

Diphtheria Immunity Status in Egypt

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The aim of this study was to determine immune status to corynebacterium diphtheria by screening for protective antibodies in a sample of Egyptian population. The study population consisted of 709 healthy subjects aged from 2 months to 105 years, inhabitants of 6 regions of Egypt. The study utilized Enzyme linked immunosorbent assay (ELISA) to measure serum levels IgG antibodies reactive with diphtheria toxoid. Levels of diphtheria toxoid antibody ≥ 0.1 IU/ml were defined as immune/protected, 23.9 % of the population were found to be susceptible to diphtheria (IgG level < 0.01 IU/ml), 43% had basic protection (0.01-0.09 IU/ml), and 33.1% were fully protected (0.1 IU/ml). The results revealed that serum levels of antitoxin antibodies decreased in old ages (< 60 y) with the females being more susceptible than males. These results recommend a booster immunization for the susceptible age groups.

Diphtheria was a major cause of death in the first half of the last century. In 1924, formalin treated toxin (toxoid) vaccine was introduced as an immunizing agent (Ramon, 1924). A significant decrease in the number of clinical cases and carriers was seen in the countries where vaccination against diphtheria was introduced (Skogen et al., 2001). The incidence and pattern of diphtheria in developed countries have changed dramatically in the last 60 years to the point that, until very recently, the occurrence of disease has become a rare event. The world health Organization (WHO 1990) reports a similar, but less dramatic, decrease in its incidence worldwide, although diphtheria remains endemic in many parts of developing countries. Since the mid-1980's there has been a striking resurgence of diphtheria in parts of Eastern Europe. This massive epidemic has now spread to all the Newly Independent States (NIS) of the former Soviet Union (Galazka and Robertson, 1996). In Russia, the reported number of diphtheria cases increased from 603 in 1989 to 15229 in 1993, and 39907 cases in 1994. Many of the diphtheria cases reported in 1993-1994 were imported from the NIS into other countries (Finland, Germany, and Poland). This

highlights the potential for the diphtheria epidemic in the NIS to spread to neighboring countries in Europe, the Middle East, and Asia (Galazka, 2000). Changes in The epidemiology of diphtheria are occurring in developed and developing countries. The shift to older age groups seems to occur in two stages; 1-the disease mainly attacks school children (Jordan 1977-1978, Algeria 1993-1996); 2-shifts to adolescents and young adults (Jordan 1982-1983, Lesotho 1989, China 1988-1989) (Galazka, 2000). These outbreaks have been characterized by high case fatality rates, a large proportion of patients with complications, and the occurrence of the disease in both young and older age groups (Galazka, 2000). Outbreak was reported in preschool children in Yemen with 14% case fatality (Galazka, 2000). Recent outbreaks have occurred in Europe and the United States and Sweden mainly in poor, socioeconomically disadvantaged groups (Galazka and Robertson, 1996). The role of cutaneous diphtheria has been emphasized by several diphtheria outbreaks in the United States (Galazka, 2000). Although there are some few reports from some countries in Middle East, but there is no complete picture for the disease similar to that in Western

world. The only available data from Egyptian Ministry of Health indicated that the diphtheria cases had dramatically decreased from 735 cases in 1981 to two cases in 1999 (Maple et al., 1995). Whether this is due to improved performance of health care and/or the immunization coverage is not clear. Most developing countries use the immunization schedule recommended by WHO with three DPT vaccine doses given at 6, 10, and 14 weeks of age. Other countries offer the first dose of DPT vaccine at beginning at 2 or 3 months of age with the second and third doses given at one or two month intervals (Galazka, 2000). In Egypt, the Ministry of health offers 4 doses of DPT vaccine given at 2, 4, 6, and 18 months of age (Redwan and El-Awady, 2002). The immunization coverage led to changes of diphtheria immunity status in the age-wise distribution. The lowest levels of diphtheria antibodies in various areas of the Soviet Union, and Japan were found in persons 20-40 years old, at present, this least protected group has shifted to persons 30-40 years old (Walory, 2000). In other countries, low-level protection was found in persons 40-50 years old in Australia, England, Germany, and Poland, and in persons >50 years old in Denmark, Finland, Sweden, and the United States (Galazka, 2000). Thus a high proportion of the adult population lacks

immunity and remains susceptible to diphtheria. A large pool of susceptible adults constitutes the potential for an epidemic, especially if this pool is connected with the presence of susceptible children. However, the age group with lowest levels of immunity varies by country; seem mostly related to the year when the childhood immunization program was implemented on a routine basis.

The aim of this study is to evaluate the status of diphtheria immunity in Egypt for first time.

Material and Methods

Samples

In the period from jan.1988-Nov 2001, a total of 709 healthy volunteers (389 males and 320 females) with age range (0.2-105) were collected. The sample corresponds to approximately 0.002% of the Egyptian population. They were randomly selected and referred to clinical laboratories for blood chemistry tests in 6 cities representing north, central, and south Egypt (Alexandria, Kalioubia, Cairo, Giza, Sohag, and Kena). All the samples were residents in these governorates or relevant suburbs and were included in the study according to sex and age, representing the majority of socioeconomic classes of the general population. The statistics of local population samples were calculated according to the significant threshold relative to the following age groups: 0-10 years, 11-20 years, 21-30 years, 31-40 years, 41-50 years, 51-60 years, and >60 years old (table 1), serum samples were frozen and stored at -75°C until antibody testing was performed.

Table 1. Population studied according to age and sex.

Age group(years)	Males	Females	Total	Mean age(years)	Standard deviation of mean (years)
0-1	19	29	38	0.49	0.31
1-2	25	23	48	1.45	0.34
2-3	12	18	30	2.50	0.39
3-10	22	25	47	5.90	2.33
11-20	28	38	66	15.72	2.97
21-30	81	54	135	26.03	2.92
31-40	79	47	126	35.69	2.79
41-50	64	28	92	45.28	3.03
51-60	37	28	65	55.25	3.19
>60	22	31	53	72.39	11.29
Total	389	320	709	29.56	21.21

Enzyme linked immunosorbent assay (ELISA)

Serum samples were tested for diphtheria toxoid immunoglobulins G by ELISA as previously described (Farzad et al., 1986; Simonsen et al., 1986; Severson and Larsen, 1997; Walory et al., 2000). The ELISA test was previously compared with the standard neutralization test and the regression analysis did not show any tendency for ELISA to overestimated antibody concentration (Bergamini et al., 1999). The microtiter plates (Corning-Costar, UK) were coated with purified diphtheria toxoid 0.3ug/50ul/well (specific activity 2020 Lf/mg protein nitrogen, VACSERA, Agouza-Cairo, Egypt), in 0.05 M carbonate/bicarbonate buffer pH 9.6. The plates were incubated for 3 h at room temperature and then at 4°C overnight. The diphtheria solution was removed from the plates, and then the plates were sealed and stored at 4°C. The plates were used over test period of 4 months without loss of sensitivity. The plates were washed with PBS pH 7.2 containing 0.05% tween 20 then blocked with 1% BSA-0.05% tween 20 in PBS for 1 h at 37°C. The diluted serum (1:100 in PBS-0.5% BSA-0.05% tween 20) were distributed in triplicate aliquots of 100 µl/well and incubated for 1 h at 37°C. After incubation, peroxidase-labelled goat anti-human IgG (H+L) conjugate (KPL, Gaithersburg, MD, USA) diluted 1:1500 in PBS-BSA-tween 20 buffer was added. Plates were incubated for 1 h at 37°C washed with PBS-BSA-tween 20, then with PBS only. The ready made TMB peroxidase substrate (Sigma, MO, USA) was added to the plates. The reaction was terminated after 10 min by adding 0.5 M HCl.

The absorbency values of the diluted sera were measured at 405 nm using automatic multiscane plate reader (EMS Labssystem Analyzer, MD, USA).

The absorbencies were converted to antitoxin titer in IU ml⁻¹ using a six point calibration curve (0.005, 0.01, 0.02, 0.04, 0.08, 0.16 IU ml⁻¹) prepared from human serum with an antitoxin level of 0.2 IU ml⁻¹ (young

volunteers 20-25 years old). This serum was calibrated against WHO diphtheria antitoxin equine serum (gift from VACSERA, Agouza Cairo, Egypt).

According to the following internationally accepted definitions of diphtheria protection (Maple et al., 1995; Edmunds et al., 2000), subjects with antitoxin concentration below 0.01 IU ml⁻¹ are considered susceptible, 0.01-0.09 IU ml⁻¹ to provide basic protection against the toxic manifestation of disease, and ≥ 0.1 IU ml⁻¹ to be fully protected. Differences in properties were evaluated using the student t-test. A *p* value <0.05 was considered significant. According to international epidemiological standards, a population with more than 30% of individuals with non-protective (susceptible) titers against *C. diphtheria* was considered at risk of diphtheria (Walory et al., 2001).

Results

Degrees of diphtheria immunity are shown in Table 2, 23.9% of the population studied was susceptible to diphtheria, 43% had basic protection and 33.1% were fully protected. The immunity decreased with increasing the age groups. Table 2 show the immunity levels of 0-10; 41.9%, 26.7%, 29.7% were susceptible, basic, and fully protected, respectively. In addition, the females (47.9%) were significantly more susceptible than males (38.5%). While 38.5% males and 23.4% females were fully protected against diphtheria.

Table 2. Diphtheria antitoxin levels according to age.

Subjects with antitoxin level DT										
Age Group Years	Number of Subjects	Susceptible (<0.01IU/ml)			Basic(0.01-0.099IU/ml)			Full(>=0.1IU/ml)		
		No	%	CI95%	No	%	CI95%	No	%	CI95%
0-10	172	75	41.9	0.003-0.004	45	26.7	0.03-0.04	52	29.7	0.18-0.26
11-21	66	9	15.2	0.001-0.004	32	45.5	0.02-0.05	26	39.4	0.13-0.21
21-30	135	16	11.9	0.003-0.005	62	45.9	0.03-0.05	57	42.2	0.17-0.23
31-40	126	18	17.5	0.003-0.005	58	44.4	0.03-0.04	48	38.1	0.14-0.22
41-50	92	8	8.7	0.001-0.003	50	54.3	0.04-0.05	34	36.9	0.12-0.23
51-60	65	9	13.8	0.002-0.007	43	66.2	0.03-0.06	13	20.0	0.12-0.14
>60	53	33	62.3	0.003-0.005	14	26.4	0.04-0.07	6	11.3	0.05-0.38
Total	709	170	23.9	0.001-0.008	303	42.7	0.02-0.08	236	33.3	0.05-0.39

However, after dividing the age group 0-10 into 0-1, 1-2, 2-3, and 3-10 years, the susceptible and the basic protection were not significantly different in both sexes of all groups. Females have more significant

($p > 0.05$) protection levels than males (Figure 1, 2). The percentage susceptibility decreased gradually from age group 0-1 year until 3-10 years old in both sexes (Table 3).

Table 3. Age susceptibility (diphtheria antitoxin level < 0.01 IU/ml) according to sexes.

Age Group year	Males				Females			
	Total	No Susceptible	%	CI95%	Total	No Susceptible	%	CI95%
0-1	19	11	47.4	0.003-0.004	29	13	44.8	0.003-0.005
1-2	25	8	32.0	0.004-0.008	23	7	30.4	0.001-0.003
2-3	12	4	33.3	0.001-0.006	18	6	33.3	0.001-0.004
3-10	22	7	31.8	0.001-0.009	24	8	33.3	0.002-0.007
11-21	28	3	10.7	0.007-0.001	38	6	15.8	0.002-0.006
21-30	81	6	7.4	0.002-0.006	54	10	18.5	0.002-0.004
31-40	79	9	11.4	0.002-0.005	47	11	23.4	0.003-0.006
41-50	64	6	9.4	0.003-0.008	28	2	7.1	0.001-0.007
51-60	37	3	8.1	0.002-0.006	28	6	21.4	0.005-0.009
>60	22	8	36.4	0.002-0.007	31	27	87.1	0.003-0.005
Total	389	65	16.7	0.002-0.090	320	107	33.4	0.001-0.009

The overall prevalence was 16.7% in males and 33.4% in females.

Figures 1 and 2 show the prevalence, with distinguished age and sex, of subjects with the three different immunity levels (susceptible, basic protection and full protection). Among the age group 21-30 y, a greater percentage of fully protected males were significantly more than females (53.1% males vs 23.9% females; $p < 0.005$). For all ages the prevalence of subjects with antitoxin antibody level > 0.1 was greater for males (37%) than females (31.3%) with no statistical significance.

There is some evidence of a sex effect (Table 3), although similar proportions of males and females belonging to the younger age groups are protected, the susceptibility increased among women from 23.4% in the age group 21-30 y to 89.1% in the age group

> 60 , and overall they were less protected than men. The difference is particularly evident and statistically significant among the age group 21-30 y (7.4% males and 28.5% females $p < 0.005$), 31-40 (11.4% males, 23.4% females $p < 0.005$) and 51-60 (18.1% males, 21.4% females $p < 0.07$).

For all ages a smaller proportion of males (16.7%) than females (33.4%) were unprotected, this difference was statistically significant $p < 0.005$ (Table 3). Unexpected result was in age 0-10 years, 38.5% males and 47.9% females ($p < 0.05$) were susceptible. While 38.5% males and 23.4% females were fully protected against diphtheria. The basic protection rate was 23% and 28.7% of males and females, respectively.

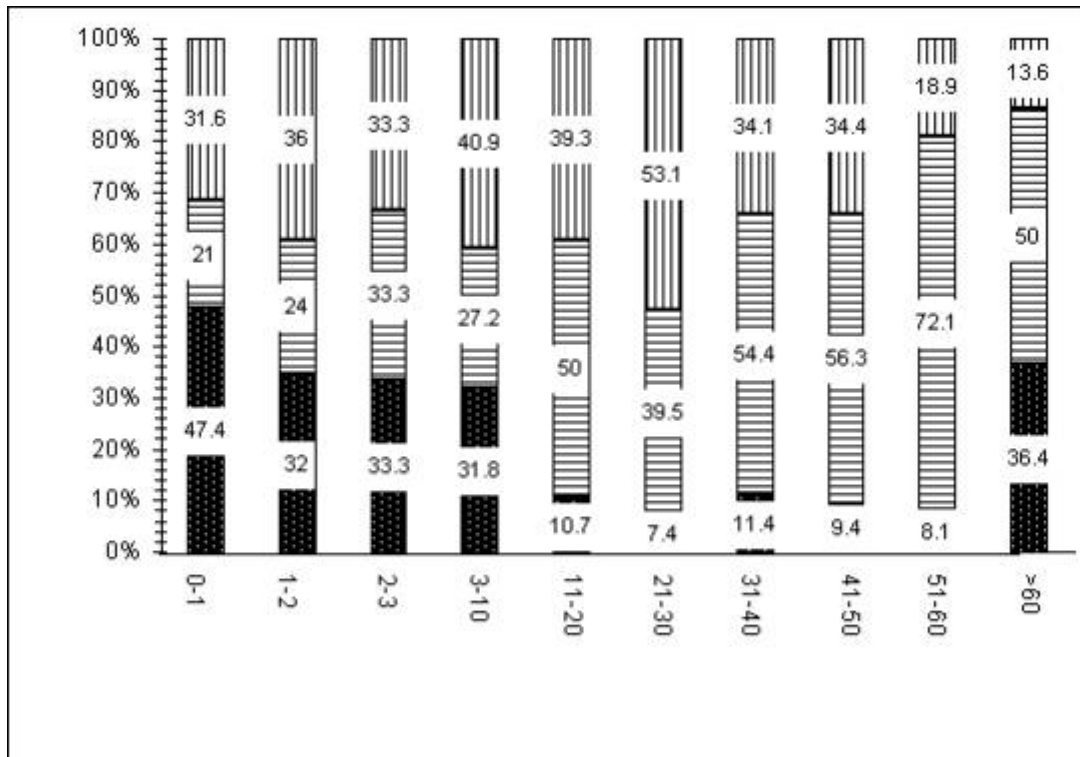


Figure 1. Age specific prevalence (%) of diphtheria antitoxin levels in males. Full protection (vertical line), Basic protection (horizontal line), susceptible (white dot).

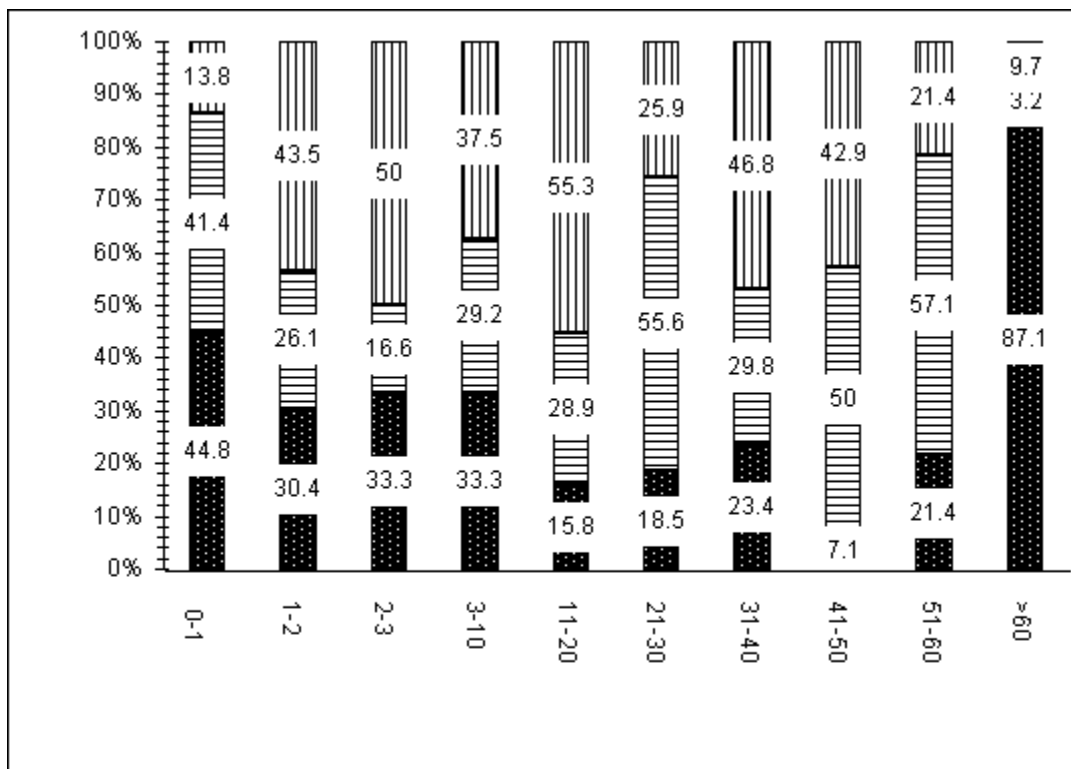


Figure 2. Age specific prevalence (%) of diphtheria antitoxin levels in females. Full protection (vertical line), Basic protection (horizontal line), susceptible (white dot).

Discussion

This is the first survey to measure diphtheria antibody levels in serum samples collected from Egyptian population. In Egypt, immunization against diphtheria is a part of the national vaccination program and consisted of a 4 dose primary schedule given at 2, 4, 6 and 18 months of age as a triple vaccine Diphtheria, Pertussis and Tetanus (DPT) (Redwan and EL-Awady, 2002). We have found low immunity in males (38.5%) and females (47.9%) in the age range 0-10 years, and that 62.3% of subjects older than 60 years have insufficient protection against diphtheria. The low immunity was observed before in France, Russia, USA and Norway, where more than 50% of age 7-10 years had insufficient protection (Skogen et al., 2001; Galazka and Robertson, 1996). The low immunity in our subjects may be explained in four ways. 1) The socioeconomic dimension of the population. Some population sectors would benefit from audio-visual campaigns to educate them about the importance of vaccination, 2) The Egyptian primary immunization schedule is unable to maintain an adequate protection level for long time, 3) some regions in Kena, Dakahlia and Giza governorates are infected with *S. mansoni* and/or *S. haematobium* (Redwan, 1995) which drastically down regulates the immune response (in mouse model) against diphtheria vaccine (Haseeb and Craig, 1997), 4) this may be due to lower levels of exposure to *C. diphtheriae* (Galazka, 2000). Although the diphtheria vaccine confers incomplete protection on an individual basis, reduction of tox C. diphtheria to almost undetectable levels in the community has virtually eliminated diphtheria from those countries in which vaccination of entire population has been achieved (Schneerson et al., 1996). At any rate, based on these results, the vaccination should be re-introduced to the Egyptian

vaccination program at school-entry and graduation and/or improve the performance of routine immunization. Generally, children above 1 year had some protection against diphtheria, but it is insufficient in age 0-1 year. This may due to the fact that immunization schemes are not complete yet (Figure 1, 2). If we exclude the 0-1 year old group, the unprotected percentages still high (33.3%) in children 2-10 years old. Whether these results are due to decreased immunization coverage among infants and children, including the socioeconomical aspects; or increased in the population number and subsequently the lack of adequate supplies for prevention; or insufficiently motivated and improperly trained health care workers, especially at the primary health care level. Comparison of the antibody levels against diphtheria between different countries is complicated because of the use of different vaccination programs, different vaccines, and different dosing schedules, in addition to the socioeconomic levels of the population. Generally, the herd immunity against Diphtheria (protection level 76%) reported in our study is moderate to greater than the level found in some western countries (Gasparini et al., 1997). For comparison, the percentage of people unprotected in the general population: Sweden 56.9% of the population between 31-40 years old, Germany 52.2% of the population aged 20-34 y, UK 50% of both female and male of age greater than 30 y, France 54% of the individuals over 40 y and 67 % of age 65 y, and only 23% of adults and 22% of the 20-30 year group in Italy had inadequate levels (Edmunds et al., 2000; Gasparinin et al., 1997). Similarly low percentage of protected adults was reported from Spain (Galazka, 2000), Nordic countries and Poland (Walory et al., 2001). In the United States, the lowest level of seroprotection was found in people over 50 years of age (Walory et al., 2001).

Our results show a gradual tendency of increasing susceptibility in ages greater than 60 y. This may be due to the immunity wanes phenomena in that age (Skogen et al., 2001). A sex effect was also observed in which fewer women were fully protected (Figure 1). This difference in the antibody level between sexes has already been observed in other countries (Gasparini et al., 1997). This is probably due to the fact that the males are serving in the army where vaccination is mandatory.

In conclusion, the immune status to diphtheria in Egypt appears alarming, specially, in light of the outbreaks of diphtheria in recent years in the former Soviet Union. We should be concerned about the possibility of re-emergence of this disease. Our results suggest that it may be prudent to re-vaccinate subjects/age groups that have inadequate protective antibodies against diphtheria.

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