



Increased risk of developing chronic HBV infection in infants born to chronically HBV infected mothers as a result of delayed second dose of hepatitis B vaccination

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ABSTRACT

This two-stage study (cross-sectional and case-control) assessed the effects of delayed second dose HB vaccination on the risk of developing chronic HBV infection in infants born to chronically HBV infected mothers. 521 infants enrolled received the first vaccination by the end of the day after birth, without HBIG. 15 of these infants were chronically HBV infected. In the case-control comparison, controlling for HBeAg in the mother, the risk of an infant becoming chronically infected was 3.74 times (95% CI = 0.97–14.39) higher if the interval between the first and the second doses exceeded 10 weeks. This finding suggests it is important that immunization programs ensure timely second dose vaccination to infants born to mothers with chronic HBV infection. Nevertheless, due to the small sample size, these findings should be verified by larger studies.

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1. Introduction

Hepatitis B virus (HBV) infection is a global public health priority. Although effective hepatitis B (HB) vaccine has been available for more than 20 years, approximately 500,000–700,000 people still succumb to hepatitis B-related disease each year [1,2]. HBV is readily transmitted by percutaneous and mucosal exposure to infected blood and other body fluids. In Asia, vertical transmission especially during perinatal period constitutes a major cause of transmission [3,4]. Without vaccination, approximately 50% of children of infected mothers become infected by them [5], and up to 90% of infected children developed chronic HBV infection [6].

To reduce the incidence of HBV infection, the World Health Organization has recommended that, starting in 1997, all countries include HB vaccine in their immunization programs [7]. Universal HB vaccination of neonates, regardless of maternal HB status, has proved very effective in reducing the rate of chronic HBV infection in HB endemic countries [8–10]. In countries with a high proportion of perinatally acquired HBV infections, it is recommended that the first dose of HB vaccine be given as soon as possible (<24 h) after birth [2]. Various studies have shown that neonatal vaccination is effective, whether vaccine alone or vaccine plus HB immunoglobulin (HBIG) is administered [11,12]. The rates of chronic HBV infection in many endemic countries (e.g. Thailand

and Taiwan) have as a result decreased to below 1% [8,13,14]. In Thailand, the rate of chronic HBV infection among children under the age of 4 was estimated at 4–8% prior to the introduction of universal HB vaccination without HBIG at birth in 1992 [15]. Surveys in several regions of Thailand in 1999 and 2004 demonstrated that the rate of chronic HBV infection among children born after 1992 had fallen to only 0.7% [8,13].

Despite the effectiveness of current HB vaccination programmes, it could take several generations to eliminate the disease, as newborns infected by their mothers are likely to be a source of infection throughout their lives. In developing countries where HBIG is not available, maximising the effectiveness of vaccination is thus particularly important. The birth dose strategy is an example of how such improved effectiveness may be achieved in practice. The question addressed by this study is whether the efficacy of the neonatal dose may be further improved by optimising the interval between it and the second dose. Intuitively, a long interval between the first and second vaccine dose should be avoided, but unequivocal data are not available. The present study was conducted to address the above issues, to provide a basis on which Thailand's National Advisory Committee on Immunization could modify the country's HB immunization schedule to reduce vertical transmission.

2. Materials and methods

This study was approved by the Ethical Review Committee for Research in Human Subjects, Department of Disease Control, Min-

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Fig. 1. Location of Chiangrai, the northernmost province of Thailand.

istry of Public Health, Thailand. The study was carried out from June to October 2006. Written informed consent was obtained from all participants.

2.1. Study location

Chiangrai, a province in the North of Thailand with some 10,000 births per year was selected as the study site, because of its particular characteristics. These included routine testing for HBsAg in all antenatal clinics, and HBIG being neither available nor recommended for newborns in public or private hospitals. The majority of the population in the province is indigenous Thai, but 12.5% consists of ethnic minority “hill tribes” most of whom live in rather basic conditions in mountainous areas. All public hospitals in the province (1 provincial and 17 district hospitals) participated in the study. The location of Chiangrai is shown in Fig. 1.

2.2. HB vaccination schedules in Chiangrai

For children of HBsAg-negative mothers, HB vaccination in the province follows the national schedule, which requires monovalent HB vaccine within 24 h of birth and combined DTPw-HB vaccine at 2, 4 and 6 months. Coverage of this three-dose course of DTPw-HB

vaccination among children age 12–17 months in Chiangrai was 93.8% in 2000 [16].

In infants born to HBsAg-positive mothers (mothers with chronic HBV infection), due to the concern that delayed second HB vaccination might increase the risk of chronic HBV infection in children, the Chiangrai Maternal and Child Health Board recommended giving an extra dose of monovalent HB vaccine at 1 month. In 17 district hospitals, the recommendation from the Board was followed, so such infants received 5 doses of HB vaccine. In provincial hospitals, due to budget constraints, the first dose of DTPw-HB vaccine was given earlier, at 6 weeks, so the HB vaccination schedule for such infants there remained 4 doses (Table 1). However, in practice, about half of all infants born to mothers with chronic HBV infection (in district and in provincial hospitals) received their second doses later than scheduled. The situation in Chiangrai thus provided an opportunity to investigate the effects of the interval between the first two doses of HB vaccine on the rate of chronic HBV infection in infants.

2.3. Stage 1: cross-sectional study

The cross-sectional study, started in June 2006, was used to estimate the overall chronic HBV infection rate in infants born to mothers with chronic HBV infection, in association with their HB vaccination history.

From hospital records, lists were made of children born in 2004 and 2005 to mothers with chronic HBV infection, i.e. those found HBsAg positive during routine antenatal testing. At the time of recruitment, the children of these mothers were aged 6 months to 2.5 years. The children concerned were then located and their parents invited to enroll them in the study. Sera from these children were tested for HBsAg, HBsAb and HBcAb, and their HB vaccination histories noted. The history was in most instances obtained from the child’s vaccination card. If the card was lost or incomplete, the history was obtained instead from health facility records.

Children were excluded from the cross-sectional study if any of the following applied: no longer residing in Chiangrai; had received HBIG; had not received the first dose of HB vaccine within by the end of the day following birth; had not received all scheduled doses of HB vaccine by at least 1 month before serum collection; or, parents refused consent.

2.4. Stage 2: case-control study

The case-control study was built upon the results of the cross-sectional study. Its objective was to identify associations between the HBV status of the children born to mothers with chronic HBV infection and the vaccination history of these children, controlling for the HBeAg status of the mothers. Cases were all children with chronic HBV infection, while controls were randomly selected non-chronically infected children identified from the cross-sectional study (Fig. 2). Mothers of all cases and controls were invited to test for HBsAg and HBeAg. Cases and controls were excluded where mothers were found to be HBsAg negative, had any disease precluding venous puncture, or refused consent.

Table 1 Recommended HB vaccination schedule for newborn children of HBsAg-positive and -negative mothers, Chiangrai, 2004–2006.

Group	Age					
	Birth	1 month	6 weeks	2 months	4 months	6 months
Children born to HBsAg-negative mothers	HB			DTPw-HB	DTPw-HB	DTPw-HB
Children born to HBsAg-positive mothers	HB	HB		DTPw-HB	DTPw-HB	DTPw-HB
	4-Dose group	HB	DTPw-HB		DTPw-HB	DTPw-HB

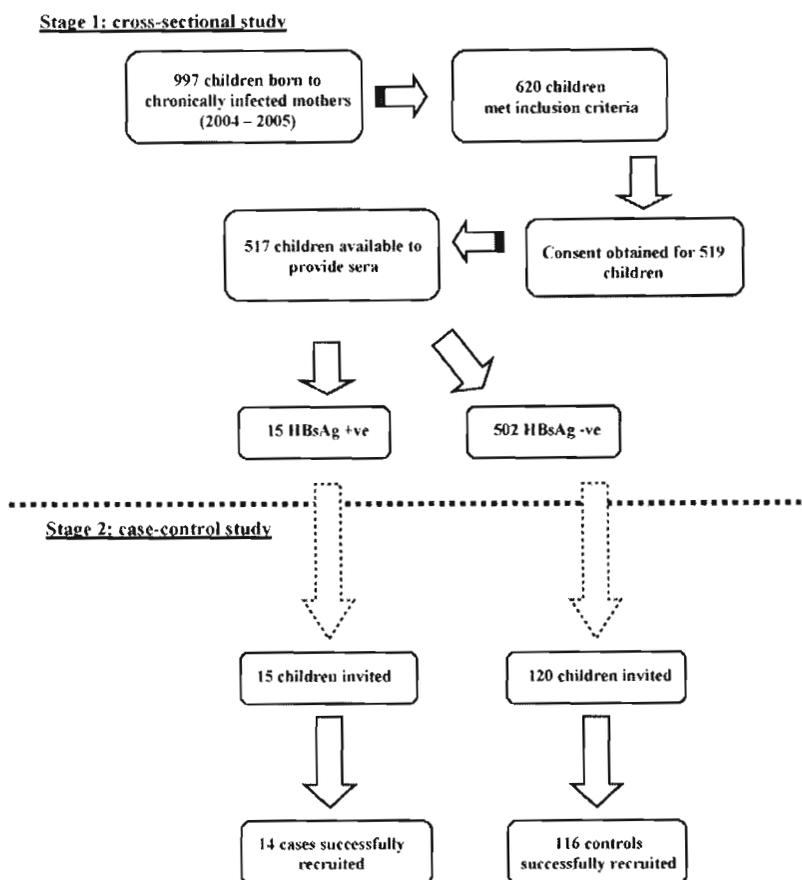


Fig. 2. Population and samples in the study.

2.5. Laboratory tests

The ELISA test kits for HBsAg, HBeAg, HBsAb and HBeAb were those produced commercially by Abbott laboratories (Murex Biotech Ltd, Dartford, UK). The positive cut-off points specified by the manufacturer were used for all tests.

2.6. Data analysis

In order to determine the effect, if any, of the interval between the first and second dose of HB vaccine (HB1–2) on perinatal transmission rates, children were divided into groups based on their total number of HB vaccinations (4-dose and 5-dose), and on the duration of their HB1–2 intervals (less than 6 weeks, 6–7 weeks, 8–9 weeks, and 10 weeks or longer).

The descriptive data were analyzed by determining the percentage of chronically HBV infected children and the corresponding 95% confidence intervals (95% CI) for each group of children. The case-control results were analyzed using the odds ratio (OR) and its 95% CI. Anti-HBs levels were determined by geometric mean titre (GMT). Statistical analysis was performed using the STATA 6.0 package.

3. Results

A total of 997 children were born to mothers with chronic HBV infection in Chiangrai in 2004 and 2005, of whom 620 met the study's inclusion criteria. Of those excluded, 194 could not be located, 125 had moved outside Chiangrai, 27 had received their

first dose of HB vaccine too late, 24 had not completed the HB vaccine regimen in time, and 7 had died from various diseases not related to the vaccine. Of the 620 children who could potentially be included, consent was obtained for 519, but sera were successfully taken from only 517 (Fig. 2).

These 517 children had a median age of 568 days (range 212–929 days) on the dates their sera were taken. Fifteen of them were found to develop chronic HBV infection, the overall rate of chronic infection being 2.9% (95% CI = 1.69–4.85). The rate of chronic infection in the 5-dose group was 1.44% (4/277), while in the 4-dose group it was 4.58% (11/240). The risk in the 4-dose group was thus greater than in the 5-dose group by a factor of 3.17 (95% CI = 1.02–9.84). In the 5-dose group, all but one child had an HB1–2 interval shorter than 8 weeks (90% less than 6 weeks). Rates of chronic HBV infection by HB1–2 interval are shown in Table 2. All of the 502 children without chronic HBV infection had anti-HBs levels above the protective level of 10 mIU/ml, the average GMT being 193.4 mIU/ml. In children whose sera were taken 91–180 days after complete vaccination, those who received 5 doses initially had higher GMT levels than those who received 4 doses, but this effect faded with time (Fig. 3).

In the case-control study, one of the 15 HBsAg-positive children had to be excluded because his mother could not be located. The remaining 14 HBsAg-positive children were recruited as cases. Of the 120 randomly selected HBsAg-negative children invited as controls (8 controls per 1 case), 116 were successfully recruited (Fig. 2). All the cases except one (i.e. 92.9%) had HBeAg positive mothers (Table 3), while 33 of the 116 (28.4%) controls had HBeAg positive mothers.

Table 2
Chronic HBV infection rates by HB1-2 interval in the cross-sectional study.

	No. of children	No. of HBsAg positive	Chronic HBV infection rate (%) and 95% CI
5 Dose group	277	4	1.44, 0.46–3.91
By HB1-2 interval			
Less than 6 weeks	249	4	1.61, 0.52–4.34
6–7 weeks	27	0	NA
8–9 weeks	1	0	NA
4-Dose group	240	11	4.58, 2.43–8.28
By HB1-2 interval			
Less than 6 weeks	21	1	4.76, 0.25–25.87
6–7 weeks	30	1	3.33, 0.17–19.05
8–9 weeks	89	2	2.25, 0.39–8.65
10 weeks and above	100	7	7.00, 3.1–14.38

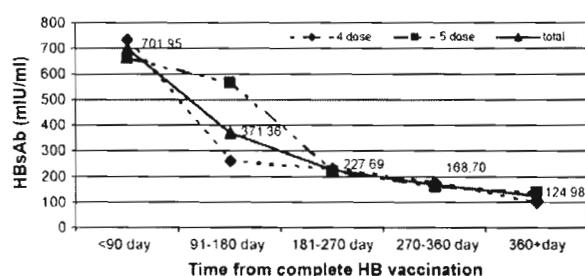


Fig. 3. GMT of anti-HBs by number of doses of HB vaccine, relative to time since complete HB vaccination.

After controlling for maternal HBeAg status using multiple logistic regression, it was found that children having intervals longer than 10 weeks between their first two HB vaccine doses were at greater risk of developing chronic HBV infection (Table 4). Their risk was 3.74 times higher than that of those with intervals shorter than 10 weeks (95% CI = 0.97–14.39). Restricting the comparison to the children having HBeAg positive mothers (13 cases and 33 controls), those who had their first two doses of vaccine more than 10 weeks apart were 4.8 times more likely to become chronically HBV infected (95% CI = 1.13–20.41, Table 5).

4. Discussion

The study reported here is believed to be the first to estimate the risk of delaying second dose vaccination. It is well known that most cases of hepatitis B vaccine failure in high risk newborns occur during the first months of life [8,17]. However, there are no rigid guidelines as to the interval between the first and second doses of vaccine. Most studies have demonstrated the high efficacy of hepatitis B vaccine in high risk neonates of vaccine doses administered at birth, 1 and 6 months [18,19], and the HB vaccination schedules recommended by the manufacturers for infants in endemic countries are consistent with this finding.

The WHO has suggested implementing universal hepatitis B vaccination in highly endemic countries, with a first dose at birth and subsequent doses added to the national immunization programme without requiring additional visits for vaccination [20]. As a result, intervals between the first two doses as recommended by different national programmes range from 4 weeks to 2 months, depending on various factors, e.g., pre-existing immunization schedule, readiness of parents to accept multiple injections in one visit, combination vaccine used etc. Additionally, operation of vaccination clinics in developing countries is often periodically not daily, so many children may in practice receive vaccine some weeks after the recommended age.

Table 3
Demographic data of HBsAg-positive children and their mothers.

No	Sex	HB dose	Days between last vaccination and serum collection	Days between 1st and 2nd HB	District	HBV marker									
						Children	Mothers	Children	Mothers	Children	Mothers				
						HBsAg	HBeAg	Anti-HBc	Anti-HBs	AST	ALT	HBsAg	HBeAg	AST	ALT
1	M	4	344	88	Mueang	+	+	+	+	47	51	+	+	28	29
2	F	4	295	41	Mueang	+	+	+	+	26	42	+	+	33	25
3	M	4	456	73	Thoeng	+	+	+	+	57	57	+	+	27	31
4	M	4	218	73	Thoeng	+	+	+	+	62	71	+	+	38	56
5	M	5	162	31	Phan	+	+	+	+	33	29	+	+	38	24
6	F	4	407	73	Mae Chan	+	+	+	+	42	25	+	+	26	35
7	F	4	134	103	Mae Sai	+	+	+	+	31	27	+	+	20	21
8	M	5	449	27	Wiang Pa Pao	+	+	+	+	45	15	+	+	75	55
9	M	4	498	54	Phaya Mengrai	+	+	+	+	41	33	+	+	20	15
10	F	5	344	30	Phaya Mengrai	+	+	+	+	56	63	+	+	28	31
11	M	5	253	34	Wiang Kaen	+	+	+	+	35	11	+	+	22	23.4
12	F	4	71	65	Wiang Kaen	+	+	+	+	423	334	+	+	116	55.4
13	F	4	253	64	Khun Tan	+	+	+	+	55	10	+	+	17	5
14	M	4	64	73	Chiang Saen	+	+	+	+	49	37	+	+	31	31
15	M	4	499	94	Mae Fa Luang	+	+	+	+	No data	No data	No data	No data	No data	No data

+: positive; -: negative.

Table 4

Risk of children becoming chronically HBV infected: adjusted odds ratios and 95% confidence intervals (by multiple logistic regression).

Risk factor	Adjusted OR	95% CI
HBeAg positive mother	34.03	4.19–276.62
HB1-2 interval of 10 weeks or more	3.74	0.97–14.39

Table 5

Number of cases (children with chronic HBV infection) and control (children without HBV infection) that were born to HBeAg positive mother by HB1-2 interval.

	Cases	Control
HB1-2 interval less than 10 weeks	7	28
HB1-2 interval 10 weeks or more	6	5

Although our main study result has not reached statistically significant due to small sample size, it suggests that, where children receive the birth dose vaccination on time, delaying the second dose vaccination beyond 10 weeks typically increases their risk of becoming chronically HBV infected four- to five-fold. This could have significant implications in Thailand, and probably in many other developing countries. In Thailand, 81.5% of newborns receive the HB vaccine birth dose on time [21], and the second dose is recommended at 2 months. However, since the immunization clinic operates once a month, some 50% of the 40,000 infants born to mothers with chronic HBV infection would have their second dose appointment at later than 10 weeks of age. Modifying Thailand's national HB immunization schedule could thus help further reduce the chronic HBV infection rate.

The overall chronic HBV infection rate among high risk infants in our study was very low, at 2.9%. This was probably influenced by the inclusion criteria, which required the children in the study to have received HB doses shortly after birth and subsequently in accordance with the HB vaccine schedule. The low chronic HBV infection rate resulted in an unexpectedly small number of cases, limiting statistical power of the study. Low sample size also prevented our assessing the effectiveness of combined DTPw-HB versus monovalent HB vaccine, although many previous studies have shown them to be equally immunogenic [22,23]. However, there was no reason to suspect other confounding factors (except for HBeAg in the mother) or bias in our study.

There was one child who was infected despite the mother being HBeAg negative, and DNA analysis of maternal HBV revealed a high viral load by real time PCR [24] (1.26×10^7 copies/ μ l). By the sequencing result, this mother has been infected by HBV precore mutants (G1896A). Due to the amount of HBV in the Thai population, and the selection pressure from vaccine use, there is always the possibility of vaccine escape mutants emerging and causing HBV failure. This research did not study the potential significance of surface gene mutants as a cause for immunization failure. Nonetheless, our previous study has shown that vaccine escape variants cannot be considered solely responsible for failed immunization [25,26].

The present study also confirmed the efficacy of combined DTPw-HB vaccine. All non-infected children whose sera were taken at 91–180 days after complete vaccination had anti-HBs levels above the protective level of 10 mIU/ml. Combine vaccines should be used where possible as they are more cost-effective, providing broader antigen cover and excellent protective levels. The majority of those vaccinated with combined DTPw-HB vaccine in Thailand appear to have remained immune for at least 7–10 years [27].

In conclusion, the findings of this study suggest that the risk of infants born to mothers with chronic HBV infection becoming chronically infected is associated with a delayed second dose of HB vaccine, even if the birth dose has been administered promptly. Further studies of this topic are needed, using larger population samples. In Thailand (where pregnant women are routinely

screened for HBsAg in antenatal clinics), the National Advisory Committee on Immunization has already decided to recommend an extra dose of monovalent HB vaccine exactly 1 month after the HB birth dose for children born to mothers with chronic HBV infection. This is to be followed in each case by DTPw-HB doses at 2, 4 and 6 months.

Disclosure statement

Appropriate informed consent was obtained, and the study was conducted in accordance with the guidelines for human experimentation specified by the Institutional Review Board of the Ministry of Public Health, Thailand.

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