

## Estimation of Ketone Bodies- An Early Indicator of Toxigenicity of C. Diphtheria

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**Abstract:** Estimation of ketone bodies in urine detects the presence of toxin of C. diphtheria. It is also seen in Diphtheritic myocarditis as the toxin causes lysis of myocardial cells. Clinical diphtheria is on rise worldwide today and leads to the development of complicated diphtheria. Whenever clinical diphtheriae is diagnosed, there is a definite need for further laboratory evidence of elevated ketone body levels in the urine samples as it indicates the presence of toxin. It is also one of the laboratory prognostic parameter of complicated diphtheria especially in diphtheritic myocarditis. This study was done on all cases of Clinical diphtheria admitted at Sir Ronald Ross Institute of Tropical and Communicable Diseases (SRRITCD), Hyderabad which is an Integrated Disease Surveillance Project (IDSP) network hospital from January 2008 to December 2013. The aims of this study were to estimate the ketone bodies in the urine samples of Clinical diphtheria patients and especially to emphasize its relation to toxin production and its relevance to complications like myocarditis. 2925 Clinical diphtheria were admitted in the isolation wards of SRRITCD. H/O complete immunization was elicited only in 41%. Most of the nonimmunized cases were ended up in complicated diphtheria mainly due to myocarditis. 82 deaths were noted most of them are due to myocarditis. This recommends more laboratory evidence like estimation of ketone bodies apart from routine identification of Klebs - Löffler's Bacilli (KLB) and culture, as there is possibility of low smear positivity and isolation of organism due to many factors. Hence an attempt was made to study the disease in a more detailed manner and to evaluate the relation between presence of ketone bodies in urine and diphtheria and also its relevance to diphtheritic myocarditis. This study emphasizes that detection of ketone bodies in urine samples not only helps in confirmation of the disease, it can be used as a prognostic indicator. It would help in the identification of toxin and in the early & easy control of the disease.

**Keywords:** Clinical Diphtheria, Myocarditis, Immunization, Ketone bodies.

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### I. Introduction

The clinical correlation with laboratory findings helps in confirmation of diagnosis and onward transmission of data helps the health authorities to spread awareness, boost up immunization programme and prevent community spread. The factors contributing to high morbidity and mortality of diphtheria include patient's immunization status, age at infection, clinical type and time of intervention.<sup>(1,2)</sup> Most of the cases of nonimmunized patients develop complications like myocarditis etc., as the disease progresses and end succumb to death. As there is a possibility of low smear positivity and isolation of organism due to various factors, further laboratory evidence like estimation of ketone bodies in the urine sample aid in diagnosis as it is the direct evidence of presence of toxin. Thus early diagnosis and timely intervention go a long way in reducing incidence, containing the infection in community and to decrease morbidity and mortality in the affected individuals.

### II. Aims

The study was aimed to analyse the importance of elevated ketone bodies in detection of toxin production and also its relevance to diphtheritic myocarditis.

### III. Material & Methods

This study was taken up at SRRITCD, Hyderabad from January 2008 to December 2013. All the cases of clinical diphtheria were included in this study. Patients who died before any therapeutic measure could be under taken and patients who left the hospital against medical advice were excluded from the study. 2925 cases of clinical diphtheria were admitted in the isolation wards of the hospital. All the cases were diagnosed as Clinical Diphtheria based on CDC Criteria.<sup>(3,4,8)</sup> They are upper respiratory tract illness with an adherent membrane of the nose, pharynx, tonsils, or larynx; and any of the following: isolation of Corynebacterium diphtheriae from the nose or throat; or epidemiologic linkage to a laboratory-confirmed case of diphtheria.

Clinical and personal data including duration of illness prior to admission, immunization status (information given by parents/attenders/Individual), laboratory findings and outcome were documented.

Anatomical extent of membrane formation, total leukocyte count and serum glutamic oxalo -acetic transaminase (SGOT) levels were analysed in fatal and non-fatal cases. Apart from these, ESR and complete urine examination including Rothera's test for the estimation of ketone bodies were done in all cases. ECG was taken soon after admission on every alternate day during inpatient stay and at the time of discharge to exclude myocarditis. Throat swabs for direct microscopy for Klebs - Löffler's Bacilli (KLB) and culture for Corynebacteria were done soon after admission and repeated on fifth day for each patient. All the admitted patients were treated at SRRITCD.

After the provisional clinical diagnosis was made and appropriate cultures were obtained, persons with suspected diphtheria were given antitoxin and antibiotics in adequate dosage and placed in isolation. Respiratory support and airway maintenance was also administered as needed.

Treatment with Erythromycin orally or by injection (40 mg/kg/day; maximum, 2 gm/day) for 14 days, or Crystalline Penicillin daily, intravenously 5 to 20 lakhs sixth hourly as per the age for 14 days. The disease is usually not contagious 48 hours after antibiotics are instituted. Elimination of the organism should be documented by two consecutive negative cultures after therapy is completed.<sup>(3,4)</sup>

Whenever patient developed systemic complications like myocarditis, stridor, nephritis etc., opinion from respective departments like Cardiology / ENT/ Nephrology were taken and transferred to the respective referral centres whenever necessary.

#### IV. Results

A total of 2925 Diphtheria cases were admitted in isolation wards at SRRITCD during the period from January 2008 to December 2013. 60% were of females, maximum affected age group was 20-30 years as shown in table 1 and figure 1. Mortality rate was comparatively high in age groups below 10 years than other age groups.

1199 (41%) cases gave history of complete immunization, whereas 236 (8%) cases were partially immunized, 1257 (43%) cases were non immunized and for 333 (8%) of the cases, immunization status was not known. For statistical analysis partially, nonimmunised are considered as nonimmunized. Patients who gave H/O complete vaccination presented early in course of illness (2-5 days) and suffered mild form of disease, and recovered completely. But patients who failed to get complete vaccination, could not approach tertiary care centre early had severe forms of disease and died due to complications. Death rate was more in partially immunized (6.2%) and nonimmunized patients (88.9%) as compared to the completely immunized patients. (4.9%) as shown in table 2 and figure 2. The most common cause of death in these cases was myocarditis.

All cases of Diphtheria were of nasopharyngeal type. Signs and symptoms suggestive of myocarditis developed in 556 (19%) cases, 1-2 days after admission. Most of the patients suffered from dyspnoea and complained of chest pain and showed ECG abnormalities in the form of conduction blocks and arrhythmias.

The patients were categorized into two groups.<sup>(5, 9, 10)</sup> Category I included clinical symptoms of clinical heart failure, fever, viral prodrome, fatigue, dyspnoea on exertion, chest pain, palpitations, pre-syncope or syncope. Category II included evidence of cardiac structural/functional perturbation in the absence of regional coronary ischaemia like echo evidence, regional wall motion abnormalities, cardiac dilation and regional cardiac hypertrophy.

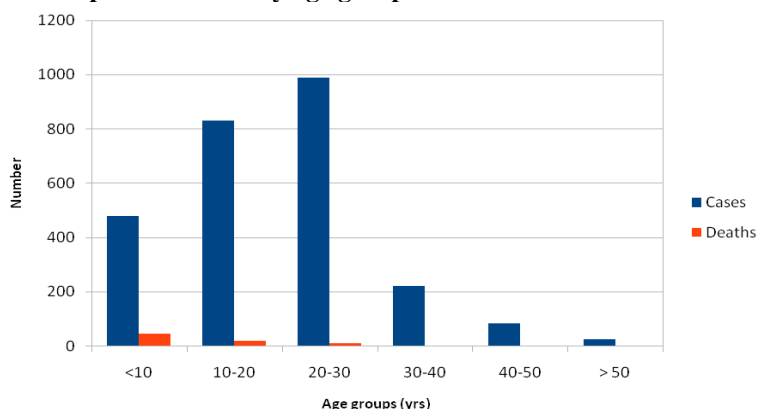
Smears for KLB were positive in 92 (3.14%) patients while culture was positive in 478 (16.34%). Complete blood picture showed leucocytosis. Ketone bodies were present in 2028 diphtheria cases (61.5%) as seen in table 3 & figure 3. They were also elevated in all complicated cases especially 556 (19%) patients of Diphthirtic myocarditis as seen in table 4 & figure 4. 2844 of the cases were cured and discharged, while 81 cases died due to myocarditis.

**Table1: Distribution of Diphtheria cases by age group and sex in relation to outcome.**

Age	Males	Females	Total	No. of deaths (%)
>10years	200	277	477	47 ( 10.51)
10-20years	337	492	829	21 (2.53)
20-30years	446	542	988	10 (1.01)
30-40years	96	124	220	2 (.09)
40-50years	36	45	81	- (0.0)
50-60years	11	13	24	2 (8.33)
< 65years	2	6	-	-

Mortality rate was comparatively high in age groups below 10 years and it was decreased in other age groups.

**Figure 1: Distribution of Diphtheria cases by age group and sex in relation to outcome**

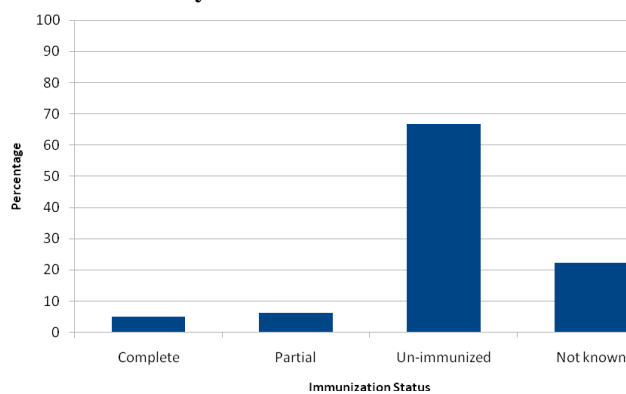


**Table 2 :Distribution of myocartitis in relation to immunization and outcome**

Immunization	No. of deaths of myocartitis	Percentage
Complete	4	4.9%
Partial	5	6.11%
Nonimmunized	54	66.6%
Not known	18	22.2%

Mortality rate is high in non immunized and partially immunized patients than completely immunized patients.

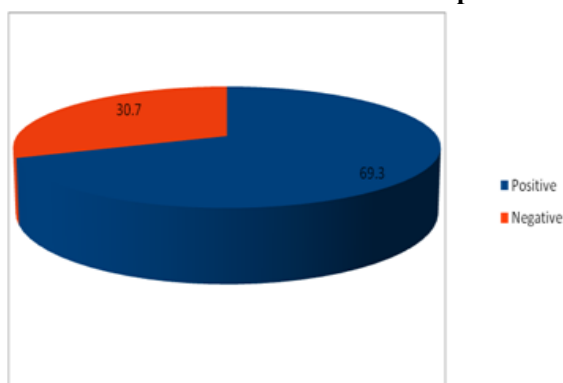
**Figure 2 Distribution of myocartitis in relation to immunization and outcome**



**Table:3 Estimation of Ketone bodies in Diphtheria cases**

No. of cases	No. of Cases	Percentage
Total No. of Diphtheria	2925	100%
Ketone bodies positive	2028	69.3%
Ketone bodies Negative	897	30.7%

**Figure 3: Estimation of Ketone bodies in Diphtheria cases**



**Table 4: Distribution of myocarditis cases in diphtheria cases in relation to ketone body positivity in myocarditis**

	No. of Cases	Percentage
Total No. of Diphtheria	2925	100%
No. of Myocarditis	556	19%
Ketone bodies positive	556	19%

**Table 5: Distribution of ketone body positivity in Diphtheric Myocarditis**

Number of Myocarditis	Ketone body positive	Ketone body negative
556	556 (100%)	0 (0.0%)

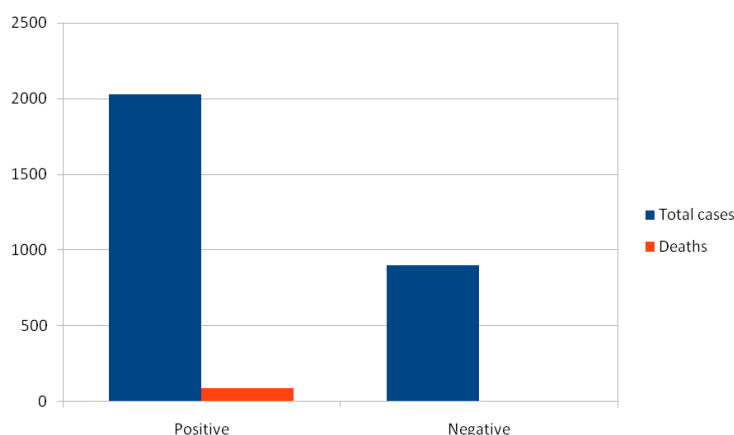
Total number of Diphtheritic myocarditis cases were 556 and all these cases showed ketone body positivity.

**Table 6: Distribution of ketone body positivity in relation to mortality**

Ketone bodies positivity	No of Cases	Total no of Death Cases (%)
Ketone body positive	2028 (69.3%)	81 (2.7%)
Ketone body negative	897(30.7%)	0.0%

Total number of diphtheria cases were 2925 out of which 69.3% i.e., 2028 cases were positive for ketone bodies and all 81 (2.7%) patients who died showed ketone body positivity. But no deaths were noted in 897(30.7%) cases in which ketone bodies were negative. Fisher's Exact Test  $P < 0.01$  which is significant.

**Figure 6: : Distribution of ketone body positivity in relation to mortality**



## V. Discussion

Diphtheria, if not detected early and treated, it can lead to significant mortality and morbidity because of critical complications like myocarditis, obstructive airway disease etc,<sup>(5,6,7)</sup>.

The clinical presentation, course of illness and outcome vary in different age groups. Mortality rate was comparatively high in age groups below 10 years it was decreased in other age groups.

Maximum incidence of deaths were due to myocarditis. Deaths noted were more in non immunized and partially immunized patients compared to completely immunized patients.

L- Carnitine (beta hydroxy trimethyl amino butyric acid) is a crucial component in fat metabolism. It is required to shuttle long chain fatty acids into mitochondria for beta oxidation and is essential for tissues dependent on fatty acid oxidation such as cardiac and skeletal muscle. Carnitine also participates in metabolism of branched chain aminoacids and stabilizes cellular membranes. It is also a free radical scavenger and is likely to take part in control of nuclear transcription.

A fragment of exotoxin of diphtheria inhibits protein synthesis and decreases the synthesis of carriers of carnitine across the cardiac cell membrane, thereby reduces the rate of uptake and increases the efflux of - [3H] carnitine in established cell line from human heart. This results in decrease in the level of intracellular carnitine to about 55% of control after exposure to 10-8mol/L diphtheria toxin for 24hours. Impairment in carnitine transport leads to loss of carnitine molecules. Indeed, carnitine depletion from myocardium is a characteristic feature of diphtheria. Intracellular carnitine levels which impairs the availability of long chain fatty acids for beta-oxidation and energy production, and also the production of ketone bodies. This impedes entry of acetyl - COA into Krebs cycle which is then converted in liver mitochondria to ketone bodies. Thus

elevated ketone bodies are seen in urine samples of most of the diphtheritic patients. Exotoxin also causes lysis of myocardial cells and is an indication for the diphtheritic myocarditis.

All deaths were due to myocarditis. Epistaxis and hematemesis and airways obstruction were associated morbid conditions. The mortality rate is higher in children as is also observed in the other studies. (11,12,13)

In this study, total number of diphtheria cases were 2925, out of which 2028 (69.3%) cases were positive for ketone bodies. It was also noted that in all the cases who died due to Diphtheritic myocarditis 81 (2.7%), the ketone bodies were positive and the p value is <0.01 which is significant. But no deaths were noted in patients with ketone body negativity.

Studies<sup>(14,15,16)</sup> done on the carnitine depletion especially in diphtheria patients concluded that it is mainly due to the exotoxin production by *C. diphtheriae* and also it is one of the factor for elevation of ketone bodies in urine. In the present study also most of the patients showed the positivity for ketone bodies(61.5%) in the urine samples of diphtheriae patients.

In the present study in all the diphtheritic myocarditis cases(19%), ketone bodies were detected in the urine samples there was no single case with negative ketone bodies in the urine. Thus it indirectly reflects the carnitine depletion in myocardium by exotoxin. Many studies have been reported on the detection of carnitine depletion diphtheritic patients<sup>(3,5,17,18,19)</sup> as well as patients with diphtheritic myocarditis.<sup>(20-26)</sup>

The ability to produce exotoxin by *C. diphtheriae* is essential if the disease is to result in major epidemics and its detection is mandatory for early and easy control of the disease. Hence the estimation of ketone bodies in urine is the simplest diagnostic test available in all tertiary hospitals to confirm the diphtheria as it indicates the toxin production especially when there is low smear positivity and isolation of organism due to various factors.

Presence of ketone bodies in the urine definitely confirms the intracellular toxin and also seen in most of the cases of diphtheria and also in all cases of diphtheritic myocarditis. Thus early clinical diagnosis and therapeutic intervention are mandatory for better patient outcome. Deaths occurred mostly commonly in younger age group, those who presented late in course of disease. Laboratory investigations help for confirmation of clinical diagnosis and surveillance.

## VI. Conclusions

Diphtheria continues to be a major health concern in a developing country like India with considerable morbidity and mortality. Detection of the elevated ketone bodies and ability to produce the exotoxin by *C. diphtheriae* which is responsible for the severe complications of Diphtheria would help in early and easy control of the mortality and morbidity of Diphtheria.

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