EVALUATION OF A HEPATITIS B VACCINATION PROGRAM IN CHIANG MAI, THAILAND

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Abstract. Chiang Mai is a province in northern Thailand that started a vaccination program for hepatitis B virus (HBV) infection in 1989. In this paper, we report the long-term efficacy of this program. Of children aged 4-9 years, 65.7% had a complete course and 3.8% had an incomplete vaccination course. Urban schoolchildren had higher percentage of HB vaccination than rural schoolchildren (89.1% *vs* 46.9% for the complete course, $p < 0.001$). The overall prevalence rate of HBsAg in Chiang Mai schoolchildren was 1.2%, with no significant differences between gender ($p = 0.496$) and school areas ($p = 0.477$). Anti-HBc antibodies were detected in 6.9% of children. Overall, 26.2% of children had protective levels of anti-HBs antibodies (≥10.0 mIU/ml), and 11.2% had low levels of these antibodies (1.0-9.9 mIU/ml). Compared to previous reports, our results show a lower percentage of anti-HBs antibodies, 33.8% of children age 4 years had protective anti-HBs antibodies, dropping to 18.4% by age 9 years. Among those anti-HBs seropositive, 9.1% were anti-HBc positive, indicating a natural infection with HBV. We found a small number of children, despite adequate immunization, developed HBV infection.

INTRODUCTION

Hepatitis B virus (HBV) infection is endemic in Southeast Asia and Africa, and is transmitted by parenteral routes, maternal-infant exposure, and horizontal spread between children (Merican *et al*, 2000). Almost all HBV-infected infants become carriers of HBV, and act as sources of the infection to their family and community. Such individuals are at significant risk for developing chronic hepatitis, liver cirrhosis and hepatocellular carcinoma. Thus, control of HBV infection and interruption of its spread are important public health goals. The most important method of prevention of HBV infection is the vaccination of newborns and children with hepatitis B (HB) vaccine. Studies showed that before immunization, about 20% of children under 5 had serologic evidence of HBV infection, rising to 70% by age 15 (Grossman *et al*, 1975). About 4% of non-immunized Thai children were carriers of hepatitis B (Luksamijarulkul *et al*, 1995), reaching 8% by adulthood (Grossman *et al*, 1975; Merican *et al*, 2000). Population-based studies have shown that the use of the HB vaccine in infants can reduce the HBV chronic carrier prevalence from high (>8%) to low (<2%) in immunized cohorts of children (Kane, 1998).

Thailand has undertaken a systematic approach toward control of HBV infection. Before systematic immunization, one study found that 5.7% of children acquired the infection in a one-year period (Kozik *et al*, 2000). The carrier rate in Thai children age 2-16 years was found to be 13% of those who were infected with HBV (Kozik *et al*, 2000). In 1989, the Thailand Ministry of Public Health (MOPH) established a pilot project of HB immunization in Chiang Mai and Chon Buri Provinces demonstrating that HB vaccine can be effectively administered along with other Expanded Program of Immunization (EPI) vaccines. In 1992, the Thai Government integrated the HB vaccine into the national EPI. Children receive 0.5 ml of HB vaccine intramuscularly within 7 days of birth, at 2 months and at 6 months of age. The HB vaccines given to older children varied with the year of inoculation. In 1989, a

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plasma-derived vaccine (the Cheil Suger, Korea), with a 3 μg per dose, was used. From 1990 to 1994, the plasma-derived vaccine produced by Korean Green Cross Corporation (Korea) was administered at 10 μg per dose. Since 1995, the 10 μg per dose of recombinant Engerix B® vaccine (SmithKline Biologicals, Belgium) has been used (Poovorawan *et al*, 2001). Long-term evaluation of this project in Chiang Mai, where the project began, has not been done. In this paper, we report the results of hepatitis B vaccination in Chiang Mai, Thailand, and estimate the efficacy of this program.

MATERIALS AND METHODS

Study population

From July 1998 to August 2000, children aged 4-9 years were randomly selected from 7 rural schools (377 children, including 185 males and 192 females) and 3 urban schools (303 children, including 147 males and 156 females) in Chiang Mai Province, northern Thailand. The purpose of the study was discussed with the parents or guardians, and written consent was obtained in all cases. A questionnaire was completed and personal vaccination records were reviewed. A total of 680 blood samples were obtained from the schoolchildren (332 males and 348 females). Serum samples were prepared on the day they were obtained, stored at 4ºC for not more than 3 days, or stored at -20ºC until tested.

Serologic studies

A qualified technician tested for the presence of HBsAg using the Monolisa Ag HBs second generation ELISA kit (Sanofi Diagnostic Pasteur, Manes la Coquette, France). Testing for anti-HBs antibodies was done using the Monolisa Anti-HBs 3.0 kit together with the 5 points calibration Monolisa Anti-HBs Standard (negative control, and 10, 50, 100, and 150 mIU/ml) (Sanofi Diagnostic Pasteur). All positive samples were retested, and all remained positive. The anti-HBs antibody titers were reported as the average value between the initial positive test and the repeated test. Levels above 10.0 mIU/ml were considered to be protective; anti-HBs and levels between 1.0-9.9 mIU/ml were considered to be low.

Data analysis

Data were analysed by determining the percentages of hepatitis B vaccine coverage and each viral marker obtained per population group. Prevalence among the different groups was compared using χ^2 or Fisher's exact test as appropriate. Results were considered statistically significant when $p < 0.05$.

RESULTS

Coverage of the HB vaccination program

Children aged 4-9 years should reflect the status of subjects born after the HB immunization program in Chiang Mai started in 1989.

Older age schoolchildren represented children at the start of the program. Younger age schoolchildren represent children under the current national EPI program. The children were divided into three groups: those who received a complete course of HB vaccination (*ie*, 3-5 doses of HB vaccine); those who received an incomplete course of HB vaccination (1-2 doses of HB vaccine) and those who did not received HB vaccination. This information was obtained by questionnaire along with review of the personal vaccination records. The results by age group and children school areas are presented in Fig 1. Of the 680 children, 447 (65.7%) had a history of complete HB vaccination, including 221 males (66.6%) and 226 females (64.9%); 26 (3.8%) had

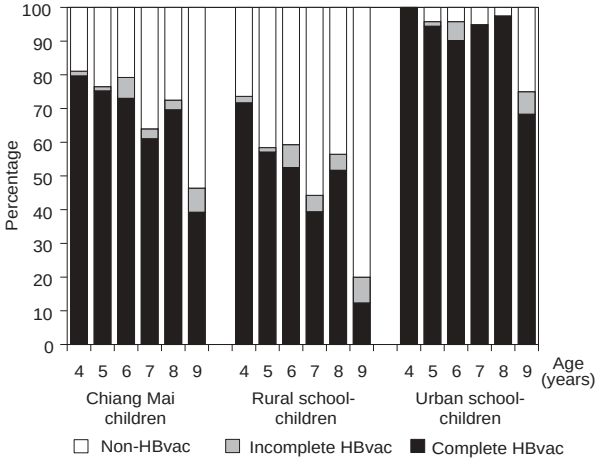

Fig 1–Coverage of hepatitis B vaccination in Chiang Mai children.

Table 1
HBsAg and anti-HBc positivity among Chiang Mai children by age and comparison between school areas.

Age (years)	HBsAg positive (%)			Anti-HBc positive (%)		
	Chiang Mai children	Rural school-children	Urban school-children	Chiang Mai children	Rural school-children	Urban school-children
4-5	1/223 (0.4%)	0/130 (0.0%)	1/93 (1.1%)	18/223 (8.1%)	16/130 (12.3%)	2/93 (2.2%)
6-7	2/230 (0.9%)	1/120 (0.8%)	1/110 (0.9%)	13/230 (5.7%)	5/120 (4.2%)	8/110 (7.3%)
8-9	5/227 (2.2%)	2/127 (1.6%)	3/100 (3.0%)	16/227 (7.0%)	10/127 (7.9%)	6/100 (6.0%)
Total	8/680 (1.2%)	3/377 (0.8%)	5/303 (1.7%)	47/680 (6.9%)	31/377 (8.2%)	16/303 (5.3%)

a history of incomplete HB vaccination, including 14 males (4.2%) and 12 females (3.4%). Children in urban schools showed a much higher prevalence in terms of HB vaccination than children in rural schools (89.1% *vs* 46.9% for a complete course, $p < 0.001$). This difference was not related to the sex of the children, $p = 0.722$. In urban schools, 87.8% of males and 90.4% of females received a complete course, and in rural schools, 49.7% of males and 44.3% of females did. The coverage of HB vaccination increased over time. For the oldest age group (9 years), 49/125 (39.2%) and the youngest age group (4 years), 59/74 (79.7%) had received a complete course of vaccination. Most of these children were from urban schools, 68.3% of the oldest age group (9 years) and 100% in the youngest age group (4 years) had undergone a complete course of vaccination, compared to 12.3% in the oldest age group and 71.7% in the youngest age group of children from rural schools.

Prevalence of HBV infection in Chiang Mai children

The results of testing for HBsAg and anti-HBc are presented in Table 1. The average prevalence rate of HBsAg in Chiang Mai schoolchildren age 4-9 years was 1.2% (8/680). There was a general increase in the prevalence rate with age, reaching 2.2% in the 8-9 years age group. The positive rate in males was 1.5% (5/332) and in females was 0.9% (3/348), which was not statistically different ($p = 0.496$). HBsAg positive rates in urban schoolchildren (1.7%) and rural schoolchildren (0.8%) were not significantly different, $p = 0.477$.

Another indicator of true infection by HBV is the development of anti-HBc antibodies. The overall prevalence of anti-HBc antibodies was 6.9% (47/680), with no significant difference between the sexes (6.9% in males and females, $p = 0.999$). When rural and urban children were considered separately, the prevalence in the rural children (8.2%) was not significantly different from urban children (5.3%), $p = 0.133$.

Surprisingly, all 5 HBsAg positive children from urban schools had a history of HB vaccination (two at 9 years of age, one at 7 years of age and one at 4 years of age had complete HB vaccination and one at 9 years of age had an incomplete HB vaccination). Most of them were anti-HBc positive, except the one 9 years of age who was anti-HBc negative (who had a history of complete HB vaccination). All 3 HBsAg positive children from rural schools were non-vaccinated and anti-HBc positive, two at 9 years of age and one at 6 years of age.

Efficacy of the vaccination program

A simple indicator of efficacy is the seroprevalence of anti-HBs antibodies. These values are presented in Fig 2. In our study group, we found that of those age 4-9 years, 178 (26.2%) had protective anti-HBs and 76 (11.2%)

Table 2
Anti-HBc positivity among Chiang Mai children with anti-HBs negative and positive by age groups and comparison between school areas.

Age (years)	Anti-HBc positive/Anti-HBs negative			Anti-HBc positive/Anti-HBs positive		
	Chiang Mai children	Rural school-children	Urban school-children	Chiang Mai children	Rural school-children	Urban school-children
4-5	8/119 (6.7%)	7/73 (9.6%)	1/46 (2.2%)	10/104 (9.6%)	9/57 (15.8%)	1/47 (2.1%)
6-7	5/152 (3.3%)	1/82 (1.2%)	4/70 (5.7%)	8/78 (10.3%)	4/38 (10.5%)	4/40 (10.0%)
8-9	11/155 (7.1%)	7/93 (7.5%)	4/62 (6.5%)	5/72 (6.9%	3/34 (8.8%)	2/38 (5.3%)
Total	24/426 (5.6%)	15/248 (6.0%)	9/178 (5.1%)	23/254 (9.1%)	16/129 (12.4%)	7/125 (5.6%)

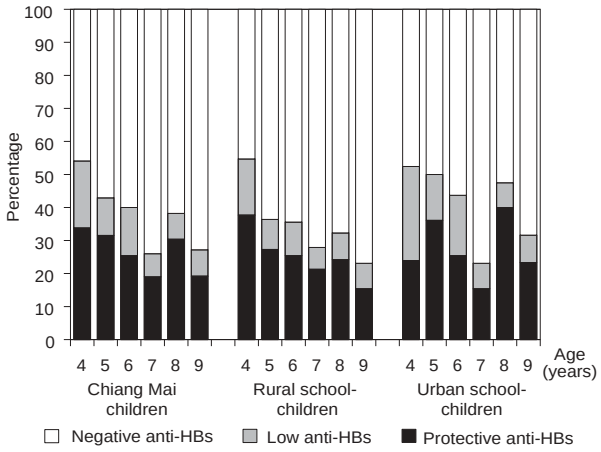

Fig 2–Seroprevalence of anti-HBs levels in Chiang Mai schoolchildren, by age and areas (rural *vs* urban).

had low levels of anti-HBs. The prevalence decreased with age, from 33.8% with protective anti-HBs and 20.3% with low levels of anti-HBs at age 4 years to 18.4% with protective anti-HBs and 8.0% with low levels of anti-HBs by age 9 years. No significant difference was observed between male and female children, p = 0.096. When separated into rural and urban groups, the seroprevalence of anti-HBs antibodies in urban children (28.1% with protective anti-HBs) was not significantly different from rural schoolchildren (24.7% with protective anti-HBs), p = 0.131.

Efficacy may be more accurately judged by the development of protective levels of anti-HBs antibodies (≥10 mIU/ml). Compared to the coverage with HB vaccination, the proportion of protective anti-HBs antibodies is quite low, demonstrated by Figs 1 and 2. The coverage of complete HB vaccination was 65.7%. The presence of protective anti-HBs in our study group was 26.2%. In the youngest age group (4 years) with 79.7% with complete HB vaccination, 33.8% had protective levels of antibodies. Even in the youngest age group (4 years) of urban schoolchildren with 100% complete HB vaccination, 23.8% had protective levels of antibodies.

The presence of anti-HBc antibodies indicates natural infection with HBV. The prevalence of anti-HBc in anti-HBs seronegative and anti-HBs seropositive individuals is presented in Table 2. The prevalence of anti-HBc among the anti-HBs seropositive group (9.1%) was not significantly different from the anti-HBs seronegative group (5.6%), p = 0.089. In the anti-HBs seropositive group, the prevalence of anti-HBc in rural schoolchildren was 12.4%, while in the urban schoolchildren it was 5.6%, which was not significantly different (p = 0.059).

DISCUSSION

From 1989 to 1992, the Thailand's HB immunization model program was conducted in Chiang Mai and Chon Buri Provinces and demonstrated that HB vaccine could be effectively administered along with other EPI vaccines. By the end of the project, overall coverage for complete HB immunization in Chiang Mai had

reached 93.1%. A minority of children received incomplete HB immunization or no immunization. HB vaccination was shown to provide over 80% protective efficacy against HBV infection (Chunsuttiwat *et al*, 1997). Stepwise expansion of HB vaccination evolved into a nationwide program in 1992. The HB vaccination coverage rate has been rapidly catching up with its EPI counterparts, with the coverage rate of the third dose ranging from 71.2-94.3% (Chongsrisawat *et al*, 2000; Poovorawan *et al*, 2001). We found that the coverage rates of Chiang Mai children age 4-9 years old, who should have received 3 doses of HB vaccine, was 65.7%. Some children received incomplete HB immunization (3.8%). In Chon Buri, after its integration into the EPI program, the complete HB vaccination rate was 71.2% and the incomplete HB vaccination rate was 12.9% (Poovorawan *et al*, 2001). There was a marked difference between the urban and rural environments. City children had a coverage rate of 89.1% compared to only 46.9% for rural children. These results are slightly less than those reported in other study. These numbers may be an underestimate, since the data were obtained by questionnaire and from health record booklets, and some of which had been lost, especially in older children and in rural schools.

The prevalence of HBsAg can be used as an indicator of true HBV infection, as opposed to anti-HBs antibodies, which develop following infection and immunization. Other studies have shown that the prevalence of HBsAg in non-immunized children is 3.64% (Luksamijarulkul *et al*, 1995), but this figure has dropped by 85% after the immunization program was instituted (Chongsrisawat *et al*, 2000), with a prevalence of 0.67% reported (Poovorawan *et al*, 2000). The current carrier rate in Thai children has been determined to be 0.55-7% (Chub-uppakarn *et al*, 1998; Poovorawan *et al*, 2000; 2001). In Chon Buri, after the integration of the HB vaccine into the EPI, the HBsAg positive rate was 0.7% (Poovorawan *et al*, 2001). In Chiang Mai, 1.2% of all children had circulating HBsAg in serum, a figure comparable to other studies. The presence of anti-HBc antibodies can also serve as an indicator of infection by HBV. Other studies have found that 5.5% of children 1-10 years old have anti-HBc antibodies (Poovorawan *et al*, 2000), compared to 6% of non-immunized children (Luksamijarulkul *et al*, 1995). In our study group, we found the prevalence rate among children 4-9 years old was 6.9%. While in Chon Buri, after the integration of HB vaccine into the EPI, the anti-HBc positive rate was 6.3% (Poovorawan *et al*, 2001).

The efficacy of immunization can be evaluated by measuring the levels of protective anti-HBs antibodies. By age 10, about 56% of immunized children have antibodies to HBsAg (Poovorawan *et al*, 2000), compared to 15% of non-immunized children (Luksamijarulkul *et al*, 1995). The prevalence rate is higher in younger children, with values of 94% at 0-2 years of age, dropping to 76% by 3-5 years of age (Chub-uppakarn *et al*, 1998). In our study, 33.8% of children 4 years old had protective anti-HBs antibodies, dropping to 18.4% by age 9 years. The prevalence of samples with anti-HBs antibodies declined as children got older, except for an increase in the 8-year old age group (30.4%). The pattern of anti-HBs present is similar to the pattern of the coverage of HB vaccination, by age and area (rural *vs* urban). Compared to previous reports, our results show a lower percentage of anti-HBs antibodies. Anti-HBs antibodies may be from natural infection with HBV. This was demonstrated by the fact that 9.1% of children with anti-HBs were anti-HBc positive.

In our study group, there were some apparently completely immunized children who had evidence of true HBV infection with circulating HBsAg and/or anti-HBc antibodies. Children in Thailand are not screened for previous HBV infection prior to immunization. The children with anti-HBc antibodies may well have acquired the natural infection before they were immunized. The three HBsAg positive children from rural schools were non-vaccinated and had anti-HBc as a result of natural infection. While the five HBsAg positive children from urban schools had a history of HB vaccination (four had complete HB vaccination and one had incomplete HB vaccination). Most of them were anti-HBc positive, except one who was anti-HBc negative (had a history of complete HB vaccination). The children with circulating HBsAg despite immuniza-

tion were true failures of vaccination. These children have the potential to become chronic carriers of HBV. These results imply failures or gaps in the immunization program. Our study did not uncover any specific source of the problem. Several factors, such as the commercial source of the vaccines, inadequate transportation and storage systems, and inefficient methods of administration might reduce the effectiveness of the vaccine. More recently, new variants of HBV have been reported that occur more frequently in vaccinated individuals (Theamboonlers *et al*, 2001). These viruses have critical amino acid differences that allow them to escape the host immune system and the protective effects of the vaccine.

Previous studies have reported seroprevalence based on any positive value for anti-HBs antibodies. In our study, we distinguished between positive serology for anti-HBs antibodies as protective levels (≥10.0 mIU/ml) and low levels (1.0-9.9 mIU/ml) of these antibodies. We found that, overall 26.2% of children had protective levels of anti-HBs antibodies, and 11.2% had low levels of these antibodies. After vaccination, the strongest antibody response was detected within the first year, and after approximately 5 years it decreased to low or undetectable levels in some individuals. Most studies in Thailand have suggested that a booster dose after the initial three doses is not necessary, and that immunologic memory provides adequate protection, even if levels of anti-HBs antibodies are below 'protective' levels (Chongsrisawat *et al*, 2000; Poovorawan *et al*, 2000). The presence of HBsAg/anti-HBc in vaccinated children from urban schools with high coverage under the HB vaccination program call for evaluation HBV infection after HB vaccination. Some children can become chronic carriers, despite adequate vaccination.

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