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Short communication

Outpatient human coronavirus associated conjunctivitis in India

N Venkatesh Prajna a, Prajna Lalitha a, Gonugunta Vishnu Teja a, Rameshkumar Gunasekaran a, Sankalp S. Sharma a, Armin Hinterwirth b, Kevin Ruder b, Lina Zhong b, Cindi Chen b, Michael Deiner c, ChunHong Huang d, Benjamin A. Pinsky d, e, Thomas M. Lietman b, c, Gerami D. Seitzman b, c, Thuy Doan b, c,*, on behalf of the SCORPIO (Seasonal Conjunctivitis Outbreak Reporting for Prevention and Improved Outcomes) Study Group

a Aravind Eye Hospital, Madurai, India
b Francis I. Proctor Foundation, University of California San Francisco, San Francisco, CA, United States of America
c Department of Ophthalmology, University of California, San Francisco, CA, United States of America
d Department of Pathology, Stanford University School of Medicine, Stanford, CA, United States of America
e Department of Medicine, Division of Infectious Diseases and Geographic Medicine, Stanford University School of Medicine, Stanford, CA, United States of America

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ABSTRACT

Background: Viral conjunctivitis (pink eye) can be highly contagious and is of public health importance. There remains significant debate whether SARS-CoV-2 can present as a primary conjunctivitis. The aim of this study was to identify pathogens associated with outpatient infectious conjunctivitis during the COVID-19 Delta surge.

Methods: This prospective study was conducted in the spring and summer months of 2021. 106 patients with acute conjunctivitis who presented to the Aravind Eye Center in Madurai, India were included. One anterior nasal swab and one conjunctival swab of each eye were obtained for each enrolled patient. Samples were subsequently processed for unbiased metagenomic RNA deep sequencing (RNA-seq). Outcomes included clinical findings and codetection of other pathogens with SARS-CoV-2 in patients with conjunctivitis.

Results: Among the 13 patients identified with human coronavirus RNA fragments in their swabs, 6 patients had SARS-CoV-2 infection, 5 patients had coinfections of SARS-CoV-2 and human adenovirus (HAdV), 1 patient had a coinfection with human coronavirus OC43 and HAdV, and 1 patient had a coinfection of Vittaforma corneae and SARS-CoV-2. 30% had bilateral disease and symptoms on presentation. Petechial hemorrhage was noted in 33% of patients with SARS-CoV-2 infection. No patients with SARS-CoV-2 or SARS-CoV-2 and HAdV infections had subepithelial infiltrates on presentation. All patients denied systemic symptoms.

Conclusions: Among the patients presented with conjunctivitis associated with human coronavirus infection, over 50% of the patients had co-infections with other circulating pathogens, suggesting the public-health importance of broad pathogen testing and surveillance in the outpatient conjunctivitis population.

1. Introduction

SARS-CoV-2 RNA has been detected in the conjunctiva and tears of patients hospitalized with moderate to severe COVID-19 disease [1,2]. Conjunctivitis associated with COVID-19 disease is documented in both the outpatient and hospitalized populations, although the prevalence varies dependent on the study and population investigated and it remains a debate whether infection with SARS-CoV-2 can present as a primary conjunctivitis [1,3-6].

In a companion paper, we presented a study in which we used unbiased metagenomic sequencing to identify pathogens known to cause conjunctivitis during the conjunctivitis season that happened to coincide with the Delta surge in India. We found that HAdV was the most common pathogen detected in patients who presented to the Aravind Eye Hospital [7]. Because all patients were subjected to unbiased testing, we were able to detect other viruses and pathogens that may have not been on the differential. In this paper, we describe a subgroup of those patients who were positive for human coronavirus (HCoV) RNA in either conjunctival or anterior nasal samples collected on presentation. Cases of presumed co-infections are also characterized. This

* Corresponding author at: 490 Illinois Street, Floor 2, San Francisco, CA, 94158 United States of America
E-mail address: thuy.doan@ucsf.edu (T. Doan).
information may assist outpatient management given that SARS-CoV-2 will likely be endemic in the setting of other known circulating pathogens causing conjunctivitis.

2. Materials & methods

Ethical approval was obtained from the Aravind Eye Hospital and University of California San Francisco (UCSF) Institutional Review Boards (IRB). The Stanford University IRB waived review. This study adhered to the tenets of the Declaration of Helsinki. All patients with presumed infectious conjunctivitis at the Aravind Eye Clinic in Madurai, India, from April 1 to May 1, 2021 and from June 1 to September 17, 2021, were included in the analysis. The gap in sample collection represented a lockdown period in which all research activities at Aravind were suspended due to the Delta surge. A conjunctival swab of each eye and an anterior nasal swab of both nares were obtained for each enrolled patient. Swabs were placed in DNA/RNA Shield (Zymo Research, Irvine, CA) and stored at \(-80^\circ\text{C}\) until processing. Samples were deidentified and all laboratory personnel were masked. RNA sequencing was performed as previously described [8]. Briefly, 5 \(\mu\text{L}\) of extracted RNA of each sample was converted to cDNA and sequencing libraries were prepared using the NEBNext ULTRA II RNA Library Prep Kit for Illumina (New England Biolabs, Ipswich, MA) according to manufacturer’s instructions and then pooled and sequenced on the NovaSeq system (NovaSeq 6000, Illumina, San Diego, CA) using 150-nucleotide paired-end sequencing.

Analysis of the sequenced data to identify pathogens was performed as described [8]. The pre-specified criteria for positive pathogen are 1) it is known to be a human pathogen and represent the most abundant reads after water background subtraction or 2) two or more unique reads covering separate regions in DNA virus genomes or 3) one or more unique reads matching RNA virus genomes. SARS-CoV-2 positive samples on RNA-seq were subjected to a laboratory-developed confirmatory SARS-CoV-2 reverse transcription-quantitative PCR (RT-qPCR) at the Stanford Clinical Virology Laboratory [9]. Testing was performed as previously described, except that RNA was extracted using the Quick-DNA/RNA MicroPrep Kit (Zymo Research, Irvine, CA) and eluted in 20 \(\mu\text{L}\) of DNase/RNase free H\(_2\)O. This multiplex RT-PCR targets the SARS-CoV-2 \(E\) gene and also includes detection of human RNase P nucleic acids as internal control in a separate fluorescence channel. The presence of RNase P at a cycle threshold (C\(_T\)) value less than 35 cycles indicates adequate specimen collection and nucleic acid extraction, as well as the absence of RT-PCR inhibitors. Samples that were positive on confirmatory RT-qPCR underwent a genotyping RT-qPCR as previously described [10,11]. Genotyping was performed using a laboratory-developed two-reaction multiplex RT-qPCR assay targeting spike gene mutations L452R, E484K, and N501Y in reaction 1 and del69–70, K417N, and T478K in reaction 2 [11].

3. Results

A total of 318 conjunctival and anterior nasal swab samples from 106 patients were included in the study. The sequencing depth, host reads, and non-host reads for all samples are shown in Fig. 1. Of those, 13 patients had at least one sample that was positive for human coronavirus (OC43 and SARS-CoV-2) RNA on RNA-seq (Table 1 and Fig. 2). Patient demographics and clinical signs and symptoms on presentation are shown in Table 1. The mean age was 40 years old and 77% were male.

![Fig. 1. Characteristics of metagenomic RNA deep sequencing. Number of sequencing read counts as a function of total, host, and non-host for 318 samples. Each dot represents a sample. Bars represent mean and standard deviation.](image-url)
Table 1
Patient demographics and clinical signs and symptoms on presentation for 13 coronavirus-positive patients with conjunctivitis. Only patient #7 had documented subepithelial infiltrates and membrane or pseudomembranes. Abbreviations: HAdV, human adenovirus; HCoV-OC43, human coronavirus OC43; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; MDS, metagenomic RNA deep sequencing.

| Pt # | Age (years) | Sex | Contact affected | Symptom Duration (days) | Eye(s) with conjunctivitis | Sore Throat | Runny Nose | Pre-auricular lymphadenopathy | Itching | Tearing | Purulent Discharge | Petechiae | MDS Right Conjunctiva | MDS Left Conjunctiva | MDS Nose |
|------|-------------|-----|------------------|-------------------------|---------------------------|-------------|------------|-----------------------------|---------|---------|---------------------|----------|---------------------|---------------------|---------|
| 1    | 22          | M   | No               | 1                       | Right                     | No          | No         | No                          | Yes     | Yes     | Yes                 | Yes      | SARS-CoV-2           | Negative            | SARS-CoV-2 |
| 2    | 50          | M   | Unknown          | 1                       | Left                      | No          | No         | No                          | Yes     | Yes     | Yes                 | Yes      | SARS-CoV-2           | SARS-CoV-2          | SARS-CoV-2 |
| 3    | 25          | M   | No               | 2                       | Right                     | No          | No         | No                          | No      | Yes     | Yes                 | No       | SARS-CoV-2           | Negative            | SARS-CoV-2 |
| 4    | 21          | M   | Yes              | 2                       | Left                      | No          | No         | No                          | No      | No      | Yes                 | No       | Negative            | Vittiforma cornea, SARS-CoV-2 | Negative |
| 5    | 63          | M   | No               | 1                       | Left                      | No          | No         | No                          | No      | Yes     | Yes                 | No       | Negative            | SARS-CoV-2          | SARS-CoV-2 |
| 6    | 22          | F   | No               | 6                       | Left                      | No          | No         | Left                         | Yes     | Yes     | Yes                 | No       | Negative            | SARS-CoV-2           | HAdV     |
| 7    | 60          | M   | No               | 3                       | Left                      | No          | Yes        | Left                         | No      | Yes     | Yes                 | No       | Negative            | HAdV                | SARS-CoV-2 |
| 8    | 39          | M   | No               | 1                       | Right                     | Yes         | No         | No                          | No      | Yes     | Yes                 | Yes      | SARS-CoV-2           | Negative            | SARS-CoV-2 |
| 9    | 24          | M   | No               | 10                      | Both                      | No          | No         | Left                         | Yes     | Yes     | Yes                 | Yes      | SARS-CoV-2, HAdV | HAdV                | HAdV     |
| 10   | 52          | M   | No               | 6                       | Both                      | No          | No         | Right                        | Yes     | Yes     | No                  | No       | SARS-CoV-2           | Negative            | HAdV     |
| 11   | 40          | M   | No               | 5                       | Left                      | No          | No         | No                          | Yes     | Yes     | Yes                 | Yes      | SARS-CoV-2, Negative | SARS-CoV-2           | HAdV     |
| 12   | 65          | F   | No               | 6                       | Both                      | No          | Yes        | Right                        | Yes     | Yes     | Yes                 | No       | HAdV                | SARS-CoV-2, HAdV   | HAdV     |
| 13   | 34          | F   | No               | 5                       | Both                      | No          | No         | No                          | No      | No      | No                  | Yes      | HAdV                | SARS-CoV-2, HAdV   | HAdV     |
Fig. 2. Identification of human coronaviruses by metagenomic RNA deep sequencing. A. Alignment of detected SARS-CoV-2 sequencing reads to the SARS-CoV-2 genome (NCBI reference sequence NC_045512) for each positive sample. B. Alignment of detected human coronavirus OC43 reads in the nasal sample of patient #7 to the NCBI reference sequence NC_006213.1. Abbreviations: OD, right eye; OS, left eye; N, nasal.
69% (95% confidence interval (CI): 42% to 87%) of the patients had unilateral disease. Of the symptoms queried, the most common symptom was tearing (77%, 95%CI: 50% to 92%). Other symptoms included itchiness (46%, 95%CI: 23% to 71%), runny nose (15%, 95%CI: 3% to 42%), and sore throat (8%, 95%CI: 0% to 33%). No patients reported coughing (0%, 95%CI: 0% to 23%) or diarrhea (0%, 95%CI: 0% to 23%). The most common clinical sign was purulent discharge (69%, 95%CI: 42% to 87%). Other signs included pre-auricular lymphadenopathy (38%, 95%CI: 18% to 64%), conjunctival petechiae (31%, 95%CI: 13% to 58%), subepithelial infiltrates (8%, 95%CI: 0% to 33%), and membranes or pseudomembranes (8%, 95%CI: 0% to 33%).

The Delta surge in the Tamil region began in March, peaked in May, and quickly tapered off by July 2021 (Fig. 3). This period coincided with the conjunctivitis season in India [12]. The presence of SARS-CoV-2 and HAdV RNA in either conjunctival or nasal samples was presumed to be pathologic as these viruses are not considered normal flora of the conjunctiva or respiratory tract. Six patients had only SARS-CoV-2 RNA detected and another five patients had co-detection of SARS-CoV-2 and HAdV RNA. One 60-year-old male patient with left eye involvement had HAdV RNA detected in the conjunctival swab of his left eye and HAdV and human coronavirus OC43 RNA detected in his nasal swab. A conjunctival swab from the left eye of a patient with unilateral conjunctivitis of the left eye was positive for *Vittaforma corneae* and SARS-CoV-2. Thus, of the 106 patients with presumed acute infectious conjunctivitis evaluated, 11% (95%CI: 7% to 19%) had SARS-CoV-2 associated conjunctivitis and 7% (95%CI: 4% to 14%) had co-infections with HAdV, SARS-CoV-2, or *Vittaforma corneae*. Representative external photos for each infectious category are shown in Fig. 4.

Fifty eight percent (7/12) of patients with detectable SARS-CoV-2 by RNA-seq were confirmed by RT-qPCR and genotyping demonstrated the Delta variant. The 5 patients not detectable by RT-qPCR had limited numbers of reads via sequencing.

4. Discussion

Unbiased pathogen detection using RNA-seq of conjunctival and anterior nasal samples of patients with acute conjunctivitis during the Delta variant surge in southern India showed not only the association of SARS-CoV-2 infection and outpatient conjunctivitis, but also the co-infections of other circulating viruses.

Most patients with SARS-CoV-2 infection or co-infection with HAdV presented with tearing and purulent discharge. Exam findings were notable for conjunctival petechial hemorrhages in 31% of patients. Subepithelial infiltrates and pseudomembranes presented in only one patient whose samples had codetection of HAdV and the human coronavirus OC43 RNA and were not present in any SARS-CoV-2 positive patients. Human coronavirus OC43 is a common HCoV that can cause respiratory symptoms, gastroenteritis, and conjunctivitis [13]. From a public health standpoint, the co-circulation of HAdV, HCoV-OC43, and SARS-CoV-2 suggests the importance of broad pathogen surveillance, particularly in the outpatient setting, where paradoxically, testing is rarely performed.

It was notable that none of the patients who presented to the Aravind Eye Center had a known diagnosis of SARS-CoV-2 infection. While 2 out of 13 patients (15%) positive for human coronaviruses had rhinorrhea, none of these patients reported other respiratory symptoms or GI symptoms. While it appeared that these participants sought medical care solely for conjunctivitis, one cannot rule out under-reporting given the perceived stigma associated with potential SARS-CoV-2 infection during this time period. Thus, as we continue to adapt to the continual presence of SARS-CoV-2, it may be prudent for the clinical staff to adhere to personal protective equipment protocols when examining conjunctivitis patients.
fungi may occur, indicating a need for the surveillance of outpatient conjunctivitis cases and the consideration of broad pathogen testing.

Nonauthor contribution

Seasonal Conjunctivitis Outbreak Reporting for Prevention and Improved Outcomes (SCORPIO) Study Group:

Aravind Eye Hospital, Madurai, India – Lalitha Prajna, N. Venkatesh Prajna, Ramesh Gunasekaran, Sankalp Singh Sharma, Vishnu Teja; B.P Koirala Lions Center for Ophthalmic Studies, Kathmandu, Nepal – Meenu Chaudhary, Sanjeeta Sitaula; centre de Recherche en Sante de Nouna, Nouna, Burkina Faso – Ali Sié, Boubacar Coulibaly, Mamadou Bountogo; Chulalongkorn University, Bangkok, Thailand – Thanapong Somkijrunggroj, Vannarut Satitpitakul; Hai Yen Vision Institute, Ho Chi Minh City, Vietnam – Huy Tran, Linh Hoang Mai, Thao Ha Xuan, Yen Tran; Hospital Clínico Universidad de Chile, Santiago, Chile – Cristian A. Urzuza, Fabian Vega, Felipe Salgado, Loreto Cuítino; Hospital Universitario Austral – Emiliano Sebastian Lopez, Federico Luengo Gimeno, Tomas Jaeschke; Instituto Mexicano de Oftalmología, Santiago de Querétaro, Mexico – Fernando Pérez, Jaime Macías Martínez, Van Charles Lansingham; Khon Kaen University, Khon Kaen, Thailand – Sukhumal Thanapaisal, Wipada Lavoijianakul; National Eye Institute – George McKie (Program Officer); Oregon Health and Science University, Portland, Oregon, USA – Kenia Chavez, Travis Redd, Winston Chamberlain; Pacific Vision Institute of Hawaii, Honolulu, Hawaii, USA – Angel Cheng, Vivien Tham; Phramongkutklao Hospital, Bangkok, Thailand – Wiwan Sansanayudh; Programme National de Santé Oculaire, Niamey, Niger – Abba Kaka Hajia Yakoura, Abdou Amza, Abdoul Salam Youssoufou Souley, Adam Nouhou Diori, Beido Nassirou, Boubacar Kadri, Boubacar Mariama, Cassé Mamadou Ibrahim, Lamyne Aboubacar Rofaye, Ramatou Boulhassane, Saley Ali, Zakou Abdou; Rabin Medical Center, Petah Tikva, Israel – Lee Goren, Ruti Sell; Sinai Hospital, Baltimore, Maryland, USA – Clare Kelíher, Laura Green; Singapore Eye Research Institute, Singapore – Hon Shing Ong, Jod Mehta, Yu-Chi Liu; Stanford University School of Medicine – Benjamin A. Pinsky; Taipei Veterans General Hospital, Taipei, Taiwan – De-Kuang Hwang, Nai-Wen Fan; The University of Sydney, Save Sight Institute, Sydney, Australia – Hong Sheng Chiong, Javier Lacorzana, Maria Cabrera-Aguas, Stephanie Watson; University of California Los Angeles Stein Eye Institute, Los Angeles, California, USA – Edmund Tsui, Joana Ramirez, Nina M. Cherian, Rachel Feit-Leichman, Reginald E. Hughes Jr, Tania Onclick; University of California San Diego Shiley Eye Institute; La Jolla, California, USA – Carol Yu, Esmeralda McClean, Iliana Molina; University of California San Francisco Francis I. Proctor Foundation, San Francisco, California, USA – Armín Hinterwirth, Cindy Chen, Danny Yu, David Liu, Elodie Lebas, Emily Colby, Gerami Seitzman, Kevin Ruder, Lina Zhong, Michael Deiner, Thomas Lietman, Thuy Doan (Principal Investigator), Travis Porco, Stephen McLeod; University of California Berkeley School of Optometry, Berkeley, California, USA – Kuniyoshi Kanai, Meredith Whiteside; University of Nebraska Medical Center Trihusen Eye Institute, Omaha, Nebraska, USA – Steven Yeh, Tolulope Fashina; University of New Mexico, Albuquerque, New Mexico – James Chodosh; University of Papua New Guinea School of Medicine and Health Sciences, Port Moresby, Papua New Guinea – Bridgit Turkap, Jambi N. Garap, Magdalene Mangot; Vanuatu Eye Program, Ministry of Health, Vanuatu – Edwin Amel, Fasihah Taleo, Johnson Kasso, Kalbule Willie, Madopule Nanu, Prudence Rymill, World Health Organization – Anthony W. Solomon

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5. Limitations

A limitation of this study is the lack of long-term follow-up. It was unclear if any of these patients subsequently developed respiratory symptoms or had a poor visual outcome. However, previous studies have shown that most SARS-CoV-2 associated outpatient conjunctivitis cases appeared mild [1] and the majority of patients’ symptoms resolved within 3 weeks [5]. Similar to many places around the world, clinical studies were placed on hold during the surge, as was the case at the Aravind Eye Center. Thus, we were unable to determine the true prevalence of SARS-CoV-2 associated conjunctivitis during the Delta surge in the region. This limitation resulted in the small sample size and is reflected in the large confidence intervals for the findings described in this study.

6. Conclusions

 Conjunctivitis may be the only presenting clinical sign of patients with SARS-CoV-2 infection. Co-infections with other DNA viruses or
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