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ISOLATION OF NOVEL CORONAVIRUS IN TEARS AND CONJUNCTIVAL SECRETIONS FROM COVID-19 PATIENTS PRESENTING WITH KERATOCONJUNTIVITIS

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ABSTRACT This study aimed to assess the isolation of novel coronavirus in tears and conjunctival secretions by reverse transcriptase polymerase chain reaction (RT-PCR) assay from novel coronavirus disease 2019 (COVID-19) patients presenting with keratoconjunctivitis.

KEYWORDS :

INTRODUCTION

An outbreak of a novel coronavirus disease (COVID-19) emerged in Wuhan, China, in December 2019 which quickly spread throughout the world[1]. World Health Organization (WHO) has declared the ongoing outbreak as a global public health emergency. Currently the research on novel coronavirus is still in the primary stages. This disease was known to be caused by a novel betacoronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Coronaviruses are single-stranded RNA viruses with a diameter of 80 to 120 nm. There are 4 types: α-coronavirus, β-coronavirus, δ-coronavirus and γ – coronavirus[2]. Prior to SARS-CoV-2, 6 coronaviruses were known to cause disease in humans, including SARS-CoV and MERS-CoV[3]. They are known to cause diseases including the Common Cold, Severe Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome (MERS) in humans. Compared with SARS and MERS, the novel coronavirus has high transmissibility and infectivity, despite of low mortality rate[4]. SARS-CoV-2 uses angiotensin converting enzyme 2 (ACE2) as its receptor according to several analysis[5]. Bats are considered to be the natural hosts of SARS-CoV-2, and pangolins and snakes are thought to be the intermediate hosts. Droplets and close contact are the most common routes of transmission of SARS-CoV-2, and aerosol transmission may be another route. In addition, researchers have detected SARS-CoV-2 in samples of stool, gastrointestinal tract, saliva and urine. SARS-CoV-2 was moreover detected in the tears and conjunctival secretions of patients with COVID-19[6]. According to an epidemiological investigational report elderly people were found to be the most susceptible age group to SARS-CoV-2 (median age at death 75 years), and most of the patients who died had comorbidities or a history of surgery before admission[7]. SARS-CoV-2 has a median incubation period of 3 days[8]. Most common symptoms of COVID-19 are fever (87.9%), cough (67.7%) and fatigue (38.1%); diarrhoea (3.7%) and vomiting (5.0%)[8,9]. There is also an evidence of ocular surface infection in patients with COVID-19, and SARS-CoV-2 RNA was detected in eye secretions of patients[6]. The detection of viral RNA by reverse transcriptase polymerase chain reaction (RT-PCR) assay is the standard for noninvasive diagnosis of COVID-19.

MATERIALS AND METHODS

This prospective observational study was conducted in the Department of Opthalmology and Department of Microbiology, LLRM Medical College and SVBP Hospital, Meerut, Uttar Pradesh, India. We included COVID-19 positive patients with complaints of conjunctival congestion, conjunctival redness/hyperemia, epiphora, photophobia.

SAMPLE COLLECTION

Before taking the sample, patient was informed about the procedure and consent for the same was taken. Sample was collected using conjunctival swab technique to collect the tears and conjunctival secretions from patients. The lower eyelid of the patient was opened and the conjunctiva of the lower eyelid fornix was wiped using a disposable sampling swab (Sterile Hiculture collecting device).

Sampling head of the swab was inserted into HiViral™ Transport Medium [Figure 2] containing 3 ml of viral transport medium and the cap was tightly closed. Patient details were entered into a prestructured form and also written on each sampling VTM tube. Each sample was triple packaged as per the guidelines by ICMR-NIV and was transported at 4°C to the Microbiology Laboratory.

The sample was visually inspected carefully and was rejected in case of leaked/broken VTM, inadequately maintained cold chain, incompletely filled patient forms, lack of patient details on the VTM tube. Two aliquots were made from the sample received. The viral transport media was first vortexed and 1.5 mL each of the viral transport media was pipetted into two separate cryovials, one for the isolation of novel coronavirus using RT-PCR and one sample was stored at -80°C as a reserve aliquot.

RT-PCR

RNA EXTRACTION:

Viral RNA nucleic acid extraction was done using Invitrogen pure link viral RNA/DNA mini kit™ according to kit instructions.

AMPLIFICATION:

Amplification of the extracted RNA was done using BGI's Real-Time Fluorescent RT-PCR Kit as per the kit instructions in Applied Biosystem Quantstudio 7 Flex Machine. Kit used is a qualitative in-vitro nucleic acid amplification assay to detect ORF1ab gene of 2019-nCoV using reverse transcription PCR.

THERMAL PROFILE:

Thermal profile of the assay was defined and caliberated in the PCR Computer Program in the following way:

STEP	DURATION AND TEMPERATURE
RNA Extraction (cDNASynthesis)	First hold 50°C for 20 minutes
Denaturation	Second hold 95°C for 10 minutes
Cycling	95°C for 15 seconds, followed by 60°C for 30 seconds and defining the data acquiring channel
Extension	72°C for 15 seconds (setting the number of cycles to 45 cycles in the cycling profile)

PCR was run and the interpretation and analysis of the generated data was done. All the samples with elevation of the PCR graph for both Open reading frame 1b (ORF1b) gene and RNase P gene (Internal Control) within 45 cycles were considered to be positive for the Novel coronavirus. Samples showing elevation of the PCR graph only for RNase P gene (Internal control) within 45 cycles showed that samples were properly collected, and it contained Human DNA in it. But these samples were considered negative for Novel coronavirus as there was no elevation of the graph for ORF 1b gene.

OBSERVATION AND RESULTS:

A total of 44 COVID-19 positive patients with ocular features of conjunctivitis were selected for this study. Tears and conjunctival secretions were collected from the affected eye of the patients with the above signs and symptoms for reverse transcriptase PCR. Of the 44 patients, a single patient (2.2%) had RNA identified in tear and conjunctival secretion.

DISCUSSION

Since novel coronavirus epidemic which started from December 2019, there have been anecdotal reports of ocular infection. Ocular implications of human Coronavirus infection have not been widely studied. On the 22nd of January 2020, Guangfa Wang, a member of the national expert on pneumonia had developed conjunctivitis during an inspection of Wuhan, the epicenter of the outbreak. He was subsequently tested positive for the SARS-CoV-2 but recovered from the infection eventually[10]. This had resulted in a call for research on the isolation of SARS-CoV-2 RNA from tears and conjunctival secretions. According to a recently published literature on COVID-19 conjunctivitis, tear sample and conjunctival swab are reported to be positive for the novel coronavirus.

Signs and symptoms of COVID-19 have thus far been described as fever, cough, myalgia, fatigue, sputum production, headache, hemoptysis, and diarrhea[11]. Interestingly, a recent update provided by the American Academy of Ophthalmology discussed conjunctivitis as a presenting symptom of COVID 19 in affected patients[12]. Xia and colleagues at the First Affiliated Hospital of Zhejiang University evaluated the conjunctival secretions of 30 confirmed cases of COVID-19. Though majority of patients who had been enrolled for the study did not present with conjunctivitis, only one (3.3%), who did had the coronavirus in tear and conjunctival swab. In this patient both tear and conjunctival secretions tested positive for the virus by RT-PCR. Conjunctivitis reported was mild, with a watery discharge. The results concluded that tears and conjunctival secretions had coronavirus in patients with conjunctivitis, but was absent in those without conjunctivitis. This also raised an alarm among the treating ophthalmologists towards the possible spread of the virus through tears, along with other body fluids. Another study in China reported conjunctivitis in 9 patients with COVID-19, out of 1099 (0.8%). Our study shows 1 patient (2.2%) of the 44 COVID-19 positive patients being tested positive for the virus by RT-PCR in both tear and conjunctival secretions[6]. Similarly one large clinical study has reported a low incidence of conjunctivitis (0.8%)[8]. The fact that a single patient in our study developed keratoconjunctivitis with positive SARS-CoV-2 tests in conjunctival swab samples indicated that SARS-CoV-2 could indeed cause ocular complications but not necessarily in the early stage of illness. ACE 2 (ACE2) is a cellular receptor for SARS-CoV-2[13]. Given that ACE2 has also been detected in the human retina,[14] vascularised retinal pigment epithelium choroid[15] and conjunctival epithelia,[16] further clinical studies are needed to more fully evaluate the clinical spectrum of ocular diseases caused by SARS-CoV-2 infection.

CONCLUSION

This study emphasizes the importance for opthalmologists to remain vigilant and consider SARS-CoV-2 as the causative agent in patients presenting with keratoconjunctivitis, particularly in high-risk patients with travel to areas of active transmission of the virus. These cases could represent an early presentation of COVID-19. Protocols should be put in place to minimize exposure risk to other patients and health care providers. Though conjunctiva might not serve as an ideal site for sampling for early diagnostic test of SARS-CoV-2 infection, but it is a potential organ of involvement in COVID-19 positive patients.

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