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Diphtheria, tetanus and pertussis immunity in newentrants of primary school, Angthong, Thailand

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The study of diphtheria, tetanus and pertussis immunity in children of age 6-8 years was done in 162 new entrants from 10 primary schools of Amphoe Muang, Angthong Province, during June-July 1988. It was found that the geometric means of antitoxins or antibody were 0.39 IU/ml to diphtheria, 0.53 IU/ml to tetanus and 1:25 to pertussis. The percentages of children with protective level were 96.91% for diphtheria, 98.15% for tetanus and 5.56% for pertussis. Of all children, 9.26% and 19.14% respectively had safety levels of diphtheria and tetanus antitoxins. Furthermore, the children were divided into 2 groups by the history of DTP immunization : 100 fully immunized (≥3 doses of DTP vaccine) and 52 partially or not immunized (0-2 doses of DTP vaccine). The immune statuses of 2 groups were compared and found that the means of antitoxins or antibody and the percentages of children with antitoxins (≥0.5 IU/ml) in the fully immunized children were significantly higher than those in the partially immunized children (P < 0.005). The distributions of antibodies and the percentages of children with protective and safety levels in the studied areas were also demonstrated. The low percentages of children with protective level against pertussis and the low titers of pertussis antibody showed that the booster doses of DTP vaccine prior to entering school might be important to enhance pertussis antibody in order to interrupt the transmission of pertussis in school age children.

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พิพัฒน์ ลักษมีจรัลกุล, เฟื่องฟ้า จารึกวงศ์สวัสดิ์. สภาวะภูมิคุ้มกันโรคคอตีบ บาดทะยัก และไอกรนของเด็กนักเรียนเข้าใหม่ โรงเรียนประถมศึกษา อำเภอเมือง จังหวัดอ่างทอง. จุฬาลงกรณ์เวชสาร 2534 มกราคม ; 35(1): 27-33

การศึกษาภูมิคุ้มกันโรคคอตีบ บาดทะยัก และไอกรน ของเด็กไทยอายุ 6-8 ปี ได้ดำเนินการในเด็กนักเรียนเข้าใหม่โรงเรียนประถมศึกษา อำเภอเมือง จังหวัดอ่างทอง 10 แห่ง จำนวน 162 ราย ระหว่าง เดือนมิถุนายน-กรกฎาคม 2531 พบว่า ระดับภูมิต้านพิษเฉลี่ยต่อโรคคอตีบ 0.39 IU/ml ต่อโรคบาดทะยัก 0.53 IU/ml และระดับแอนติบอดีเฉลี่ยต่อโรคไอกรน 1:25 ร้อยละของเด็กที่มีภูมิคุ้มกันเพียงพอในการป้องกันโรคคอตีบ ร้อยละ 96.91 โรคบาดทะยัก ร้อยละ 98.15 และโรคไอกรนร้อยละ 5.56 เด็กนักเรียนร้อยละ 9.26 และ 19.14 มีระดับภูมิต้านพิษต่อโรคคอตีบและบาดทะยัก ในระดับที่ปลอดภัยต่อโรค เด็กนักเรียนกลุ่มที่ได้รับวัคซีน ดีทีพี ครบถ้วน (≥ 3 ครั้ง) มีจำนวน 110 ราย และกลุ่มที่ได้รับวัคซีน ดีทีพี บางส่วน หรือไม่ได้รับ (0-2 ครั้ง) มี 52 ราย สภาวะภูมิคุ้มกันโรคของเด็ก 2 กลุ่ม แตกต่างกันอย่างมีนัยสำคัญทางสถิติ ($P < 0.005$) เฉพาะระดับภูมิคุ้มกันเฉลี่ย และร้อยละของเด็กที่มีระดับภูมิต้านพิษตั้งแต่ 0.5 IU/ml ขึ้นไป โดยเด็กนักเรียนที่ได้รับวัคซีนดีทีพีครบถ้วน มีสัดส่วนที่สูงกว่าเด็กนักเรียนที่ได้รับวัคซีนดีทีพีไม่ครบถ้วน สำหรับระดับแอนติบอดีต่อโรคไอกรน และร้อยละของเด็กนักเรียนที่มีระดับแอนติบอดีที่เพียงพอในการป้องกันโรคไอกรนมีระดับต่ำมาก แสดงให้เห็นถึงความจำเป็นที่จะต้องเน้นการฉีดวัคซีนดีทีพีกระตุ้นก่อนเข้าโรงเรียน เพื่อให้เด็กนักเรียนมีระดับภูมิคุ้มกันสูงขึ้น สามารถยับยั้งการแพร่กระจายของโรคในโรงเรียนได้

Diphtheria, tetanus and pertussis are EPI target diseases in Thailand. However the trend of these diseases, especially diphtheria and pertussis, have been decreasing morbidity rates. During 1980-1988,[1] there were several outbreaks in certain provinces of Thailand including Nakhonratchasima[2,3], Chiangrai[4], Phayoa[5], Samutprakan[6], Ratchaburi[7], Lampang[8] and Chiangmai.[9] DTP immunization in infants and young children is an important strategy in controlling the diseases. In recent years, the coverage of DTP vaccination (3 doses of DTP vaccine) has increased from 21.22% in 1982 to 75.09% in 1987[10], but in fact, some children were out of step with schedules of routine immunization and lacked the booster doses. The outbreaks of diphtheria and pertussis in the recent period were reported in children of primary schools[3,4] and the incidence rates shifted from pre-school to early school-age.[11,12] The older children whose naturally aquired immunity or by immunization-may have faded, may become ill with atypical diseases. These unrecognized patients may be the source of infection to others or even to younger siblings at home before immunization.[13,14] Therefore, the informations about the immunity status of the early school-age children are important for health planning in order to provide valuable enlightenment on ways that the present immunization schedules can be modified for suitable disease control.

Materials and methods

Studied population and studied design.

The cross-sectional study of immune status to diphtheria, tetanus and pertussis in new entrants of primary school was conducted in 162 children of age 6-8 years. The children were randomized, from 10 primary schools in Amphoe Muang, Angthong Province, during June-July 1988. Blood specimens were collected from the fingertips. The sera were separated and stored at-20°C until the assay of antibodies. The DTP vaccination status and some social informations were taken individually.

Methods for determination of antibodies

The antitoxins against diphtheria and tetanus were determined by an indirect ELISA adapted from Saunder and Clinard (1976).[15] The purified diphtheria and tetanus toxoid (1.22 Lf/ml and 1.62 Lf/ml) were used as coating antigens. The 1:500 dilution of peroxidase-conjugated goat anti-human polyvalent immunoglobulin (from chequerboard titration) was used, and OPD/H_2O_2 substrate was added. The reaction was allowed to proceed for 15 minutes at room temperature, then stopped with 4 N H_2SO_4. The optical density (OD) of the colored solution was measured by a spectrophotometer Minireader II at the wave length of 490 nm. Each assay included a high-positive serum (25 IU/ml or 1:25,600), low-positive serum (0.1 IU/ml or 1:200) and a negative serum used as control sera. The optical density values of the tested sera above 0.29 at the 1:200 dilution or $\geq$ 0.1 IU/ml (the upper limit of 95% confidence interval of the mean OD of low-positive sera) were considered positive for diphtheria and tetanus antitoxins.[16-19]

The pertussis antibodies in serum samples were tested by the microagglutination method (Manclark and Meade, 1980)[20] using vaccine strains of *B.pertussis* (phase I) as antigens. The standard anti-pertussis serum (1:1,280) provided by the Division of Biological Standards NIH, Bethesda, Maryland, USA and the normal rabbit serum were used as positive and negative control sera. The antibody titer of 1:320 or greater was considered for protective level against pertussis.[21,22]

Statistic analysis

The t-test was applied for significant difference of antibody titers and the proportional Z test was used for significant difference of percentages of immune children between the 2 groups of children. The critical level of $\alpha = 0.05$ was used for statistic significance.

Results

The immunities against diphtheria, tetanus and pertussis in 162 primary school new entrants, Amphoe Muang, Angthong, Thailand were analysed. Results showed that the geometric means of antitoxins or antibody were 0.39 IU/ml, 0.53 IU/ml and 1:25 against diphtheria, tetanus and pertussis, respectively. The percentages of children with protective level ($\geq$0.1 IU/ml) for diphtheria and tetanus were 96.91% and 98.15%. When the cut-off level of 0.5 IU/ml or greater was used, the percentages of protected children were reduced to 45.68% against diphtheria and 64.82% against tetanus (Table 1). The protection rate to pertussis was 5.56% when the titer of 1:320 or greater was used for protective level; 9.26% and 19.14% of children had safety levels ($\geq$1.0 IU/ml) for diphtheria and tetanus. Furthermore, the children were divided into 2 groups by the history of DTP vaccination, 110 fully immunized ($\geq$3 doses of DTP vaccine) and 52 partially or none immunized children (0-2 doses of DTP vaccine). The immune statuses of the 2 groups against diphtheria, tetanus and pertussis are demonstrated in Table 1. The geometric means titer of antitoxins or antibody and the percentages of children with antitoxins $\geq$ 0.5 IU/ml in fully immunized children were significantly higher than those in partially immunized children ($P<0.005$).

Table 1. Immune status to diphtheria, tetanus and pertussis in new entrants of primary school, Amphoe Muang, Angthong Province.

Variables	History of DTP Immunization	No. of Children	Immune status of children against		
			Diphtheria	Tetanus	Pertussis
Geometric Mean Titers (95% Confidence interval)	Fully immunized	110	0.45 IU/ml* (0.13-1.62)	0.61 IU/ml* (0.15-2.42)	1:33* (1:2-1:825)
	Partially immunized	52	0.30 IU/ml (0.07-1.35)	0.40 IU/ml (0.07-2.27)	1:13 (1:1-1:208)
	Total	162	0.39 IU/ml (0.10-1.58)	0.53 IU/ml (0.11-2.47)	1:25 (1:1-1:625)
% of children with protective level[1]	Fully immunized	110	98.18	99.09	6.37
	Partially immunized	52	94.23	96.15	3.84
	Total	162	96.91	98.15	5.56
% of children with antitoxin ≥ 0.5 IU/ml	Fully immunized	110	55.45*	73.64*	
	Partially immunized	52	24.99	46.15	ND
	Total	162	45.68	64.82	
% of children with safety level[2]	Fully immunized	110	9.09	21.82	
	Partially immunized	52	9.61	13.46	ND
	Total	162	9.26	19.14	

*Statistically significant difference between fully immunized children and partially immunized children at $\alpha = 0.05$
[1]Protective level for diphtheria and tetanus antitoxins ≥ 0.1 IU/ml and for pertussis titer ≥ 1:320
[2]Safety level for diphtheria and tetanus antitoxins ≥ 1.0 IU/ml
ND = Not done

The distribution of antibodies in the studied areas (sub-districts) is also demonstrated (Table 2). The geometric mean of diphtheria antitoxin in children at Ban-ru (0.55 IU/ml) was the highest and that at Mahatthai subdistrict (0.30 IU/ml) was the lowest. The antitoxin to tetanus in various areas was rather higher than to diphtheria, and most of them had the level of 0.5 IU/ml or greater. On the other hand, pertussis agglutinin was rather low in titer in every sub-district. The lowest titer (1:9) was demonstrated at Ban-it and the highest titer (1:64) at saladaeng subdistrict (Table 2).

Table 2. The geometric means of diphtheria and tetanus antitoxins and pertussis agglutinin, in the studied areas.

Studied areas (Subdistricts)	No. of children	GMT (95% Confidence interval) of antitoxin or antibody to disease		
		Diphtheria (IU/ml)	Tetanus (IU/ml)	Pertussis (Titer)
Huaphai	33	0.39 (0.11-1.41)	0.60 (0.15-2.51)	1:25 (1:2-1:398)
Ban-ru	21	0.55 (0.20-1.51)	0.63 (0.22-1.82)	1:35 (1:2-1:871)
Ban-it	21	0.31 (0.05-1.78)	0.33 (0.08-1.32)	1:9 (1:1-1:81)

Table 2 (Continued)

Studied areas (Subdistricts)	No. of children	GMT (95% Confidence interval) of antitoxin or antibody to disease		
		Diphtheria (IU/ml)	Tetanus (IU/ml)	Pertussis (Titer)
Pa-nguai	20	0.44 (0.15-1.26)	0.65 (0.16-2.57)	1:23 (1:2-1:363)
Mahatthai	26	0.30 (0.06-1.51)	0.41 (0.07-2.57)	1:20 (1:2-1:316)
Ban-hae	20	0.39 (0.07-2.14)	0.53 (0.07-3.98)	1:25 (1:1-1:631)
Saladaeng	21	0.47 (0.19-1.17)	0.67 (0.23-1.95)	1:64 (1:2-1:2344)
Total	**162**	**0.39 (0.10-1.58)**	**0.53 (0.11-2.47)**	**1:25 (1:1-1:625)**

The percentages of children with protective and safety levels against diphtheria, tetanus and pertussis distributed in the studied areas are shown in table 3. Most of them had antitoxin levels of 0.1 IU/ml or greater, but some (3.03-20.00% to diphtheria and 4.76-25.00% to tetanus) had the safety level (≥1.0 IU/ml). Low percentages of children (0-23.81%) had the protective titer against pertussis (≥ 1:320).

Table 3. Number and percentages of children with antitoxins or antibody against diphtheria, tetanus and pertussis in the studied areas (subdistricts).

Studied areas (subdistricts)	No. of children	No. of children with antitoxin or antibody levels				
		Diphtheria		Tetanus		Pertussis
		≥0.1IU/ml*	≥1IU/ml**	≥0.1IU/ml*	≥1IU/ml**	≥1:320***
Hua-phai	33	32 (96.97%)	1 (3.03)	33 (100.00)	6 (18.18)	0 (0.00)
Ban-ru	21	21 (100.00)	3 (14.29)	21 (100.00)	5 (23.81)	2 (9.52)
Ban-it	21	20 (95.24)	3 (14.29)	20 (95.24)	1 (4.76)	0 (0.00)
Pa-nguai	20	20 (100.00)	1 (5.00)	20 (100.00)	5 (25.00)	1 (5.00)
Mahatthai	26	24 (92.31)	2 (7.69)	24 (92.31)	4 (15.39)	0 (0.00)
Ban-hae	20	19 (95.00)	4 (20.00)	20 (100.00)	5 (25.00)	1 (5.00)
Saladaeng	21	21 (100.00)	1 (4.76)	21 (100.00)	5 (23.81)	5 (23.81)
Total	**162**	**157 (96.91)**	**15 (9.26)**	**159 (98.15)**	**31 (19.14)**	**9 (5.56)**

*Protective levels of antitoxin to diphtheria and tetanus
**Safety levels of antitoxin to diphtheria and tetanus
***Protective titer of antibody to pertussis

Discussion

The present study demonstrated that immunities to diphtheria and tetanus in Thai rural children of age 6-8 years, at primary schools are adequate. They showed that the percentages of children who had the protective levels of 0.1 IU/ml or greater to diphtheria and tetanus were 96.91% and 98.15% respectively. However, the protective rates were reduced to 45.68% against diphtheria and 64.82% against tetanus when the antitoxin of 0.5 IU/ml or greater were used for protective levels as in the study of Chasangbong (1988).[23] The previous study of Pechclai et al (1978) showed 89.4% of fully DTP immunized children and 40% of partially DTP immunized children who visited Ramathibodi Hospital had adequate immunity to diphtheria and tetanus.[24] This study reported that the percentages of children with protective levels of diphtheria and tetanus antitoxins ($\geq$0.1 IU/ml) in the fully immunized and partially immunized children were not significantly different ($P > 0.05$). However it was significantly different ($P < 0.005$) when the percentages of children with antitoxins $\geq$ 0.5 IU/ml were compared. The geometric means of antitoxin levels against diphtheria and tetanus in fully DTP immunized children (0.45 IU/ml and 0.61 IU/ml, respectively) were significantly higher than those in partially DTP immunized children (0.30 IU/ml and 0.40 IU/ml, respectively), $P < 0.005$. The mean of diphtheria antitoxins in all children (0.39 IU/ml) was lower than in the previous studies in Chainat Province (0.94 IU/ml)[25] and Mukdahan Province (0.86 IU/ml).[23] It may have been due to the natural booster of diphtheria infection in those endemic areas.

The immunity to pertussis is frequently inadequate, 6.37% for fully DTP immunized children and 3.84% for partially DTP immunized children. The geometric mean of pertussis agglutinins in fully immunized children (1:33) was significantly higher than that in partially immunized children ($P < 0.005$) as in the study at Bua-Yai Community, Nachonratchasima Province.[26] Although, the protective level of pertussis was reported at 1:320 or greater, the lower titers ($\leq$1:160) did not indicate susceptibility.[21,22] Evidences showed that children with titer of less than 1:320 were not ill when they contacted pertussis patients. The determination of pertussis agglutinin was only an indirect measurement of pertussis immunity.[20] However, most previously vaccinated individuals were susceptible a few years after receiving the 3 doses of DTP vaccine.[27] In the recent period, school-age children were the most common source of pertussis infection to younger siblings at home. Therefore, the booster doses of DTP vaccine prior to entering school may be important to enhance the immunity in order to interrupt this chain of transmission.

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