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Conjunctival polymerase chain reaction tests (RT-PCR) of COVID-19 patients in Shenyang, China

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Abstract

Introduction

We report the laboratory results of conjunctival RT-PCR tests and some clinical features of these patients infected with COVID 19 in Shenyang, China.

Methods

We collected conjunctival samples of the patients to do the laboratory tests by RT-PCR. Medical observed patients were enrolled if they had clinical symptoms. Then we analyzed the RT-PCR results and clinical features in order to find some relationships.

Results

The study includes 14 confirmed diagnosed cases, 16 suspected cases and some medical observed patients. One of these patients who was suspected as COVID-19 case at initial visit diagnosed positive finally, and 22 medical observed cases were excepted with negative clinical and RT-PCR results. All the conjunctival results of RT-PCR test were negative.

Discussion

The reasons of the negative detection results of RT-PCR in conjunctival swabs were discussed.

Conclusion

Conjunctiva may be a transmission medium of COVID 19 and ocular conjunctival swabs could be a diagnostic method for identifying the infection of COVID-19. Emphasis on the false negative results is vital.

Keywords: COVID-19, Conjunctival swabs, RT-PCR

Introduction

Recently, a new strain of coronavirus, designated as COVID-19 by WHO, firstly emerged in Wuhan, Hubei, China, and has spread to multiple cities rapidly. Coronaviruses are enveloped non-segmented positive sense RNA viruses belonging to the family Coronaviridae and the order Nidovirales, which are widely detected in humans and other mammals, and commonly denoted in etiologies of respiratory tract infections in humans [1-3]. With similarity to SARS and MERS, they are all dangerous zoonotic coronaviruses which may have the potential to cause a threatening pandemic situation among human beings [4-5].

Based on clinical experience, COVID-19 is mainly disseminated by person to person through inhalation of respiratory droplets. However, some researchers reported that the viruses could also transmitted by direct contact [6] and digestive tract [7], and conjunctiva may also be possible transmission route without validation [8]. As previously reported, two clinical doctors were also infected with COVID-19 who wore N95 masks during his work, but they did not wear goggles at that time leaving their ocular surface directly to the room air.

In this study, we make an effort to testify whether conjunctiva is a possible way for the transmission of COVID-19 and whether it could be an effective diagnostic approach to detecting the infection of COVID-19.

Methods

Data Collection

In this cross-sectional non-randomized study, 14 identified cases, 16 suspected cases and 22 medical observed cases that infected with COVID-19 from January, 2020 to February, 2020 were included. The study was approved by local ethics committee, sponsored by Ministry of Shenyang science and technology and in accordance with Declaration of Helsinki. At the time of collecting conjunctival samples, every patient was informed of purposes and methods, and informed consent was obtained from all patients. Fundamental information on age, gender, underlying systemic disease and severity of diseases was collected. Ophthalmic information on ocular signs and symptoms, history of ocular surgery and the results of conjunctival RT-PCR tests were recorded. Detailed research methods were listed as follows-

Nucleic acid extraction and real time RT-PCR

After obtaining conjunctival samples by sterile swabs, the samples were placed in EP tubes filled with virus sampling fluid. Then the samples were transported immediately to the -80°C freezer in order to preserve and isolate COVID-19 subsequently.

Nucleic acid extraction and PCR analysis

Specimens were extracted using a viral nucleic acid detection kit (QIAGEN). Briefly, 200 μL sample was used to prepare reaction mixture. After 30 minutes 'standing', the reaction mixture was centrifuged for 10 seconds. The reaction system consisted of 13 μL Cov test buffer and 5 μL reaction fluid. After extracting the viral DNA, the samples were used for COVID-19 RT-PCR analysis. The assay was performed in the laboratory with a real time Light Cycler ABI7500). Reactions were set up and performed according to the manufacturer's instructions. Real time RT-PCR was performed using the Light Cycler and targeted agents (made by Jienuo Biotechnology Limited Company in Shanghai) to amplify the COVID-19 at 42°C for 10s followed by 95°C for 10 s, and finally 40 cycles for 10s at 95°C and for 45 s at 60 s at 60°C .

The results mainly depended on the CT values of two targets, the ORF (Open reading frame) and core-shell protein gene.

Results

We retrospectively analyzed 30 patients who were diagnosed as infected of COVID-19 including 14 identified cases and 16 suspected cases. One suspected case has converted to identified case recently. Further 22 medical observed cases who had intimate contact with identified or suspected cases were included. However, because none of medical observed cases have converted to identified cases and related detection of samples were negative, they are removed from this study. As displayed in the Table 1, the mean age of the identified group is 48 ± 13.4 years old and that of the suspected group is 40 ± 16.2 years old. The numbers of male and female are nearly equal. As for systematic diseases, 3 of them have diabetic mellitus, 4 of them have hypertension and 1 of them has hepatitis B. None of them have the history of general or ocular surgery. Just 1 identified case is complicated with macular degeneration and another 1 identified case felt eye itching before the onset.

With regard to the severity of disease, common pneumoniae accounts for most of identified cases and mild cases are the most common type of the suspected cases. In the identified group, there is just one severe pneumonia case, whereas another one converted to severe pneumonia from a suspected case.

The test results and related time are shown in Table 2.

Discussion

Generally speaking, deducted from the fundamental

condition of our cases, we found that the severity of disease in our patients is relatively moderate. In addition, the affected age group is young and without underlying diseases. There are only 2 cases manifested as severe pneumonia, and one of them converted from suspected case. By investigating the possible reasons, we speculated this consequence may be caused by the delayed confirmation of infection. However, the condition seems to contradict the epidemiological study done by a cluster of Chinese researchers, in which they found that COVID-19 infection could cause severe respiratory illness similar to severe acute respiratory syndrome and was associated with ICU admission and high mortality [9]. It may be due to the fact that the included cases are those who recently have visited to Wuhan or contacted with the identified cases, compared with those who were invaded directly by viruses.

RT-PCR test has been widely used to confirm the viral infection targeted to the virus specific nucleic acid sequences, but in our study, we found that after isolation for presumed infection of COVID-19, the 2 cases were finally diagnosed by repeated pharynx swabs tests. We postulated that maybe it caused by false sampling position, which means that the viruses are likely to exist in lower respiratory tract other than the upper. So the false negative rate of RT-PCR may mislead the clinical doctors and even miss the optimal opportunity for treatment. Some Chinese clinicians also found similar phenomenon and reported 5 cases. By evaluating radiographic characteristics of 5 patients with confirmed 2019-nCoV infection and initial negative or weakly positive RT-PCR, they found that the patients presented characteristic radiographic features of COVID-19 pneumonia from the first scan and then were confirmed by positive repeat swabs test during the isolated observation or treatment. They ascribed the possible reasons to laboratory error or insufficient viral material in the specimen [10].

In our cases, all of the conjunctival results of PCR were negative no matter in identified or suspected cases. We analyze and summarize the reasons as follows:

1. The shedding loads of the COVID-19 were below the sensitivity of the test or some individuals were actually not shedding viral DNA at that time. This opinion correlates with Burr's study, where she obtained 28 conjunctival swabs during the outbreak of a cute hemorrhagic conjunctivitis in the Gambia, West Africa, caused by the epidemic of coxsackievirus, and 25% of the RT-PCR test showed negative results [11].

2. In our study, the conjunctival sampling time of the cases was after identification. The administration of drugs, such as corticosteroid and antiviral drugs, and the mutation of viruses could all affect the results.

3. The sensitivity of the viral nucleic acid kits is low.

4. The conjunctiva lacks related receptors. A pivotal factor for efficient person to person transmission is the ability of the virus to attach to human cells because coronaviruses use a spike protein for attachment to host cells [12].

As previously reported, both COVID-19 and SARS-nCov use the same receptor called ACE2, which has been verified to locate mainly on lung alveolar epithelial cells and enterocytes of the small intestine [13]. However, whether ACE2 receptors exist on the conjunctival surface and the level of conjunctival ACE2 expression still need further investigation. Given that ACE2 expression is extremely rare on the ocular surface, the viruses could not attach to the conjunctiva and they may transfer to any organs in our bodies through lacrimal ductile. In our study, a 29 year old female without any systematic disease converted to identified case from suspected case. She was a moderate suspected case, whose result of pharynx swab was positive at first, and after treatment, the result changed negative. However, the result represented as positive after 3 days. So we could speculate that present therapies may only resist the viruses temporarily, and the COVID-19 may seek any opportunity to dominate when the human immunity weakens. What's worse, they may get transferred anywhere to find suitable hosts. In addition, we found that the sampling time contributed to obtain a reliable result, pharynx and conjunctiva swab should be collected in meantime. In our study, the two swabs were obtained meantime in 3 identified cases, but the results were inconsistent. The coexistence of positive pharynx specimen and negative result of conjunctiva specimen reminded that the viruses may be more likely to attach to respiratory tract rather than conjunctiva.

Conclusion

Undoubtedly, the regular samples for RT-PCR tests, such as pharynx and sputum swabs have their advantage in some facets, but considering their errors and the viral transmission characteristics, conjunctival swabs for RT-PCR may become convenient and noninvasive with simple diagnostic methods. Whether conjunctiva is a possible transmission route of COVID-19 still needs further research. Sampling time is also a key factor as well. A more uniformed scheme should be established to guide the clinical practitioners make correct judgments avoiding the cross infection.

Study Limitations

Restricted by reality, there are some limitations in our study regarding the limited number of cases and lack of ocular manifestations of all cases except one. Firstly, no positive result was obtained from identified and

suspected cases. Secondly, we lack of swabs collected from individuals with normal eyes during the sampling process.

Abbreviations

RT-PCR: Reverse Transcription Polymerase Chain Reaction;
 COVID-19: coronavirus disease 2019

Conflict of Interest

The authors declare that they have no competing interests.

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Tables

Table 1: Demographics and baseline characteristics of identified and suspected cases infected with COVID-19

Characteristics	All patients(N=30)	Identified(n1=14)	Suspected(n2=16)
Age, years	43.7±14.3	48±13.4	40±16.2
Sex			
Men	14 (46.7%)	7(50%)	7(43.75%)
Women	16(53.3%)	7(50%)	9(56.25%)
Earliest Contact Time		2020.01.04	2020.01.13
Last Contact Time		2020.01.31	2020.01.31
Onset Time, numbers			
January	16(55.2%)	10	6
February	13(44.8%)	4	9
Identifying Time, numbers			
January	4(18.2%)	4	0
February	18(81.8%)	10	8
Underlying Systematic Disease			
Diabetes	3(37.5%)	3	0
Hypertension	4(50%)	4	0
Hepatitis	1(12.5%)	1	0
Ocular or General Surgery History	None	None	None
Ocular Symptoms			
eye itching	1	1	0
Ocular Comorbidity			
macular degeneration	1	1	0
Severity of Disease	(8 cases were removed from suspected group without abnormalities)		
mild	6(27.3%)	2	4
moderate	3(13.6%)	2	1
ordinary	4(18.2%)	3	1
severe	1(4.5%)	1	0
common pneumonia	6(27.3%)	5	1
severe pneumonia	2(9.1%)	1	1
Total	22(100%)	14(63.6%)	8(36.4%)

