Section: Microbiology

Original Research Article

MOLECULAR ANALYSIS OF OUTBREAK OF ACUTE SEVERE CONJUNCTIVITIS ASSOCIATED WITH ENTEROVIRUS IN A TERTIARY CARE HOSPITAL REGIONAL INSTITUTE OF OPHTHALMOLOGY, GOVERNMENT OPHTHALMIC HOSPITAL, MADRAS MEDICAL COLLEGE, CHENNAI

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Abstract

Background: Viral conjunctivitis, a highly contagious eye infection predominantly caused by adenoviruses (65–90%), spreads via contact, droplets, or contaminated water. While adenoviruses are usually suspected, this study aimed to identify the etiological agents of severe conjunctivitis cases during a recent outbreak using molecular diagnostic methods at the Regional Institute of Ophthalmology, Government Ophthalmic Hospital, Madras Medical College, Chennai. **Materials and Methods:** A total of 157 patients with severe conjunctivitis were referred to the Microbiology department for RT-PCR testing at the Regional institute of Ophthalmology, Government Ophthalmic Hospital, Madras Medical College, Chennai between October and December 2023. Conjunctival swabs were collected and analyzed using real-time polymerase chain reaction (RT-PCR) to identify viral pathogens. **Result:** Of 157 patients, 101 (64.33%) were male and 56 (35.66%) were female. The age distribution showed that the 14–30 years' groups was most affected and 30-45 years' group was the next commonly affected. Among all cases, 31 (19.74%) were positive for viral conjunctivitis via RT-PCR, of which 23 (74.19%) were male and eight (25.80%) were female. Enterovirus was identified as the predominant pathogen in the RT-PCR-positive cases. **Conclusion:** Our study identified enterovirus, rather than adenovirus, as the primary cause of severe conjunctivitis outbreak, highlighting the importance of RT-PCR for accurate pathogen identification. Early diagnosis, targeted management, and adherence to hygiene practices are critical for controlling the spread of these outbreaks.

INTRODUCTION

Conjunctivitis, commonly known as pinkeye, may have viral, bacterial, or non-infectious allergic origins. Viral infections constitute over 80% of all infectious illnesses, followed by bacterial infections.[1-3] It is a highly contagious, acute viral infection of the conjunctiva, primarily caused by adenoviruses (65-90%), followed by enteroviruses HSV-1, HSV-2, and varicella-zoster virus (VZV). Viral conjunctivitis is disseminated by direct contact, respiratory droplets, fecal-oral transmission, and exposure to unchlorinated or inadequately chlorinated water.[4-6] Viral conjunctivitis typically manifests with symptoms, such as foreign body sensation, pruritus, photophobia, impaired vision, and bulbar conjunctival erythema. Severe cases exhibit petechial or subconjuctival hemorrhage.[7]

Immune cell infiltration and localized inflammatory lesions in the subepithelial layer are associated with viral conjunctivitis. Determining the etiological agents of conjunctivitis in a particular geographical area may facilitate the selection of suitable treatment procedures. Laboratories can employ multiple techniques to identify viral infections, including viral culture, antigen detection, serology, nucleic acid detection, direct immunofluorescence, and rapid antigen detection immunoassays. Viral cell cultures of conjunctival specimens can confirm the presence of the virus; however, they are difficult to perform owing to the need for sophisticated equipment, skilled laboratory personnel, and considerable delays in result acquisition.

Nucleic acid detection is more sensitive than alternative methods and does not rely on the presence of a living virus or suitably infected cells. The diagnosis of viral conjunctivitis is typically based on

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patient history and clinical observations; however, serological and molecular diagnostic tests are also accessible. Unwarranted antibiotic treatment is often prescribed owing to unidentified causes. Consequently, polymerase chain reaction (PCR) is becoming recognized as the "gold standard" for identifying viral conjunctivitis.[8-11]

Aim: This study aimed to identify the current etiological agents of viral conjunctivitis in severe cases using molecular diagnostic methods at the Regional Institute of Ophthalmology and Govt Ophthalmic Hospital, Madras Medical College, Chennai.

MATERIALS AND METHODS

This observational and cross-sectional study included 157 patients with severe conjunctivitis who were referred to the microbiology department for RTPCR for viral study at the Regional Institute of Ophthalmology, Government Ophthalmic Hospital, Madras Medical College, Chennai, between October and December 2023. Written informed consent was obtained from all patients.

Inclusion Criteria

- Patients with severe conjunctivitis were referred for RT-PCR analysis.
- Cases were reported between October and December 2023.

Exclusion Criteria

Patients with mild or moderate conjunctivitis were excluded.

Methods: Conjunctival swabs were collected from the inferior palpebral conjunctiva of clinically suspected severe viral conjunctivitis cases. The swabs were immediately placed in a viral transport medium (VTM) and transported in a cold chain to the virology laboratory at the King Institute of Preventive Medicine and Research, Chennai, Tamil Nadu.

The samples were processed in the virology laboratory by initial vortexing and centrifugation at 10,000 rpm for 10 min at 4°C. The supernatant was subjected to a real-time polymerase chain reaction (PCR) for viral analysis. The data were entered into a Microsoft Excel spreadsheet and presented as frequencies and percentages.

RESULTS

Among 157 patients, males are 101 (64.33%), females are 56 (35.66%) patients. The age distributions of the total cases, < 14 years were 17 males and 7 females, 14-30 years of age were 28 males and 26 females, 31-45 years of age were 20 males and 12 females, ages between 46-60 years were 23 males and 6 females, and in age >60 years, 13 patients were males and 5 were females.

Among 157 severe conjunctivitis cases, 31 (19.74%) had total RTPCR-positive conjunctivitis, of which 23 were male positive (74.19%) and 8 were female positive (25.80%) for viral study [Table 1].

Among the 31 RTPCR-positive cases, 26 (83.87%) were enterovirus positive, 3 (9.67%) were adenovirus positive, and 2 (6.45%) were HSV-positive [Table 2].

Table 1: Age, gender wise distribution of severe conjunctivitis cases and identification of viral etiology.

		Gender		Viral Etiology						
		Male	Female	Total tested	Total positive	Male	Female	Enterovirus	Adenovirus	HSV
Age groups (years)	< 14	17	7	24	1	0	1	1	0	0
	14-30	28	26	54	10	8	2	10	0	0
	31-45	20	12	32	11	8	3	6	3	2
	46-60	23	6	29	7	5	2	7	0	0
	> 60	13	5	18	2	2	0	2	0	0
Total		101 (64.3%)	56 (35.7%)	157 (100%)	31 (19.7%)	23 (74.2%)	8 (25.8%)	26 (83.9%)	3 (9.7%)	2 (6.5%)

Table 2: RT-PCR positive virus distribution of severe conjunctivitis cases

Total positive RT-PCR	Enterovirus	Adenovirus	HSV
31 (19.74%)	26 (83.87%)	3 (9.67%)	2 (6.45%)

DISCUSSION

Conjunctivitis outbreaks typically go unnoticed in standard disease surveillance systems unless they garner media attention locally. Identifying conjunctivitis clusters and halting their spread is crucial for preventing a population-wide outbreak.[11]

In our study, 157 cases of severe conjunctivitis were analyzed. Of these, 101 (64.33%) were male and 56 (35.66%) were female. The age-wise distribution showed that the 14-30-year age group was the most affected, with 54 (34.39%) cases, followed by the 31-45-year age group, which accounted for 32 (20.38%) cases. This finding aligns with a prior study by Azari and Barney,[12] which emphasizes that youth are more sensitive to acute conjunctivitis due to increased exposure to social gathering venues, such as schools, workplaces, social events, and outdoor activities. Furthermore, Gordon-Shaag et al,[13] proposed that susceptibility among the 31-45 age demographic may be attributed to occupational exposure and lifestyle factors. These trends underscore the influence of environmental exposure, social interactions, and lifestyle factors on the onset of conjunctivitis.[14]

In contrast, pediatric and elderly participants were the least affected, likely because of reduced exposure to

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high-risk environments and behavioural differences, such as more controlled hygiene practices in children and decreased communal activities in older adults.[14,15]

RT-PCR analysis revealed that 31 (19.74%) patients were positive for viral conjunctivitis. Of these, 23 (74.19%) were male and eight (25.80%) were female, reflecting a significantly higher prevalence among males. This pattern aligns with a study conducted by Fredrick et al., which reported a male-to-female ratio of 1.34:1 and attributed the higher prevalence in males to occupational and environmental risk factors. Men often engage in outdoor activities, thereby increasing their exposure to viral transmission risks.[16,17]

Among RT-PCR-positive severe conjunctivitis cases, enterovirus was identified in 26 (83.87%) cases, adenovirus in 3 (9.67%) cases, and herpes simplex virus (HSV) in 2 (6.45%) cases. These findings highlight the predominance of enteroviruses as the primary etiological agents. Enteroviruses, which belong to the Picornaviridae family, can cause a range of symptoms, the severity of which is influenced by factors such as viral load, exposure time, and host conditions. The use of RT-PCR, the most sensitive diagnostic method, allows the accurate detection of these pathogens and provides insights into the viral load on the conjunctival surface, which is relatively sparse compared to other mucosal surfaces.[17-19] These findings collectively emphasize the critical role of molecular diagnostics in identifying the etiological agents of conjunctivitis outbreaks and understanding epidemiological trends related to demographic and behavioural factors.

The findings from this study are further supported by our understanding of enterovirus epidemiology and clinical manifestations, such as the study by Nishimura et al., which highlights the diversity and pathogenic potential of enteroviruses. Enteroviruses can cause a wide range of diseases, including acute hemorrhagic conjunctivitis and neurological complications. Their persistence in the environment and ability to adapt to various host conditions underscores their potential to cause widespread outbreaks in susceptible populations.[20,21]

Furthermore, global trends in enterovirus outbreaks reflect periodic and seasonal variations, which may explain the epidemiological dynamics observed in our study. These trends emphasize the importance of public health surveillance and targeted interventions to mitigate the spread of enterovirus-associated conjunctivitis. Public health strategies, including enhanced hygiene practices, proper waste management, and educational campaigns, are essential for controlling outbreaks. Future research on the molecular characteristics and transmission dynamics of enteroviruses can provide deeper insights and inform the development of targeted preventive and therapeutic strategies.

CONCLUSION

In conclusion, our study identified enterovirus as the predominant etiological agent in severe conjunctivitis cases during this outbreak, verified through RT-PCR, underscoring the value of molecular diagnostics in managing viral outbreaks in tertiary care settings. The demographic trends observed, such as the higher prevalence in males and middle-aged individuals, highlight the impact of occupational and social exposure on disease vulnerability. These findings call for targeted public health interventions and enhanced preventive measures to mitigate the risk in high-exposure groups. Further research on the molecular characteristics and transmission dynamics of enterovirus-associated conjunctivitis may provide deeper insights and inform effective control strategies.

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