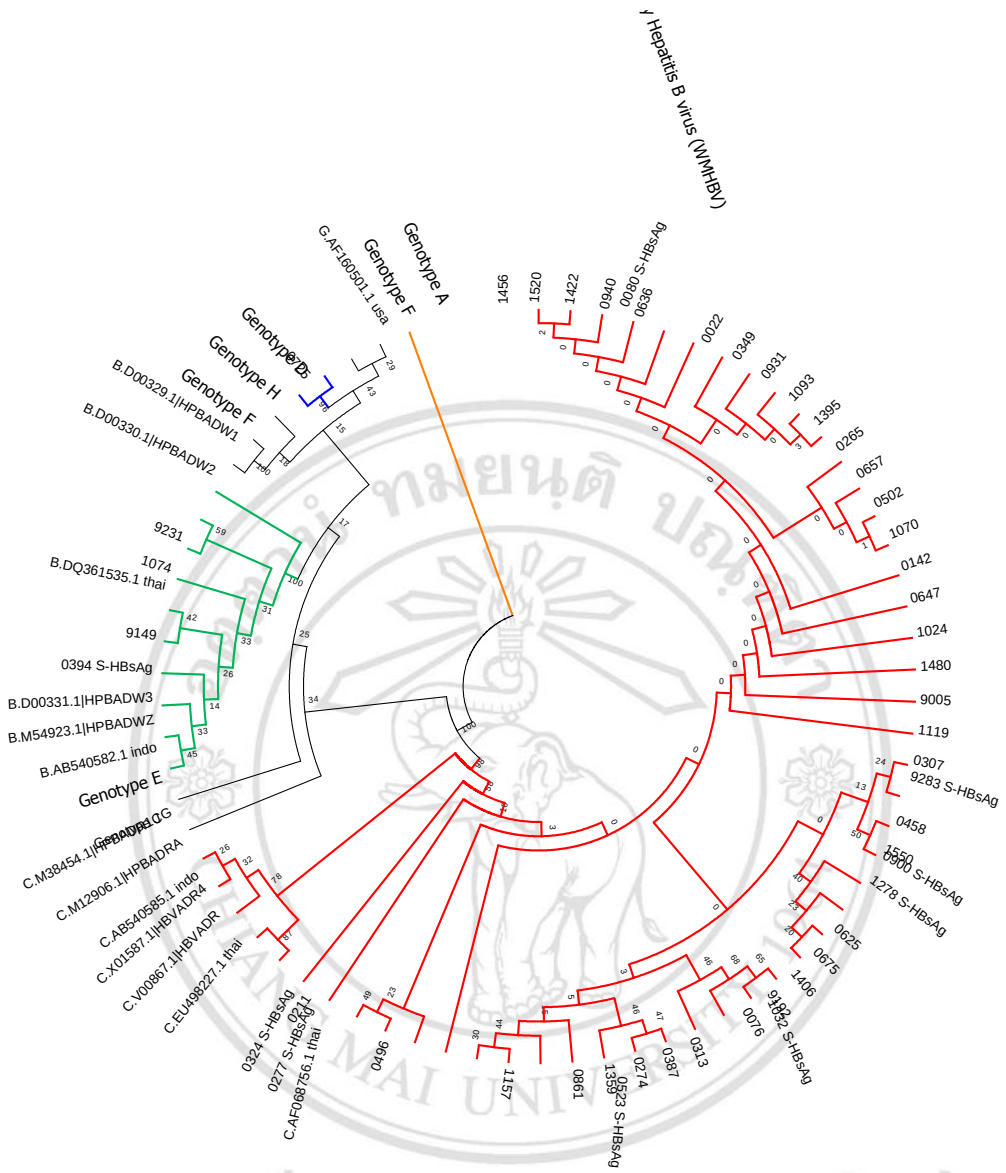

Figure 3.3 Neighbor joining phylogenetic tree based on surface region of human HBV with 37 reference sequences derived from GenBank and 49 HBV isolates from HIV-1 co-infected pregnant women. The sample sequences are identified with the symbol▲, followed by the sample number. Green line branch indicated genotype B. Red line branch indicated C. Blue line branch indicated G. The reference sequences are identified by genotype followed, by the accession numbers. Woolly monkey Hepatitis B virus (WMHBV) was utilized as an out-group. Bootstrap values (%) are shown at the nodes.

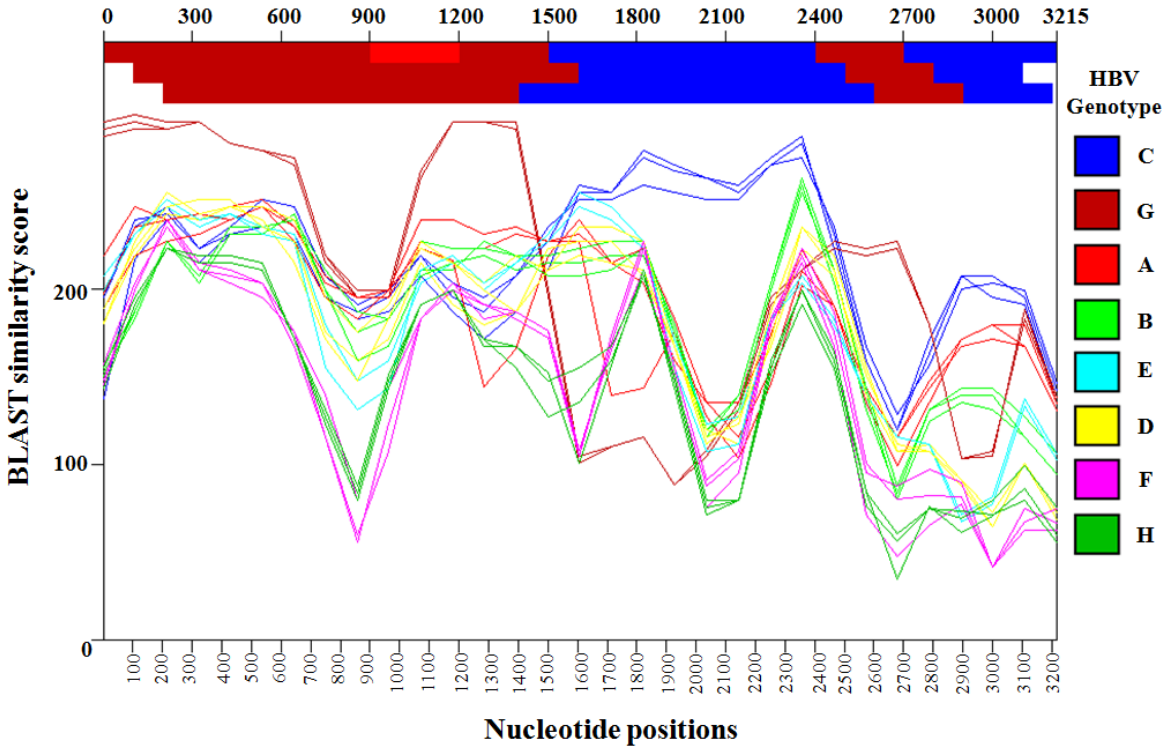

Figure 3.4 HBV genotypic identification using NCBI Genotyping tool. The y-axis on the graph shows BLAST similarity scores for the alignments between the sample sequence and HBV reference and x-axis shows genome nucleotide positions. The dominant red and blue color of the horizontal top bar suggests recombinant genotype G/C for this HBV sequence.

3.3 Sub-study 1: Analysis of relationship between HBV genetic diversity and perinatal HBV transmission

3.3.1 Characteristics of HBV transmitting- and HBV non-transmitting pregnant women

Among 131 pregnant women, 9 women had HBV infected infants, so called "HBV transmitting pregnant women" (TM) [24] and women who did not transmit HBV to their offspring, so called "HBV non-transmitting pregnant women" (NTM)

Transmitting pregnant women were matched to non-transmitting pregnant women on a ratio of 1: 2 according to their baseline HBV DNA levels and HBeAg status. Those 27 women had median age of 27 (IQR: 22-30) years and median HBV DNA was 7.8 (IQR: 6.3-7.9) $\log_{10}$ IU/mL. Their baseline characteristics between both groups were not different, except a greater body weight found in non-transmitting women (Table 3.3).

Table 3.3 Baseline characteristics of HBV transmitting- and HBV non-transmitting pregnant women

Baseline characteristics	Overall		TM		NTM		[a]*p*-value
	N	Median (IQR) or N (%)	N	Median (IQR) or N (%)	N	Median (IQR) or N (%)	
Age at enrollment; years	27	27 (22-30)	9	23(22-28)	18	28 (24-31)	0.14
Body weight; kgs	26	56.0 (51.3-61.2)	8	49.8 (46.5-56.3)	18	58.3 (54.0-63.5)	**0.02**
White blood cell count; cells/mm^3	26	7,970 (6,900-9,300)	8	7,650 (6,850-8,720)	18	8,850 (6,900-10,900)	0.32
Absolute CD4+ T-cell; cells/mm^3	27	353 (228-462)	9	276 (228-449)	18	377 (228-500)	0.63
Absolute CD8+ T-cell; cells/mm^3	25	840 (670-1,170)	8	870 (610-1,173)	17	840 (682-1,170)	0.64
Serum ALT; IU/L	26	19 (13-38)	8	25 (15-36)	18	17 (12-38)	0.37
Serum creatinine; mg/dL	26	0.7 (0.5-0.8)	8	0.7 (0.5-0.9)	18	0.7 (0.5-0.8)	0.84
Anti-HCV antibody positive	27	1 (4)	9	0 (0)	18	1 (6)	1.00
HIV RNA load; log_{10} copies/mL	25	4.1 (3.5-4.7)	8	4.2 (3.8-4.9)	17	4.0 (3.4-4.5)	0.41
Anti-syphilis antibody positive	27	2 (7)	9	1 (11)	18	1 (6)	1.00
HBV DNA; log_{10} IU/mL	27	7.8 (6.3-7.9)	9	7.8 (6.5-7.9)	18	7.8 (6.3-7.9)	0.88
HBeAg positive	27	24 (89)	9	8 (89)	18	16 (89)	1.00
HBV Genotype B:C	26	2:24 (8:92)	9	2:7 (22:78)	17	0:17 (0:100)	0.11

Note: IQR: Interquartile range; TM: HBV transmitting pregnant women; NTM: HBV non-transmitting pregnant women; ALT: Alanine transaminase; [a]Fisher's exact test or Wilcoxon rank-sum test were used

3.3.2 Comparison of HBV genetic diversity between HBV transmitting pregnant women and non-transmitting pregnant women

To assess the relationship between HBV genetic diversity and perinatal HBV transmission, the nucleotide and amino acid diversity were analyzed and compared between transmitting group and non-transmitting group using three parameters: 1) Shannon entropy score, 2) the mean genetic distance, 3) the number of synonymous substitutions per synonymous site (dS) and the number of non-synonymous substitutions per non-synonymous site (dN). Of 27 samples, 9 transmitting women and 17 non-transmitting women had full-length HBV genome successfully amplified and sequenced. (Figure 3.5)

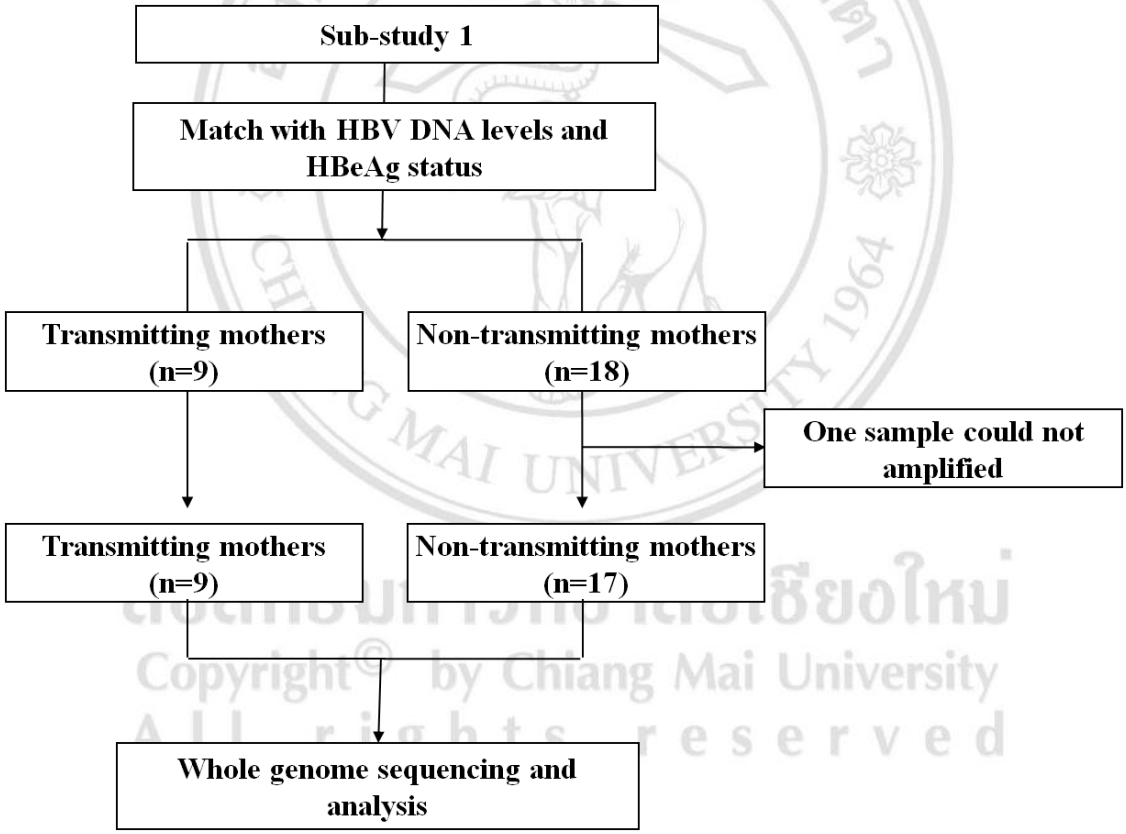

Figure 3.5 Schematic diagram of sub-study 1

1) Comparison of Shannon entropy at single nucleotide position and amino acid levels between HBV transmitting pregnant women and non-transmitting pregnant women

The Shannon entropy score of each nucleotide position was determined in transmitting group (n=9) and non-transmitting group (n=17). The entropy score of each position along the full-length HBV genome and 4 ORFs are shown in Figure 3.6. In *pol* region overlapped with the entire *preS/S* region (nucleotide 1035-2237) , especially *preS1* at nucleotide 1145-1256, the entropy score of transmitting group was higher than non-transmitting group. This result indicates that an increased divergence of *preS1* region may associate to HBV perinatal transmission in transmitting group. The *preS1* region encodes the preS1 surface protein which is an immunogenic epitope targeting of T and B cell. Conversely, the entropy score of non-transmitting group tended to be higher in X region overlapping with *pol* region at nucleotide 2686-2822.

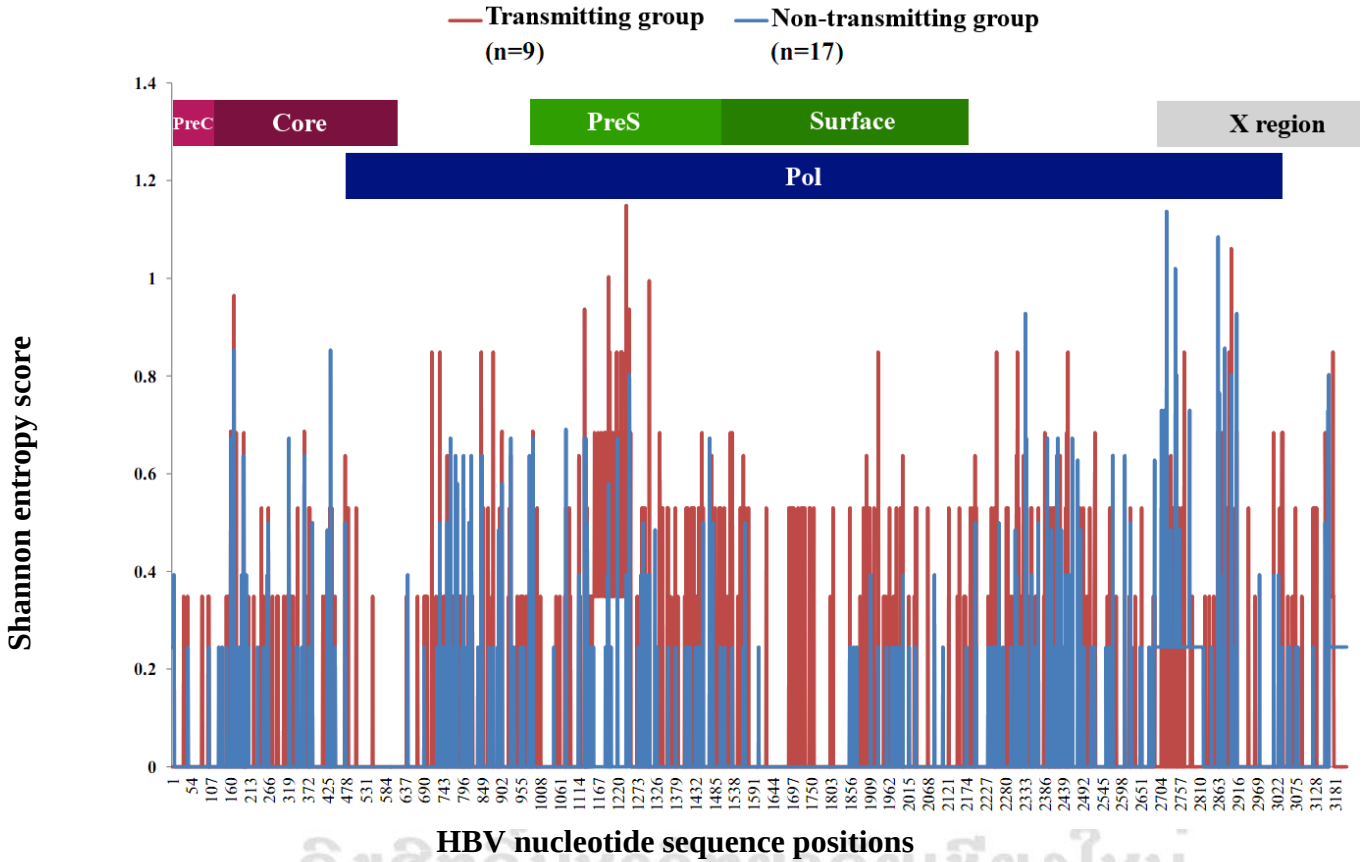

Figure 3.6 Comparison of nucleotide diversity in transmitting group and non-transmitting groups using Shannon entropy. The HBV genome sequence positions are numbered on the x-axis and four ORFs of HBV are shown at the top. The y-axis reports the Shannon entropy scores. The red line on the graph represented the scores of transmitting group, whereas the blue line was non-transmitting group in nucleotide position.

The average overall entropy score was higher in transmitting group than non-transmitting group in almost regions. By region, transmitting group had high diversity in *pol* and *preS/S* region. Shannon entropy score was mostly correlated at both nucleotide and amino acid levels (Table 3.4). These results suggest that more viral adaptation in transmitting group compared to non-transmitting group.

Table 3.4 Shannon entropy score of transmitting and non-transmitting groups

Shannon entropy	**Transmitting group (n=9) Mean (95% CI)**	**Non-transmitting group (n=17) Mean (95% CI)**	**[a]*p*-value**
Nucleotide level			
- Whole genome	0.067 (0.061-0.073)	0.043 (0.039-0.047)	**<0.0001**
- Pol	0.071 (0.064-0-078)	0.042 (0.037-0.047)	**<0.0001**
- PreC/C	0.033 (0.024-0.043)	0.023 (0.015-0.031)	**0.01**
- PreS/S	0.085 (0.074-0.096)	0.019 (0.014-0.024)	**<0.0001**
- X	0.061 (0.046-0.077)	0.097 (0.082-0.112)	**0.001**
Amino acid level			
- Pol	0.084 (0.070-0.098)	0.048 (0.039-0.056)	**<0.0001**
- PreC/C	0.014 (0.003-0.024)	0.016 (0.005-0.027)	0.78
- PreS/S	0.104 (0.083-0.125)	0.022 (0.012-0.031)	**<0.0001**
- X	0.102 (0.067-0.136)	0.125 (0.092-0.158)	0.39

Note: [a]Fisher's exact test or Wilcoxon rank-sum test were used

2) Comparison of mean genetic distances between HBV transmitting pregnant women and non-transmitting pregnant women

The mean genetic distances (GD) among HBV transmitting pregnant women and non-transmitting pregnant women were calculated by Pairwise comparison of nucleotide sequences using the Kimura 2-parameter model [152] (Table 3.5, 3.6 and 3.7). The mean GD values were highest in sample ID 9149 (0.098) and 0394 (0.073) (sequences identified as genotype B) than the other samples (genotype C) (Table 3.5). Interestingly, the mean intra-genotype genetic distance of genotypes B was seem higher than genotype C, as shown in the table 3.5

Table 3.5 The genetic distances (GD) of whole genome in transmitting group

Patient ID	Genotype	0625	0387	0022	1395	0349	0657	9149	1550	0394
0625	C									
0387	C	0.017								
0022	C	0.014	0.014							
1395	C	0.013	0.013	0.005						
0349	C	0.017	0.016	0.008	0.007					
0657	C	0.013	0.012	0.004	0.002	0.006				
9149	B	0.084	0.082	0.081	0.079	0.082	0.080			
1550	C	0.020	0.020	0.018	0.017	0.020	0.017	0.084		
0394	B	0.067	0.067	0.064	0.063	0.064	0.062	0.051	0.071	
	Mean GD	0.025	0.025	0.019	0.019	0.021	0.018	0.098	0.027	0.072

Note: ID: Identification Number; GD values of HBV genotype B were underlined

Table 3.6 The genetic distances (GD) of whole genome in non-transmitting group

Patient ID	Genotype	0496	1359	0675	1520	1406	9005	0142	0274	1157	9192	0307	1093	1480	0080	9283
0496	C															
1359	C	0.028														
0675	C	0.013	0.027													
1520	C	0.010	0.025	0.009												
1406	C	0.023	0.017	0.021	0.020											
9005	C	0.013	0.022	0.011	0.009	0.018										
0142	C	0.012	0.021	0.012	0.009	0.019	0.003									
0274	C	0.026	0.020	0.024	0.022	0.015	0.019	0.019								
1157	C	0.015	0.025	0.013	0.012	0.020	0.009	0.009	0.021							
9192	C	0.024	0.020	0.024	0.021	0.006	0.020	0.020	0.017	0.022						
0307	C	0.025	0.022	0.022	0.021	0.019	0.018	0.019	0.021	0.021	0.020					
1093	C	0.014	0.024	0.014	0.011	0.020	0.006	0.006	0.022	0.011	0.021	0.021				
1480	C	0.014	0.024	0.012	0.011	0.018	0.008	0.008	0.019	0.007	0.020	0.020	0.010			
0080	C	0.012	0.023	0.011	0.009	0.019	0.005	0.005	0.019	0.009	0.020	0.019	0.007	0.007		
9283	C	0.027	0.021	0.027	0.025	0.016	0.022	0.022	0.016	0.023	0.018	0.022	0.024	0.022	0.022	
	Mean GD	0.018	0.023	0.017	0.015	0.018	0.013	0.013	0.020	0.016	0.020	0.021	0.015	0.014	0.013	0.022

Note: ID: Patient Identification Number

Table 3.7 Mean genetic distance for each HBV genetic region of transmitting and non-transmitting groups

Genetic distance	Transmitting group (n=9) Mean (95% CI)	Non-transmitting group (n=17) Mean (95% CI)	[a]*p*-value
Whole genome	0.038 (0.023-0.052)	0.017 (0.015-0.019)	**0.0005**
Pol	0.036 (0.014-0.058)	0.024 (0.022-0.027)	0.08
PreC/C	0.020 (0.010-0.029)	0.014 (0.013-0.016)	0.09
PreS/S	0.034 (0.009-0.059)	0.020 (0.018-0.022)	0.06
X	0.028 (0.012-0.043)	0.021 (0.019-0.023)	0.21

Note: [a]Fisher's exact test or Wilcoxon rank-sum test were used

3) Comparison of dN/dS ratio between HBV transmitting pregnant women and non-transmitting pregnant women

Genetic diversity was also determined by the number of synonymous substitutions per synonymous site (dS) and the number of non-synonymous substitutions per non-synonymous site (dN) using the Tamura-Nei model [154] as implemented in MEGA6 software[153]. The ratio between synonymous (dS) and non-synonymous (dN) mutations of each ORFs (dN/dS) were calculated. For all regions, the dN/dS were smaller than 1 (Table 3.8), indicating that there is no positive selection and all regions are highly conserved. There is no statistically significant difference of the dN/dS ratio between two groups.

Table 3. 8 The dN/ dS ratio for each HBV genetic region of transmitting and non-transmitting groups

dN/dS	Transmitting group (n=9) Mean (95% CI)	Non-transmitting group (n=17) Mean (95% CI)	[a]*p*-value
Pol	0.080 (0.043-0.116)	0.065 (0.030-0.099)	0.57
PreC/C	0.048 (0.000-0.096)	0.067 (0.019-0.152)	0.68
PreS/S	0.141 (0.041-0.242)	0.071 (0.027-0.170)	0.36
X	0.243 (0.016-0.470)	0.042 (0.000-0.134)	0.13

Note: [a]Fisher's exact test or Wilcoxon rank-sum test were used

In order to exclude the effect of inter-genotype diversity, the genetic diversity of only HBV genotype C was then investigated (Table 3.9). At nucleotide level, average overall entropy scores were significant higher in non-transmitting group than transmitting group. By region, non-transmitting group had high diversity in *pol*, *preS/S* and X region. Shannon entropy score was correlated at both nucleotide and amino acid levels, except entropy score of pol region. These results indicated that HBV in non-transmitting group had higher diversity than those transmitting group, which were discordant to the earlier results. Non-transmitting group had also higher mean genetic distance than transmitting group. Whereas dN/dS ratio was not different between the two groups and this was concordant with the earlier results.

Table 3.9 Genetic diversity of HBV genotype C in transmitting and non-transmitting groups

Genetic diversity Parameter	ORFs	Transmitting group (n=7)	Non-transmitting group (n=17)	[a]*p*-value
		Mean (95% CI)	Mean (95% CI)	
1) Shannon entropy				
- Nucleotide level	Whole genome	0.020 (0.017-0.024)	0.043 (0.039-0.047)	**<0.0001**
	Pol	0.056 (0.050-0.062)	0.042 (0.037-0.047)	**0.0005**
	PreC/C	0.015 (0.008-0.022)	0.023 (0.015-0.031)	0.14
	PreS/S	0.012 (0.008-0.017)	0.019 (0.014-0.024)	**0.03**
	X	0.033 (0.022-0.044)	0.097 (0.082-0.112)	**<0.0001**
- Amino acid level	Pol	0.063 (0.051-0.074)	0.048 (0.039-0.056)	**0.039**
	PreC/C	0.002 (0.000-0.006)	0.016 (0.005-0.027)	**0.019**
	PreS/S	0.011 (0.004-0.019)	0.022 (0.012-0.031)	0.10
	X	0.055 (0.031-0.079)	0.125 (0.092-0.158)	**0.0008**
2) Genetic distance	Whole genome	0.013 (0.010-0.016)	0.017 (0.015-0.019)	**0.014**
	Pol	0.022 (0.019-0.025)	0.024 (0.022-0.027)	0.25
	PreC/C	0.018 (0.009-0.027)	0.014 (0.013-0.016)	0.23
	PreS/S	0.018 (0.015-0.021)	0.020 (0.018-0.022)	0.28
	X	0.018 (0.015-0.020)	0.021 (0.019-0.023)	0.05
3) dN/dS	Pol	0.041 (0.005-0.076)	0.065 (0.030-0.099)	0.36
	PreC/C	0.000 (0.000-0.000)	0.067 (0.000-0.152)	0.21
	PreS/S	0.000 (0.000-0.000)	0.071 (0.000-0.170)	0.29
	X	0.000 (0.000-0.000)	0.042 (0.000-0.134)	0.65

Note: [a]Fisher's exact test or Wilcoxon rank-sum test were used

3.3.3 Analysis of mutations of HBV transmitting- and HBV non-transmitting pregnant women

Mutations of the 4 coding regions along the full-length HBV genome were analyzed in 24 sequences. Two samples were amplified just only in surface region. Of these 26 sequences, 9 sequences were derived from transmitting women and 17 sequences from non-transmitting women

1) Mutations in BCP, PC and core region of transmitting and non-transmitting groups

Nucleotide substitutions of basal core promoter (BCP), precore (PC) and core regions of HBV are shown in Table 3.10.

In BCP region, the mutation at nucleotide 1762 from adenine (A) to thymine (T) together with mutation at nucleotide 1764 from guanine (G) to adenine (A) mutations (A1762T/G1764A) was detected in three non-transmitting pregnant women. Of these three, two women were HBeAg-negative and one was HBeAg positive. The G1764A mutation alone was also found in one non-transmitting mother with HBeAg positive.

In the case of mutations in precore region, the G1896A mutation was only found in one case of transmitting mother HBeAg positive. High frequency of nucleotide substitution was found at the following nucleotide position: G1975T (n=10, 38%), G1984A/T (n=11, 42%), G2011A/C (n=7, 27%), C2134T (n=8, 31%) C2176T (n=9, 35%), A2248G/T (n=7, 27%) and C2288A (n=6, 23%). However, there was one sequence (ID 1395) from transmitting group showed no mutation, as compared to consensus sequences.

Table 3.10 Nucleotide substitutions in BCP, PC and core region of transmitting and non-transmitting groups

Genotype	Patient ID	Nucleotide substitutions in BCP, PC and core region
Transmitting group		
C	0022	G1915A, G2146A
C	0349	T1963C, C2078T, G2260A
C	0387	G1984A, C2009T, G2011A, C2078T, C2176T, C2246T, T2318C
C	0625	C1969T, G1975T, G1984A, C2009T, C2176T, T2227C
C	0657	A2234C
C	1395	None
C	1550	G1975T, G1984T, C2009T, G2017A, A2137C, C2176T, A2185G, C2191T, C2246T, C2288A
B	0394	C1857T, G1897A, G1984A, C1990G, A2038G, T2089A, A2120G, C2134T, C2136A, C2176T, C2198T, A2248T, T2287C
B	9149	A1846T, G1984A, **G1896A**, C1913G, C1990T, G2011C, C2023A, A2065C, C2102T, G2104A, G2128A, C2134T, A2140T, C2191T, T2318C, T2363A
Non-transmitting group		
C	0080	A1763G, A2075G
C	0142	**A1762T/G1764A**
C	0274	G1915A, T1963G, G1975T, G1984A, C1990A, G2011A, C2134T, A2248G, G2260C
C	0307	T1858C, G1975T, G1984T, C2176T, A2248T
C	0496	**A1762T/ G1764A**, C1999T, C2078T, A2159G, A2189C, C2198T, A2239C, T2242A, C2288A
C	0675	C2078T, C2198T, C2289T
C	1032	N/A
C	1093	**A1762T/G1764A**, C2049A, A2239C, C2288A
C	1157	T2005A, G2017A, A2065C, C2134T, C2246T
C	1278	N/A
C	1359	G1942A, G1975T, G1984A, G2011A, C2023A, T2170C, C2176T, C2191T, A2248T
C	1406	G1975T, G1984A, G2011A, C2134T, C2176T, A2248T
C	1480	C2002T, T2005A, G2017A, C2134T
C	1520	A2075G, C2078T, C2198T
C	9005	G1764A, A2155C
C	9192	G1975T, G1984A, G2011A, C2134T, C2176T, A2248T
C	9283	T1951G, G1975T, G1984A, G2011A, C2134T, A2137C, C2176T, A2248T, C2288A

Note: Letters in bold denote well-known mutations related to HBeAg production N/A: Not available due to unsuccessful amplification of region of interest

2) Mutations in preS/S region of transmitting and non-transmitting groups

All nucleotide sequences of *preS/S* gene were translated into the preS/S open reading frame to analyze the presence of amino acid variants. The analysis showed that the highest number of amino acid variations was found in the preS1 region (Table 3.11). The number of mutations was high at amino acid position G27D/S (n=6, 23%), G35R (n=15, 19%), and S73N/G (n=13, 50%) in preS1 region and T5A/S (n=4, 15%) in the surface region.

Several variants in "a" determinants were detected in this study including I126T, T131N, M133T, F134L, K141N, S143T and D144A. Amino acid variation could be not detected in samples of three transmitting pregnant women (i.e. patient ID 0022, 0657 and 1395). (Table 3.11)

Table 3.11 Amino acid mutations in preS/S region of transmitting and non-transmitting groups

Genotype	Patient ID	preS1	preS2	S
Transmitting group				
C	0022	None	None	None
C	0349	G27D, G35R, S73N	None	None
C	0387	S73N	None	S3N
C	0625	S38Q, S62A, S73N, I84T	S124T	None
C	0657	None	None	None
C	1395	None	None	None
C	1550	G27D, S101T	None	None
B	0394	I84M, S101V	R48T	S154L, A159V, M198I
B	9149	Q10K, G27D, G35K, N39D, F45L, N48H, Q51N, A54D, A55S, Q57K, S62A, S73G	H9L, R48K, A53V	L110I, K122R, I126T, T131N, M133T, T140I
Non-transmitting group				
C	0080	G27S, S73N	None	None
C	0142	W4C	None	None
C	0274	S73G, I84T, T97A	P41H	S3N
C	0307	G27D, S38L, N56H, S73I	None	None
C	0496	None	None	**K141N**, G185E
C	0675	G35R, Q51H, S73N	S6N, R48K	A17E
C	1032	N/A	N/A	T5A
C	1093	None	Q10K	**I126T**

Table 3.11 (Continued)

Genotype	Patient ID	preS1	preS2	S
C	1157	S73N	None	None
C	1278	N/A	N/A	None
C	1359	S73G	None	T5A
C	1406	G35R, S73G	None	T5A
C	1480	S73N, T97S	None	None
C	1520	None	P15S	None
C	9005	G27D, G35R	None	None
C	9192	None	None	T5A
C	9283	S73G, I84T	None	None

Note: Letters in bold denote mutation in the "*a*" determinant of HBsAg (amino acid position 124 to 147), which is associated with vaccine/immunoglobulin escape; N/A: Not available due to unsuccessful amplification of the region of interest

3) Mutations in reverse transcriptase region of transmitting and non-transmitting groups

Antiviral drug resistant variants are localized in the reverse transcriptase (RT) region of the HBV polymerase gene. Mutations were observed in 3.11 samples while 9 samples show no mutation (Table 3.12). High frequency of amino acid substitutions was found at position rtH13R/Y/L (n=6, 23%), rtS223A (n=5, 19%), and rtL269I (n= 4, 15%) of RT region. Mutation detected in "a" determinant region of HBsAg caused mutation in overlapping RT region, sT131N to rtN139K, was observed in one sequence from transmitting group (patient ID 9149). No well-known antiviral drug resistant-mutation was detected in this study.

Table 3.12 Amino acid mutations in reverse transcriptase of *pol* region of transmitting and non-transmitting groups

Genotype	Patient ID	Amino acid mutations in reverse transcriptase
Transmitting group		
C	0022	None
C	0349	None
C	0387	None
C	0625	rtS223A, rtQ267L
C	0657	None

Table 3.12 (Continued)

Genotype	Patient ID	Amino acid mutations in reverse transcriptase
C	1395	None
C	1550	rtY124H
B	0394	rtE1D, rtL91I, rtV207M, rtS256G, rtI278T, rtC332T
B	9149	rtT7A, rtT118N, rtN139K, rtV214I, rtI278V, rtC332N
Non-transmitting group		
C	0080	None
C	0142	None
C	0274	rtL269I
C	0307	rtH13Y, rtS223A, rtI224V, rtN238T, rtQ267H, rtL269I, rtQ319R,
C	0496	rtT150P, rtR153W, rtH156Y, rtI163F, rtA317S
C	0675	rtS223A, rtI278V, rtQ319L
C	1032	rtH13R, rtA97S, rtK149Q
C	1093	rtV112A, rtN238H
C	1157	None
C	1278	rtS223A
C	1359	rtH13R, rtS223A, rtV291A, rtM309L
C	1406	rtH13R, rtT128A, rtA186S, rtL269I, rtA329T
C	1480	None
C	1520	rtQ316H, rtC332R
C	9005	rtQ316H
C	9192	rtH13R, rtP109S, rtT128A, rtV142A, rtA186S, rtQ215S, rtL269I
C	9283	rtH13Y, rtL115V, rtK241R, rtI278T

Note: N/A: Not available due to unsuccessful amplification of region of interest

4) Mutations in X region of transmitting and non-transmitting groups

Of 154 amino acids of X region, mutations were observed in 30 amino acids. Mutation frequencies in the X region were high at position I30L/V, S42A/P and T47A/ P. Double mutations K130M, V131I of X region overlapped with double mutations in BCP at nucleotides 1,762 and 1,764 were observed in three sequences from non-transmitting groups. (Table 3.13)

Table 3. 13 Amino acid mutations in X region of transmitting and non-transmitting groups

Genotype	Patient ID	Amino acid mutations in X region
Transmitting group		
C	0022	None
C	0349	I30L, P38S, S42A
C	0387	G22S, R26C, T47A
C	0625	T47A, R86C
C	0657	S42A
C	1395	S42A
C	1550	I30L, T36P, P40T, T47A, T106I
B	0394	C6Y, I30L, F34T, V45L, T47A, V88T, L98I
B	9149	I30L, S31P, F34L, V88N, I127M
Non-transmitting group		
C	0080	S42A, K130R
C	0142	I30L, S42A, T47P, K130M, V131I
C	0274	I30V, F34L, T47A
C	0307	I30V, T36A, T47A
C	0496	I30L, S31P, S101P, K130M, V131I
C	0675	G32R
C	1032	N/A
C	1093	S42A, H94Y, K130M, V131I
C	1157	V88I
C	1278	N/A
C	1359	I30L, T47A
C	1406	I30V, S31A, T36S, A85T
C	1480	None
C	1520	E24K, I30L, G32R, A44T
C	9005	S42A, T47P, V131I
C	9192	I30V, S31A, G35R, T36S, A85T
C	9283	I30V, S31P, T36P, S42P, T47A

Note: N/A: Not available due to unsuccessful amplification of region of interest

3.4 Sub-study 2: Analysis of relationship between HBV genetic diversity and HBe antigen

3.4.1 Characteristics of HBeAg negative- and HBeAg positive-pregnant women

Among 131 pregnant women with HBV DNA levels equal to or greater than 3.5 $\log_{10}$ IU/ mL, 17 women had HBeAg negative. Fifteen HBeAg negative pregnant women with sample available were selected for this study. Samples from 15 women HBeAg positivity were also randomly selected as controls. Baseline characteristics between HBeAg negative- and HBeAg positive-pregnant women were not different, except HBV DNA levels was higher in HBeAg positive pregnant women as expected (Table 3.14).

Table 3.14 Baseline demographic and clinical characteristics of HBeAg negative and HBeAg positive pregnant women

Baseline characteristics	Overall		HBeAg negative		HBeAg positive		[a]*p*-value
	N	Median (IQR) or N (%)	N	Median (IQR) or N (%)	N	Median (IQR) or N (%)	
Age at enrollment; years	30	24 (21-29)	15	24 (21-30)	15	22 (20-29)	0.52
Body weight; kgs	30	55.2 (51.0-61.0)	15	55.4 (51.0-62.1)	15	55.0 (50.0-61.0)	0.92
White blood cell count; cells/mm^3	30	8,800 (6,900-10,300)	15	9,000 (7,500-10,300)	15	7,900 (6,700-10,300)	0.31
Absolute CD4+ T-cell; cells/mm^3	29	380 (246-486)	15	420 (324-500)	14	292 (180-486)	0.19
Absolute CD8+ T-cell; cells/mm^3	27	850 (658-1,240)	14	860 (614-1,121)	13	850 (810-1,392)	0.56
Serum ALT; IU/L	30	17 (13-22)	15	15 (13-20)	15	19 (13-23)	0.35
Serum creatinine; mg/dL	29	0.7 (0.5-0.8)	15	0.7 (0.5-0.9)	14	0.6 (0.6-0.7)	0.35
HBV DNA; log_{10} IU/mL	30	6.33 (4.03-7.63)	15	4.03 (3.68-5.49)	15	7.49 (7.23-7.99)	**<0.001**
HBV Genotype B:C:G	24	2:21:1 (8:88:4)	9	1:7:1 (11:78:11)	15	1:14:0 (7:93:0)	NS
HIV RNA load; log_{10} copies/mL	30	4.10 (3.64-4.66)	15	4.10 (3.64-4.72)	15	4.11 (3.39-4.66)	0.84
Anti-HCV antibody positive	30	0	15	0	15	0	NS
Anti-syphilis antibody positive	29	0	15	0	14	0	NS

Note: IQR: Interquartile range; ALT: Alanine transaminase; NS: Not Statistically Significant; [a]Fisher's exact test or Wilcoxon rank-sum test were used

3.4.2 Comparison of HBV genetic diversity between HBeAg negative- and HBeAg positive-pregnant women

Relationship between HBV genetic diversity and absence of plasma HBeAg in high HBV DNA level was assessed and compared among 6 women with HBeAg negative and 15 women with HBeAg positive by three parameters: 1) Shannon entropy, 2) the mean genetic distance, and 3) the ratio dN/dS. (Figure 3.7)

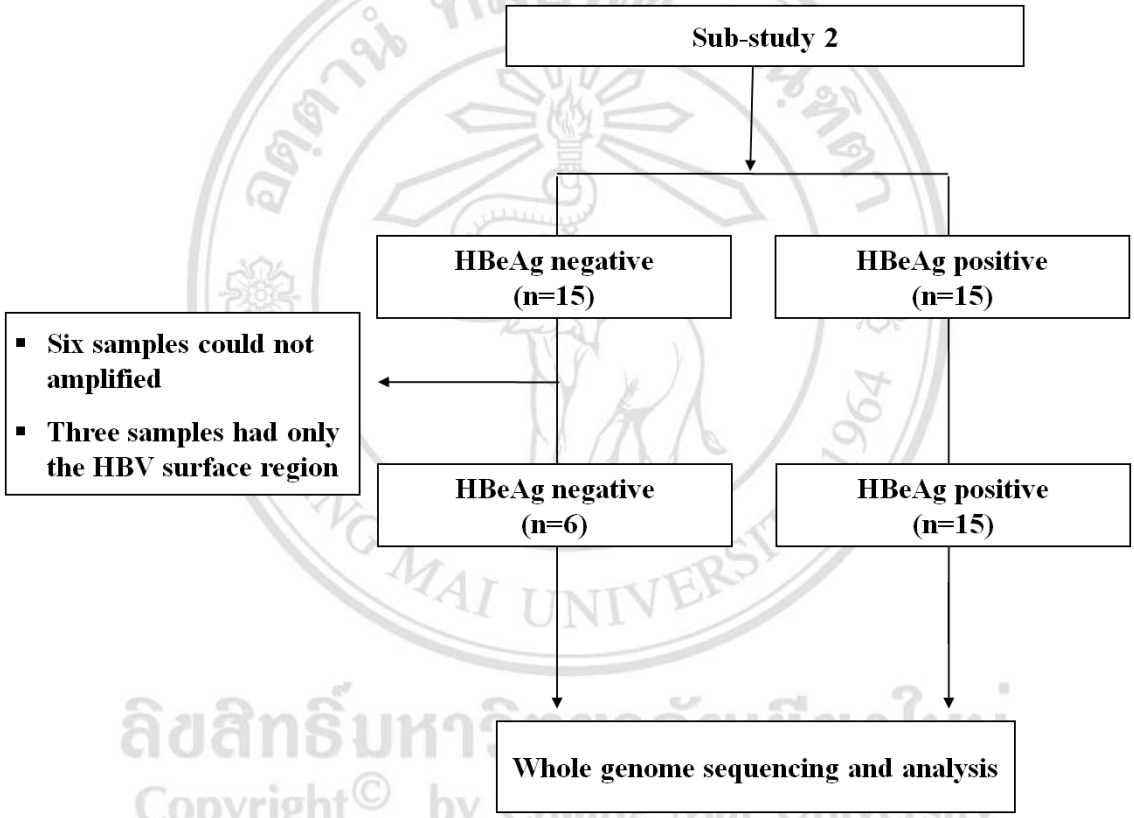

Figure 3.7 Schematic diagram of sub-study 2

1) Comparison of Shannon entropy at single nucleotide position and amino acid levels between HBeAg negative- and HBeAg positive-pregnant women

The Shannon entropy score of each nucleotide position was determined in HBeAg negative (n=6) and HBeAg positive group (n=15). The entropy score of each position along the full-length HBV genome and 4 coding regions are illustrated in Figure 3.8. In general, the entropy score of HBeAg negative group seem to be greater than HBeAg positive group.

The mean entropy scores at nucleotide level of whole genome and of each coding region were compared between HBeAg negative and HBeAg positive groups. Shannon entropy score of whole genome was significant higher in HBeAg negative than that in HBeAg positive groups. HBeAg negative group had entropy scores in *pol* and *preS/S* regions significant higher than that of HBeAg positive group but HBeAg negative group had significant lower entropy score in X region than HBeAg positive group. At amino acid level, the results were similar to nucleotide level. These results indicate that HBV had more genetic divergence among HBeAg negative women when compared to HBV among HBeAg positive, except in X region. (Table 3.15)

Table 3.15 Shannon entropy scores of HBeAg negative and HBeAg positive groups

Shannon entropy	**HBeAg negative (n=6) Mean (95% CI)**	**HBeAg positive (n=15) Mean (95% CI))**	**[a]*p*-values**
Nucleotide level			
- Whole genome	0.063 (0.057-0.069)	0.045 (0.041-0.049)	**<0.0001**
- Pol	0.069 (0.062-0.077)	0.042 (0.037-0.046)	**<0.0001**
- PreC/C	0.029 (0.020-0.039)	0.025 (0.018-0.031)	0.459
- PreS/S	0.068 (0.058-0.078)	0.033 (0.027-0.038)	**<0.0001**
- X	0.040 (0.028-0.053)	0.088 (0.073-0.102)	**<0.0001**
Amino acid level			
- Pol	0.086 (0.071-0.100)	0.050 (0.042-0.059)	**<0.0001**
- PreC/C	0.025 (0.007-0.044)	0.006 (0.001-0.011)	**<0.0001**
- PreS/S	0.096 (0.076-0.116)	0.042 (0.032-0.053)	**<0.0001**
- X	0.086 (0.052-0.120)	0.125 (0.093-0.158)	0.103

Note: [a]Fisher's exact test or Wilcoxon rank-sum test were used

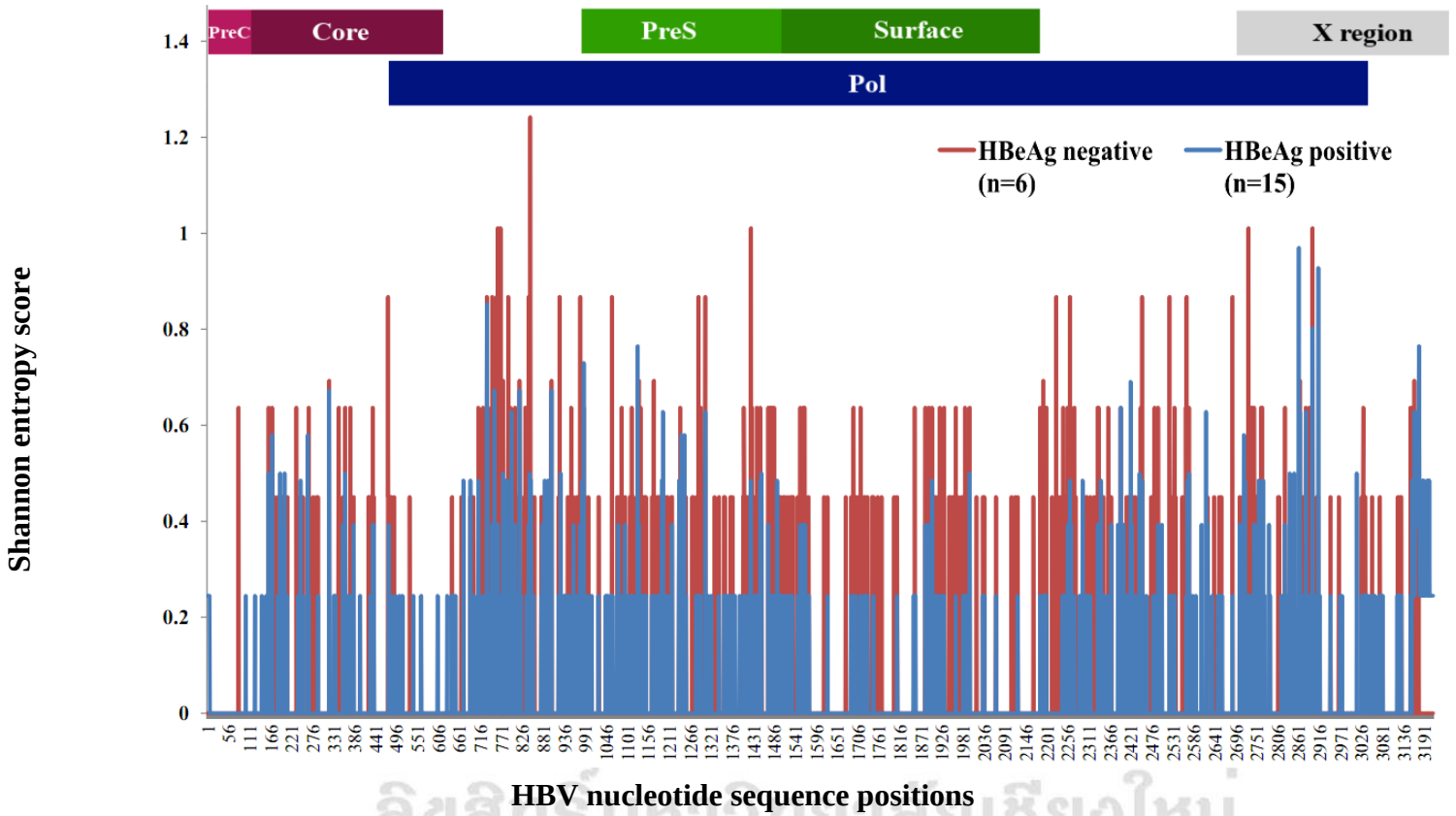

Figure 3.8 Comparison of nucleotide diversity in HBeAg negative and HBeAg positive groups using Shannon entropy. The HBV genome sequence positions are numbered on the x axis and four coding regions of HBV are labelled at the top. The y axis reports the Shannon entropy scores. The red line on the graph represented the scores of HBeAg negative group, while the blue line was HBeAg positive group.

2) Comparison of mean genetic distances between HBeAg negative- and HBeAg positive-pregnant women

Genetic diversity was calculated among HBeAg negative and HBeAg positive groups using the mean genetic distances of whole genome (Table 3.16 and 3.17). The mean genetic distance was greater in sample no. 0725 (genotype G), no. 1074 and 9231, (genotype B).

Generally, the inter-genotype mean genetic distance was greater than the intra-genotype. For intra-genotype, the mean genetic distances were not different in both HBeAg negative and positive groups.

Table 3.16 The genetic distances (GD) of whole genome in HBeAg negative groups

Patient ID	Genotype	0496	1456	0725	0647	1074	0265
0496	C						
1456	C	0.015					
0725	G	0.064	0.060				
0647	C	0.013	0.008	0.059			
1074	B	0.090	0.086	0.090	0.085		
0265	C	0.013	0.009	0.060	0.008	0.086	
Mean GD		0.039	0.036	0.066	0.034	0.087	0.035

Note: ID: Identification Number; GD values of HBV genotype B and G were underlined

Table 3.17 The genetic distances (GD) of whole genome in HBeAg positive groups

Patient ID	Genotype	0076	0861	9231	0211	1422	0931	0940	1070	0636	1119	1024	0313	0458	0502	0277
0076	C															
0861	C	0.025														
9231	B	0.086	0.090													
0211	C	0.023	0.017	0.089												
1422	C	0.021	0.015	0.090	0.009											
0931	C	0.020	0.016	0.091	0.012	0.009										
0940	C	0.021	0.016	0.089	0.012	0.009	0.006									
1070	C	0.021	0.017	0.090	0.012	0.010	0.007	0.007								
0636	C	0.019	0.015	0.089	0.010	0.008	0.006	0.006	0.007							
1119	C	0.022	0.017	0.089	0.014	0.012	0.012	0.012	0.013	0.010						
1024	C	0.023	0.018	0.094	0.018	0.015	0.015	0.014	0.016	0.014	0.018					
0313	C	0.022	0.016	0.090	0.010	0.007	0.011	0.011	0.012	0.010	0.014	0.016				
0458	C	0.023	0.016	0.091	0.014	0.011	0.011	0.010	0.012	0.009	0.013	0.017	0.012			
0502	C	0.021	0.017	0.086	0.012	0.011	0.007	0.007	0.008	0.007	0.012	0.016	0.012	0.012		
0277	C	0.022	0.017	0.090	0.012	0.010	0.009	0.009	0.010	0.007	0.012	0.016	0.012	0.012	0.010	
Mean GD		0.026	0.022	0.090	0.019	0.017	0.016	0.016	0.017	0.016	0.019	0.022	0.018	0.019	0.017	0.018

Note: ID: Identification Number; GD values of HBV genotype B were underlined

The mean genetic distances at nucleotide level of whole genome and each coding region were compared between HBeAg negative and HBeAg positive groups (Table 3.18). Whole genome of HBeAg negative had mean genetic distances greater than HBeAg positive groups. In 4 coding regions, the mean genetic distances were similar in two groups. These results suggest that genetic divergence is higher among HBeAg negative group than that among HBeAg positive group.

Table 3.18 Mean genetic distance for each HBV genetic region of HBeAg negative and HBeAg positive groups

Mean genetic distance	HBeAg negative (n=6) Mean (95% CI)	HBeAg positive (n=15) Mean (95% CI)	[a]*p*-values
Whole genome	0.050 (0.026-0.073)	0.024 (0.013-0.034)	**0.012**
Pol	0.035 (0.012-0.058)	0.027 (0.016-0.038)	0.43
PreC/C	0.019 (0.010-0.028)	0.015 (0.013-0.017)	0.18
PreS/S	0.034 (0.010-0.057)	0.023 (0.012-0.033)	0.29
X	0.029 (0.014-0.045)	0.026 (0.018-0.034)	0.65

Note: [a]Fisher's exact test or Wilcoxon rank-sum test were used

3) Comparison of dN/dS ratio between HBeAg negative- and HBeAg positive-pregnant women

The synonymous mutations rate (dS) and non-synonymous mutations rates rate (dN) of 4 open reading frames were compared between HBeAg negative and HBeAg positive (Table 3.19). All regions had dN/dS values lower than 1, indicative of negative selection. The selection pressure was similar in HBeAg negative and HBeAg positive groups.

Table 3.19 The dN/dS ratio for each HBV genetic region of HBeAg negative and HBeAg positive groups

dN/dS	HBeAg negative (n=6) Mean (95% CI)	HBeAg positive (n=15) Mean (95% CI)	[a]*p*-value
Pol	0.095 (0.059-0.131)	0.248 (0.064-0.561)	0.33
PreC/C	0.062 (0.067-0.192)	0.013 (0.013-0.039)	0.34
PreS/S	0.107 (0.050-0.163)	0.136 (0.60-0.212)	0.54
X	0.942 (0.881-2.766)	0.196 (0.070-0.322)	0.20

Note: [a]Fisher's exact test or Wilcoxon rank-sum test were used

In order to exclude the effect of inter-genotype diversity, the genetic diversity of HBV genotype C was investigated (Table 3.20). Average overall entropy scores were significant higher in HBeAg positive group than HBeAg negative group. By region, HBeAg positive group had high diversity in *pol* and X region. Shannon entropy score was correlated at both nucleotide and amino acid levels, except entropy score of preC region. These results indicated that HBV in HBeAg positive group had higher diversity than HBeAg negative group, which were discordant to the earlier results when all genotypes were analyzed . HBeAg positive group also had higher mean genetic distance of whole genome than HBeAg negative group but mean genetic distances by coding regions were not different between the two groups. In addition, dN/dS ratio was not different between the two groups and this was concordant with the earlier results.

Table 3.20 Genetic diversity of HBV genotype C in HBeAg negative and HBeAg positive groups

Genetic diversity Parameter	ORFs	HBeAg negative group (n=4)	HBeAg positive group (n=14)	[a]*p*-value
		Mean (95% CI)	Mean (95% CI)	
1) Shannon entropy				
- Nucleotide level	Whole genome	0.013 (0.010-0.017)	0.028 (0.025-0.032)	**<0.0001**
	Pol	0.012 (0.009-0.016)	0.023 (0.019-0.026)	**<0.0001**
	PreC/C	0.013 (0.006-0.020)	0.022 (0.015-0.028)	0.06
	PreS/S	0.009 (0.005-0.013)	0.014 (0.010-0.017)	0.10
	X	0.014 (0.006-0.022)	0.076 (0.062-0.091)	**<0.0001**
- Amino acid level	Pol	0.019 (0.012-0.026)	0.026 (0.019-0.032)	0.13
	PreC/C	0.019 (0.003-0.035)	0.006 (0.001-0.011)	0.12
	PreS/S	0.013 (0.005-0.022)	0.015 (0.008-0.022)	0.77
	X	0.037 (0.015-0.060)	0.107 (0.075-0.140)	**0.0005**
2) Genetic distance	Whole genome	0.009 (0.004-0.014)	0.013 (0.011-0.015)	**0.03**
	Pol	0.021 (0.018-0.025)	0.022 (0.021-0.024)	0.58
	PreC/C	0.014 (0.006-0.023)	0.014 (0.012-0.016)	0.98
	PreS/S	0.018 (0.014-0.022)	0.018 (0.017-0.019)	0.88
	X	0.020 (0.016-0.024)	0.022 (0.020-0.025)	0.36
3) dN/dS	Pol	0.129 (0.000-0.312)	0.039 (0.010-0.068)	0.11
	PreC/C	0.000 (0.000-0.000)	0.015 (0.000-0.045)	0.66
	PreS/S	0.000 (0.000-0.000)	0.044 (0.000-0.110)	0.47
	X	0.000 (0.000-0.000)	0.112 (0.000-0.243)	0.51

Note: [a]Fisher's exact test or Wilcoxon rank-sum test were used

3.4.3 Analysis of mutations of HBeAg negative- and HBeAg positive-pregnant women

Amplification of the full-length HBV genome were 100 percent successful for samples from women HBeAg positive (15/15), whereas full-length HBV genome were successfully amplified in 40 percent (6/15) of samples from women HBeAg negative and 20 percent (3/15) in surface region and 40 percent (6/15) could not be amplified. Nucleotide substitutions in *preC/C* region and amino acid mutations in preS/S, *pol* and X were identified.

1) Mutations in BCP, PC and core region of HBeAg negative- and HBeAg positive-pregnant women

The A1762T/ G1764A double mutations were found in both HBeAg negative (n=2) and HBeAg positive groups (n=1). The G1764A mutation alone was also found in one case with HBeAg negative and another case with HBeAg positive. In precore region, G1896A was detected in two cases with HBeAg negative. In core region, C2134T mutation was predominantly found (9/30). Mutations in core region leading to change of HBeAg serostatus were found at nt C2048G in 2 cases HBeAg negative. Other mutations found in the precore and core region were mostly silent and not significant (Table 3.21).

Table 3.21 Nucleotide substitutions in BCP and *preC/C* region of HBeAg negative and HBeAg positive groups

Genotype	Patient ID	Nucleotide substitutions in BCP, PC and core region
HBeAg negative		
C	0265	**A1762T/G1764A**, C2048G, T2101C, C2134T, A2159G, T2170C, A2189C, C2288G
C	0324	N/A
C	0496	**A1762T/G1764A**, C1999T, C2078T, A2159G, A2189C, C2198T, A2239C, T2242A, C2288A
C	0523	N/A
C	0647	None
G	0725	**G1896A**, G1975T, T1978C, G1984A, G2011A, T2012C, T2024C, G2080A, C2134T, C2176T, G2237C, A2248T, G2345A
C	0900	N/A

Table 3.21 (Continued)

Genotype	Patient ID	Nucleotide substitutions in BCP, PC and core region
B	1074	**G1896A**, G1975T, G1984A, C1990T, C2048G, A2059G, G2080A, G2092, G2104A, C2134T, A2158C, C2176T, C2289T, C2296T
C	1456	G1764A
HBeAg positive		
C	0076	G1975T, G1984A, G2011A, C2134T, T2150C, C2176T, A2248T
C	0211	C1999T, T2005A, G2017A, C2134T, G2251A
C	0277	G2080A, G2146A
C	0313	T2005A, G2017A, C2134T
C	0458	G1915A, G1939A, C1969T, C2053T, C2078T
C	0502	G2021T, T2170C
C	0636	None
C	0861	G1764A, G1984A, C2009T, C2078T, C2176T, C2246T, C2290T, T2308C, T2318C, T2443C
C	0931	T2215C
C	0940	None
C	1024	**A1762T/G1764A**, G1975T, T1978C, G1984A, C2009T, T2014C, A2326G, G2418A
C	1070	G1957A, C2023A, C2134T, C2191T, A2375C
C	1119	A2059C, C2078T, A2185G, C2198T, T2242A
C	1422	T2005A, G2017A, C2078T, C2134T
B	9231	G1975T, G1984A, A2059G, G2104A, C2134T, T2170C, C2176T, C2198T, C2354A

Note: Letters in bold denote well-known mutations related to HBeAg production

N/A: Not available due to unsuccessful amplification of region of interest

2) Mutations in preS/S region of HBeAg negative- and HBeAg positive-pregnant women

Analysis of direct sequences of *S* genes showed that several amino acid substitutions were detected (Table 3.22). Mutation at amino acid position G27D in preS1 was found in HBV genotype B, C and G. Six out of nine HBeAg negative women had I126T in S region. This variant was also found in two HBeAg positive women. One woman with HBeAg negative harbored the K141I together with I126T.

Table 3.22 Amino acid mutations in preS/S region of HBeAg negative and HBeAg positive groups

Genotype	Patient ID	preS1	preS2	S
HBeAg negative				
C	0265	G27D	None	**I126T**
C	0324	N/A	N/A	G44E, Q54P, S61L, **G145R**
C	0496	None	None	**K141N**, G185E
C	0523	N/A	N/A	F20S, P62Q, L77R, **I126T, K141I**, **C147Y**
C	0647	None	None	None
G	0725	Q10E, G27D, G35R, A81S, V88L, A91D	S6T, T7A, D14N, R16K, F22L, T31I, T38I, P41H, T49I	S3N, Q51L, T63I, I68T, L110I, T113S, **I126T, T131N, F134Y,** S204N, L213II
C	0900	N/A	N/A	I92T, Q101K, I126T, F134I, R160S, P203R, N207T
B	1074	Q10K, G27D, G35K, S73G, Q80L, I84L	S6T, V35A, P41A, I45T, A53V	S3N, T5A, F8L, L21S, N40S, L110I, T113S, K122R, I126T, M133L, R160K, L213IM, V224A
C	1456	G27D	P54Q	I126T, L213II, F220L
HBeAg positive				
C	0076	S73G	R16K	T5A
C	0211	S73N, P94T	T38S	K122R, **D144E**
C	0277	None	V35G	F8S, L110I, **G145R**
C	0313	S73N	None	S3N
C	0458	None	None	None
C	0502	None	None	None
C	0636	None	S47L	None
C	0861	S73N	None	None
C	0931	Q10H	None	None
C	0940	None	None	None
C	1024	S6G, R9G, G23R	None	None
C	1070	None	None	None
C	1119	None	None	T113S
C	1422	S73N, T97S	None	None
B	9231	Q10K, G27D, G35K, T68I, S73G	S6T, V35A, A39V, P41A, F46L, A53V	S3N, T5A, F8L, L110I, T113S, **I126T**, R160K, L213IM

Note: Letters in bold denote mutation in the "*a*" determinant of HBsAg (amino acid position 124 to 147), which is associated with vaccine/immunoglobulin escape; N/A: Not available due to unsuccessful amplification of the region of interest

3) Mutations in reverse transcriptase region of HBeAg negative- and HBeAg positive-pregnant women

Analysis of direct sequences of *pol* gene showed no known drug resistance mutation. Variation of amino acid was observed in almost all samples from HBeAg negative women. High number of amino acid substitutions was found at position rtP109S/Q (n=5, 24%), rtS223A (n=4, 19%) and rtF221Y (n=3, 14%). No mutation in overlapping surface and RT regions was detected (Table 3.23).

Table 3.23 Amino acid mutations in reverse transcriptase of pol region of HBeAg negative and HBeAg positive groups

Genotype	Patient ID	Amino acid mutations in reverse transcriptase
HBeAg negative		
C	0265	rtN337H
C	0324	rtR153Q, rtV191I
C	0496	rtT150P, rtR153W, rtH156Y, rtI163F, rtA317S
C	0523	rtK149N
C	0647	None
G	0725	rtE1Q, rtY9H, rtT54A, rtH55R, rtL91I, rtP109S, rtT118D, rtN121I, rtI122L, rtY124H, rtH126Y, rtM129L, rtD131N, rtN139Q, rtL145M, rtF221Y, rtS223A, rtI224V, rtV266I, rtL269F, rtI278V, rtL336M
C	0900	rtP109Q, rtV142D, rtI169L, rtQ215H, rtS223A
B	1074	rtY9H, rtH13C, rtH55R, rtP109S, rtT118N, rtN121I, rtY124H, rtD131N, rtY141F, rtL145M, rtF151Y, rtR153W, rtF221Y, rtS223A, rtI224V, rtT259S, rtQ271L, rtI278V, rtL336M
C	1456	rtF221Y, rtL229V
HBeAg positive		
C	0076	rtH13R, rtT128A, rtL269I, rtP325S
C	0211	None
C	0277	rtT118N, rtR153Q
C	0313	None
C	0458	rtH13Y, rtP109S, rtY124H
C	0502	rtY9H
C	0636	None
C	0861	None
C	0931	rtK318R
C	0940	None
C	1024	rtV253I
C	1070	rtK333Q
C	1119	rtN121I, rtI122L
C	1422	rtQ271H

Table 3.23 (Continued)

Genotype	Patient ID	Amino acid mutations in reverse transcriptase
B	9231	rtY9H, rtH13R, rtH55R, rtP109S, rtT118N, rtN121I, rtY124N, rtD131N, rtD134N, rtL145M, rtF151Y, rtF221Y, rtS223A, rtI224V, rtS256G, rtL269I, rtQ271M, rtI278V, rtP325S,

Note: N/A: Not available due to unsuccessful amplification of the region of interest

4) Mutations in X region of HBeAg negative- and HBeAg positive-pregnant women

The mutations in X region commonly found in this population were S42A/P (n=10, 48%) and T47A/P (n=7, 33%). Double mutations K130M/V131I (n=3, 14%) of region overlapped with double mutations in BCP at nucleotides 1,762 and 1,764 were observed in two sequences from HBeAg negative women and one from HBeAg positive woman (Table 3.24).

Table 3.24 Amino acid mutations in X region of HBeAg negative and HBeAg positive groups

Genotype	Patient ID	Amino acid mutations in X region
HBeAg negative		
C	0265	I30L, S42A, H94Y, K130M, V131I
C	0496	I30L, S31P, S101P, K130M, V131I
C	0647	I30L, S42A, R86H
G	0725	A12S, G22S, R26S, I30L, S31P, T36A, P38S, S42P, T47A, A85S, R86S
B	1074	I30L, S31P, T36A, S42P, S65P, R86H
C	1456	I30L, S41P, S42A, V131I, F132Y
HBeAg positive		
C	0076	I30V, S31A, T36S, A85T
C	0211	I30L, A102V, S144A,
C	0277	V133S
C	0313	R26C,
C	0458	V21F
C	0502	I30L, S42A, T47P, L129V, H139D, V142A, C143R
C	0636	None
C	0861	G22S, R26C, I30L, T47A, V131I, R138T
C	0931	R26C, S42A, T47P, D48V
C	0940	V15I, T36A, S42A, T47P, D48N

Table 3.24 (Continued)

Genotype	Patient ID	Amino acid mutations in X region
C	1024	G22S, T47A, V116L, K130M, V131I
C	1070	S42A, T47P
C	1119	I30L, S31P, P38S
C	1422	None
B	9231	G22S, I30L, S31P, T36A, S42P, S43P, A44G, V45I, A66T, R86H, A102T, M103T

Note: N/A: Not available due to unsuccessful amplification of the region of interest