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Acute Conjunctiviti? Cause: A study at Tertiary Care Center During Conjunctivitis Outbreak at Dhule, Maharashtra

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ABSTRACT
Conjunctivitis is one of the most frequent reason for visiting to general medical and ophthalmology clinics. Acute conjunctivitis can be caused by viruses like epidemic adenoviruses, coxsackievirus A24 and enterovirus 70 (EV-70). According to state officials total cases of acute conjunctivitis in Maharashtra during outbreak were 3,57,265 on 8th August 2023. Acute conjunctivitis can be managed effectively if diagnosed and treated appropriately in time. To identify the causative agent for recent outbreak of acute conjunctivitis in Dhule. 2. To determine the antimicrobial sensitivity pattern of bacteriological agents if found. The Present study was conducted from 1st July 2023 to 10th August 2023. Two Conjunctival swabs were taken from each patient with clinically diagnosed Acute conjunctivitis. One swab was collected in viral transport media for SARS COV 2 real time PCR testing and further transportation to NIV PUNE for viral PCR testing. Other swab was processed immediately for bacterial culture, Gram staining in Microbiology and Giemsa staining at Pathology department. Fourty samples were collected out of which 10 samples showed viral nuclear inclusions on Giemsa stain. Three Samples were confirmed as enteroviruses by NIV Pune out of 10 randomly sent samples. 10 samples showed significant growth of β hemolytic Staphylococcus aureus on Blood agar. Twenty one samples showed growth of *Staphylococcus aureus* but colonies were less than 5. There was no growth in 9 samples. Though Recent outbreak in Dhule was suggestive of viral etiology secondary bacterial infections occurred in significant number of cases.

INTRODUCTION

Conjunctivitis is one of the most frequent reasons for visiting to general medical and ophthalmology clinics. It is the inflammation of the conjunctiva due to infection (viral, bacterial or parasitic), mechanical irritation, neoplasia, toxic substances or allergies[1]. Conjunctivitis is characterized by dilatation of the conjunctival vessels, resulting in hyperemia and edema of the conjunctiva. Bacterial Conjunctivitis is usually diagnosed by Gram staining and culture which is useful in recurrent conjunctivitis and conjunctivitis with severe mucopurulent discharge. Nucleic acid amplification techniques (PCR),requiring special swabs, may be used in diagnosing viral infections[2]. It is very much difficult to come across accurate diagnosis just based on the presenting signs and symptoms[1]. In several studies clinical inaccuracy ranging from 40-75 % has been demonstrated[3].

In 1969 an outbreak of AHC (Acute hemorrhagic conjunctivitis) was first reported from Ghana which was referred to as Apollo conjunctivitis. Another outbreak of AHC occurred in Delhi, north India, during the rainy season(August and September) in 1996. Acute conjunctivitis can be caused by viruses like epidemic adenoviruses, coxsackievirus A24, and enterovirus 70 (EV-70)[4]. For 1969 outbreak a new enterovirus (EV-70) was identified as the etiologic agent[4] subsequently it spread to other parts of Africa and Asia including India. The conjunctivitis outbreaks due to viruses as well as bacteria are common and are frequently linked to crowding of people in settings like hostels, classrooms, shared accommodations[5] According to state officials total cases of acute conjunctivitis in Maharashtra were 3,57,265 on 8th August 2023 making it one of the most severe outbreak in recent years[6]. Among infectious conjunctivitis cases viral conjunctivitis is responsible for up to 80% of all acute conjunctivitis cases[7]. Secondary Bacterial infection superimposed on the viral infection may occur by exogenous bacteria or normal commensals of the conjunctiva due to decrease local immunity[8]. Acute conjunctivitis can be managed effectively if diagnosed and treated appropriately in time. In a view of this we conducted a study to identify the etiologic agent and antibiotic sensitivity pattern in cases of secondary bacterial infection.

Research question: What is the etiological agent in recent outbreak of acute conjunctivitis in Dhule, Maharashtra?

Objectives:

- To identify the etiological agent for recent outbreak of acute conjunctivitis in Dhule
- To determine the antimicrobial sensitivity pattern of bacteriological agent if found

MATERIAL AND METHODS

The Present study was conducted from 1st July 2023 to 10th August 2023, during conjunctivitis outbreak period, in the department of Microbiology at JMF'S ACPM Medical College which is a tertiary health care center at Dhule.

As per information collected from Ophthalmology department only 55 patients attended ophthalmology OPD. Out of these 55 patients 40 gave consent to get enroll in the study. Taking all aseptic precautions, sterile swabs were used to collect discharges from lid margins and inferior fornices of affected eyes. Two Samples were collected from each patient one for bacteriological identification process and another sample was collected in VTM for identification of viral etiological agents. These samples were immediately transported to microbiology and pathology department. These were smeared on two slides for Gram and Giemsa staining. The conjunctival discharges were also inoculated on blood agar. The identification was done with the help of Gram stain and biochemical tests on samples which showed growth on blood agar. The antibiotic susceptibility testing was done by Clinical and Laboratory Standards Institute (CLSI) recommended Kirby-Bauer disc diffusion testing on Mueller-Hinton agar. Methicillin resistance was tested by standard Microbiological techniques by using cefoxitin disk (30µg) by Kirby-Bauer's disk diffusion method on Mueller Hinton agar as per CLSI guidelines. Strains with inhibition zone size of $\geq$22 mm were read as sensitive whereas zone size of $\leq$21 mm was interpreted as resistant[9]. All the disc for sensitivity testing were procured from Hi-Media Laboratories. Real time RTPCR testing for SARS CoV2 was also performed on the samples which were collected in viral transport media. Out of these 40 samples 10 Randomly selected samples were sent to NIV pune for Viral PCR testing to find out viral etiological agent.

RESULTS

Out of the 40 samples which were collected from cases of clinically diagnosed conjunctivitis 10 samples showed viral nuclear inclusions on giemsa stain. (Table 1) All the samples were negative for SARS Cov2 by real time PCR testing. Three Samples were confirmed as Enteroviruses by NIV Pune out of 10 randomly sent samples for identification of viral etiological agent by PCR testing. All the 40 samples were cultured on Blood Agar. Ten samples showed significant growth of β hemolytic *Staphylococcus aureus*. Twenty one samples showed growth of *Staphylococcus aureus* but colonies were less than 5.

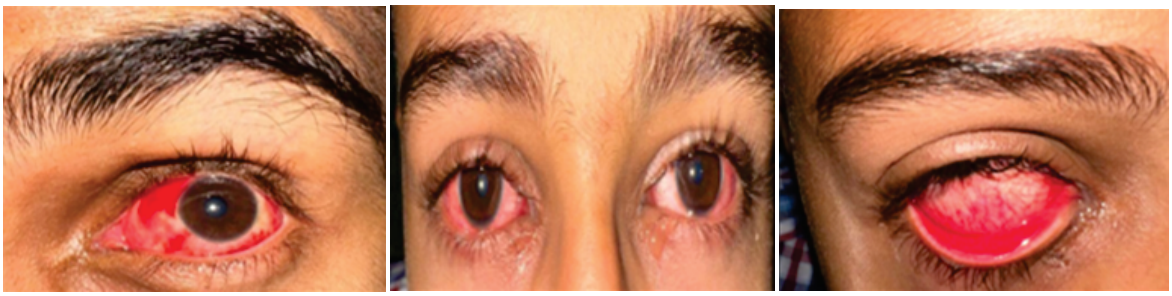

Fig. 1: Red Eye, Watering and Purulent Discharge

Table 1: Conjunctivitis cytology report by Giemsa stain

Findings	No of patients
Squamous epithelial cells and nuclear inclusions	
S/O Viral infection	10
Sqamous epithelial cells with degenerative changes and cytoplsmic vacuolations S/O reactive changes	18
Polymorphonulclear cells with degenerated squamous epithelial cells and nuclear inclusions S/O viral with secondary bacterial infection	12

Table 2: Antibiotic sensitivity pattern for *Staphylococcus aureus* (n = 10)

Antibiotic disc	Sensitive	Resistant
Amikacin	8	2
Clindamycin	6	4
Ceftazidime	10	0
Cephazoline	10	0
Tetracycline	7	3
Erythromycin	6	4
Chloroamphenicol	7	3
Moxifloxacin	10	0
Ofloxacin	10	0
Cefoxitin	10	0
Vancomycin	10	0

There was no growth in 9 samples. All the strains were sensitive to ofloxacin. No strain was found to be Methicillin resistant *Staphylococcus aureus* when tested with Cifoxitin disc. The sensitivity pattern of 10 isolates was shown as per (Table 2).

DISCUSSIONS

As per information collected from Ophthalmology department only 55 patients attended ophthalmology OPD. with complaints of itching, watering, redness of eyes, serous discharge and pain (Fig. 1-3). Fever was absent in all these patients. Matting of conjunctiva was complained by few patients. Very few cases attended OPD as most of the cases may have took treatment over the counter.

According to ophthalmologist's observations, in the present epidemic of acute conjunctivitis clinical symptoms were suggestive of viral etiology. In One of the study conjunctivitis was confirmed as a symptom of COVID-19 infection[10]. The prevalence rate of acute conjunctivitis in COVID-19 was reported ranging from 1.1 to 31.6% of patients[11,12]. In our study all the samples tested for SARS COV 2 real time PCR testing were negative. We sent only 10 random samples to NIV Pune for viral PCR testing considering the load on referral laboratory. Out of the 10 randomly sent samples to NIV Pune for viral PCR testing 30% were positive for enterovirus. Acute hemorrhagic conjunctivitis or enteroviral hemorrhagic conjunctivitis is a highly contagious ophthalmic infection that first appeared in Ghana (1969). Besides enterovirus 70 (EV-70), coxsackievirus A24 can also cause acute hemorrhagic conjunctivitis[13].

In India EV-70 infection was first reported in 1971-72 serologically and first isolation was reported in 1975 from south India from a single case during a small epidemic. In North India 2 outbreaks occurred in 1986 and 1996 almost 1 decade apart. In Pune (Maharashtra) also similar outbreak occurred in 1991[4]. As per the state official reports the present outbreak was most severe outbreak in recent years.[6] Though Patient's history, ocular symptoms, focused ocular examination regarding type of ocular discharge are very much important but is not sufficient to determine the cause of the conjunctivitis. Most of the time bacterial conjunctivitis is characterized by a purulent or mucopurulent discharge while a watery discharge is present in viral conjunctivitis. However, the clinical presentation is often nonspecific[1].

Secondary Bacterial infection superimposed on viral conjunctivitis by exogenous bacteria or normal commensals of the conjunctiva may be caused due to a local decrease in the Immunity. So we tested all the conjunctival swabs for Microscopy with Gram and Giemsa stain and culture- sensitivity testing to find out secondary bacterial infection. Twenty five percetage

of the swabs from the patient showed growth of β hemolytic *Staphylococcus aureus* on blood Agar and 30% of the swabs showed Polymorphonulcler cells with degenerated squamous epithelial cells and nuclear inclusions on Giemsa stain S/O viral with secondary bacterial infection.

One of the study showed growth of Proteus, Diphtheroides and Micrococci in viral conjunctivitis cases with secondary bacterial infection[8]. In our study all the isolates grown were *Staphylococcus aureus*. Mild bacterial conjunctivitis is usually self-limiting within 1-2 weeks of presentation but for severe cases characterized by purulent discharge, pain and marked inflammation of the eyes antibiotic treatment is needed. Topical antibiotics seem to be more effective in patients who have positive bacterial culture results[14,1]. In the present study no staphylococcal isolate was methicillin resistant and all were sensitive to ofloxacin (fluoroquinolone) which is commonly prescribed topical eyedrops in conjunctivitis cases.

As per the information from Ophthalmologist, Patients were treated with decongestants and antihistaminic eye drops, Chlorpheniramine Maleate IP 0.03% w/v and Tetrahydrozoline hydrochloride USP 0.01% w/v. For prevention and treatment of secondary bacterial infection ofloxacin/Moxifloxacin eyedrops were advised.

CONCLUSION

Though Recent outbreak in Dhule was suggestive of viral etiology secondary bacterial infections occurred in significant number of cases. Microscopy and culture sensitivity can support the clinical diagnosis and help to treat the conjunctivitis cases judiciously.

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