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Diphtheria Anti-toxoid Antibody Levels Among Pre-clinical Students and Staff in an Institute of Higher Learning in Malaysia: Are They Protected?

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ABSTRACT

Introduction: Little is known about the sero-prevalence of diphtheria anti-toxoid antibody levels among medical students in Malaysia. They too, just like other health care workers (HCWs) are at risk of contracting and transmitting diphtheria. Fortunately, this can be prevented by giving a specific vaccine: the diphtheria, tetanus and pertussis (DTP) vaccine. Nonetheless, data from local or regional surveys are needed before any decision is made by the respective authorities. **General objective:** We studied the epidemiology of diphtheria anti-toxoid antibody levels and vaccination history amongst medical students and staff in Faculty of Medicine and Health Sciences, Universiti Putra Malaysia. **Specific objectives:** We determined the level of diphtheria anti-toxoid antibodies amongst pre-clinical students and staff. **Methodology:** A total of 152 sera were collected from subjects aged 19 to 63, and diphtheria anti-toxoid levels were measured by an enzyme-linked immunosorbent assay. **Results:** One hundred and fifty-two (94.4%) blood samples out of 161 participants were successfully withdrawn, which comprised 105 (69.1%) and 47 (30.9%) medical students and staff, respectively. A total of 77.6% and the other 22.4% of the subjects had full and basic protection, respectively. Higher levels were predominant amongst males and they were 1.3 times more protected than females in 20-29 year-old group (85.1% vs 66.2%; odd ratios 1.25 [95% CI 1.03-1.50]; P=0.03). No significant difference in the levels of immunity among subjects for ethnicity and academic position (P>0.05). **Recommendations:** Level of full protection against diphtheria toxin should be clearly defined by broad population based studies using several comparable detection methods. Medical students and staff with basic protection should be closely monitored or should be given a booster dose for those who are at high risk of acquiring the disease. Thus, a standard degree of coverage should be clearly determined for health workers to prevent a potential outbreak. **Conclusion:** Students and staff possess immunity towards diptheria toxin however the level of full protective antibody is yet to be determined in future.

Keywords: Diphtheria, diphtheria anti-toxoid antibody, full protection, basic protection, medical students, staff

INTRODUCTION

Greatest concerns on the re-emergence of diphtheria epidemics came from a large-scale outbreak in the Newly Independent States (NIS) of the former Soviet Union, which involved 140 000 cases and 400 deaths during 1990-1995. [1] This was further stigmatized by subsequent outbreaks of varying degrees in other parts of Western Europe and other countries through out the world. [2, 3] Recommendations and control strategies proposed by World Health Organization (WHO) have been successful for some countries in combating the disease. One of them was to asses diphtheria immunity levels among adults worldwide. [4, 5] However, data reported from the South East Asia region are very much lacking. As a result, our knowledge on the epidemiology of diphtheria is still inadequate. In a similar context, knowledge on the immunization policies of "high-risk" occupational groups in diphtheria outbreaks is also hampered by limited available data. In addition, critical issues on vaccine-preventable diseases in health-care workers (HCWs) and other high-risk groups have been extensively discussed. Recently, major improvements have been made by Centers for Disease Control and Prevention (CDC) and Advisory Committee on Immunization Practices (ACIP) in promoting tetanus, diphtheria and pertussis vaccine and other types of vaccines to all categories of health care workers.[6]

However, teachers, medical students or other important high-risk groups in health-related fields have been mistakenly underemphasized; and often marginalized because they are not considered hospital staff. [7, 8, 9] Furthermore, only a small number of systematic immunization programs exist for medical students and staff in medical institutions

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worldwide. Special consideration is needed for these target groups as they are often exposed to infectious diseases directly or indirectly during clinical training. Now, it is recommended a one-time dose of Tdap for all HCWs including physicians and other primary-care providers such as nurses, aides, respiratory therapists, radiology technicians, students (e.g., medical, nursing, and other) that have direct contact with patient. This was supported by Healthcare Infection Control Practices Advisory Committee (HICPAC). [10]

However, experts propose that groups at risk should be clearly defined and evidence-based before any decision is made by the respective authorities. [2, 3] Thus, our aim in this study was to evaluate the immune status of medical students and staff members towards diphtheria in relation to the selected socio-demographical variables. We do hope that our findings would at least, in part, complement our national immunization coverage reports that have been submitted annually to WHO/UNICEF. Also, to provide additional input for policy-makers before embarking on new immunization programs for students who enter medical school.

MATERIALS AND METHODS

Study population

The cross-sectional study was conducted from January 2008 to July 2008 at the Faculty of Medicine and Health Sciences, University Putra Malaysia (UPM)—a public university in Peninsular Malaysia, approximately 30 kilometres from Kuala Lumpur. The participants consisted of pre-clinical (medical) students, and staff members. Informed consent was obtained and the following data was recorded in the standard Pro Forma: age, sex and ethnicity. Students and staff were ensured that the results would be kept confidential, and would be personally informed; advice about the risks and benefits of immunization were also provided. A standard questionnaire was also given to obtain the data on the history of DPT vaccination. The protocol and consent procedures were approved by the Ethical Committee of Universiti Putra Malaysia.

Serological method

Three millilitres of venous blood samples were obtained from the participants. The samples were allowed to clot naturally at room temperature, and the sera were collected and stored at –80 C for analyses. Measurement of specific IgG antibody against diphtheria toxoid levels in International Unit (IU/ml) was performed by using ELISA (enzyme-linked immunosorbent assay, VaccZymeTM Diphtheria Toxoid IgG, The Binding Site Ltd., United Kingdom). The relative sensitivity and specificity of this kit as compared with the gold standard Vero Cell Assay (VCA) are 93.06% and 91.82%, respectively.

All procedures and interpretations were done according to the manufacturer's guidelines. Briefly, 100 µl of each calibrator, control and 1: 100 diluted sample were added to the appropriate wells, all of which were coated with diphtheria toxoid antigen derived from *Corynebacterium diphtheriae*. Following incubation for 30 minutes; all unbound proteins in the wells were discarded by washing three times. One hundred (100) µl of purified peroxidase-labeled rabbit anti-human IgG used as conjugate were added and again re-incubated for another 30 minutes. The wells were washed three times to remove excess unbound conjugate. Finally, 100 µl of tetramethylbenzidine (TMB) were added as substrate and subsequently incubated in a dark room for 30 minutes to give blue reaction product. The intensity of the reaction was determined by using DYNATEX Microplate Reader (USA) at optical density (OD) of 450 nm. The measurement was done within 30 minutes of stopping the reaction using 100 µl of phosphoric acid. All procedures were carried out at room temperature.

The results were interpreted as follows based on the manufacturer's guideline:

Diphtheria immunity status:

< 0.01 IU/ml:	No protection
0.01 to 0.1 IU/ml:	Basic protection
> 0.1 IU/ml:	Full protection

Statistical analysis

Data analysis was carried out by using SPSS version 16.0 software (SPSS Inc., Chicago, IL, USA). Pearson chi square and Fisher's exact tests were used to analyze the association of the various socio-demographical variables with the prevalence of diphtheria antitoxins. P values < 0.05 were considered to be statistically significant.

RESULTS

One hundred and fifty-two (94.4%) blood samples out of 161 participants were successfully withdrawn, of whom 100 (65.8%) and 52 (34.2%) were females and males, respectively. Their ages ranged from 19 to 63 years (median 21.0 years, standard deviation [SD] 6.2 years). The remaining 9 participants were excluded, as their blood could not

be taken for various reasons. Of 152, 105 (69.1%) comprised medical students and 47 (30.9%) staff. The ethnicity distribution was as follows: 99 (65.1%) Malays, 44 (28.9%) Chinese, 6 (3.9%) Indians and the remainder, 3 (2.0%) were of other ethnic groups. Overall, 147 (97.2%) received diphtheria, tetanus and pertussis (DTP) vaccine as primary immunization and a booster dose at school entry. All of the participants (100%) were serologically immune. One hundred and eighteen (77.6%) and 34 (22.4%) of them had full and basic protection, respectively. The prevalence of diphtheria immunity status according to socio-demographic characteristics is shown in Table 1. The median diphtheria anti-toxoid antibody concentration reached 0.21 IU/ml (quartiles Q25–Q75; 0.1 to 0.37; Table 2).

Table 1. Diphtheria immunity status of participants according to socio-demographic characteristics

Socio-demographic profiles	Diphtheria immunity status		P-value
	Basic protection (0.01–0.1 IU/ml) No. (%)	Full protection (> 0.1 IU/ml) No. (%)	
Age-group (in years)			
< 20	0 (0.0)	3 (100.0)	0.084
20–29	34 (25.8)	98 (74.2)	
30–39	0 (0.0)	14 (100.0)	
>39	0 (0.0)	3 (100.0)	
Gender			
Male	7 (13.5)	45 (86.5)	0.057
Female	27 (27.0)	73 (73.0)	
Ethnicity			
Malay	22 (22.2)	77 (77.8)	0.549
Chinese	11 (25.0)	33 (75.0)	
Indian	0 (0.0)	6 (100.0)	
Others	1 (33.3)	2 (66.7)	
Academic position			
Medical lecturer	0 (0.0)	10 (100.0)	0.266
Tutor	0 (0.0)	4 (100.0)	
Science officer	1 (33.3)	2 (66.7)	
Laboratory personnel	7 (31.8)	15 (68.2)	
Administrative staff	3 (37.5)	5 (62.5)	
Medical student	23 (21.9)	82 (78.1)	
Vaccination among subjects[a]			
Completed	34 (23.1)	113 (76.9)	0.446
Not sure	0 (0.0)	5(100.0)	

[a] History of diphtheria vaccination was considered completed if three doses of toxoid given at 3, 4, and 5 months of age as primary immunization in combined vaccine against DPT, followed by a fourth dose at 18 months, and a booster dose at school entry, between 6 and 7 years of age were documented.

In general, 86.5% of the males had full protective diphtheria anti-toxoid levels whereas; only 73.0% of the females were fully protected. The difference was not statistically significant (P=0.06; Table 1). The median diphtheria anti-toxoid antibody concentration in males was 0.18 IU/ml (0.12–0.32) compared with 0.22 IU/ml (0.09–0.39) in females (Table 2). However, when gender in the 20–29 year-old category was analyzed on those with full protective diphtheria anti-toxoid levels (Figure 1), significant difference was observed (P =0.03, data is not shown). Males (85.1%) had higher percentages of full protective diphtheria anti-toxoid levels than females (66.2%) among this group, and males

were the most predominant and 1.3 times more protected than females in 20-29 year-old group (odd ratios 1.25 [95% CI 1.03-1.50]).

Table 2. Diphtheria anti-toxoid antibody concentration (IU/ml) according to academic position, gender and ethnicity

Characteristics	Diphtheria anti-toxoid concentration (IU/ml): median (Q25–Q75)
Total	0.21 (0.10–0.37)
Academic position	
Staff	0.31 (0.10–0.44)
Medical student	0.18 (0.10–0.38)
Gender	
Male	0.18 (0.12–0.32)
Female	0.22 (0.09–0.39)
Ethnicity	
Malay	0.22 (0.10–0.39)
Non-Malay	0.17 (0.10–0.32)

Figure 1. Distribution of full protective diphtheria anti-toxoid levels in 98 participants of 20 to 29-year-old age group according to gender

With reference to ethnicity, levels of full protection were found in 77 (77.8%), 33 (75%), 6 (100%) and 2 (66.7%) of Malays, Chinese, Indians and others, respectively. However, neither ethnicity nor academic position had statistically significant difference (Table 1; $P > 0.05$). In general, the median diphtheria anti-toxoid antibody concentration in Malays (0.22 IU/ml; quartiles Q25–Q75; 0.10–0.39) was slightly higher than non-Malays (0.17 IU/ml; quartiles Q25–Q75; 0.10–0.32; Table 2). Overall, medical students and staff seemed to have equal percentages of full protective diphtheria anti-toxoid levels, i.e., 82 (78.1%) and 36 (76.6%) of students and staff, respectively (Figure 2). However, median diphtheria anti-toxoid antibody concentration in staff members (0.31 IU/ml; quartiles Q25–Q75; 0.10–0.44)

was higher than medical students (0.18 IU/ml; quartiles Q25–Q75; 0.10–0.38; Table 2). Nevertheless, small number of subjects (staff) may contribute to this finding.

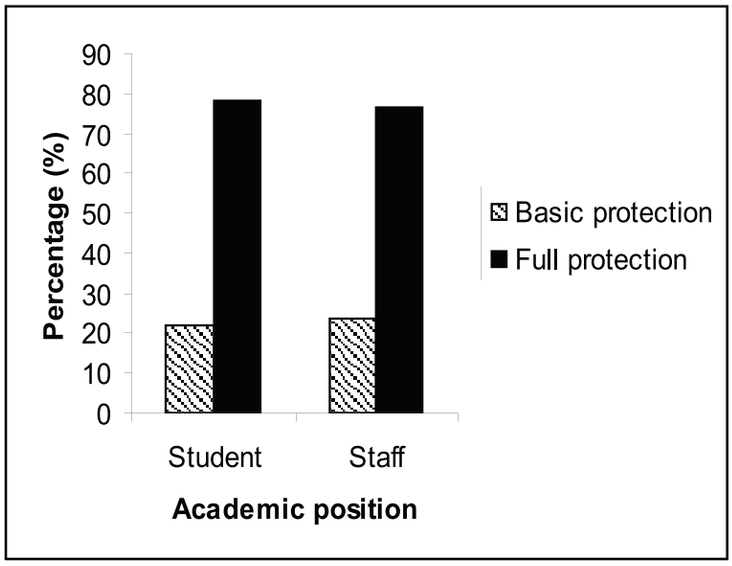

Figure 2. Distribution of diphtheria immunity status according to academic position (basic protection: 0.09–0.1 IU/ml; full protection: > 0.1 IU/ml)

DISCUSSION

In our present study, we documented that 78.1% of medical students had full protective diphtheria anti-toxoid levels (> 0.1 IU/ml). The remaining 21.9% had basic protective levels (0.01—0.1 IU/ml). Importantly, all of them (100%) had immunity to diphtheria. This is not surprising, as most of them were born after the year of commencement of Extended Program on Immunization (EPI) [11], which was started in 1989. And also, this probably reflects highly sustainable immunization coverage of diphtheria, tetanus and pertussis (DTP) of more than 90% in our country since 1990. [12] Our findings are also comparable to previous studies, which reported 68.3 % and 78.4% of medical students in London [13] and Poland [14] respectively, were fully protected. As high protective levels are vital in this group, a booster dose might be required in those who had basic protection. Several studies have reported that high immunity levels were required for full protection. [15] In a different scenario, it is well documented that at least 70% of children must be immunized in order to prevent major outbreaks of diphtheria in the community. [16] However, so far, no general consensus has been proposed for HCWs, including medical students and staff as to what percentage to be achieved in order to prevent nosocomial or institutional outbreaks.

As for staff members, 76.6% were fully protected and 23.4% had basic protection. Again, all of them (100%) were immune to diphtheria toxin. Surprisingly, higher concentration of anti-toxoid antibody was found in our staff (0.31 IU/ml) than medical students (0.18 IU/ml; Table 2) in general. As for comparison, our prevalence rate of full protection is very much higher than reported in other studies. Lower prevalence rates of 26%, 51% and 57% were reported in Germany [17], United Kingdom [18] and Australia [19], respectively. Recently, only 36.3% of infection control staff in Japan was reported to have full protective levels against diphtheria antitoxin. [20] However, different values of these percentages were attributed to the different study designs and methodologies, vaccination programs and types of serological test used. Our result may also probably signify the effectiveness of vaccine surveillance networks and good public health infrastructures. These are very important and possibly explain lower levels of full protection in underprivileged and minority populations in Thailand despite persistently high immunization coverage, which was more than 90% reported annually in that country. [21]

In addition, for adults in highly developed countries, a booster dose is given to them before they are recruited into the army, which might probably explain high levels of protective immunity. [22,23] As for our case, it may be related to our national immunization policy. In Malaysia, the national policy of diphtheria immunization includes three doses of toxoid given at 3, 4, and 5 months of age as primary immunization in combined vaccine against DPT, followed by a fourth dose at 18 months, and a booster dose at school entry, between 6 and 7 years of age. [24] Because of the boostering effects, this may probably contribute to higher prevalence of full protection in our study. It is well known

that booster effects can be maintained for up to 30 years. [25] One study reported 90% of respondents produced more than or equal to 1 IU/ml of antitoxins following a booster after one-month period. [26] Also, the acquisition rate of fully protective level was higher among young adults as reported in Japan. [20] This was also supported by a study in Israel that reported adults acquired higher protective antitoxin levels following a booster dose injection given to them at 18 years of age. [27]

However, experts postulated that other factors rather than vaccination alone could possibly play a role. Changes in socioeconomic and lifestyle would give different epidemiological patterns of the disease. [20] Based on these observations, higher percentages of full protection found in both medical students and staff may also be related to these factors. Malaysia is now recognized as a rapidly industrialized country with high standard of living. Also, our socio-economic stability might have provided equal opportunities of vaccination among our participants. However, local data on age-related difference in diphtheria cases was not available for further analyzes. The limitation of this study is that we did not conduct a population based randomized study, and therefore a recruitment bias is possible. However, as immunity levels in HCWs are of the main priority, this can be accepted.

In this present study, males (86.5%) had significantly higher percentage of full protection than females (73%) in general (Table 1). However, the difference was not statistically significant (P=0.06). Interestingly, in the 20-29-year-old group, males were 1.2 times more likely to have full protective antibodies than females (odd ratios 1.25 [95% CI 1.03-1.50]). Forty (85.1%) out of 47 males in this group, were fully protected as compared to females (68.2%); (data not shown). The difference was statistically significant (P=0.03; Figure 2). With regard to gender-related difference, many conflicting results were documented; and multiple contributing factors were proposed. [27] Some of them remained poorly understood. [28, 29] In several studies, routine administration of Tetanus-Diphtheria toxoids vaccine (Td) for injury cases—men were more prone, was responsible for higher protection rates in males [3, 30, 31], as well as, a booster dose given prior to army's recruitment (mostly males). [2] Different immunization responses in females following vaccination were also responsible for the finding. Lower antibody titres and shorter period in maintaining immunity levels in females were reported in several studies. [22, 29] In our case, the concentrations of anti-toxoid in males (0.18 IU/ml) and females (0.22 IU/ml) were almost equal (Table 2). In contradiction, few studies reported that males had lower protection rates than females. [32, 33] Nevertheless, neither Td vaccination nor booster dose is recommended for such occasions in Malaysia. In our study, ethnicity of different origin was not statistically associated with diphtheria immunity status. This was in accordance with previous studies conducted in Israel. [27]

CONCLUSION

In summary, our study revealed that medical students and staff members possess immunity to diphtheria toxin. Hence, the risk for contracting and transmitting the disease is relatively minimal. For this reason, it might not be necessary to institute a specific vaccination program for diphtheria in our medical school and institution. However, our findings will not be similar to medical schools in other developing countries and the full protective immunity is yet to be further determined. Each country should analyze data gathered from its local and national surveys before formulating specific recommendations on diphtheria vaccination.

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