RECENT CASES OF DIPHTHERIA IN A METROPOLITAN CITY

Microbiology

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ABSTRACT

Introduction: Diphtheria is an acute, highly infectious and previously endemic disease. Due to the vaccine preventable nature of the disease and initial steady decline, not much attention had been given towards this disease. Recent increase in the number of clinically suspected and laboratory confirmed cases of diphtheria have been observed in our laboratory.

Aims and objectives: To study the status of resurgence of the disease in a tertiary care hospital of Mumbai.

Materials and methods: 76 throat swabs and 1 slough material from 57 clinically suspected cases of diphtheria over a period of one and a half year from July 2018 to December 2019 were obtained and were processed for microscopy and culture for *Corynebacterium* species by standard microbiological techniques.

Results: Eighteen (23.37%) out of 77 samples yielded *Corynebacterium* species on culture. Out of 57 suspected patients, 11 were culture confirmed diphtheria (19.29%). None of the patients were less than 5 years of age, 55% were between 5 and 9 years and 45% were above 10 years of age with a total female preponderance of 64%. The immunization status was unclear in 27% cases. All the smear and/or culture positive cases were informed immediately to the clinicians so that the patients could be transferred to the Infectious disease hospital for further management.

Conclusion: The study has shown an increase in the number of Diphtheria cases in a metropolitan city, which is a cause of concern in healthcare. There is also a shift in age to older children (> 5 years). The study also highlights the need for confirmation of toxin production to facilitate early information to the treating clinicians

KEYWORDS

Diphtheria, throat swabs, Infectious disease hospital, toxin

INTRODUCTION:

Diphtheria is an acute, highly infectious, vaccine preventable and previously endemic disease, the etiologic agent being *Corynebacterium diphtheriae*. Diphtheria may manifest as an upper respiratory tract infection, a cutaneous infection or as an asymptomatic carrier state¹. The cornerstone of treatment for respiratory diphtheria is diphtheria antitoxin. Early treatment is critical as the degree of protection by the antitoxin is inversely proportional to the duration of the illness before its administration¹.

With the start of universal immunisation programme (UIP) since 1985, most of the vaccine preventable diseases have shown a decline. Among all vaccines, diphtheria toxoid is one of the most effective and safest². Despite the success of mass immunization in many countries worldwide, diphtheria continues to play a major role as a lethal resurgent infectious disease and is sixth in the list with highest mortality rate in India^{3,4}.

Re-emergence of diphtheria in the country seems mainly due to low vaccine coverage in children^{3,5}. There are reports of re-emergence of the infection in adolescents which may be due to waning of immunity with age³. Improved immunization rates in children reduces the circulating wild organisms and thereby provide the opportunities for natural boosting through subclinical infections^{6,7}.

An accurate microbiological diagnosis of diphtheria is crucial and is always regarded as being complementary to clinical diagnosis, because diphtheria is often confused with other conditions, such as severe streptococcal sore throat, Vincents angina, or glandular fever¹.

According to the WHO 2018 guidelines, "Two temporally and geographically linked cases, of which at least one is laboratory-confirmed, is considered as an outbreak of diphtheria"⁸.

N Khan et al reported steady increase in the number of cases from the year 2000 to 2005 at the Infectious Diseases Hospital, Mumbai⁹. A recent increase in the number of clinically suspected and laboratory confirmed cases of diphtheria have been observed in our laboratory. Hence this study was conducted to know the status of re-emergence of the infection in the metropolitan city of Mumbai and also to observe any changes in the age distribution of diphtheria cases.

MATERIALS AND METHODS:

A retrospective analysis was conducted from June 2018 to December 2019 at a tertiary care hospital of Mumbai. 76 throat swabs and 1 slough material from 57 clinically suspected cases of diphtheria were included in the analysis. Analysis of the repeat samples from the same patient was not included in the study. Standard microbiological techniques were employed for the laboratory identification of *Corynebacterium* species (Figure 1).

FIGURE 1: Laboratory identification of *Corynebacterium* species

GRAM STAIN: Cuneiform arrangement of gram positive bacilli

ALBERT STAIN: Bluish black granules (Babes Earnst granules) at the tips of the bacilli

Early growth on Loeffler's Serum Slope

Black colonies on Potassium tellurite agar

Cultures from the first 2 patients were sent for confirmation to the Vaccine Preventable and Invasive Bacterial Diseases (VPIBS) WHO reference laboratory, Vellore, following the WHO guidelines for safe transport of biohazard material.

Chi-square test was used as the statistical tool for significance of association.

OBSERVATIONS:

A total of 77 samples from 57 clinically suspected Diphtheria cases were sent to the Microbiology department from June 2018 to December 2019. Out of the 57 clinically suspected patients, 11 were culture confirmed Diphtheria (19.29%). No case was observed in the dry summer months of April and May.

None of the case was below 5 years of age. 55% of the patients were between 5-10 years and the rest were above 10 years of age (45%). A coincidental finding of female preponderance was observed (64%).

On history and clinical examination, a significant number of all the suspected patients had fever (84%). History of sore throat and dysphagia was given by 53% and 32% patients respectively. Clinical examination revealed tonsillar membrane in 39% patients with cervical lymphadenopathy in 19% cases. Neuropathy (vocal cord palsy) was reported in 12% patients (Figure 2).

FIGURE 2: Clinical signs and symptoms of culture positive patients

Only one patient presented to the emergency with classical neck swelling (bull's neck). The immunization history revealed that complete immunization (primary as well as booster doses) was reported in 37% cases in the 5-10 years age group whereas it was reported in only 9% cases in the age group of more than 10 years, which was statistically significant by Chi square test ($p > 0.5$) (Table 1).

TABLE 1: Immunization history of Diphtheria patients

Immunization status	5-10 years (n= 7)	Above 10 years (n=4)	Statistical correlation
Primary Immunization	2 (18%)	1 (9%)	Statistically not significant
Complete Immunization (Primary+Booster)	4 (37%)	1 (9%)	$p > 0.5$ by Chisquare test Statistically significant
Unclear immunization status	1 (9%)	2 (18%)	Statistically not significant

Complete immunization: p value = $0.99 > 0.5$ (Chisquare test)

The initial two culture isolates were from 2 different patients who were negative on primary smear but were culture positive. Hence for confirmation, the isolates were sent to the Vaccine Preventable and Invasive Bacterial Diseases (VPIBS) WHO reference laboratory, Vellore. The cultures were confirmed as *Corynebacterium diphtheriae* (tox_A). Rest of the 7 culture confirmed cases were reported positive also on primary smear findings. All the culture confirmed cases were reported to IDSP.

The antibiotic susceptibility test was not reported for the patients as the MIC testing facility was not available for all the recommended drugs in this laboratory. Furthermore, all the smear and/or culture positive patients were referred to the Infectious diseases hospital immediately for further management.

The data was compared with that of the previous 3 years (June 2015 to May 2018) of our department and it was observed that there has been a

definite increase in the number of clinically suspected and laboratory confirmed cases of Diphtheria which is a health concern for the financial capital of India (Table 2).

TABLE 2: Comparison of the number of cases over a period of 3 years

Year	Total Swabs received for <i>Corynebacterium diphtheriae</i>	Culture positive <i>Corynebacterium</i> species
June 2015 to May 2016	03	02
June 2016 to May 2017	02	00
June 2017 to May 2018	04	00
June 2018 to December 2019 (Current study)	57	11 (19.29%)

DISCUSSION:

Diphtheria is a highly infectious disease which if not detected early and treated promptly with antibiotics and antidiphtheritic serum (ADS), can lead to significant mortality and morbidity. The WHO 2018 global figures reveal 16,648 reported cases of Diphtheria¹⁰.

In our study there were 57 clinically suspected patients of Diphtheria out of which 11 cases were culture confirmed (19.29%). Bhagat et al (2015) and Parande et al (2017) have reported a culture positivity of 23.2% and 8.79% respectively in their studies^{3,4}.

Diphtheria mainly affects children between 1 to 5 years. However, due to good vaccine coverage worldwide, a shift in age incidence has been observed from preschool to school age (5-15 years) with more and more cases now being reported in adults^{3,6,11,12}. In our study, 55% of the patients were between 5-10 years which is very close to that reported by Krishnan et al (2018) (48.3% in age group of 6 to 10 years)⁷. The rest of the cases were above 10 years of age (45%). None of the patients was less than 5 years age. Parande et al (2017) reported an age distribution of 55.26% for children between 1 and 5 years of age and 36.84% for children between 5 and 10 years⁴.

Almost all children develop protective levels of antibodies following primary immunization. In the absence of boosters the titres drop and over 20-65% of adults may become susceptible⁶. In our study, complete immunization (primary as well as booster doses) was reported in 37% cases in the 5-10 years age group whereas it was reported in only 9% cases in the age group of more than 10 years. Due to lack of adult vaccination and natural immunity a high proportion of adults become susceptible to diphtheria¹³.

DT boosters throughout adult life at ten year intervals and minimum two booster doses over 40 years of age are recommended⁴. Few authors have given recommendations like monitoring the anti-Diphtheria IgG antibody level in population. A high level of anti-diphtheria toxoid IgG antibody ≥ 1.0 IU/ml confers long-term protection whereas levels between 0.1 and 1 IU/ml require immediate immunization⁷.

Clinically, tonsillar membrane was documented in 39% of patients. Phalkey et al (2013) reported tonsillar patch and a pseudomembrane in 36% and 64% patients respectively⁸. Neurotoxic manifestation in the form of vocal cord palsy was seen in 12% patients. Mustafa et al (2016) mentioned in their study that about 3/4th of the patients with severe disease develop neuropathic changes¹⁴.

The first 2 culture isolates were reported from 2 different patients treated on OPD basis. These were negative on primary smear. Hence for confirmation, these 2 isolates were sent to the WHO reference laboratory, Vellore as there are no facilities for toxin detection or confirmation of culture isolates at our hospital. The culture isolates from rest of the suspected patients were reported positive also on primary smear and the patients were immediately referred to the Infectious disease hospital. The patients were successfully treated there and discharged. With the increase in new infections every year, setting up of molecular laboratories at all the tertiary care hospitals is the need of the hour in our country. This will help in early confirmation of isolates and better patient care.

The data from the previous three years (June 2015 to May 2018) was retrieved and compared with the current data which has shown an increase in the number of throat swabs from clinically suspected patients in the current year. Bhagat et al (2015) have reported persistence in Diphtheria cases from 2012 to 2014 in Delhi and NCR region⁵.

CONCLUSION:

Re-emergence of diphtheria in the country seems mainly due to low coverage of primary immunization as well as boosters doses³. Age shift, is considered an indicator of good under five immunization⁶. The Government of India has made changes in the UIP by replacing TT vaccination with Td (TT and low dose diphtheria toxoid) vaccine without any additional burden to immunisation program¹⁵. This was also recommended by Bhagat et al³(2015).

Samples from all the clinically suspected patients should be sent to the microbiology laboratory for smear and culture confirmation. The use of multiplex PCR is becoming increasingly important in the diagnosis of infectious diseases and could be a simple and fast alternative procedure for identification of *C. diphtheriae*, including screening for toxigenic and non-toxigenic strains, which was not done in this study for all the isolates¹³.

A good surveillance is needed to document the impact of vaccination and cover the information about primary vaccinations as well as boosters⁵. Reporting the cases under the IDSP is important as it helps in tracing the contacts and taking preventive measures.

ACKNOWLEDGEMENT:

We sincerely thank the Vaccine Preventable and Invasive Bacterial Diseases (VPIBS) WHO reference laboratory, Vellore, India for confirming few of our isolates as *C.diphtheriae*.

Conflicts of interest: None

Financial support: None

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