

REPORT

Seroprevalence of *Corynebacterium diphtheriae* among vaccinated population of Rawalpindi/Islamabad, Pakistan

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Abstract: Diphtheria is a communicable disease of global significance, and its outbreaks have to be reported to the world community under the International Health Regulations (IHR). A pilot seroepidemiological survey was conducted to assess immunity status of diphtheria among healthy individuals of Rawalpindi/Islamabad (Pakistan), who had been administered at least one dose of the vaccine against the disease, as part of childhood vaccination. The study group comprised of 128 healthy subjects, grouped according to the decade representing their age. Antidiphtheria IgG levels were measured by Enzyme Linked Immunosorbent Assay (ELISA) method. The studied sample showed 100% prevalence of diphtheria antitoxin, confirming prior vaccination; however 49.2% exhibited only minimal protection against diphtheria. Full protection was observed in a significantly higher ($p=0.013$) percentage of males (54.45%) as compared to female subjects (33.33%). Maximum level of serum antibodies were seen in 1-10 year age group (0.195 ± 0.031 IU/mL), which was significantly higher than that recorded in the age group of 11-20 ($p=0.024$) and above 30 years ($p=0.0064$). The present results emphasize the need for periodical booster immunization in adolescents and adults, after primary childhood immunization.

Keywords: Diphtheria; seroepidemiology; vaccine; Pakistan

INTRODUCTION

Diphtheria is a serious upper respiratory tract disease, with high morbidity and mortality rates. It is one of the diseases that are monitored globally under the International Health Regulations, and all countries are expected to report any outbreak. Vaccination, via administration of diphtheria endotoxin, has significantly reduced the incidence of the disease in most of the developed world (Maple *et al.*, 1995). However, the disease remains endemic in many developing countries, including Pakistan, where vaccination against *Corynebacterium diphtheriae* is included in the Extended Programme on Immunization (EPI), which was initiated in 1976. Recommended vaccination schedule by EPI requires its administration as a combined vaccine, diphtheria-tetanus-pertussis (DTP), in 3 doses at 6, 10 and 14 weeks after birth, but a high proportion of children fail to complete the three doses of DTP. The general conception about diphtheria among the Pakistani population is that it is a childhood disease with a single dose capable of inducing lifelong immunity, which is only reinforced through the two boosters in the first year of the neonate's life. No booster dose is recommended in the present programme after DTP3, which is administered at the age of 14 weeks. Immunization coverage levels in

Pakistan have been consistently lower than the socio-economically comparable WHO member nations of the Eastern Mediterranean Region, of which Pakistan is a part, and the neighbouring Southeast Asia region. Recent studies conducted in Pakistan are related to estimation and improvement of immunization coverage (Chandir *et al.*, 2010; Hasan *et al.*, 2010). In Pakistan, during 2008-2009, the DTP3 coverage was recorded to be 73–85% (WHO Vaccine Preventable Diseases Monitoring System, 2011). Monitoring of diphtheria IgG antibodies in the population is, therefore, an important means of evaluating the effectiveness of vaccination, and controlling the potential risk of diphtheria infection. The aim of the present study was to assess seroepidemiology of diphtheria among selected urban vaccinated Pakistani population, and to evaluate the need for a timely booster dose.

METHODS

Study population

This pilot cross-sectional seroepidemiological study was conducted on healthy vaccinated population of Rawalpindi/Islamabad, Pakistan. A sample of 128 was selected by simple random method. The subjects were stratified by age: Group A (1-10), Group B (11-20), Group C (21-30) and Group D (above 30). The data related to age, gender and vaccination status was obtained through an interview. All participants had received at least one

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dose of diphtheria-tetanus-pertussis, provided by WHO, under EPI programme and the individuals neither suffered from diphtheria at the time of sampling, nor had any prior history of the disease. This study was approved by Ethical Review Committee of COMSATS institute of Information Technology, Islamabad, and informed consent was obtained from all study participants.

Serological investigation

Venous blood samples (5-ml) were collected, and serum was separated by centrifugation and stored at -80°C until used. Anti-diphtheria IgG levels were determined by Enzyme Linked Immunosorbent Assay (ELISA), according to instructions provided by manufacturer (Serion Immunodiagnostica, Germany). Seroimmunity was classified as susceptibility (<0.01IU/mL), insufficient protection (0.01<0.1IU/mL) and full protection (≥0.1 IU/mL) against the disease.

STATISTICAL ANALYSIS

Gender and age distribution, along with mean levels of anti diphtheria IgG, in the studied subjects were determined. Difference in seroimmunity percentages were computed using the chi-square test. Quantitatively, Student’s ‘t’-test was used for comparing mean IgG levels between the sub-groups. Significance level was set at p<0.05.

RESULTS

Of the subjects, 27 (21%) were female and 101 (79%) were male. Age ranged between 1 to 44 years, with a mean value of 17.74 +1.33 years. Although all subjects had been administered diphtheria vaccine at least once, it was found that 63 (49.2%) had only minimal protection against diphtheria, while 65 (50.8%) had full protection. Mean IgG levels in males and females were 0.142±0.011 and 0.133±0.026 IU/mL, respectively (table1). A majority of females (67.66%) had insufficient or minimal protection in contrast to males (44.55%), indicating a

significant difference among the genders (p=0.024), in terms of seroimmunity. While comparing protection according to age groups (table2) significantly lower levels of IgG were observed in subjects who were 11-20 years old (p=0.013), and those that were over 30 years old (p=0.0064), in comparison to the youngest group.

DISCUSSION

This is a first report on seroprevalence of diphtheria among selected population of Rawalpindi/Islamabad (Pakistan), where literacy rate is high and residents are have better health care facilities. The study was designed to determine protection against diphtheria among vaccinated population to assess efficacy of DPT vaccination. Our results indicated that although there was a protective titre, but half of the studied population had minimal level of protection, which is indicative of inadequate vaccination. High proportion of females showed low level of serum antibodies as compared to men. Similar results were obtained in many countries such as Holland, Sweden, England, Israel and the United States of America, where the protection rates against diphtheria in men were higher than in women (McQuillan *et al.*, 2002; Edmunds *et al.*, 2000;Cohen *et al.* 1991; Kjeldsen *et al.*, 1998). Authors have proposed that either vaccination is less efficient in women, or that diphtheria immunity following vaccination might be less long lasting in women (Egemen *et al.* 2000). About 50% of the female subjects showing low level of protection were pregnant at the time of sampling in the present study. This finding is in contrast to studies conducted in Russia, where pregnant women showed high protective titre, which might be due to the fact that in Pakistan there is no programme for diphtheria booster dose administration during pregnancy (Za'tsev *et al.*, 2010). Mean diphtheria antibodies were recorded to be maximum in 1-10 age group, followed by decreases of titre in adolescence. It has been reported that administration of all three doses of the DPT vaccine in recent years has encompassed more than three fourth of the target population (WHO Vaccine Preventable Diseases

Table 1: Comparative protection across genders

Gender	Protected population (%)	Ig G IU/mL (Mean±SEM)	p-value
Male	54.45	0.142±0.011	0.024
Female	33.33	0.133±0.026	

Table 2: Immunity status at different age groups

Age Group (Years)	Ig G IU/mL(Mean±SEM)	Immunity	Booster Required
1-10	0.195±0.031	Full	After 5 years
11-20	0.098±0.018	Insufficient	Immediately
21-30	0.131±0.021	Full	After 5 years
Over 30	0.097±0.007	Insufficient	Immediately

Monitoring System, 2011). Decrease in proportion of antibody against diphtheria has also been reported in United States (McQuillan *et al.*, 2002) and Spain (Pachon *et al.*, 2002). This decrease can be attributed to the fact that for vaccinated persons, immunity begins to wane after some years and opportunities for natural boosting are less common than in the past (Simonsen *et al.*, 1996; Galazka *et al.*, 1995), therefore, in many countries, diphtheria booster after every 10 years is included in adult immunization programs (Levy-Bruhl, 1993; Centers for Disease Control and Prevention, 2003). Low levels of IgG in subjects between 11-20 years of age could be attributed to low compliance of parents in completing the full dose of three DPT injections, and in above 30 year old subjects, it might be related to the fact that they were born before initiation EPI programme in Pak (Chandir *et al.*, 2010). Better seroimmunity in 21-30 years group coincides with the launch of EPI, and with regular immunization days for polio, public awareness for other immunizations has also increased, resulting in more protection in under 10 years old children and increase in coverage of DPT3 upto even 85% of the target population (WHO Vaccine Preventable Diseases Monitoring System, 2011).

This pilot study recorded seroprevalence status of diphtheria among urban population of Rawalpindi/Islamabad. Majority the subjects have minimal protection requiring immediate booster dose, while rest had safe levels, still needing booster every five years. Childhood vaccination does not warrant complete protection to *Corynebacterium diphtheriae* for life, therefore, a booster dose is recommended depending upon the existing protection level of the individual, at a given point of time. An important point is that with nearly half of the population sampled in the capital requiring an immediate booster dose, the situation can be worse in the rural areas, where there is low level of compliance to the three doses of DPT. This work indicates a need to conduct a broad based study, and to address the problem to prevent eventuality of an epidemic.

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