Seroprevalence of Diphtheria IgG Antibody in Relation with Socio-demographic Change at a Tertiary Care Hospital in Bangladesh

Khandaker Md. Tasnim Sajid, Premananda Das, Tarana Jahan, Shib Prasad Sinha, Toufique Tania Prity, Md. Arifur Rahman, Siddika Khatun

1Assistant Professor, Department of Microbiology, Cox’s Bazar Medical College, Cox’s Bazar, Bangladesh; 2Associate Professor, Department of Microbiology, Syllhet MAG Osmani Medical College, Syllhet, Bangladesh; 3Assistant Professor, Department of Microbiology, Monno Medical College, Manikganj, Bangladesh; 4Lecturer, Department of Virology, Syllhet MAG Osmani Medical College, Syllhet, Bangladesh; 5Medical Officer, Department of Medicine, Ibrahim General Hospital, Dhaka, Bangladesh; 6Associate Professor, Department of Microbiology, Brahmanbaria Medical College, Brahmanbaria, Bangladesh; 7Assistant Professor, Department of Biochemistry, Ibn Sina Medical College, Dhaka, Bangladesh

[Received on: 22 April 2021; Accepted on: 12 May 2021; Published: 1 July 2021]

Abstract
Background: Seroprevalence of diphtheria antibody may vary in different socioeconomic people. Objective: The purpose of the present study was to determine the seroprevalence of diphtheria IgG antibody and to identify the relationship between diphtheria seroprevalence and several sociodemographic characteristics. Methodology: This cross-sectional study was conducted in the Department of Microbiology at Syllhet MAG Osmani Medical College, Syllhet, Bangladesh from July 2016 to June 2017 for duration of one year. Apparently healthy individuals were enrolled in this study as study population by following a set of inclusion and exclusion criteria. For laboratory procedure, anti-diphtheria antibody titer was measured by ELISA method. Result: A total number of 200 healthy individuals were recruited for this study. The mean serum IgG level was 0.23±0.26 IU/mL in participants of aged between 18 to 25 years which was much lesser in participants of aged between 26 to 32 years and 33 to 38 years group (p=0.001). Mean serum IgG level was 0.12±0.12 IU/mL in male participants. Majority participants have been come from lower middle class having mean serum IgG level of 0.10±0.21 IU/mL. Conclusion: In conclusion diphtheria IgG level is most commonly found among young male adult person in upper middle class socioeconomic condition.

Keywords: Diphtheria; seroprevalence; IgG antibody; socioeconomic condition

Introduction
Diphtheria is an acute infection caused by Corynebacterium diphtheriae. The primary lesion usually occurs in the throat or nasopharynx and is characterized by the presence of a spreading grayish pseudomembranous growth. As the organism multiplies at this site, they elaborate a potent exotoxin that is transported by the blood to remote tissues of the body causing hemorrhagic and necrotic damage in various organs. This occurs primarily in the tropics but can occur worldwide in indigent persons with poor skin hygiene. 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. Although diphtheria is now reported infrequently in the world, in the pre-vaccine era, the disease was one of the most common causes of illness and death among children. Today diphtheria evolves from children’s disease into disease affecting...
transported by the blood to remote tissues of the body. Pseudomembranous growth, as the organism multiplies by the presence of a spreading grayish occlusion in the throat or nasopharynx and is characterized by the disease was one of the most common causes of transmission of this disease. Although diphtheria is now diphtheria and are risk factors associated with immunocompromised states facilitate susceptibility to conditions, incomplete immunization and socio-demographic change. Seroprevalence of Diphtheria IgG Antibody in Relation with Socio-demographic Change. Sajid et al. Bangladesh number of diphtheria cases were serially continuous occurrence of few cases of diphtheria in subclinical infection, carrier state or by immunization can be induced by natural toxin during clinical or primary immunization as well as boosters. 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. In Bangladesh however there is a continuous occurrence of few cases of diphtheria. The disease, which is common among under five children in the past, is now affecting older children (5-19 years) and adults. Persistence or resurgence of diphtheria in the country was mainly due to low coverage of primary immunization.

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. In Bangladesh however there is a continuous occurrence of few cases of diphtheria. The disease, which is common among under five children in the past, is now affecting older children (5-19 years) and adults. Persistence or resurgence of diphtheria in the country was mainly due to low coverage of primary immunization.

In this study majority participants were student which 69 (34.5%) participants were student 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. After proper aseptic precaution 5 ml of venous blood was collected in a vacutainer 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 centrifuge tubes, properly capped, labeled and stored in -20°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 anti-diphtheria 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 Immunosorbent Assay) technique. Before assay, all samples should be diluted 1+100 with IgG sample diluents and 10 µl sample and 1 µl IgG sample diluents into tubes was dispensed to obtain a 1+100 dilution and thoroughly mixed with vortex. All data were processed and analyzed with the help of SPSS (Statistical package for Social Sciences) Version 21.0. Quantitative data were expressed as mean and standard deviation and qualitative data as frequency and percentage. Association was analyzed by Pearson’s Chi square (X²) test. A probability (p) value of <0.05 was considered statistically significant.

Results
A total number of 200 respondents were recruited after fulfilling the inclusion and exclusion criteria. The age of the participants ranged from 18 to 38 years with the mean age of 27.15 (SD ± 5.24) years. Majority participants aged between 18-25 years which was 77(38.5%) participants followed by 26-32 years and

Methodology
This descriptive cross-sectional study was conducted in the Department of Microbiology at 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. 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. After proper aseptic precaution 5 ml of venous blood was collected in a vacutainer 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 centrifuge tubes, properly capped, labeled and stored in -20°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 anti-diphtheria 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 Immunosorbent Assay) technique. Before assay, all samples should be diluted 1+100 with IgG sample diluents and 10 µl sample and 1 µl IgG sample diluents into tubes was dispensed to obtain a 1+100 dilution and thoroughly mixed with vortex. All data were processed and analyzed with the help of SPSS (Statistical package for Social Sciences) Version 21.0. Quantitative data were expressed as mean and standard deviation and qualitative data as frequency and percentage. Association was analyzed by Pearson’s Chi square (X²) test. A probability (p) value of <0.05 was considered statistically significant.

Results
A total number of 200 respondents were recruited after fulfilling the inclusion and exclusion criteria. The age of the participants ranged from 18 to 38 years with the mean age of 27.15 (SD ± 5.24) years. Majority participants aged between 18-25 years which was 77(38.5%) participants followed by 26-32 years and
33-38 years which was 71 (35.5%) participants and 52 (26.0%) participants (Figure I).

The mean serum IgG level was highest in participants of aged between 18 to 25 years which was 0.23 ± 0.26 IU/mL, followed by participants of aged between 26 to 32 years & 33 to 38 years which were 0.06 ± 0.10 IU/mL and 0.04 ± 0.03 IU/mL. There was a statistical significant difference of mean serum IgG level among the different age groups (F=25.100; p<0.001).

Among 200 respondents male participants were predominant than female which was 103 (51.5%) and 97 (48.5%) respectively. Figure II showed the frequency distribution of participants according to gender.

The mean serum IgG level was 0.12 ± 0.12 IU/mL in male participants and 0.12 ± 0.22 IU/mL in female participants. There was no statistical significant difference of mean serum IgG level male and female (t=-0.022; p=0.982).

In this study majority participants were student which was 69 (34.5%) respondents followed by service holder, physician, house wife, other occupations, & businessman which were 64(32.0%), 23(11.5%), 22 (11.0%),12 (6.0%) and 10 (5.0%) participants(Table 1).

Table-1: Showing distribution of participants according to occupation (n=200)

| Occupation  | Frequency | Percent |
|-------------|-----------|---------|
| Student     | 69        | 34.5    |
| House wife  | 22        | 11.0    |
| Service     | 64        | 32.0    |
| Business    | 10        | 5.0     |
| Physician   | 23        | 11.5    |
| Others      | 12        | 6.0     |
| **Total**   | **200**   | **100.0**|

The mean with SD of serum IgG level was predominant among business which was 0.19 ± 0.30 IU/mL followed by other occupation, student,
physicians, housewife, service holder which was 0.18 ± 0.49 IU/mL, 0.18 ± 0.20 IU/mL, 0.08 ± 0.06 IU/mL, 0.07 ± 0.07 IU/mL, 0.06 ± 0.08 IU/mL. There was significant difference of mean serum IgG level among different occupation (F=3.788; p=0.003) (Table 2).

Table 2: Showing serum IgG level among different occupation

| Occupation    | Mean±SD  | P value |
|---------------|----------|---------|
| Student       | 0.18±0.20|         |
| Housewife     | 0.07±0.07|         |
| Service       | 0.06±0.08| 0.003   |
| Business      | 0.19±0.30|         |
| Physician     | 0.08±0.06|         |
| Others        | 0.18±0.49|         |

Data were presented as mean and standard deviation; One way NOVA was performed to see the association; P≤0.05 was determined as level of significance.

In this study most of the participants came from lower middle class 118 (59.0%), from upper middle class 80 (40.0%) participants and from poor class 2 (1.0%) participants (Table 2).

Table 3: Showing distribution of socioeconomic status of the participants (n=200)

| Socioeconomic status | Frequency | Percent |
|----------------------|-----------|---------|
| Poor class           | 2         | 1.0     |
| Lower middle class   | 118       | 59.0    |
| Upper middle class   | 80        | 40.0    |
| Total                | 200       | 100.0   |

The mean serum IgG level was found high among upper middle class 0.16 ± 0.18 IU/mL followed by lower middle class & poor class which was 0.10 ± 0.21 IU/mL and 0.04 ± 0.01 IU/mL. There was no statistical significant difference of mean serum IgG level among different socioeconomic status (F=2.317; p=0.1017).

Figure V: Bar diagram showing serum IgG level among different socioeconomic status (Data were presented as mean and standard deviation; One way ANOVA was performed to see the association; P≤0.05 was determined as level of significance).

Discussion

The seroprevalence of diphtheria IgG antibody in relation with sociodemographic change 200 participants have been selected according to inclusion and exclusion criteria. The age of the participants ranged from 18 to 38 years with the mean age of 27.15 (SD ± 5.24) years. Majority 77 (38.5%) participants has been age between 18 to 25 years, 71 (35.5%) participants of age between 26 to 32 years and 52 (26.0%) participants of age between 33-38 years. The mean serum IgG level was 0.23 ± 0.26 IU/mL in participants of aged between 18 to 25 years, which was much less in participants of age between 26 to 32 years and 33 to 38 years group. It revealed that IgG level was higher in younger group and declined as age increased. This finding was also significant statistically (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. A study on China reported that adult population was generally unprotected against diphtheria. Among 200 respondents male participants has been predominant which is 103 (51.5%) respondents and female participants has been 97 (48.5%) respondents. Mean serum IgG level has been found 0.12 ± 0.12 IU/mL in male participants and 0.12 ± 0.22 IU/mL in female participants. There was no significant difference of mean serum IgG level male and female (p=0.982). Therefore, sex difference was not effective in changing antibody titer against diphtheria. The studies done in Turkey have shown almost the same outcome. In this study majority 69 (34.5%) participants were student and mean serum IgG level has been found 0.18 ± 0.20 IU/mL, 64 (32.0%) participants service holder and mean serum IgG level 0.0 ± 0.08 IU/mL, 23 (11.5%) participants physician and mean serum IgG level 0.08 ± 0.06 IU/mL, 22 (11.0%) participants housewife and mean serum IgG level 0.07 ± 0.07 IU/mL, 10 (5.0%) participants businessman and mean serum IgG level 0.19 ± 0.30 IU/mL and 12 (6.0%) participants has been other occupations and mean serum IgG level 0.18 ± 0.49 IU/mL. There was significant difference of mean serum IgG level among different occupation (p=0.003).

In this study 118 (59.0%) participants has been come from lower middle class having mean serum IgG level 0.10 ± 0.21 IU/mL, 80 (40.0%) participants from upper middle class with 0.16 ± 0.18 IU/mL and 2 (1.0%) participants from poor having mean serum IgG level 0.04 ± 0.01 IU/mL. There was no significant difference of mean serum IgG level among different occupation.
socioeconomic status (p=0.1017). Socio-economic condition has an impact on an individual’s nutritional status, health education and awareness about vaccination that ultimately influences immune status. McQuillan et al\textsuperscript{18} noted that in the United States, higher percentage of participants had protective antibody to diphtheria toxin with increasing level of education.

**Conclusion**

In conclusion diphtheria IgG level is most commonly found among young age group. Male participants are predominant. Majority is student however IgG level found high among business occupation. Adult person in upper middle class diphtheria IgG level is found high. A nationwide study with larger sample size is required to reach a comprehensive conclusion to settle the issue of declining antibody level below protective threshold.

**References**

1. Levinson W, Chin-Hong P, Joyce EA, Nussbaum J, Schwartz B. Review of medical microbiology and immunology. Estados Unidos: McGraw-Hill;2010;141-3
2. Clarke KE. Review of the epidemiology of diphtheria 2000-2016. US Centers for Disease Control and Prevention. 2017.
3. Gavin L, Pazol K, Ahrens K. Update: providing quality family planning services—recommendations from CDC and the US Office of Population Affairs, 2017. Morbidity and Mortality Weekly Report. 2017 Dec 22;66(50):1383
4. Paveenkittiporn W, Sripakdee S, Koobkratok O, Sangkikorn S, Kerdsin A. Molecular epidemiology and antimicrobial susceptibility of outbreak-associated Corynebacterium diphtheriae in Thailand, 2012. Infection, Genetics and Evolution. 2019 Nov 1;75:104007
5. Dandinarasaiah M, Vikram BK, Krishnamurthy N, Chetan AC, Jain A. Diphtheria re-emergence: problems faced by developing countries. Indian Journal of Otolaryngology and Head & Neck Surgery. 2013;65(4):314-8
6. Damasco PV, Pimenta FP, Filardy AA, Brito SM, Andrade AF, Lopes GS, Hirata R, Mattos-Guarauchi AL. Prevalence of IgG diphtheria antitoxin in blood donors in Rio de Janeiro. Epidemiology & Infection. 2005;133(5):911-4.
7. MacQuillan GM, Kruszon-Moran D, Deforest A, Chu S, Wharton M. Serologic immunity to diphtheria and tetanus in the United States. Ann Intern Med 2002; 136: 660–6.
8. WHO. Vaccine preventable diseases: Monitoring system. 2016 global summary (Update 1 december 2016) Web page at:apps.who.int/imunization_monitoring/globalsummary/countrie s?countrycriteria%5Bcountry%5D%5B5D=BGD&commit=OK [Accessed on 15 December 2016].
9. World Health Organization. Expanded programme on Immunization (EPI) factsheet 2019: South-East Asia Region.
10. EPI Sahaika 8th ed Dhaka: DGHS 2012.: 16-33
11. World Health Organization. Expanded programme on Immunization (EPI) factsheet 2016: South-East Asia Region.
12. WHO. Diphtheria. (Update 21 May 2015) Web page at: www.who.int/biologicals/vaccines/diphtheria/en/ [Accessed on 15 January 2017].
13. Trucchi C and Zoppi G. Decennial diphtheria-tetanus adult boosters: are they really necessary? J prev med hyg 2015; 56: 44-8.
14. Galazka A. The changing epidemiology of diphtheria in the vaccine era. J Infect Dis 2000; 181: 2–9.
15. Cavus SA, Oguz VA, Yuce A. The seroprevalence of diphtheria among adults in Izmir–Turkey. Vaccine 2007; 25: 3851–4.
16. Gardner P. Issues related to the decennial tetanus-diphtheria toxoid booster recommendations in adults. Infectious disease clinics of North America. 2001 Mar 1;15(1):143-53.
17. Zasada A A, Rastawicki W, Rokosz N et al. Seoprevalence of diphtheria toxoid IgG antibodies in children, adolescents and adults in Poland: BMC inf dis 2013;13:551.
18. McQuillan GM, Kruszon-Moran D, Deforest A, Chu SY, Wharton M. Serologic immunity to diphtheria and tetanus in the United States. Annals of internal medicine. 2002;136(9):660-6.