Albert’s Stained Smear Examination And Culture in Management of Clinical Diphtheria

Dr. Meera M  
H. No. 16-11-405/37, SBI Officer’s colony, Musarambagh, Malkpet, Hyderabad, Telangana-50036

Dr. Taruni S  
H.No.12-11-1495, Boudha nagar, Near Arts college railway station, Sec’Bad-61

Dr. Kola Sujatha  
10-5-7/10, Uco Bank colony, Masab tank, Hyd-28

Dr. Kavitha latha ML  
Flat No.102, “A” Block, Natarajan residency, Padmarao nagar-25

ABSTRACT

Diphtheria is an acute, contagious, febrile illness caused by Corynebacterium diphtheriae and can lead to significant morbidity and mortality in all age groups. Seven hundred and thirty three cases of Clinical diphtheria, admitted from August to March 2016 at a tertiary care hospital were included in the study. All patients presented with fever, difficulty in swallowing and patch in the throat. On admission, before starting treatment, two throat swabs were collected; one for KLB examination and the other for Culture. The results were analyzed and it was concluded that Diphtheria being a contagious disease and associated with high morbidity and, needs to be treated as an emergency, based on high index of clinical suspicion. The results of KLB and Culture are subject to several limiting factors and negative report does not rule out diphtheria.

KEYWORDS

Diphtheria, KLB, Albert’s stain, Corynebacterium, toxigenicity

Introduction:

Diphtheria is an acute, toxin mediated, contagious febrile illness caused by locally invasive, gram positive bacterium, Corynebacterium diphtheriae. The disease continues to occur randomly leading to significant morbidity and mortality in all age groups, especially in the nonimmunized and in the partially immunized i.e. those who either did not complete the three primary doses and/booster dose. Even In the completely immunized, milder form of the disease was observed.

Globally, the overall case fatality rate is 5-10% with higher death rates at extremes of age. Most of the time, the diagnosis is based on clinical signs and symptoms which include fever, sore throat, difficulty in swallowing and swelling in the neck. There may be a dirty white patch covering one or both tonsils on examination. Throat swabs are collected for Klebs-Loeffler’s Bacillus (KLB) by direct microscopy and for culture and sensitivity of the organism. Anti Diphtheric Serum (ADS) is given to neutralize circulating toxin if smear is reported positive and sometimes on strong clinical suspicion even if it is reported negative. Both KLB and culture of the organism, its identification along with toxigenicity testing which is regarded as the gold standard for confirmed diagnosis, are subject to a number of limiting factors. In the present study, an attempt has been made to correlate clinical diagnosis of diphtheria with smear and culture positivity at a tertiary care infectious diseases hospital.

Materials and methods:

The retrospective study done at a tertiary care hospital included seven hundred and thirty three cases with suspected diagnosis of Clinical diphtheria.

Inclusion criteria: All patients admitted with fever, sore throat and a patch in the throat were included in the study.

Exclusion criteria: Patients who died before any therapeutic measure could be undertaken and those who left the hospital against medical advice.

The study period was six months from September 2015 to March 2016.

The age, gender, occupation, address, history of the present illness, antibiotic usage, precipitating factors, if any, immunization status and presenting symptoms and signs were noted.

Age wise patients were categorized into four categories. Table 1:

<table>
<thead>
<tr>
<th>Category</th>
<th>&lt;10 years</th>
<th>10-20 years</th>
<th>20-40 years</th>
<th>&gt;40 years</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>214</td>
<td>214</td>
<td>264</td>
<td>41</td>
<td>733</td>
</tr>
<tr>
<td>II</td>
<td>29.2%</td>
<td>29.2%</td>
<td>36.1%</td>
<td>5.5%</td>
<td>100%</td>
</tr>
</tbody>
</table>

ECG was taken for all the patients and changes, if any, noted.

Two throat swabs were collected from each patient before starting treatment. In children, parents were asked to hold the child on the lap and extend the head of the child with one hand while holding both hands of the child with the other for better visualization of throat.

First swab was inoculated on specific culture media i.e. Loeffler’s serum slope followed by incubation in Hartley’s broth for enrichment as per the standard laboratory guide lines. The second swab was inoculated on Potassium tellurite, Diphtheria virulence agar and Blood agar in that order and the same swab used for smear preparation for direct microscopy by Albert’s staining. Preliminary identification done on the same day based on KLB and growth on Loffler’s serum while identification of toxigenic strains took 48-72 hrs.

The sugar media used for biochemical identification of the isolate include serum sugars - glucose, maltose, sucrose, trehalose, urease and starch.

All isolates that showed Glucose, Maltose, Starch and Trehalose fermentation were identified as Corynebacterium diphtheriae (CD) and the others as Corynebacterium other than diphtheriae (COD).
Antibiotic susceptibility testing by Kirby-Bauer’s disc diffusion method was done against Penicillin, Erythromycin, Azithromycin, Ciprofloxacin, Ceftriaxone and Cefotaxime.

Specific treatment initiated based on clinical diagnosis and later modified based on sensitivity report and clinical response.

Results:
Out of seven hundred and thirty three cases of Clinical diphtheria, three hundred and forty (46.3%) were males and three hundred and ninety three (53.6%) were females.

Table 1 shows age wise distribution of cases.

All patients were from Hyderabad and the neighboring districts of Nalgonda, Nizamabad and Karimnagar of Telangana State.

All of them presented with fever, sore throat and a patch in the throat.

Additionally, patients presented with lymphadenopathy or bull neck, rhinorhea, vomiting and abdominal pain, laryngitis, stridor and pneumonia.

All patients of clinical diphtheria were given specific treatment which included full course of Crystalline Penicillin, dosage based on body weight and age, ADS 20000 units for adults and 10000 for children after skin test dose along with symptomatic and general measures for a period of one week with repeat KLB and culture on 3rd and 5th day.

While on treatment, two patients in group I died; others recovered.

Five patients developed myocarditis changes on ECG and recovered after follow up.

In group II, two out of sixteen cases died while others recovered without any complications.

All patients in group III recovered completely at the time of discharge.

In group IV, two cases died while three developed pneumonia.

They were followed with specific treatment until recovery.

Discussion:
Diphtheria is a condition if not detected and treated early can lead to significant morbidity and mortality due to associated complications like respiratory obstruction, myocarditis, secondary pneumonia, adrenal insufficiency and polyneuritis 5.

All clinical diphtheria cases (733) admitted to tertiary care hospital from September 2015 to March 2016 were of no-saprophyngial type and presented with fever of moderate severity, sore throat and membrane in the throat, in addition to malaise, cough, dysphagia, vomiting, abdominal pain and palpitations.

A slight female preponderance was observed in the present study 6.

Age wise, an equal number of patients in the age groups <10 yrs and 10-20 yrs (214) were found to be affected in the present study while maximum number of cases were observed in the age group 20-40 yrs.

KLB was reported positive in 42 cases (5.7%) while CD could be isolated from 53 (7.2%) cases.

Both KLB & culture was positive in 16 cases

KLB positive and culture negative in 26 cases

KLB negative and culture positive 37

Both KLB and culture negative in 654 cases.

Out of total number of KLB positive (42) and culture positive (53) only 16 were both KLB and culture positive i.e., only 2.17% out of 733 clinical diphtheria cases. Both KLB and culture negative constituted 89% of cases. While KLB was negative, culture alone was positive in 5% of cases. And KLB was positive, culture negative in 3.5% of cases.

The reasons for such wide variations can be multifold 7.

Clinical diphtheria- KLB and culture positive (2.1%) 8:

Clinical diphtheria, KLB and culture negative (89%) 9: could be due to

- inappropriate sample due to faulty collection
- Patient uncooperative-not able to open mouth widely
- Has taken an appropriate antibiotic earlier to admission
- Inappropriate laboratory technique in processing and reporting

KLB positive, culture negative (3.5%): Due to

- Non viable organisms due to antibiotic usage prior to admission

KLB negative, culture positive (5%): could be due to

- Faulty swab collection
- Dry swab
- Faulty technique

Out of 733 cases, 727 recovered with treatment. It was observed in some of these cases, though both KLB and culture negative, patients improved clinically soon after ADS administration. It was also observed that clinical illness was more severe in culture positive cases especially those with CD infection than COD which are known to produce mild disease 9.

Conclusions:
The study concludes that diphtheria still remains a condition where an early clinical diagnosis is mandatory so far as management is concerned. Since neutralizing the circulating toxin is the mainstay of treatment, ADS needs to be administered after skin sensitivity testing wherever clinical judgment applies, as early as possible without waiting for either KLB or culture report. This is very important to prevent complications or to decrease morbidity and mortality. Both KLB and culture are to be given due weight age in confirmation of diagnosis from community point of view and for prevention of spread of the infection by implementing appropriate public health guidelines.

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