ORIGINAL ARTICLE

Decline of Tetanus Antitoxin Level with Age in Taiwan

*Chi-Jung Wu,[1,3] Huei-Chen Ko,[4] Hsin-Chun Lee,[1,2,3] Wei-Chuan Tsai,[2,3] Min-Gi Li,[5] Yu-Zhen Pao,[5] Nan-Yao Lee,[3] Chia-Ming Chang,[3] Hsin-I Shih,[3] Wen-Chien Ko[2,3]**

Background/Purpose: Tetanus is caused by *Clostridium tetani*, and is a vaccine-preventable infectious disease. The purpose of this study was to investigate the degree of protective tetanus immunity among adolescents and adults in Taiwan, which may provide valuable information for recommendations for tetanus vaccination strategy.

Methods: Individuals aged 16 years or older who were visiting a local hospital for health examinations were invited to participate in the study. Participants' serum levels of tetanus antitoxin were measured. A standard questionnaire was used to collect demographic data and information about risk factors. The prevalence of protective tetanus immunity in various age groups was described and sociodemographic factors that potentially influenced the degree of tetanus immunity were analyzed.

Results: Overall, 326 persons were included. Of these, 217 (67%) had never received a toxoid booster, while 109 (33%) had received a booster at least once. Among all participants, 95% had protective tetanus antitoxin levels (≥ 0.11 IU/mL), and 60% had protective antitoxin levels without the need of an immediate booster, i.e. ≥ 0.51 IU/mL. Among 70 participants aged > 60 years, 89% had protective antitoxin levels ≥ 0.11 IU/mL, and 31% had protective antitoxin levels ≥ 0.51 IU/mL. Tetanus antitoxin levels declined with age. Male gender, birth after 1955, and prior receipt of toxoid booster(s) were independently associated with protective tetanus immunity (≥ 0.51 IU/mL) by multivariate analysis. Compared with those without tetanus toxoid boosters, individuals with a prior booster had higher antitoxin levels. The percentage of people with protective immunity declined if the interval between the last toxoid booster increased.

Conclusion: Waning immunity to tetanus was observed after primary tetanus vaccination or toxoid booster. The public health policy that one dose of toxoid booster after primary vaccination should be emphasized for continuing protection against tetanus. [*J Formos Med Assoc* 2009;108(5):395–401]

Key Words: seroprevalence, Taiwan, tetanus, tetanus antitoxin, vaccine

Tetanus is a vaccine-preventable, global infectious disease caused by *Clostridium tetani*, with significant morbidity and mortality. The global status of serological immunity against tetanus varies between countries as a result of different national vaccination policies and methods, and the criteria used for determination of serum levels of tetanus antitoxin. For example, protective immunity was noted in 15.7% of people in Turkey and 23–32% of those in Denmark aged > 60 years, while 53–80% of people aged > 60 years residing in the United States, England, Wales and Sweden, and < 75% of Australians aged > 50 years were immune to tetanus.[1–6]

[1]Graduate Institute of Clinical Medicine and [2]Department of Medicine, National Cheng Kung University, Medical College; [3]Department of Internal Medicine, National Cheng Kung University Hospital, Tainan; Departments of [4]Internal Medicine and [5]Pathology, National Cheng Kung University Hospital, Douliou Branch, Taiwan.

Received: July 20, 2008
Revised: September 24, 2008
Accepted: November 2, 2008

***Correspondence to:** Dr Wen-Chien Ko, Department of Internal Medicine, National Cheng Kung University Hospital, 138 Sheng Li Road, Tainan 704, Taiwan.
E-mail: winston@mail.ncku.edu.tw

Tetanus toxoid for post-traumatic prophylaxis was introduced in Taiwan after 1953. In Taiwan, according to the National Children's Vaccine Program, diphtheria–pertussis–tetanus (DPT) vaccine was given routinely to 2-, 4-, 6- and 18-month-old children as primary vaccination since 1955. As a result of implementation of DPT vaccination programs and improvement in obstetrical practices and neonatal care, tetanus has occurred mainly in people older than 65 years, rather than neonates or children, in recent decades.[7,8] From 2000 to 2005, according to data from the Centers of Disease Control (CDC) Taiwan, there were 102 reported cases of tetanus.[9] Patients aged ≥ 60 years accounted for 60% of tetanus cases. The mortality rate of tetanus among these patients was substantial, ranging from 10% to 22%.[7,8] Waning immunity to tetanus in the elderly and poor wound management practices by primary care physicians were suspected to be contributory factors.[7] The CDC in the United States recommended that tetanus toxoid boosters should be given every 10 years after primary vaccination, to maintain protective immunity against tetanus.[10] However, in Taiwan, most adults did not receive booster doses unless they had physical trauma. At present, there is no regular vaccine program against tetanus for the elderly.

The latest data in Taiwan about serum levels of tetanus antitoxin among neonates and children were reported in 1987.[11] However, the information about tetanus immunity in adults is scant. The purpose of this study was to investigate the degrees of protective tetanus immunity among adolescents and adults, which may provide valuable information for recommendations for tetanus vaccination strategy.

Materials and Methods

A prospective study was performed in National Cheng Kung University Hospital (NCKUH), Douliou Branch, between March 2006 and November 2006, which was approved by the Institutional Review Board of NCKUH. Individuals aged ≥ 16 years who were visiting a local hospital for health examinations were invited to participate. Participants' serum levels of tetanus antitoxin were measured. Those not sure of their status of toxoid vaccination were excluded. After obtaining informed consent, blood samples were collected, and sera were stored at –20°C until use. Meanwhile, a standard questionnaire, including items of residency, occupation, previous soil or water exposure, previous histories of military service, injury or tetanus, and the status of toxoid boosters in adolescence or adulthood, was filled in based on face to face interviews. Wounds caused by puncture with rusted iron materials or animal bites, or those contaminated by soil were regarded as dirty. For convenience, among participants who did not receive primary tetanus vaccination in infancy, such as those born before the initiation of the DPT vaccination program in 1955, toxoid injection later in life was also considered as a booster dose.

Serum level of tetanus antitoxin was measured by an enzyme-linked immunosorbent assay (ELISA) (Novatec, Dietzenbach, Germany). Diluted serum was dispensed into each well, and incubated for 1 hour at 37°C, followed by three washes in the washing solution. Tetanus anti-IgG conjugate was then added, and the wells were incubated for 30 minutes at room temperature in the dark, and washed again. Tetramethylbenzidine substrate was then added to each well, and incubated for 15 minutes at room temperature in the dark. The absorbance of each specimen at 450/620 nm was measured by an ELISA microwell plate reader (Murex Diagnostics, Dartford, UK).

The serum tetanus antitoxin levels were interpreted as defined previously by Schroder and Kuhlmann.[12] An antitoxin concentration ≥ 0.11 IU/mL was regarded as protective immunity. According to the manufacturer's instructions, an immediate booster and antitoxin measurement 4–6 weeks later is recommended if antitoxin level is 0.11–0.5 IU/mL. A booster is not required immediately and antitoxin measurement 2 years later is suggested if antitoxin level is 0.51–1.0 IU/mL. Long-term protection can be achieved and antitoxin measurement 10 years later is recommended if antitoxin level is > 5.0 IU/mL.

The prevalence of protective tetanus immunity was described in various age groups. Sociodemographic factors potentially influencing the degree of tetanus immunity were analyzed.

The χ^2 test was used for qualitative data, and Student's t test for quantitative data. Linear regression was used for correlation analysis. A value of $p < 0.05$ was considered to be significant.

Results

Demographic characteristics of participants

Between March 2006 and November 2006, 336 individuals agreed to participate in this study initially. Eleven persons who were not sure about their immune and tetanus booster status were excluded from this study. Finally, 325 individuals, including 147 men and 179 women, were included. One woman received a toxoid booster because of trauma and her serum was obtained 30 days later, and she was counted twice. Therefore, there were a total of 326 participants. Those aged 40–59 years were divided into two age groups: 40–50 and 51–59 years, because the DPT National Children's Vaccination Program in Taiwan was initiated among participants aged ≤ 51 years old. The case numbers in different age groups are shown in Table 1. One third (109, 33.4%) of 326 participants recalled receiving at least one toxoid booster. Of these, 86 (78.9%) had received one booster, 18 (16.5%), two, and five (4.5%), three or more. Overall, 55% of men and 76% of women, which accounted for two thirds of all participants, denied the receipt of a toxoid booster after childhood. None of the participants enrolled had a history of tetanus.

Tetanus immunity among participants

Among 326 participants, 95% had a protective antitoxin level (≥ 0.11 IU/mL), and 60% had a protective antitoxin level that did not require an immediate booster (≥ 0.51 IU/mL). Among 70 participants aged > 60 years, 89% had protective antitoxin levels (≥ 0.11 IU/mL), and 31% had protective antitoxin level (≥ 0.51 IU/mL) (Table 2). Mean antitoxin level declined with age, from 4.83 IU/mL in those aged 16–19 to 0.82–0.87 IU/mL in those aged > 51 years (Figure 1).

Tetanus immunity among participants without toxoid booster

Among 217 participants without toxoid boosters after childhood, 96–100% of those aged ≤ 50 years had protective antitoxin levels (≥ 0.11 IU/mL), while 83–88% of those > 51 years had protective antitoxin levels (Figure 2). The proportion of people with protective immunity and no need for an immediate booster, i.e. antitoxin levels ≥ 0.51 IU/mL, declined with age. The ratio decreased from 100% in those aged 16–19 years, to 52% in those aged 40–50 years, and to 19% in those aged ≥ 70 years (correlation $r = 0.9$, $p = 0.001$). Likewise, a similar trend in decline of mean antitoxin level with age was observed. The level decreased from 3.31 IU/mL in those aged 16–19 years to 0.31–0.81 IU/mL in those aged > 51 years (Figure 1).

Table 1. Demographic characteristics and history of tetanus vaccine booster

Age (yr)	Total case no. (case no. with booster, %)		
	Males	Females	All
16–19	15 (3, 20%)	8 (3, 38%)	23 (6, 26%)
20–29	53 (29, 55%)	56 (9, 16%)	109 (38, 35%)
30–39	15 (10, 67%)	25 (5, 2%)	40 (15, 38%)
40–50	15 (6, 40%)	36 (12, 33%)	51 (18, 35%)
51–59	17 (7, 41%)	16 (3, 19%)	33 (10, 30%)
60–69	13 (5, 38%)	13 (4, 31%)	26 (9, 35%)
≥ 70	19 (6, 32%)	25 (7, 28%)	44 (13, 30%)
Total	147 (66, 45%)	179 (43, 24%)	326 (109, 28%)

Table 2. Host factors associated with protective tetanus immunity

	Total case no.	Case no. with protective immunity (anti-toxin ≥ 0.11 IU/mL), (%)	$p^{\dagger}$	Case no. with protective immunity (anti-toxin ≥ 0.51 IU/mL), (%)	$p^{\dagger}$
All participants	326	311 (95)		195 (60)	
Gender			0.1187		$<0.001^{\ddagger}$
Male	147	143 (97)		108 (73)	
Female	179	168 (94)		87 (49)	
Previous military service			0.781		0.016
Yes	105	101 (96)		73 (70)	
No	221	210 (95)		122 (55)	
Year of birth*			<0.001		$<0.001^{\ddagger}$
Before 1955	102	89 (87)		34 (33)	
After 1955	224	222 (99)		161 (72)	
Tetanus booster			0.401		$<0.001^{\ddagger}$
Yes	109	106 (97)		85 (78)	
No	217	205 (95)		110 (51)	
Soil/garden work			0.015		0.018
Yes	93	84 (90)		46 (49)	
No	233	227 (97)		149 (63)	
History of dirty wounds			0.793		<0.001
Yes	148	142 (96)		106 (72)	
No	178	169 (95)		89 (50)	

**Diphtheria–pertussis–tetanus vaccine program commenced since 1955; †univariate analysis; ‡multivariate analysis, male gender, birth after 1955, and having ever received a tetanus booster were associated with protective tetanus immunity that did not require an immediate booster dose (tetanus antitoxin levels ≥ 0.51 IU/mL) ($p < 0.001$ for each factor).*

Host factors associated with protective immunity

Men born after 1955, who had a history of toxoid boosters, military service, dirty wounds, or frequent soil exposure, were significantly more likely to have antitoxin levels ≥ 0.51 IU/mL, by the univariate analysis (Table 2). However, only male gender, birth after 1955, and prior receipt of tetanus toxoid boosters were independently associated with protective antitoxin levels (≥ 0.51 IU/mL), by multivariate analysis. In addition, people who were born before 1955 were involved more frequently with soil or garden work than those born after 1955 (61% *vs.* 14%, $p < 0.001$).

Immunity after booster injection

Compared with 217 participants without toxoid boosters after childhood, 109 who had ever received a toxoid booster had higher antitoxin levels ($p < 0.05$) in all age groups (Figure 1). The ratio of participants with protective immunity declined, as the interval between the last toxoid booster and the time of antitoxin determination increased. Among 109 people with tetanus toxoid boosters, 59 received the last booster within 5 years, and 27, 16, and 7 people received it 6–10, 11–20, and 21–30 years ago, respectively. Of people receiving the last toxoid booster within 5 years, 6–10, 11–20, and 21–30 years ago, 86, 81, 50 and 57%, respectively, of them had antitoxin levels ≥ 0.51 IU/mL. Only 13% of people who received their last toxoid booster 11–20 years ago had antitoxin levels ≥ 5 IU/mL, which signified long-term protection of > 10 years. In addition, tetanus antitoxin level in a 39-year-old woman increased from 0.4 to 17.9 IU/mL at 30 days following a toxoid booster.

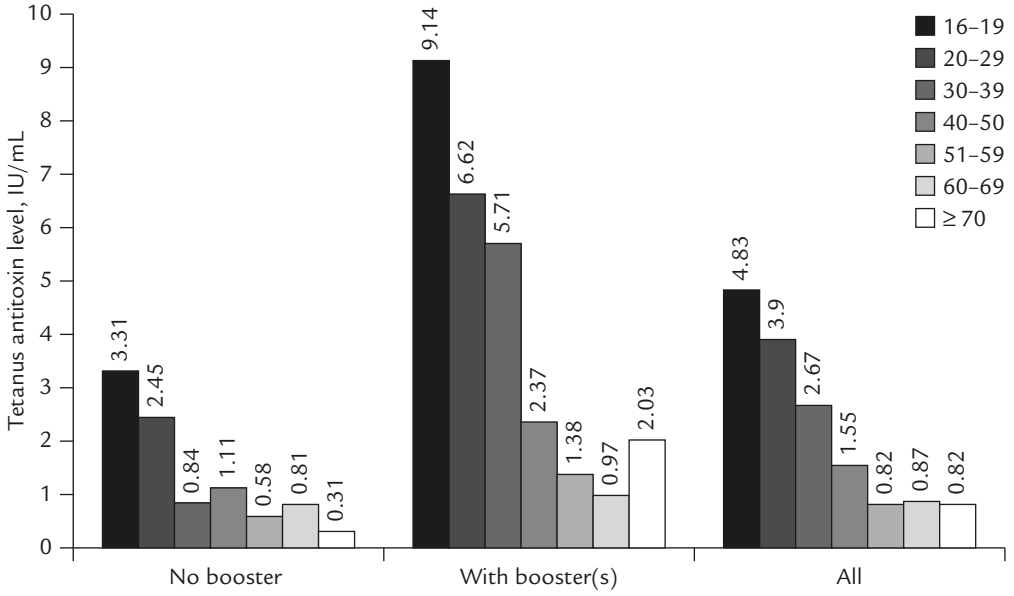

Figure 1. Comparison of tetanus antitoxin levels between participants with or without toxoid booster(s) and among all participants. Bars represent mean values. Participants who had ever received a toxoid booster were associated with significantly higher antitoxin levels than those who had not, in all age groups except 60–69 years ($p < 0.05$).

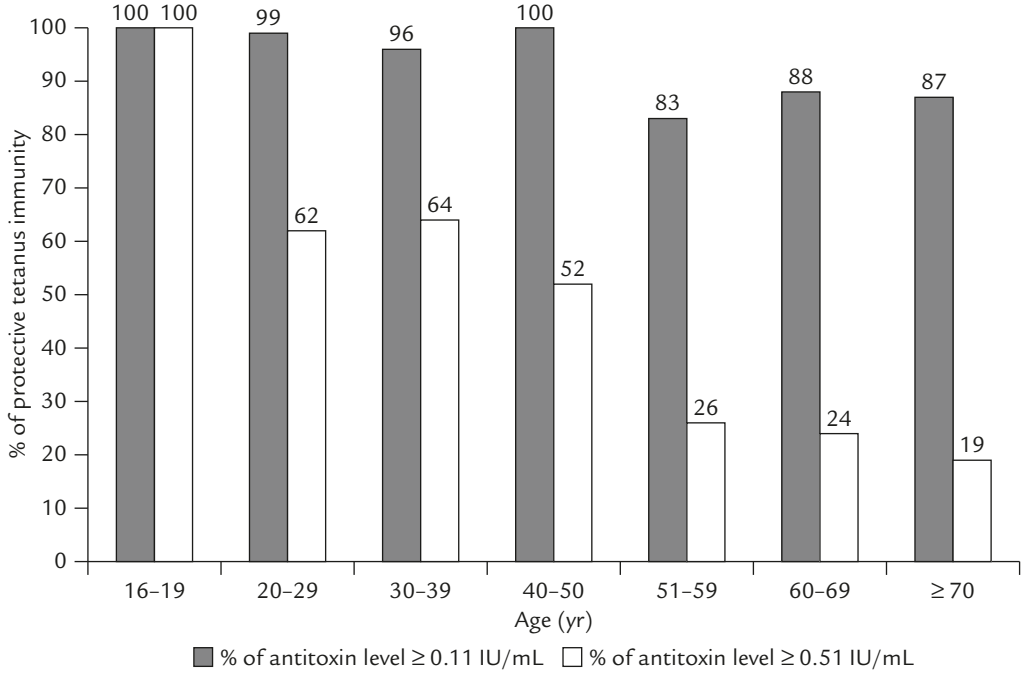

Figure 2. Serum tetanus antitoxin levels in different age groups among 217 participants without a booster, and the ratio of protective tetanus immunity defined as serum antitoxin ≥ 0.11 IU/mL or ≥ 0.51 IU/mL.

Discussion

Methods for *in vitro* determination of serum tetanus antitoxin include the passive hemagglutination test, ELISA and radioimmunoassay. ELISA was used in our study because it is simple, sensitive, rapid, and inexpensive.[12] Several ELISAs have been described in the literature, and the cut-off values for antibody titers differ between kits. The serum tetanus antitoxin levels determined by ELISA in our study were interpreted as previously defined by Schroder and Kuhlmann,[12] in which antitoxin

levels ≥ 0.11 IU/mL were considered as protective. However, a toxoid booster is still suggested if the level falls within the range 0.11–0.5 IU/mL. Immediate booster is not required if the level is ≥ 0.51 IU/mL. As a result, both protective levels (≥ 0.11 IU/mL and ≥ 0.51 IU/mL) were analyzed in our study, and the latter represented protective immunity that did not require an immediate booster.

We demonstrated that tetanus toxoid booster was associated with higher tetanus antitoxin levels. First, among all participants, those who had ever received a toxoid booster were shown more frequently to have protective antitoxin level (≥ 0.51 IU/mL) by multivariate analysis. Second, the antitoxin levels among individuals who had received a toxoid booster during childhood or adolescence were higher than those without a booster in different age groups. Furthermore, we found in a 38-year-old woman that tetanus antitoxin level increased from 0.4 IU/mL to 17.9 IU/mL at 30 days following a toxoid booster. All of the above findings implied that the toxoid booster helped to achieve an adequate antitoxin level. However, even with a toxoid booster, the protective effect was not life-long and waned with time. The duration of protection with a booster varied between individuals. In general, we found that > 80% of them had protective antitoxin levels that did not require an immediate booster, i.e. ≥ 0.51 IU/mL, if they had received the latest booster within 10 years, while only half had protective levels if they received their latest booster > 10 years ago. The waning immunity after 10 years highlighted the importance of receiving toxoid boosters every 10 years after primary vaccination, as recommended by the CDC in the USA for continuing protection against tetanus.[10]

Participants who were born after 1955 when the National Children's Vaccination Program in Taiwan was initiated were more immune to tetanus. Whether this phenomenon resulted from the long-term protective effect of primary DPT vaccination could not be clarified in the present study. This is because aging *per se* was a factor associated with lower antitoxin level, which was shown by the waning immunity with age after primary vaccination among those who did not receive a booster. However, the efficacy of tetanus toxoid has been demonstrated in studies from many countries, and ranges from 80% to 100%.[13] In Taiwan, the high efficacy of the initial three doses of primary DPT vaccination was demonstrated by the fact that 99% of 104 children aged 12–23 months possessed tetanus antitoxin concentrations above protective levels.[14] However, antitoxin level declined with age regardless of whether the subjects had received a toxoid booster. As a result, primary tetanus vaccination followed by subsequent toxoid booster is warranted to achieve adequate, life-long immunity against tetanus. The older a person is, the greater the necessity of receiving a tetanus booster because of waning immunity in the older age group.

We showed that male subjects had higher immunity against tetanus, which has also been shown in previous studies.[4,15] This may be because higher accident rates among men increase the probability of receiving a tetanus booster, which results in higher protective immunity, as seen in a study in the United States.[4] Another possible explanation is immunization during military service.[15,16] However, toxoid booster was not given routinely during military service in our country, and men were not found to experience more trauma or receive more toxoid booster doses than women in our study. Neither factor was significant in multivariate analysis. Further work is needed to elucidate the reasons for greater tetanus immunity among men than woman.

People with frequent exposure to soil and garden work are at risk of contact with *C. tetani* spores through puncture or scratch wounds, and of developing tetanus, if they do not have adequate immunity. From 2000 to 2005, according to data from CDC Taiwan, there were 102 reported cases of tetanus.[9] Patients aged ≥ 60 years accounted for 60% of tetanus cases, and 89% resided in rural areas. We found that participants who were exposed frequently to soil seemed to be associated with lower rather than increasing antitoxin levels. Such an association was absent in the multivariate analysis. This may have been because a greater

number of older people were involved with soil and gardening in the present study. The tetanus antitoxin levels were lower in older people, and age was a possible confounder of the association between low antitoxin levels and frequent soil exposure. Elderly people tended to lose their protection against tetanus because tetanus antitoxin levels declined with time, which may explain why older people accounted for most of the tetanus cases in Taiwan. This highlights the importance of maintaining adequate tetanus immunity by toxoid boosters in older people who are involved with soil and gardening.

Some authors have reported natural immunity to tetanus, because of the presence of tetanus antitoxin in the sera of persons who have not received tetanus toxoid.[17,18] Likewise, we found that some people born before the initiation of childhood tetanus vaccination, who never received tetanus toxoid, did have protective antitoxin levels. It has been proposed that natural tetanus immunity can be induced by sub-lethal doses of tetanus toxin or by fragments of toxin released from *C. tetani* located in the digestive tract, as a result of ingesting tetanus spores.[17] However, the clinical implications of natural immunity remain unresolved.

In conclusion, waning immunity to tetanus was observed after childhood tetanus vaccination or toxoid boosters. A vaccination strategy that involves a toxoid booster after primary vaccination should be emphasized to maintain protective tetanus antitoxin levels during adulthood, especially among older people at high risk for tetanus.

Acknowledgments

The study was supported by a grant (95-NCKUH-75) from National Cheng Kung University Hospital, Taiwan.

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