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Status of Protective Immunity against Diphtheria among Apparently Healthy Adult Population in Sylhet Region of Bangladesh

Khandaker Md. Tasnim Sajid[1*] Tarana Jahan[2] Md. Arifur Rahman[3]
Md. Badrul Islam[4] Sonia Afroz[5] Jasmin Akhtar[6]

ABSTRACT

Background: The epidemiological studies of recent years show that there is a change in incidence of diphtheria in different age group. Adults become more susceptible to diphtheria due to reduced opportunities to keep high immunity through subclinical infection. The purpose of the present study was to see the status of protective immunity againstdiphtheria among apparently healthy adult population.

Materials and methods: This cross sectional descriptive study was conducted in the Department of Microbiology, Sylhet MAG Osmani Medical College, Sylhet, Bangladesh during the period from July 2016 to June 2017 for duration of one year. By following a set of inclusion and exclusion criteria, a total 200 apparently healthy individuals were enrolled in this study. For laboratory procedure, anti-diphtheria antibody titer was measured by ELISA method following theinstructions provided by manufacturer's package insert.

Results: A total of 200 individuals were included in this study. Among them 71 (35.5%) participants had protective immunity and 129 (64.5%) participants had no protective immunity against diphtheria. Protective immunity was higher in age group 18 to 25 years 63(81.8%) and it was declined as age increases. Protective immunity against diphtheria was found in 40 (38.8%) participants of male and 31 (32.0%) participants of female. Protective immunity against diphtheria was found in 43 (55.8%) participants of upper middle class & 44 (63.8%) participants of student.

Conclusion: This study yielded the fact that significant number of participants remains unprotected against diphtheria. Where large-scale immunization against diphtheria has been implemented, the incidence of the disease has dropped dramatically.

KEY WORDS

Anti-diphtheria antibody; Diphtheria toxin; Diphtheria; Protective immunity; Vaccine.

INTRODUCTION

Diphtheria is a highly-contagious life threatening disease caused by toxigenic strains of *Corynebacterium diphtheria*. It is an aerobic Gram-positive bacterium, which are transformed by a bacteriophage carrying the toxin gene. Diphtheria causative agent and its major virulence factor diphtheria toxin are well studied, but outbreaks of disease still occur worldwide.[1] Diphtheria Toxin (DT) is responsible for the local cell damage at site of bacterial colonization as well as for distant toxic effect on peripheral nervous system, kidneys and heart. DT also helps bacteria to evade immune defense mechanisms and to escape from phagocytosis. Small amounts of toxin can impair protein synthesis in both polymorphonuclear leukocytes and mononuclear cells. DT also enters into the blood circulation via damaged epithelia and thus cause severe systemic toxic effects.[1,2] The organism can also infect the skin at the site of a pre-existing skin lesion. This occurs primarily in the tropics but can occur worldwide in indigent persons with poor skin hygiene.[2] Overcrowding, poor health, substandard living conditions, incomplete immunization and immunocompromised states facilitate susceptibility to diphtheria and are risk factors associated with transmission of this disease.[3] Now a day'sdiphtheria evolves from children's disease into disease affecting predominantly, adults, with severe respiratory forms of infection.[4] Despite the widespread use of immunization, diphtheria remains endemic in several regions including Africa, India, Bangladesh, Nepal, Indonesia, Vietnam,

1. Assistant Professor of Microbiology
Bangabandhu Sheikh Mujib Medical College, Faridpur.
2. Assistant Professor of Microbiology
Monno Medical College, Manikganj.
3. Associate Professor of Microbiology
Brahmanbaria Medical College, Brahmanbaria.
4. Associate Professor of Microbiology
Dhaka National Medical College, Dhaka.
5. Assistant Professor of Microbiology
Abdul Malek Ukil Medical College, Noakhali.
6. Assistant Professor
National Institute of Traumatology & Orthopaedic Rehabilitation, Dhaka.

*Correspondence : Dr. Khandaker Md. Tasnim Sajid
Email: sajidtasnim32 @gmail.com
Cell : +88 01715 51 25 56

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the tropics and areas of South America including Brazil.[5,1] However, the majority of the adult populations in Europe, Australia and the United States have no immune protection against this infection. Diphtheria remained endemic in some states of United States through the 1970s, with reported incidence rates of greater than 1.0 per million populations in Alaska, Arizona, Montana, Mexico, South Dakota and Washington. Most of these infections were attributed to incomplete vaccination[3].In the last 10 years, there have been a number of reports of either re-emergence or persistence of diphtheria from several Indian states including Andhra Pradesh, Delhi, Assam, and West Bengal etc. Persistence or resurgence of diphtheria in the country was mainly due to low coverage of primary immunization as well as boosters.[6]

Diphtheria antibody production, primarily of IgG type, can be induced by natural toxin during clinical or subclinical infection, carrier state or by immunization with diphtheria toxoid.[7] Booster immunization in every ten years is important to maintain antitoxin level in adults. Large populations of older adults may be susceptible to diphtheria, in both developed as well as in developing countries.[8] Diphtheria vaccination of the elderly population has been recommended as a routine in some countries like USA, Iran, Brazil, Italy etc.[8-10] Assessing immunity to diphtheria in the elder persons is necessary as antibody level decreases with increasing age. Changes in the epidemiology of diphtheria are occurring worldwide. A large proportion of adults in many industrialized and developing countries are now susceptible to diphtheria.[5] Immunity to diphtheria wanes with time after vaccination, and many older adults may not have received either a primary vaccination series or a recommended tetanus-diphtheria toxoid booster every 10 years.[11] In current EPI schedule of Bangladesh, Diphtheria Toxoid is given as a part of penta-valent vaccine (Diphtheria, Pertussis, Tetanus, Haemophilus Influenza and Hepatitis-B). The vaccine consists of 3 doses, at the 6th, 10th and 14th weeks of age as was during the commencement of vaccination in 1979.[12] Bangladesh has already achieved UN award in 2010 for fulfilling all the parameters of MDG goal including EPI coverage. So to achieve Sustainable Development Goal (SDG) it is important to focus on maintaining the immune status against communicable diseases like diphtheria.

MATERIALS AND METHODS

This cross sectional descriptive study was conducted in the Department of Microbiology, Sylhet MAG Osmani Medical College, Sylhet, Bangladesh. This study was carried out during the period from July 2016 to June 2017 for duration of one year. All 18 to 38 years aged healthy adult persons in Sylhet region fulfilling the enrollment criteria were selected as study population. Inclusion criteria were apparently healthy adult in the age group of 18 to 38 years and irrespective of gender who have undergone primary vaccination against diphtheria. Persons were excluded who unable to provide history about vaccination, have history of diphtheria, having chronic illness, taking immunosuppressant drugs or steroid therapy and immunocompromised persons. After selection of study population who were mostly available, easily accessible and convenient to include were identified against a serial number. Sample population was selected by lottery by hand. All the participants were thoroughly informed about their roles and the procedure of this research work. Data were collected by predesigned data collection sheet. Informed written consents were obtained from all the subjects. All information was kept confidential with due respect to the participants wish and without any force or pressure. Approval of the research protocol and ethical permission were obtained from the Ethics Review Committee of MAG Osmani Medical College, Sylhet. All the ethical committee guidelines were followed during the study period. After proper aseptic precaution 5 ml of venous blood was collected in a vaccutainer tube and was allowed to clot. Then it was centrifuged at 2000 rpm for 10 minutes and then 0.2 ml of serum was transferred carefully into eppendorf tubes, properly capped, labeled and stored in -20 0 C and analysis was done later. All reagents were kept in proper temperature before use. All steps of procedure were completed without interruption. Estimation of Antidiphtheria antibody (IgG) was done using ELISA kits manufactured by DRG GmbH, Germany. The quantitative immunoenzymatic determination of IgG-class antibodies against Corynebacterium diphtheriae toxin is based on the ELISA (Enzyme Linked Imunosorbent Assay) technique. Before assaying, all samples should be diluted 1+100 with IgG sample diluents and 10µ lsample and 1 µl IgG sample diluents into tubes was dispensed to obtain a 1+100 dilution and thoroughly mixed with vortex.

RESULTS

A total number of two hundred healthy subjects were recruited after fulfilling the inclusion and exclusion criteria. In this study 71 (35.5%) participants had protective immunity and 129 (64.5%) participants had no protective immunity against diphtheria.Distribution of the participants according to protective immunity against diphtheria was shown in Figure-I.

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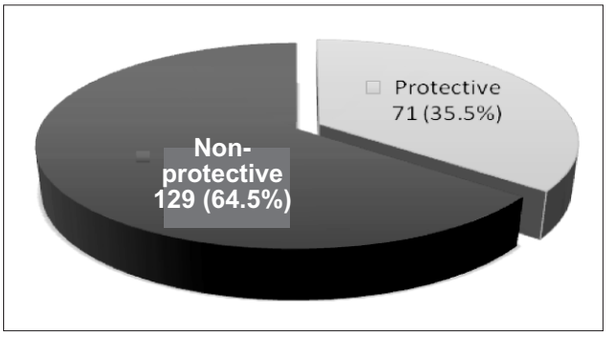

Figure 1 Pie chart showing distribution of the participants according to protective immunity against diphtheria (n=200)

Protective immunity against diphtheria was found in 63 (81.8%) participants of aged between 18 to 25 years, 7 (9.9%) participants of aged between 26 to 32 years and 1 (1.9%) participants of aged between 33 to 38 years. Protective immunity did not differ significantly (p<0.001). Protective immunity against diphtheria in different age group was shown in Table I.

Table I Showing protective immunity against diphtheria in different age group

Age Group	Protective Immunity	No Protective Immunity	Total	p Value
18-25 years	63 (81.8%)	14 (18.2%)	77(100.0%)	
26-32 years	7 (9.9%)	64 (90.1%)	71(100.0%)	p<0.001
33-38 years	1 (1.9%)	45 (98.1%)	52(100.0%)	

*Chi-Square (χ^2) test was performmed to see the association. p≤0.05 was determined as level of sigificance.

Protective immunity against diphtheria was found in 40 (38.8%) participants of male and 31 (32.0%) participants of female. Protective immunity did not differ significantly (p=0.310). Protective immunity against diphtheria between male and female was shown in Figure 2.

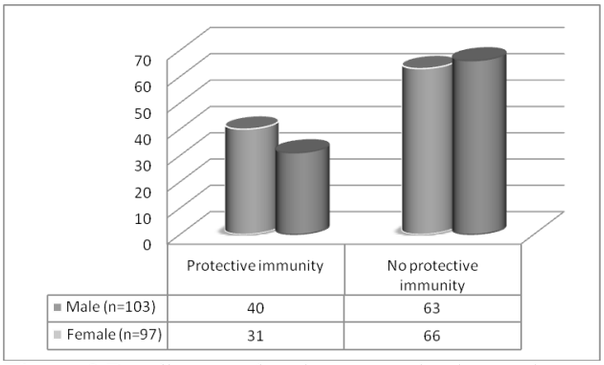

Figure 2 Bar diagram showing protective immunity against diphtheria between male and female

Protective immunity against diphtheria was found in 43 (55.8%) participants of upper middle class, 28 (23.7%) participants of lower middle class and none of poor class of socioeconomic status. Protective immunity did not differ significantly (p<0.001). Protective immunity against diphtheria in different socioeconomic status was shown in Table II.

Table II Showing protective immunity against diphtheria in different socioeconomic status

Socioeconomic status	Protective Immunity	No Protective Immunity	Total	p Value
Poor	0 (0.0%)	2 (100.0%)	2 (100.0%)	p<0.001
Lower middle	28 (23.7%)	90 (76.3%)	118 (100.0%)	
Upper middle	43 (55.8%)	37 (46.2%)	80(100.0%)	

*Chi-Square (χ^2) test was performmed to see the association. p≤0.05 was determined as level of significance.

Protective immunity against diphtheria was found in 44 (63.8%) participants of student, 6 (27.3%) participants of house wife, 11 (17.2%) participants of service holder, 3(30.0%) participants of businessman, 5 (21.7%) participants of physician and 2 (16.7%) participants of other occupation. Protective immunity against diphtheria in different occupation was shown in Figure 3.

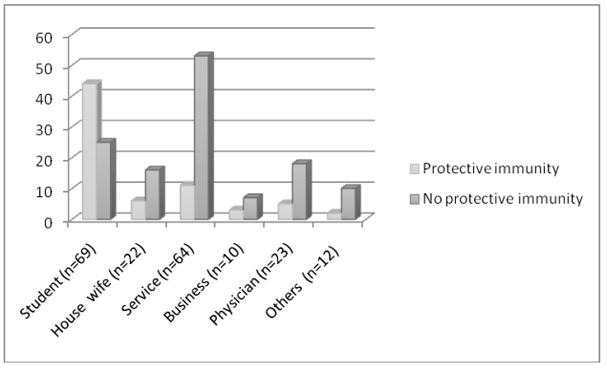

Figure 3 Bar diagram showingProtective immunity against diphtheria in different occupation

DISCUSSION

To see the status of protective immunity against diphtheria among apparently healthy adult population 200 participants have been selected according to inclusion and exclusion criteria. The immune status is represented by certain level or titer of antibody, called antitoxin, against C. diphtheriae and is primarily of the IgG type. So a level of antibody concentration 0.1 IU/ml or higher titer may be needed for full protection. In this study 71 (35.5%) participants have protective immunity against diphtheria and 129 (64.5%) participants

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have no protective immunity against diphtheria. In North Kerala, India immunity rates were found 46.6% & in Catalonia, Spain 68.3%.[13,14] The finding in Iran was remarkable where immunity rates were 99.6%.[9]

Regarding the age distribution of the study population minimum age are 18 years and maximum age are 38 years among 200 participants. The age of the participants (n=200) ranged from 18 to 38 years with the mean age of 27.15 (SD ± 5.24) years. There are 77 (38.5%) participants in the age group of 18-25 years, 71 (35.5%) participants in the age group of 26-32 years and 52 (26.0%) participants in the age group of 33-38 years. Protective immunity against diphtheria was found in 63 (81.8%) participants of aged between 18 to 25 years, 7 (9.9%) participants of aged between 26 to 32 years and 1 (1.9%) participants of aged between 33 to 38 years. Protective immunity did not differ significantly ($p<0.001$). According to a research, younger people were more likely to have protective antibody level than older people which ultimately revealed the fact that protective level of antibody decreases gradually with increasing age.[10]

There are 103 (51.5%) male participants and 97 (48.5%) female participants (n=200). Protective immunity against diphtheria has found in 40 (38.8%) participants of male and 31 (32.0%) participants of female. Protective immunity did not differ significantly ($p=0.310$). So sex difference does not effective in changing antibody titer against diphtheria. A study conductedin Thailand has shown almost the same outcome.[15] Some dissimilar findings were also seen. The antibody levels were significantly higher in males from ≥25 years age, due to military service with administration of booster.[10]

In this study 118 (59.0%) participants comes from lower middle class among them protective immunity against diphtheria was found in 28 (23.7%) participants, 80 (40.0%) participants from upper middle class among them protective immunity 43 (55.8%) participants and 2 (1.0%) poor participants and none have protective immunity. Protective immunity in different socio-economic condition did not differ significantly ($p<0.001$). Socio-economic condition has an impact on an individual's nutritional status, health education and awareness about vaccination that ultimately influences immune status. In the Chaina, higher percentage of participants had protective antibody to diphtheria toxin with increasing level of education.[16]

The distribution of participants according to occupation are 69 (34.5%) student, 64 (32.0%) service holder, 23 (11.5%) physician, 22 (11.0%) house wife, 10 (5.0%) businessman and 12 (6.0%) other occupations. Protective immunity against diphtheria was found in 44 (63.8%) participants of student, 6 (27.3%) participants of house wife, 11 (17.2%) participants of service holder, 3(30.0%) participants of businessman, 5 (21.7%) participants of physician and 2 (16.7%) participants of other occupation. Protective immunity was differ significantly among different occupation ($p<0.001$). A study conducted in Spain among adult individuals shown that almost the same outcome.[14]

LIMITATION

The limitations of the study are as it one- centered study, sample size was small due to limitation of time and resource, data was collected based only on recalled memory of participants and parents. No documents to support childhood immunization claim were available.

CONCLUSION

Diphtheria was a major cause of childhood mortality in the pre-vaccination era but now diphtheria evolves from children's disease into disease affecting predominantly, adults. Today it is clear that high immunization coverage, prompt diagnosis and rapid identification of close contacts are principal things in control of diphtheria outbreaks. Waning immunity to diphtheria was observed over time after childhood vaccination. This study had shown the necessity of administering additional doses of vaccine among adult population.

RECOMMENDATION

Immunization programme for diphtheria should be extended to adults, as present the EPI programme does not cover adult vaccination.Further multi-centered study should be done with a larger population and longer duration.

DISCLOSURE

All the authors declared no competing interest.

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