Original Article

Immune status against diphtheria in healthy adults

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Abstract

Outbreaks of diphtheria continue to occur in Kerala with an age shift to older population. Antibody seroprevalence studies are essential to understand the immune status of the community and to develop an effective immunization strategy. **Aim:** To assess the necessity of diphtheria vaccination among adults. **Settings and Design:** Cross-sectional study, among 152 healthy adults (>18 years). **Methods and Materials:** Diphtheria IgG antibody detection was performed by using ELISA technique. **Results:** Out of 152 study population, 14 (9.2%) individuals had very low antibody levels, requiring basic immunization, 123 (80.9%) needed booster vaccination, 12 (7.9%) would need a booster dose in 5 years and 3 (2%) would need a booster dose in 7 years to maintain adequate antibody levels. Out of the total, 131 (86.2%) individuals had completed childhood immunization and 21 (13.8%) had incomplete or no immunization during childhood. In the population who had completed childhood immunization, 4 (3%) had very low antibody levels requiring basic immunization and 113 (86%) had antibody levels needing booster vaccine soon, with the remaining 14 (10.6%) individuals requiring a booster vaccine after 5 years and 7 years. In the partially immunized/unimmunized population, 10 (47.6%) had antibody levels requiring basic immunization and another 10 (47.6%) had antibody levels low enough to warrant a booster vaccine. **Conclusions:** Majority of the subjects who had completed childhood immunization showed an inadequate immunity against diphtheria during adulthood. This indicates waning immunity against diphtheria. Hence, modifying the present diphtheria vaccination strategy to include booster doses during adulthood is essential. **Context:** Even in developed countries where nearly 100% universal immunization is achieved, diphtheria outbreaks are known to occur. Several seroprevalence studies have been conducted in those regions to determine whether those populations have adequate levels of antibodies against diphtheria. In India, sporadic outbreaks occur, and an increasing number of diphtheria cases are being reported over the last few years. Large outbreaks in Kerala 2016 were about 533 cases. Recent outbreaks in 2019, in Trivandrum, about 175 cases were suspected and 19 cases were confirmed in laboratory. However, Indian studies to determine whether the adult population has adequate protective antibody levels are lacking. Knowing the immune status of the population and devising an appropriate strategies to prevent outbreaks of diphtheria are the integral parts of primary care. These concerns are the basis and evaluation of the seroprevalence of IgG antibody levels against diphtheria antitoxin among healthy adults in our region in this study.

Keywords: Adults, diphtheria, immune status

Introduction

Diphtheria has increasingly been reported in Kerala over the last few years. Immunity against diphtheria primarily depends on the presence of antibody to the diphtheria toxin. Several studies done in developed countries to determine the serum levels of antibodies against diphtheria infection have shown inadequate levels of antibodies, which also decline over time[1-3]

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Received: 26-07-2019 **Revised:** 21-08-2019 **Accepted:** 19-09-2019

Access this article online

Quick Response Code:

Website: www.jfmpc.com

DOI: 10.4103/jfmpc.jfmpc_589_19

However, hardly any such data is reported from the Indian population. In the context of recent outbreaks of diphtheria among adults in our region, information gained from this study would be useful to improve current strategies to prevent outbreaks in future.

Aims and Objectives

- To determine the diphtheria toxin IgG antibody levels in healthy adults and compare their levels in vaccinated and unvaccinated groups.
- To assess the necessity of booster vaccine in adults.

How to cite this article: Kutty JM, RajanBabu B, Thiruvoth S. Immune status against diphtheria in healthy adults. J Family Med Prim Care 2019;8:3253-7.

Materials and Methods

Study population

A total of 152 adults above 18 years of age coming for preemployment health check-up in the institute.

Study design

Cross-sectional, observational, hospital-based study.

Study duration

One year (January 2017 to January 2018)

Inclusion criteria

Asymptomatic adults (age >18 years) presenting to hospital for a routine preemployment health check-up.

Exclusion criteria

Adults who had recently taken diphtheria booster vaccine.

Adults with severe illness.

Those who refused consent to participate in the study.

Method of Measurement of Outcome of Interest

A written informed consent was obtained from the study participants. A semistructured proforma was prepared and demographic details of the patient such as the biographical data and childhood vaccination status were recorded. Candidates were requested to produce their own childhood immunization records if possible. In cases where no immunization cards were produced, history was sought by recall method.

Sample collection

Blood was collected aseptically by venepuncture in a sterile, dry test tube from each study subject primarily for their preemployment health check-up purpose. The leftover serum was used for our study purpose. These samples were stored at a temperature of below −20°C in the laboratory as per the instructions provided in the ELISA kit. The samples were tested within 2 weeks of storage.

Diphtheria IgG antibody detection was performed by the investigator using ELISA kit (IBL International GmbH, REF – RE56191). The procedures were done with strict adherence to the instructions provided in the kit under the guidance of the consultant microbiologist in the laboratory. Diluted serum or plasma specimens (1:100) were incubated at 25°C for 60 min to allow specific antibodies to diphtheria to bind to the antigen-coated wells. After washing away the unbound antibodies and other serum constituents, enzyme conjugate was added to each well. After 30 min of incubation, unbound conjugate was removed by washing, and an enzyme substrate was added to each well and incubated at room temperature for 20 min in the dark. A blue color developed if antibodies to diphtheria were present. Addition of stop solution changed the color from blue to yellow. Optical densities (OD) of controls and samples were read using a photometer at 450 nm. OD ratio obtained is converted to IU value using a linear log graph. Results were interpreted according to Table 1 given along with the ELISA kit.

The following definitions were used in our study:

A. Fully vaccinated during childhood: Subject who had completed the recommended EPI Immunization schedule of BCG, DPT, and OPV (3 doses) and at 1.5 and 5 years.
B. Partially vaccinated during childhood: Subject had not completed the doses of vaccine for his/her age as per schedule or could not recollect the vaccination history reliably.

Statistical methods

Data was analyzed using SPSS 21.0. Categorical Variables was summarized as frequency with percentage. Continuous variables were summarized as mean with standard deviation or median with interquartile range. Categorical variables were tested using Chi-square/Fischer's exact test and continuous variables were tested using independent sample *t*-test/Mann–Whitney U-test in case of two groups and ANOVA/Kruskall–Wallis test in case of more than two groups. *P* value was considered statistically significant if it was less than 0.05.

Sample size calculations

Sample size was calculated using the equation $n = 4*P*Q/L^2$, where P is the expected percentage of individuals at risk (low titer) and Q is $100 - P$, and L is the allowable error in P. Expecting 20% at risk with an allowable error of 7%, sample size required was 131.

Results

a) Age distribution of the study population

The mean age of the study population was 27 years with a standard deviation of 6.53. The minimum age and maximum age of the study population were 20 and 61 years, respectively. Median age was 25. Age categories have been represented in Figure 1 with frequencies and percentage.

b) Gender distribution of the study population

Out of the total 152 study population, majority 124 (81.6%) were females and rest 28 (18.4%) were male.

Table 1: Interpretation of antibody level

IU/ml	Interpretation
< 0.1 IU/ml	Basic immunization recommended
0.1-1.0 IU/ml	Booster vaccination recommended
1.0-1.5 IU/ml	To be boosted in 5 years
1.5-2.0 IU/ml	To be boosted in 7 years
>2.0 IU/ml	To be boosted in 10 years

c) Distribution of religion in study population

Out of 152 study population, majority belonged to Hindu religion 84 (54.3%), 48 (31.6%) from Christian religion, and the rest 20 (13.2%) belonged to Muslim religion.

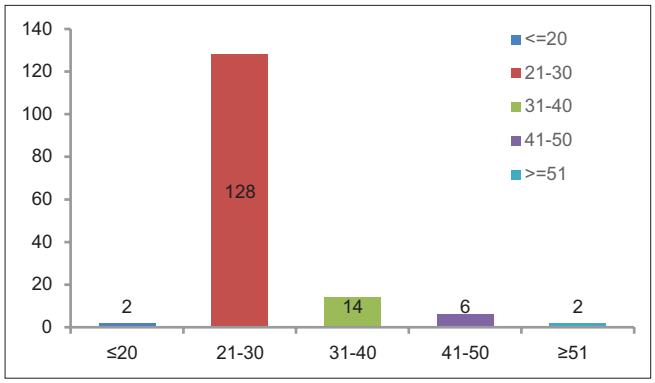

Figure 1: Age distribution

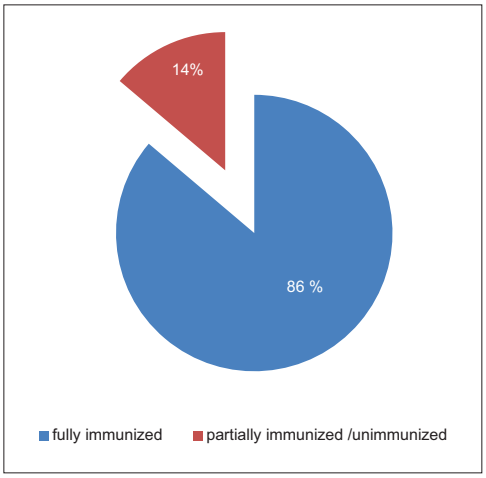

Figure 3: Distribution of immunization status

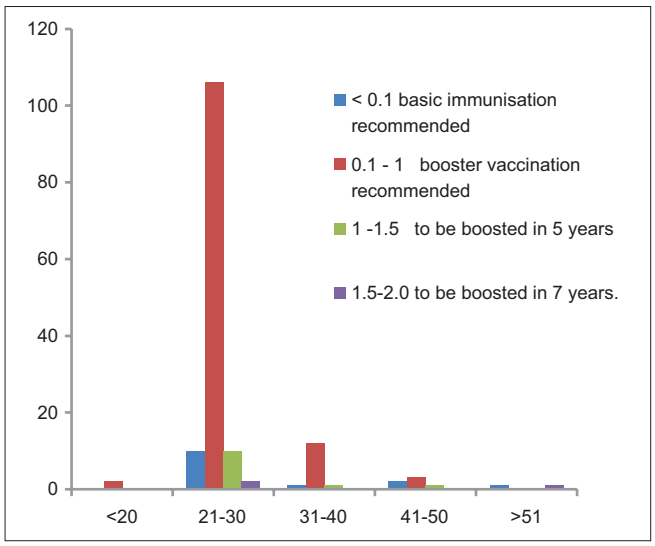

Figure 5: Interpretation of Diphtheria IgG antibody level in various age groups

d) Distribution of places in study population

Out of 152 study population, majority 89 (58.6%) belonged to Calicut district, followed by 26 (17.1%) from Kannur, 17 (11.2%) from Malappuram, and 20 (13.1%) individuals from other districts. This is depicted in Figure 2.

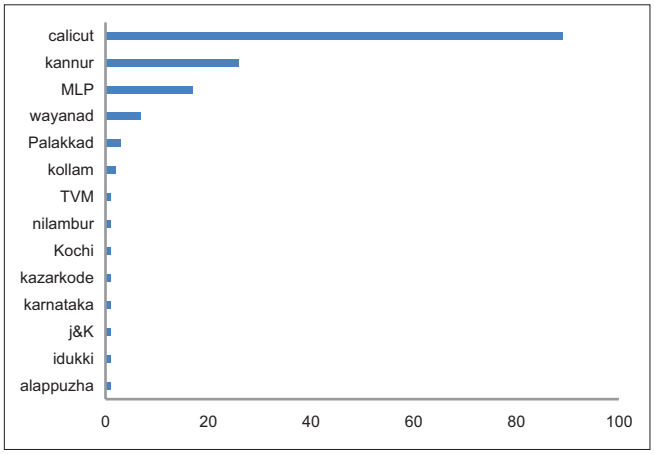

Figure 2: Distribution of place

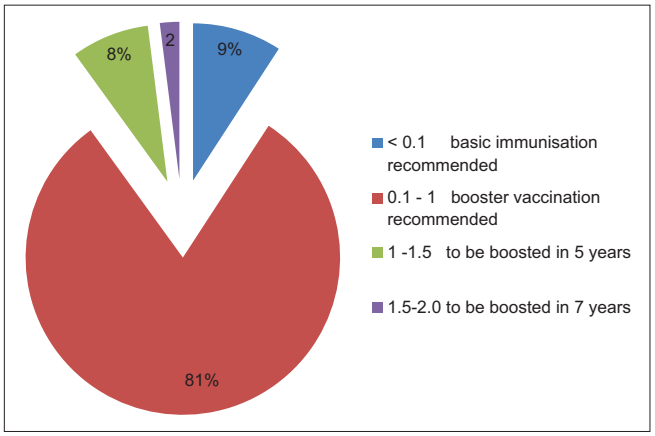

Figure 4: Distribution of Diphtheria IgG antibody titer

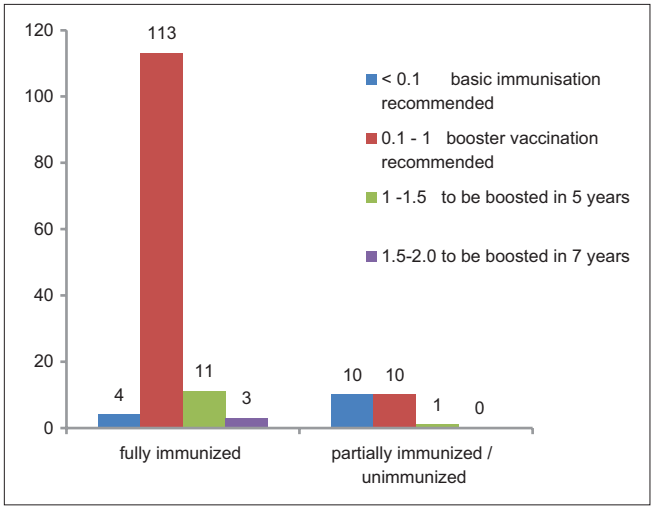

Figure 6: Immunization status and Diphtheria IgG titer value

e) Socioeconomic status of the study population

Out of 152 study population, 79 (52%) belonged to lower middle class and 73 (48%) belonged to upper middle class according to Modified Kuppuswamy scale.

g) Distribution of immunization status of the study population

Out of 152 subjects studied, 131 (86.2%) had completed childhood vaccination and 21 (13.8%) had incomplete or absent childhood vaccination status. This is represented in the following pie chart in Figure 3.

h) Distribution of awareness about diphtheria infection and vaccine

Out of 152 study population, 140 (92%) were aware about the disease and its vaccination, whereas 12 (8%) were unaware about the disease and vaccination.

i) Distribution of Diphtheria IgG antibody titer in the study population

Out of 152 study population, 14 (9.2%) required basic immunization, 123 (80.9%) needed booster vaccination, 12 (7.9%) would need to be boosted in 5 years, and 3 (2%) would need to be boosted in 7 years. This is represented in Figure 4.

A. Age versus Diphtheria IgG antibody level

In the study, (128) 84% of the population belonged to the age group 21–30 years, out of which (106) 82% would need a booster vaccine soon and (10) 8% needed basic immunization. Diphtheria IgG antibody level in various age groups is represented in the Figure 5.

B. Immunization status and Diphtheria IgG titer value

In the study, around 131 (86%) had completed childhood immunization, out of which 3% (4) had antibody levels requiring basic primary series of immunization and 86% (113) had antibody levels needing booster vaccine, while the rest 14 would require a booster vaccine after 5 years and 7 years.

Among the 21 (14%) individuals who were partially immunized or un-immunized, 10 (47.6%) had antibody levels requiring basic immunization schedule and another 10 (47.6%) had antibody levels needing booster vaccine. This is represented in Figure 6.

Discussion

In India, there are periodic outbreaks of diphtheria[4] with an increasing number of diphtheria cases being reported over the last few years.[5] In spite of fairly good childhood immunization rates, 82% primary immunization coverage reported in NFHS-4 2015–2016, seroprevalence studies regarding diphtheria toxin antibody levels are lacking in our country. Hence, such studies are essential to know the current immunity levels against this disease in any community. These concerns and lacunae in the literature formed the basis of this study.

As study population were those who came for preemployement health check-up in hospital, mainly was of nursing staff and majority were females of mean age group 27 years.

Most of the study subjects hailed from Calicut district (59%), followed by Kannur (17%) and Malappuram (11%) districts. The latest article about diphtheria outbreak in 2016 in North Kerala had reported 522 diphtheria cases from three districts—Calicut, Kannur, and Malappuram.[4] In our study, majority of the study population hailed from the same districts, and have remarkably low protective antibody levels against diphtheria toxin. Hence, these geographical areas need to be focused for further outbreaks and epidemics in our country.

As per DLHS4, Malappuram, Calicut, and Kannur had DTP3 coverage of 89, 97, and 95% respectively. In our study population, 86% had completed childhood immunization, which is actually similar to contemporary DLHS4 figures.

In our study, 86% of the subjects who had completed childhood immunizations had inadequate diphtheria toxin antibody levels. As per the recommendations mentioned in the antibody testing kit, these individuals require a booster vaccine. Three percent of the vaccinated subjects had diphtheria toxin antibody level immeasurably low, i.e. <0.1IU/ml, indicating that these individuals require basic primary immunization. Thus, a total of 89% of the study subjects who have completed their childhood vaccination are at an increased risk of acquiring or suffering from diphtheria infection.

In populations who were partially immunized or unimmunized, nearly half of them (47.6%) needed basic immunization and the remaining half required a booster vaccine. Thus, in all, about 90% of the study population was found to be susceptible to the disease. These findings were similar to the various seroprevalence studies done in Poland, Brazil, UK, and Singapore.[6-9] This indicates that the protection provided by the diphtheria vaccine may decline with the passage of time, and mass populations may be in need of periodic booster vaccination even in the background of good immunization coverage during childhood.

The low levels of diphtheria toxin antibody levels in adults can explain the recently increasing trend of diphtheria cases occurring in this part of Kerala, predominantly among adults, as compared to earlier times, when diphtheria was more common among children.[10,11] Adults who have neither been exposed to diphtheria nor received booster doses of diphtheria toxoid after their primary childhood immunization can become susceptible to diphtheria as a result of waning immunity.[12]

This study was done in subjects hailing from Calicut, who were mostly para-medical workers and belonged to socio-economic classes in which vaccination coverage is known to be high. These factors may have contributed to their good immunization coverage during their childhood. If a study similar to the present one were to be conducted in areas of low immunization coverage of the same district, or in a district like Malappuram, where pockets of very low vaccination coverage and antivaccine lobbies are predominant, the results could be eye opening.

The previous studies have shown that a fall in booster dose coverage has led to diphtheria outbreaks.[9] Hence appropriate interventions to prevent outbreaks suggested by CDC, i.e. a booster dose of Td vaccine every 10 years after primary immunization, must be emphasized. And opportunities to provide immunization services to protect adolescents and adults should be encouraged, such as a booster dose at school leaving age, pregnancy, etc., Tetanus toxoid (TT) vaccination, which is already a part of immunization schedule for adolescents (10 and 16 years of age) and pregnant women could be replaced with Td vaccine (TT and low-dose diphtheria toxoid) to provide protection against diphtheria infection also, hopefully without any significant burden to the existing immunization programs or public health concerns related to the vaccine itself.

DT vaccine, which contains a 3-4 fold higher dose of diphtheria toxoid and is commonly used in children below 7 years of age, may probably boost the levels of antibodies higher when compared to adult Td vaccine.[13] But studies are required to definitively demonstrate the efficacy of DT over Td in maintaining the immunity levels with the passage of time.

The findings of this study, coupled with the rising trend of diphtheria cases even in the face of adequate vaccine coverage, demonstrate the need for large-scale studies regarding the diphtheria toxin antibody levels in mass populations in our region. This would be of paramount importance toward strengthening the present immunization practices to rein in a fatal, vaccine-preventable disease like diphtheria.

Limitations

This is a hospital-based study involving a small proportion of population, that too among health workers. Larger studies are recommended involving all age groups to obtain a better understanding of the susceptibility to diphtheria in our community.

Conclusions

Majority of the population (89%) who have completed childhood immunization do not have protective levels of antibody against the diphtheria infection during adulthood. This indicates the declining immunity levels among adults in the community despite adequate immunization during childhood. Therefore, strengthening the current vaccination strategy along with decennial booster dose of diphtheria vaccine is essential.

Ethical considerations

The study was approved by the scientific committee and ethical committee of the hospital.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patients have given their consent for their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

Financial support and sponsorship

All three investigators contributed to buy the ELISA kit through microbiology laboratory of the hospital.

Conflicts of interest

There are no conflicts of interest.

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