Original

Diphtheria-antitoxin level and effect of the immunization with the toxoid in Chiang Mai in 1977~1982

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Summary

Seroepidemiology of diphtheria in Chiang Mai, North Thailand through 1977 to 1982 and development of the antitoxin in the blood of children immunized with the toxoid were studied. Antitoxin titers of 763 sera with unknown immunization history and of 1,059 sera with known immunization history were determined by using the microneutralization-cell culture method.

Among persons with unknown immunization history, the percentage of the antitoxin-positive in 10~14 years old subjects in 1979 was higher, as compared with the same age group in 1977, while that in 20 or more years old subjects in 1977 and 1980 were lower than in 1979. Percentage of the antitoxin-positive sera increased according to age. Geometric mean titer of the antitoxin-positive sera in 15~19 year-old subjects was higher in 1979 than in 1977, and higher also than in 5~9 year-old subjects in 1979.

All of the children being immunized in accordance with strict regulations of the Comprehensive Child Care Clinic, Chiang Mai University had the positive antibody. In experiments with this group, a significant increase in the antitoxin titer was observed one month after the booster immunization, and a gradual decrease of the antitoxin titer was observed through 1 to 37~71 months after the last booster immunization in the group of 5~9 years old. Decline of the titer was not observed in the group of 10~17 years old through 16~100 months after the last booster immunization, and the geometric mean titers of this group were 0.580 or more IU/mL.

In Wat Kau Khum school in the suburbs of Chiang Mai, one shot of the immunization produced the seroconversion in 88.2% of the children, and the geometric mean anti-
toxin titer of the antibody-positive sera was 4.491 IU/ml after the one shot.

**Key words:** Diphtheria, toxoid, antitoxin, seroepidemiology, Thailand

**Introduction**

South-East Asia is referred to as a reservoir of diphtheria which is a major infectious disease in Thailand[1-3], whereas the case number of the disease has greatly decreased in developed countries during the last two decades[4-6]. Immunization with diphtheria toxoid has been carried out for nearly 30 years therewith markedly reducing diphtheria-related morbidity and mortality. However, Petchlai et al.[7] demonstrated that diphtheria and tetanus antitoxin levels in the maternal and cord blood of Thai infants were frequently too low for protection against the disease.

In this paper, seroepidemiology of diphtheria in Chiang Mai, North Thailand and development of the antitoxin in the blood of children immunized with the toxoid were studied.

**Materials and Methods**

(1) Human sera

In Chiang Mai area 1,822 serum specimens were collected in 1977~1982. They are shown in Table 1. Of them 763 are from those with unknown immunization history to diphtheria and 1,059 from those with known immunization history. Of the latter group, 774 were from the children, who had been enrolled in Comprehensive Child Care Clinic (CCCC) since their birth. In no cases, clinical signs of diphtheria were manifest.

(2) Vaccine

Vaccines used were the Adsorbed Diphtheria-Tetanus-Pertussis (DTP) Vaccine produced by Swiss Serum and Vaccine Institute, Berne and the Adsorbed DT Toxoid produced by the Department of Pharmacy, Yod-Se, Bangkok. DTP was administered to children younger than 6 years old, and DT was given to children aged 6 or more years. Immunization was administered intramuscularly in a dose of 0.5 ml.

(3) Serologic test

Diphtheria-antitoxin neutralization titers in the sera were determined by a modification of the microneutralization-cell culture-method developed by Miyamura et al.[8,9]. Briefly, eight MCD (minimum cytopathic doses) of diphtheria toxin in 25 µl was added to 25 µl of serum dilution on the transfer
plate. After the incubation at room temperature for one hour, the toxin-serum mixture was transferred to the microculture plate medium. Then, Vero cell suspension (2×10⁴ cells/50 µl) was added to each well. The monolayer culture of Vero cells on the plate was observed microscopically for the cytopathic effect during four to five days’ incubation at 37°C in 5% CO₂. The culture medium comprised of Eagle’s minimum essential medium with a decreased (1/2) amount of sodium bicarbonate and 2% calf serum. In all the neutralization tests, neutralization of the toxin by a series of dilution of a standard antitoxin was carried out to calculate the International Diphtheria Antitoxin Unit (IU) per ml of the test samples. The diphtheria toxin (Lot M-46; 16,000 MCD/ml) and the standard antitoxin (10 IU/ml) were kindly supplied by Dr. S. Kondo, NIH, Tokyo. In this study, 0.02 IU/ml or more was tentatively referred to as the positive antibody, though Nelson et al. stated that 0.1 IU of the antitoxin per ml was a protective concentration of antibodies¹¹,¹².

(4) Statistics

Chi-square analysis was used to compare discrete variables, and the Student’s t test to determine the significance of differences between means.

Results

1. Diphtheria-antitoxin level with unknown immunization history

Seven hundreds and sixty-three sera collected during 1977 through 1982 were tested in this study. In this group, precise record of the artificial diphtheria immunization was not available, but the specimens could represent average population in Chiang Mai area which covered from the cord blood to adults of 20 or more years old.

Percentage of sera containing a protective level of antibody (0.02 IU/ml) in different years and age groups were showed in Table 2. Statistically significant differences (p<0.05) were observed between 10~14 years old in 1977 and 10~14 years old in 1979; 20 or

<table>
<thead>
<tr>
<th>Year of collecting sera</th>
<th>Age of year</th>
<th>Number of sera</th>
<th>Antitoxin-positive</th>
<th>Number</th>
<th>%</th>
<th>GM</th>
<th>log₁₀(GM×100)+SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1977</td>
<td>5~9</td>
<td>3</td>
<td>3</td>
<td>100</td>
<td>0.101</td>
<td>3.333±2.082</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10~14</td>
<td>64</td>
<td>42</td>
<td>65.6</td>
<td>0.147</td>
<td>3.881±2.155</td>
<td></td>
</tr>
<tr>
<td></td>
<td>15~19</td>
<td>53</td>
<td>41</td>
<td>77.4</td>
<td>0.145</td>
<td>3.854±1.667</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20~</td>
<td>73</td>
<td>68</td>
<td>93.2</td>
<td>0.113</td>
<td>3.550±1.419</td>
<td></td>
</tr>
<tr>
<td>1978</td>
<td>5~9</td>
<td>54</td>
<td>30</td>
<td>55.6</td>
<td>0.197</td>
<td>4.300±2.136</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10~14</td>
<td>86</td>
<td>63</td>
<td>73.3</td>
<td>0.215</td>
<td>4.429±1.663</td>
<td></td>
</tr>
<tr>
<td>1979</td>
<td>CB</td>
<td>68</td>
<td>54</td>
<td>79.4</td>
<td>0.182</td>
<td>4.194±1.897</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0~4</td>
<td>14</td>
<td>7</td>
<td>50.0</td>
<td>0.301</td>
<td>4.929±2.573</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5~9</td>
<td>73</td>
<td>45</td>
<td>61.6</td>
<td>0.148</td>
<td>3.889±1.761</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10~14</td>
<td>154</td>
<td>122</td>
<td>79.2</td>
<td>0.210</td>
<td>4.394±2.025</td>
<td></td>
</tr>
<tr>
<td></td>
<td>15~19</td>
<td>7</td>
<td>5</td>
<td>71.4</td>
<td>0.463</td>
<td>5.600±1.557</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20~</td>
<td>62</td>
<td>47</td>
<td>75.8</td>
<td>0.163</td>
<td>4.032±1.730</td>
<td></td>
</tr>
<tr>
<td>1982</td>
<td>20~</td>
<td>52</td>
<td>48</td>
<td>92.3</td>
<td>0.122</td>
<td>3.604±2.394</td>
<td></td>
</tr>
</tbody>
</table>

GM : Geometric mean antitoxin titer, SD : Standard deviation
Table 3  Diphtheria-antitoxin level with known immunization history in Chiang Mai

<table>
<thead>
<tr>
<th>Project</th>
<th>Age of year</th>
<th>Number of sera</th>
<th>History of immunization</th>
<th>Antitoxin positive</th>
<th>Time of collecting sera</th>
<th>Month after the last immunization</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (TF)</td>
<td>0</td>
<td>12</td>
<td>No</td>
<td>2</td>
<td>16.7 0.135</td>
<td>Mar. 1980</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>2 shots of P</td>
<td></td>
<td>5</td>
<td>71.4 0.735</td>
<td>Aug. 1980</td>
</tr>
<tr>
<td>2 (CCCC)</td>
<td>2~10</td>
<td>45</td>
<td>PI - P  I  II III</td>
<td>45</td>
<td>100 0.540</td>
<td>Aug. 1980</td>
</tr>
<tr>
<td></td>
<td>3~11</td>
<td>19</td>
<td>PI  II - P  I  II III</td>
<td>19</td>
<td>100 0.889</td>
<td>Aug. 1981</td>
</tr>
<tr>
<td></td>
<td>4~12</td>
<td>16</td>
<td>P I  II  III</td>
<td>16</td>
<td>100 0.474</td>
<td>Aug. 1982</td>
</tr>
<tr>
<td>3 (WKK)</td>
<td>8~14</td>
<td>102</td>
<td>1 shot</td>
<td>90</td>
<td>88.2 4.91</td>
<td>Aug. 1980</td>
</tr>
<tr>
<td></td>
<td>9~14</td>
<td>89</td>
<td>1 shot</td>
<td>87</td>
<td>97.8 1.066</td>
<td>Aug. 1981</td>
</tr>
<tr>
<td></td>
<td>10~15</td>
<td>75</td>
<td>1 shot</td>
<td>73</td>
<td>97.3 0.593</td>
<td>Aug. 1982</td>
</tr>
<tr>
<td>4 (CCCC)</td>
<td>5~7</td>
<td>37</td>
<td>PI  I II</td>
<td>37</td>
<td>100 1.144</td>
<td>Aug. 1981</td>
</tr>
<tr>
<td></td>
<td>5~8</td>
<td>30</td>
<td>PI  II - P  I  II III</td>
<td>30</td>
<td>100 6.912</td>
<td>Sep. 1981</td>
</tr>
<tr>
<td></td>
<td>6~8</td>
<td>15</td>
<td>P I  II  III</td>
<td>15</td>
<td>100 0.305</td>
<td>Aug. 1982</td>
</tr>
<tr>
<td>5 (DT)</td>
<td>5~9</td>
<td>405</td>
<td>P I  II - P  I  II III</td>
<td>405</td>
<td>100 1.101</td>
<td>Aug. 1982</td>
</tr>
<tr>
<td></td>
<td>10~17</td>
<td>207</td>
<td>P I  II  III</td>
<td>207</td>
<td>100 0.916</td>
<td></td>
</tr>
</tbody>
</table>

a) 44 children received the IInd booster 11 to 38 months before Aug. 1980, and one child received the IIIrd booster two months before Aug. 1980. b) All of the 19 children received the IIIrd booster 12 months before Aug. 1981. c) 12 children who did not respond to the first shot received one more shot in Dec. 1980. d) P: Primary immunization. I: First booster. II: Second booster. III: Third booster. e) GM: Geometric mean antitoxin titer. SD: Standard deviation.

more years old in 1977 & in 1982 and 20 or more years old in 1979; 10~14, & 15~19 years old in 1977 and 20 or more years old in 1977; 5~9 years old in 1978 and 10~14 years old in 1978; 0~4 & 5~9 years old in 1979 and 10~14 years old, & cord blood in 1979.

Geometric mean titers of the antibody-positive sera varied from 0.101 to 0.483 IU/mL among different years and age groups. Significant differences (p<0.05) were observed between 15~19 years old in 1977 and 15~19 years old in 1979; 5~9 years old in 1979 and 15~19 years old in 1979.

2. Diphtheria-antitoxin level with known immunization history

This experiment included ① 774 serum samples from 694 children under the care of CCCC since their birth at Chiang Mai University Hospital, ② 19 samples from 12 infants of 0 year of age at Tumbon Faham (TF), and ③ 266 samples from 102 pupils at Wat Kau Khum school (WKK) as shown in Table 3.

(1) Diphtheria-antitoxin level at CCCC

All of the serum samples contained the protective level of the antitoxin. Geometric mean antibody titers were 0.305 to 6.912 IU/mL as illustrated in Project 2, 4, and 5 of Table 3.

Serum samples of Project 2 were

Table 4 Immunization schedule to diphtheria in CCCC

<table>
<thead>
<tr>
<th>Immunization</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary immunization</td>
<td>2, 4, and 6 months after birth</td>
</tr>
<tr>
<td>1st booster</td>
<td>1.5 years of age</td>
</tr>
<tr>
<td>2nd booster</td>
<td>3 years of age</td>
</tr>
<tr>
<td>3rd booster</td>
<td>6 years of age</td>
</tr>
</tbody>
</table>
collected in 1980, 1981 and 1982 from the same children as shown in Table 3. Of 45 children studied in 1980, 19 appeared for blood drawing in 1981, and 16 appeared in 1982. Significant difference of the geometric mean antitoxin titer was not observed between serum samples collected in 1980, 1981, and 1982. This means that there was no significant change of the antibody titer between just before and one or two years after the booster immunization, because the children received booster immunizations just after collecting serum in August, 1980.

Serum samples of Project 4 were collected in August and September, 1981, and August, 1982 from the same children. Thirty seven children received the booster immunization in August, 1981. The serum samples were obtained from 30 out of the 37 children in September, 1981, and from 15 out of them in August, 1982. Serum samples collected one month after the booster immunization showed a significantly higher geometric mean antitoxin titer than either before the immunization in August, 1981 (p<0.01) or one year after the immunization in 1982 (p<0.001).

Serum samples of Project 5 were collected from 612 children and teen-agers in August, 1982. Significant difference was not observed between two different age groups described in Table 3.

Even except serum samples collected one month after the booster immunization which showed as high as 6.912 IU/ml of the geometric mean antitoxin titer, geometric mean antitoxin levels of children under the care of CCCC ranged from 0.305 to 1.144 IU/ml, as far as the grouping of the samples in this study was concerned. Actually the lowest titer of the individual specimens was 0.04 and the highest was 10.24 or more IU per ml. High levels of the antitoxin of more than 10 IU/ml were observed in 37 of 106 inoculated persons in Copenhagen (2).

(2) Effect of primary immunization

Infants aged 0 year at Tumbon Faham received two shots of DTP vaccine in March and June, 1980, and blood was drawn before the first shot in March and two months after the second shot. Antitoxin levels are shown in Project 1 of Table 3. Significant increase of percentage of the antibody positive sera was observed after the two shots of the immunization (p<0.05), but 2 out of 7 sera after the immunization did not contain the protective level of the antibody. Geometric mean antitoxin titer of the antibody positive sera collected two months after the two shots was 0.735 IU/ml.

(3) Effect of a single shot of the immunization

Seventy-five children at Wat Kau Khum school (WKK) in the suburbs of Chiang Mai did not have any record of the artificial immunization until July, 1980, when an epidemic of diphtheria was observed in Northern Thailand (2). They received one shot of DT vaccine in July, 1980, and blood specimens taken in August, 1980, 1981, and 1982 were tested for the diphtheria antitoxin.

Results are summarized in Table 3 as project 3. The majority (88.2%) of the children had 4.491 IU/ml of geometric mean titer of the antitoxin one month after the one shot of the immunization. Geometric mean antitoxin titer of the antitoxin-positive indivi-
duals decreased to 1.066 and 0.593 IU/ml one and two years after the single shot, respectively (p < 0.005).

Out of 12 children who did not have 0.02 IU/ml of the antitoxin one month after the single shot, nine (75%) acquired 0.16–0.4 IU/ml of antitoxin after a reimmunization in December, 1980 and still had 0.04–0.32 IU/ml in August, 1982, but two out of the 12 children did not respond to the reimmunization, (one child did not appear).

3. Duration of the antitoxin titer after the scheduled immunization

Children under the control of CCCC received the immunization in accordance with the schedule described in Table 4 and 100% of them had 0.02 or more IU/ml of antitoxin in their sera.

Six hundred and twelve children included in Project 5 (DT) (Table 3) were analyzed for the duration of the antitoxin level after the last immunization.

Results are described in Table 5. In the case of the children aged 5 to 9 years old, the antitoxin titer steadily decreased from 5–12 to 37–71 months after the last immunizations (p < 0.001–0.05), but 0.784 IU/ml of the geometric mean antitoxin titer was maintained after 37–71 months. In the case of the group aged 10 to 17 years old, no significant decrease of the antitoxin titer was observed at different intervals (16–24 to 61–100 months) after the last immunization, and the geometric mean titers were 0.580 or more IU/ml.

Discussion

The diphtheria-antitoxin level was tested by using the micro cell culture method on 1,222 serum samples, collected by the Department of Preventive and Community Medicine, Chiang Mai University and the Society for Medical Research in South-East Asia, Nippon Medical School (Chairman, Prof. A. Munakata) in Chiang Mai, Thailand through 1977 to 1982. The volunteers covered a variety of ages and immunization histories.

Among persons with unknown immunization history, percentage of the antitoxin-positive sera in 10–14 years old subjects increased from 1977 to 1979, while that in 20 or more
years old subjects in 1977 and 1980 were lower than in 1979. This suggests a prevalence of the disease among children through 1977 to 1979. Percentage of the antitoxin-positive sera increased according to age, i.e. from 0 year of age (16.7%, Table 3) to 5~9 years (50.0%), 10~14 years (65.6~79.2%), 15~19 years (71.4~77.4%), and 20 or more years as well as cord bloods (75.8~93.2%) (Table 2). These figures for adults and cord blood are higher than those reported by Petchlai et al. Namely according to them, 57.8% of mothers and 31.2% of cord blood had adequate antibody levels (≥0.03 Lf unit/ml). But our results for school children with unknown immunization history agree with those reported by Petchlai et al. Geometric mean titer of the antibody-positive sera in 15~19 year-old subjects was higher in 1979 than 1977, and higher also than in 5~9 year-old subjects in 1979. These results also suggest a prevalence of the disease among teen-agers during the period of 1977 to 1979.

This study included 774 serum samples from children being immunized in accordance with strict regulations of the CCCC, Chiang Mai University. All of them had the positive antibody. In experiments with this group, a significant increase of the antitoxin titer was observed one month after the booster immunization, and the gradual decrease of the antitoxin titer was observed through 1 to 37~71 months after the last booster immunization in the group of 5~9 years old. Necessity of the reinforcing immunization to maintain adequate titers of diphtheria antitoxin in children up to 15 years of age was suggested by experiments in Britain. Decline of the titer was not observed in the group of 10~17 years old through 16~100 months after the last booster immunization, and the geometric mean titers were 0.580 or more IU/ml. Nelson et al. stated that 0.1 IU of the antitoxin per ml was a protective concentration of the antibody. This suggests that the immunization schedule against diphtheria was sufficient in the CCCC, and no adverse reactions were reported among the children of the group despite of the omission of the Molonie test. But Rubin et al. encouraged booster immunization at 10 year intervals to maintain immunity.

In Wat Kau Khum school in the suburbs of Chiang Mai, one shot of the immunization produced the seroconversion in 88.2% of the children, and the geometric mean antitoxin titer of the antibody-positive sera was as high as 4.491 IU/ml after the one shot. This suggests a limitation of the effect of one shot, and a prevalence of the disease in the group before the immunization. A low rate (less than 25%) of seroconversion after one shot of the toxoid was reported also in developed countries. Rubin et al., who tested sera from elderly Americans (mean age 80 years) found that before the toxoid injection 59% of them had protective antitoxin levels (≥0.01 AU/ml), after one dose the percent protected rose to 88, and after the second dose all persons had protective levels.

This study was supported in part by a grant from the International Medical Foundation of Japan. Authors wish to express their thanks to Dr. Nadhirat Sangkawibha who kindly supplied serum specimens kept at the Virus Research Institute, Bangkok.

References


(Received for publication, May 16, 1985)