Detection of Coronavirus in Simultaneously Collected Tear and Throat Swab Samples from the Patients with 2019- new Coronavirus Infection Disease: A Single Center Cross-sectional Study

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Abstract

Objective: This study aimed to evaluate whether the coronavirus can be detected in the tears in the eyes of 2019-nCoV infected patients and compare the detection consistency of two simultaneously collected samples, from the tears and throat swabs, of these patients.

Methods: A total of 35 diagnosis confirmed patients with 2019-nCoV infection disease, from January 26 to February 19, 2020, at central theater Wuhan general hospital of PLA, were included in this cross-sectional case series study. The diagnosis was confirmed by both clinical and laboratory ways. Throat samples from all enrolled patients were collected with sampling swab, and simultaneously, tear samples were collected with sampling swab from 9 patients (No.1-9) and with Schirmer's strip from the remaining patients (No.10-35) (bilateral eyes for all patients). Sample collecting and testing were performed in three separate time points: first from patients No.1-9, second from patients No.10-29, and third from patients No.30-35. Reverse-transcriptase-polymerase-chain-reaction (RT-PCR) assay was performed by the same technician (Researcher Zhang), who was blind to the patients profile, within 4 hours after each collection.

Results: Among enrolled patients, 29 (No.1-29) had mild or moderate clinical symptoms and 6 (No.30-35) had severe symptoms. The mean time interval from the sample collection day to diagnosis confirmation day was 9.71 ± 6.50 days (ranged from 3 to 29 days). None of the patients had conjunctivitis. Nineteen out of 35 (54.3%) throat samples presented positive Rt-PCR results. Three (no.13,21,31) out of 35 (8.6%) tear samples presented positive RT-PCR results. Two (no.21, 31) of these three patients were throat swab positive and one (No. 13) was negative. The consistency analysis indicated that tears and throat samples showed poor consistency ($\text{Kappa} = -0.009$, $P = 0.9$). The cycle threshold value (Ct-value) of tears samples collected by sampling swab was significantly higher than that by Schirmer's strip ($t = 2.288$, $P = 0.03$).

Conclusion: In spite of the low 2019-nCoV positive detection rate of tear samples from 2019-nCoV infected patients, we cannot fully rule out the transmission by ocular surface. Whether tear testing can be used as an aid in judging of 2019-nCoV infection need further investigation.

Authors Ying Yan and Bo Zeng contributed equally to this study

Introduction

A novel coronavirus-infected disease was first reported in Wuhan, a city in central China in December 2019[1]. Up to March 10, 2020, a total of 80928 2019-nCoV infected cases in China have been confirmed[2]. The outbreak of the disease has become a global health threat, and almost 24 countries and 5 continents have been afflicted with 2019-nCoV infected disease[3].

On January 7, 2020, the 2019 novel coronavirus (2019-nCoV) was isolated from the clinical specimens of bronchoalveolar lavage fluid and was confirmed as the cause of the disease[4], and a rapid human to human transmission has been confirmed [5]. Although respiratory droplets or contact has been identified

as the route of human-to-human transmission [6], other potential routes including ocular surface secretions transmission have not been investigated[7].

It is reported that, the tears, being one of the body fluids, may potentially harbor the coronavirus. In the past, researchers detected many influenza viruses, including Ebola virus[8], severe acute respiratory syndrome coronavirus[9], and human immunodeficiency virus[10], in tears using polymerase chain reaction (PCR) technique. Therefore, we conducted this study to investigate whether 2019-nCoV can be detected in the tears of patients with 2019-nCoV infection.

Methods

This is a cross-sectional case series study. Thirty-five patients with confirmed 2019-nCoV infection by both the clinical way (includes symptoms, blood routine test and chest computed tomography) and the laboratory way (the reverse-transcriptase-polymerase chain reaction (RT-PCR) from throat swab sample), were recruited at the time of admission in the Central Theater General Hospital, Wuhan, China, from January 26 to February 19, 2020. Approval was obtained from the Ethics Committee of the Central Theater General Hospital (No. [2020]009-1). The patients' signed consents were obtained.

Throat samples and tears of all patients were collected simultaneously. Throat samples were obtained by cotton swab scraping the epiglottis, and tear samples from both eyes were collected with sampling swab (No. 1-9) and with Schirmer's strip (No. 10-35), respectively. For sampling swab, the samples were taken by putting sterile cotton wool sticks into the deep lower fornix of patients' eyes. For Schirmer's strip collection, a Schirmer's strip (Schirmer Tear Test Strips; Liaoning Meizilin Pharmaceutical Co., Ltd) was placed at the temporal canthus of each eye for 5 min without anesthetic and then removed from the eye.

The amount of wetting in millimeters was recorded by observing the location of the leading edge of moisture on the printed millimeter marks. All samples were placed into disposable virus sample tubes. Gloves were changed when collecting specimens in between patients to minimal contamination.

Samples were placed in -4°C freezing box and transported immediately (within 1 hour) to the laboratory in the virology section of Academy of Military Medical Sciences. Rt-PCR

tests were performed within 4 hours after sample collection.

RT-PCR protocol: The samples were collected in an Eppendorf tube. Nucleic acid was extracted using LabServ Prefilled Viral Total NA Kit (Cat. KFRPF-805296, Fisher Scientific,

Shanghai, China) on KingFisher™ Flex Purification System (Thermo Fisher Scientific, MA

USA). Basically, 300 µL lysis buffer was added into the tube with the test paper, then mixed by vortex for 3 minutes, and the nucleic acid was purified following the instruction of the kit. The 2019-nCoV was tested

by real-time RT-PCR using 2019-nCoV Nucleic acid Diagnostic Kit (Sansure Biotech, Changsha, Hunan Province, China), and two targets (open reading frame 1ab, nucleocapsid protein) were tested.

If either of the two targets (open reading frame 1a or 1b, nucleocapsid protein) was detected by specific real-time RT-PCR, the case would be considered as laboratory-confirmed. A cycle threshold value (Ct-value) less than 39 was defined as a positive test, and a Ct-value of 39 or more was defined as a negative test.

Results

Among the 35 cases, 29 (No.1–29) were mild or moderate and 6 (No.30–35) were severe.

The mean age was 58.9 ± 15.5 years, ranged from 20 to 89 years, and 17 (48.6%) were female. The mean time interval from the sample collection day to diagnosis confirmation day

was 9.71 ± 6.50 days (ranged from 3 to 29 days). Details of the characteristics of the patients were provided in Table 1.

All patients have been free of conjunctivitis since the onset and have not used any eye drops.

Nineteen out of 35 (54.3%) patients presented positive Rt-PCR results. This nineteen patients consists of 13 mild or moderate and all of the 6 severe cases. The baseline characteristics stratified by positive and negative throat swab Rt-PCR results of the mild and

moderate patients (No. 1–29) were compared in Table 2. The average age of positive patients was larger than that of negative patients (63.5 vs. 50.4 years, $p = 0.017$). The average time

interval between the sample collection and diagnosis confirmation day of positive patients was shorter than that of negative patients (6.15 vs. 8.44 days, $P = 0.034$). There was no difference in sex, body temperature, cough, expectoration symptoms, degree of blood oxygen

saturation, and the use of antiviral drugs.

Three (no.13,21,31) out of 35 tear samples (8.6%) presented positive RT-PCR results, 2 of which were throat swab positive and one (No. 13) was negative. Two of the these 3 were mild and moderate patients, both were 51 years old. And, the sample collection interval from diagnosis confirmed was 8 days and 7 days, respectively.(Table 3).

The consistency analysis indicated that tears and throat samples showed poor consistency. (Kappa = – 0.009, $P = 0.9$).

Mean cycle threshold value (Ct-value) of tear samples collected by conventional

sampling swab was significantly higher than that collected by Schirmer's strip (32.68 ± 1.30 Vs 31.39 ± 1.45 , $P = 0.03$).

The tear volumes of Schirmer's strip were negatively associated with the Ct-value for the 6 severe patients (coefficient value -0.928 , $P = 0.008$) (Table 4.).

Discussion

The transmission of 2019- novel coronavirus through eyes has recently attracted the researchers' attention. There were sporadic cases of conjunctivitis reported in infected patients whose conjunctival swabs were positive for nucleic acid of coronavirus[11–13]. However, for all infected patients, the positive rate of conjunctival swabs was very low, lower than that of throat swabs[11–13], which was consistent with the results of our study. It seemed that the possibility of virus existence in the ocular surface is low. However, more detailed analysis of the eye sample collection process and the laboratory nucleic acid test results can help to obtain more information.

In previous studies, conjunctival swabs were used to collect eye secretions including the tears, which were usually insufficient for test[14]. It was reported that the amount of tears collected by Schirmer's strip at one time was greater than that collected by other methods such as capillary tubes and surgical sponges [15]. In the first nine patients (no.1–9) of this study, conjunctival swabs were used to collect samples and all the test results were negative. In the subsequent patients (no.10–29), Schirmer's strips were used to collect sample, and 2 samples were detected positive. This result suggested that the positive rate of the virus detection may be higher when Schirmer's strip was used. In addition, through the analysis of the volume of tear and the value of nucleic acid detection CT in 6 severe patients, it was found that there was indeed a quantity effect relationship between the two. This, to some extent, indicated that, the larger the amount of tear samples collected, the greater the probability of positive detection.

Another potential factor that affected the test results was the viral load in the sample. In theory, the more viral load, the higher the detection rate. The low positive rate of this study also indicated that the viral load in the ocular surface was relatively limited.

Due to the influence of multiple factors, the interpretation of the detection results should be more cautious—even throat swabs may have a high negative rate. In this study, throat swabs showed a positive detection rate of 54.3%, which was far lower than that in recently published online study. Different from ours, the sputum (non throat swab) samples of patients were taken for nucleic acid detection, so a high positive detection rate (91.7%) was obtained [13]. In practice, we found that most patients did not have sputum available. In addition to the way of sampling from bronchial secretion, the patients profile may also affect the nucleic acid detection results. For the patients with mild and moderate disease, we compared the baseline characteristics stratified by positive and negative throat swab Rt-PCR results (Table 2). Since the average age of the positive patients was greater than that of the negative patients, and the average interval between the sampled and diagnosis confirmed day for positive patients was shorter than that for negative patients, we suggested that the older the patients were, the earlier the

detections were performed, the easier it was to detect nucleic acid fragments from throat swabs. However, for severe patients, because of their 100% positive rate, the possibility of the test results affected by the factors mentioned above should be small.

The fact that the consistency between the test results of throat swab and Schirmer's strip was low and that only two of three patients with tear Rt-PCR positive result had throat swab

positive result, suggested that the tear test results were not related to throat swab test results. Moreover, the result that one patient (No. 13) showed positive result of tear but negative of throat swab reminded us that Schirmer's strip detection may be used as an auxiliary means to identify patients with false negative throat swabs test result. Although the positive rate of tear test was very low, when facing this highly infectious disease, any additional screening for the control of disease transmission is of practical significance.

Limitation of this study was the relatively small sample size, especially the few severe patients included. In practice, in the face of the more infectious severe patients, sample collection performed in a single center had certain challenges. Another limitation also came from the small number of samples: no conjunctivitis patients could be included. Therefore, it was impossible to judge whether it was more likely to detect virus from the tears of conjunctivitis patients. Furthermore, we did not repeatedly collect and test samples, neither tears nor throat swab. Hence, we could not reduce the random errors occurred during the sample collection and testing process.

However, one of the advantages of this study was the simultaneous collection of throat swabs and tears from patients, which was helpful for us to compare the detection results of throat swab and tear. Another advantage came from the use of Schirmer's strip, which could collect larger volume of tear samples. From this perspective, Schirmer's strip outweighs the conjunctival swab. Most importantly, this study confirmed the practical value of Schirmer's strip by demonstrating the positive tear test result from patient no.13 who had negative throat test result.

In the future, we should pay more attention to the virus detection from ocular surface in different stages of the disease and different degrees of infection. Isolating the virus and determining the virus load, if exists, from the ocular surface, and investigating the existence of eye-body infection pathway will help us to clarify the human-human transmission route via eyes.

Conclusions

We cannot fully rule out the transmission by ocular surface. Whether tear testing can be used as an aid in judging of 2019-nCoV infection need further investigation. Moreover, we propose the use of Schirmer's strips that allow tear collection more efficiently.

Declarations

Acknowledgment Section

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Conflict of Interest

The authors declare that they have no conflicts of interest.

Statements

1. All methods were carried out in accordance with relevant guidelines and regulations.
2. All patients provided written informed consent before participation in this study.

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Tables

Table 1. Profile of 35 patients and viral detection results of throat and tears

Patients No.	Age	Sex	Interval from sample collection to diagnosis confirmation [days]	Severity of disease	Methods of tears sample collection	Rt-PCR for throat swab	Rt-PCR for tears
1	68	F	3	mild or moderate	swab	☐	☐
2	69	F	4	mild or moderate	swab	☐	☐
3	57	F	12	mild or moderate	swab	☐	☐
4	66	M	6	mild or moderate	swab	☐	☐
5	68	M	9	mild or moderate	swab	☐	☐
6	70	F	3	mild or moderate	swab	☐	☐
7	70	F	14	mild or moderate	swab	☐	☐
8	70	M	11	mild or moderate	swab	☐	☐
9	66	M	6	mild or moderate	swab	☐	☐
10	55	F	10	mild or moderate	Schirmer's strip	☐	☐
11	57	F	11	mild or moderate	Schirmer's strip	☐	☐
12	88	M	4	mild or moderate	Schirmer's strip	☐	☐
13	51	M	8	mild or moderate	Schirmer's strip	☐	☐
14	51	F	10	mild or moderate	Schirmer's strip	☐	☐
15	52	F	7	mild or moderate	Schirmer's strip	☐	☐
16	35	M	10	mild or moderate	Schirmer's strip	☐	☐
17	20	M	5	mild or moderate	Schirmer's strip	☐	☐
18	35	M	6	mild or moderate	Schirmer's strip	☐	☐

19	56	F	6	mild or moderate	Schirmer's strip	0	0
20	63	F	10	mild or moderate	Schirmer's strip	0	0

21	51	F	7	mild or moderate	Schirmer's strip	00
22	31	F	7	mild or moderate	Schirmer's strip	00
23	34	M	9	mild or moderate	Schirmer's strip	00
24	50	M	10	mild or moderate	Schirmer's strip	00
25	40	M	3	mild or moderate	Schirmer's strip	00
26	57	M	7	mild or moderate	Schirmer's strip	00
27	68	F	7	mild or moderate	Schirmer's strip	00
28	67	F	6	mild or moderate	Schirmer's strip	00
29	67	F	4	mild or moderate	Schirmer's strip	00
30	68	F	28	severe	Schirmer's strip	00
31	54	M	29	severe	Schirmer's strip	00
32	89	M	21	severe	Schirmer's strip	00
33	78	M	24	severe	Schirmer's strip	00
34	72	M	11	severe	Schirmer's strip	00
35	68	M	12	severe	Schirmer's strip	00

Table 2. The baseline characteristics stratified by positive and negative throat swab Rt-PCR results of the mild and moderate patients (No.1-29)

	Positive throat swab Rt-PCR results	Negative throat swab Rt-PCR results	P Value
	(n=13)	(n=16)	
Age(yrs)	63.54±11.50	50.38±15.37	0.017*
(mean±SD)			
Female,sex, no (%)	8(61.54)	8(50.00)	0.534
The average interval between the sample collected and diagnosis confirmed day(days)	6.15±3.05	8.44±2.45	0.034*
(mean±SD)			
Body temperature(°C)	36.35±0.19	36.72±0.52	0.054
(mean±SD)			
Cough, no (%)	4(30.77)	7(43.75)	0.474
Expectoration symptoms	1(7.69)	3(18.75)	0.390
no (%)			
Degree of blood oxygen saturation, %	97.08±2.10	97.19±1.56	0.872
(mean±SD)			
the use of antiviral drugs, no (%)	13(100)	16(100)	1.000

* :p value of less than 0.05 was considered to indicate statistical significance.

Table 3. Profile of three cases with positive RT-PCR test result for tears samples

Case			
Patient no.	13	21	3
Age(yrs)	51	51	54
Sex	Male	Female	Male
Date of confirmed CNIP	February 6,2020	February 7,2020	January 28.2020
Course of disease	Day 8	Day 7	Day 29
Severity of disease	mild or moderate	mild or moderate	severe
Sample test result			
Tears	positive	positive	positive
Throat	negative	positive	positive
Date of collection samples	February 14,2020	February 14,2020	February 26,2020
Key indication			
Cough	+	+	-
Expectoration symptoms	-	+	-
Body temperature(°C)	37.0	36.2	36.5
Degree of blood oxygen saturation, %	97	96	89
State of consciousness	awake state	awake state	coma
Using Extracorporeal Membrane Oxygenation	-	-	+

Table 4. The relationship between a Ct-value and tears volume of 6 severe patients

Patients no.	tears length of Schirmer's strip(mm)	Ct-value of tears sample (RNase P)
	left eye/right eye	
30	5/7	31.168
31	20/25	26.215
32	12/12	27.240
33	6/7	29.262
34	8/9	26.417
35	7/6	29.634

The tears volume of Schirmer's strip were negatively associated with the Ct-value of tears sample of 6 severe patients.
(Spearman correlation coefficient , coefficient value = -0.928, P =0.008).