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Original Article

Role of Conjunctival Swab in Diagnosis of Patients with COVID-19

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ABSTRACT

Purpose: To investigate the role of conjunctival swab in diagnosing COVID-19 and to find out correlation between COVID-19 severity and conjunctival swab positivity.

Study Design: Analytic Correlational study.

Place and Duration of Study: Primasatya Husada Citra Hospital Surabaya, Indonesia in March 2021.

Methods: Forty-seven patients diagnosed with COVID-19 and admitted in the hospital during the month of March 2021 were included. Sociodemographic data, symptoms, ocular manifestations, and result of reverse transcription-polymerase chain reaction (RT-PCR) from nasopharyngeal and conjunctival swabs for SARS-CoV-2 were collected. Conjunctival swab was taken 1 – 4 days after the nasopharyngeal swab. Patients were divided into two groups; group with ocular symptoms and the other group with ocular signs. Patients with ocular signs had chemosis, conjunctival hyperemia, and eye discharge. This group also included patients who were on Ventilator and could not complain about their ocular problems. Data were analyzed using Spearman correlation analysis with p-value less than 0.05 considered as statistically significant.

Results: Conjunctival swab PCR was negative in 95.7% patients. Correlation between COVID-19 severity and conjunctival swab was also not significant ($P = 0.589$). Only 2 subjects (4.3%) who had ocular signs and symptoms revealed positive PCR ($p > 0.05$). Similarly, severity of the disease was also not correlated with positivity of conjunctival swab ($p > 0.05$).

Conclusions: The limited sensitivity of conjunctival specimens based on the result of this study showed that the diagnostic value of conjunctival specimens for the detection of SARS-CoV-2 is low.

Key Words: COVID-19, SARS-CoV-2, Polymerase chain reaction.

How to Cite this Article: Titiek E. Role of Conjunctival Swab in Diagnosis of Patients with COVID-19. Pak J Ophthalmol. 2023;39(3):213-217.

Doi: 10.36351/pjo.v39i3.1620

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Received: March 30, 2023

Accepted: June 12, 2023

INTRODUCTION

It is now a well-known fact that COVID-19 is caused by Severe Acute Respiratory Syndrome Coronavirus 2.¹ World Health Organization declared COVID-19 as

a threat to the international community on January 30, 2020 and then as a pandemic on March 11, 2020.² The global number of new cases had been on the increase with sequence of waves. In August 2021, over 4.4 million cases were reported. There was large number of deaths caused by the deadly virus. In August 2021, the Government of the Republic of Indonesia reported 3.967.048 persons with confirmed COVID-19 and 125.342 deaths related to COVID-19.⁴

Individuals infected with COVID-19 release respiratory droplets and microscopic particles (bioaerosols) via breath, cough, sneeze, or speech.

These droplets contact the host's ocular and extra-ocular mucus membranes. The virus particle gains entry into the body through interaction with an epithelial cell using its SARS-CoV-2 spike (S) glycoprotein, which binds to the cell membrane protein angiotensin-converting enzyme 2 (ACE2).^{5,6} There are reports which indicate that detection rate via nasal swab is only 63% and via pharyngeal swab is slightly more than half at 32%.⁷

Ocular symptoms range from mild conjunctival injection to acute anterior and posterior uveitis, retinitis, and optic neuritis. The role of ocular surface as a source of transmission and retrograde transmission has been investigated in several studies and remains an unresolved issue.^{8,9} COVID-19 can be transferred through mucous membranes, including conjunctiva. Many cases have been reported in which ophthalmologists were infected.¹⁰ Given that the virus is present in body fluids, tear fluid can be a source of spread of infection.¹¹

It is debatable whether the virus is present within tears or not. Xia et al found the presence of COVID-19 within tear film in only one patient with conjunctivitis among persons infected with SARS-CoV-2, using real-time reverse transcription-polymerase chain reaction (RT-PCR) assays.¹¹ Seah et al, on the other hand, proposed a low risk of ocular transmission because neither viral culture nor reverse transcription were able to detect virus particles in their tested samples.¹² An Italian research organization of Spallanzani Group, described a case of concomitant NPs negativity versus conjunctival swab positivity in a SARS-CoV-2 patient.¹³ This finding opens questions about correct diagnosis in clinical practice, highlighting the potential role of conjunctival testing. Similarly, the way of transmission via the conjunctiva and tears cannot be excluded.

The primary goal of our study was to investigate the positivity of COVID-19 in conjunctival mucosa, as a possible diagnostic technique. The secondary goal was to find correlation between the conjunctival positivity and the disease-related systemic and local impairment. We also correlated COVID-19 severity with conjunctival swab and conjunctival swab positivity rate with ocular and systemic manifestation.

METHODS

This was an analytic, correlational study conducted in Surabaya, Indonesia. All patients with diagnosed

COVID-19 and admitted at Hospital in Surabaya during the month of March 2021 were included. Diagnosis of COVID-19 was based on the guideline released from Ministry of Health Republic of Indonesia. We obtained and analyzed sociodemographic data, patients' symptoms, ocular manifestations and result of reverse transcription-polymerase chain reaction (RT-PCR) from nasopharyngeal and conjunctival swabs for SARS-CoV-2. Conjunctival swab was performed by an ophthalmologist and was taken 1 – 4 days after the nasopharyngeal swab. Swab were taken from both eyes, by putting a sterile cotton wool stick into the deep lower fornix and then sent to laboratory.

Patients were divided into two groups; group with ocular symptoms and the other group with ocular signs. Patients with ocular signs had chemosis, conjunctival hyperemia, and eye discharge. This group also included patients who were on ventilator and could not complaint about their ocular problems. Data were analyzed using Spearman correlation with p-value less than 0.05 considered as statistically significant. Data analyses were done using SPSS 24.0 version. The study was approved by the Health Research Ethics Commission number 107/WM12/KEPK/DOSEN/T/2020 based on WHO-CIOMS International Ethical Guidelines for Health-related Research Involving Humans.

RESULTS

Forty-seven patients were enrolled in study. Table 1 shows baseline characteristics of the participants. Mean age was 51.63 ± 14.71 years. There were more male patients than females. Conjunctival swab PCR was positive only in 4.3% and positive PCR was not correlated with the severity of COVID-19 ($p = 0.589$).

Table 3 shows that only 2 subjects (4.3%) who had positive ocular signs or symptoms had positive result on PCR examination. Out of 11 patients who had ocular symptoms, only one patient had positive conjunctival PCR showing the correlation was not significant ($p = 0.428$). Similarly, only one out of seven subjects who had abnormal ocular examination findings gave positive result on conjunctival PCR and the correlation between ocular examination findings with conjunctival swab was not significant ($p = 0.213$).

Table 1: Baseline Characteristic of Patients.

Baseline Characteristic	Value
Sex	
Male N (%)	28 (59.6%)
Female N (%)	19 (40.4%)
Age Mean± SD	51.63 ± 14.71
Severity of COVID-19	
Asymptomatic	3 (6.4%)
Mild-Moderate	28 (59.6%)
Severe	8 (17.0%)
Severe on Ventilator	8 (17.0%)
Ocular complaints	
Yes	12 (25.5%)
No	35 (74.5%)
Conjunctival Hyperemia	
Yes	8 (17.0%)
No	39 (83.0%)
Epiphora	
Yes	10 (21.3%)
No	37 (78.7%)
Eye discharge	
Yes	2 (4.3%)
No	45 (95.7%)
Burning eye sensation	
Yes	1 (2.1%)
No	46 (97.9%)
Itchy eye sensation	
Yes	1 (2.1%)
No	46 (97.9%)
CT Value PCR Naso-pharyngeal	28.29 ± 4.98
CT Value PCR Conjunctival	32.11 ± 0.89

SD = standard deviation; CT= Cycle Threshold; PCR=Polymerase chain reaction

Table 2: COVID-19 Severity and Conjunctival swab correlation.

	Positive RT PCR 2 (4.3%)	Negative RT PCR 45 (95.7%)	P- value
Covid-19 Severity			
Asymptomatic	0 (0.0%)	3 (6.7%)	0.589
Mild-moderate	1 (50.0%)	27 (60.0%)	
Severe	0 (0.0%)	8 (17.8%)	
Severe on ventilator	1 (50.0%)	7 (15.6%)	

Table 3: Ocular complaints and ocular examination findings with conjunctival swab correlation.

	Positive RT PCR 2 (4.3%)	Negative RT PCR 45 (95.7%)	P- value
Ocular symptoms			
Yes	1 (50.0%)	11 (24.4%)	0.428
No	1 (50.0%)	34 (75.6%)	
Ocular signs			
Normal	1 (50.0%)	38 (84.4%)	0.213
Abnormal	1 (50.0%)	7 (15.6%)	

DISCUSSION

Coronavirus disease (COVID-19) which originated from Wuhan and caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) can be transmitted via direct and indirect contact.^{14,15} Although respiratory droplets are the main mode of transmission for SARS-CoV-2, it is hypothesized that SARS-CoV-2 can transmit indirectly through mucosal membranes such as the eyes.¹⁶ Some previous studies have reported RT-PCR positive results on conjunctival specimens. In our study, there were 47 confirmed COVID-19 patients and among them, only two (4.3%) patients with ocular signs or symptoms had conjunctival swabs positive for SARS-CoV-2 on RT-PCR.

A previous study by Xia et al. found that only 1 out of 30 patients with conjunctivitis yielded positive result in conjunctival samples and there was no viral RNA detected in the tear fluids and conjunctival secretions in patients without conjunctivitis.¹¹ According to Hadrawi et al, only 4.9% patients tested positive for SARS-CoV-2 in conjunctival specimens among 164 patients.¹⁷ As shown in a Chinese study, 2 out of 28 confirmed COVID-19 patients (5.2%) had positive results on the conjunctival specimens.¹⁵ Kiran Kumar et al. also reported that only 1 out of 45 confirmed COVID-19 patients (2.23%) had positive SARS-CoV-2 RT-PCR findings on conjunctival specimens.¹⁸ However, contrary to that, another study reported that all of 64 ocular samples from their 17 confirmed COVID-19 patients were negative.¹⁹ Based on the findings of our study and previous study, the sensitivity of conjunctival specimens was poor. Therefore, the conjunctival specimens had a weak diagnostic value for detecting SARS-CoV-2.¹²

The low positive rate RT-PCR test results from conjunctival specimen can be caused by multiple factors, such as the small number of samples, the time of sampling, the low viral load in the eye or some patients had started systemic antiviral therapy before the examination so it could not be detected on RT-PCR.²⁰⁻²² Although the positive rate is low, it does not rule out the possibility of ocular transmission. Therefore, health workers are required to wear personal protective equipment at all times at work.

Our study found no correlation between ocular complaints and conjunctival swab findings (P = 0.428). These findings were similar to Zhou Y et al., who found that out of 8 patients with eye symptoms, only 1 had a positive SARS-CoV-2 RT-PCR result in

the conjunctiva without a significant relationship ($p = 0.39$).²³

The strength of this study was that we analysed correlation between the severity of COVID-19 with the SARS-CoV-2 RT-PCR results from conjunctival specimens. We also analysed correlation between ocular examination results with SARS-CoV-2 RT-PCR results from conjunctival specimens and the correlation between ocular examination results with the severity of COVID-19. Further studies are needed to evaluate correlation between the severity of COVID-19 and ocular examination findings.

In conclusion, the limited sensitivity of conjunctival specimens based on the result of this study showed that the diagnostic value of conjunctival specimens for the detection of SARS-CoV-2 was quite low.

However, there were several limitations to our study. First, this study had a small sample size. Secondly, it was an observational study which did not have follow up of the patients. The results might have become positive after some time. Further studies are needed to address this question.

ACKNOWLEDGEMENT

We would like to acknowledge the support of Medical Faculty, Widya Mandala Surabaya Catholic University, for this study as an approved research proposal and Primasatya Husada Citra Hospital for their support and assistance.

Conflict of Interest: Authors declared no conflict of interest.

Ethical Approval

The study was approved by the Institutional review board/Ethical review board (07/WM12/KEPK/DOSEN/T/2020).

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