Effect of throat washings on detection of 2019 novel coronavirus

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Abstract:

The 2019 novel coronavirus was detected in the self-collected throat washings. Positive testing rate of throat washing was much higher than that of Nasopharyngeal swabs. Throat washing is a promising candidate for 2019-nCoV screening and monitoring due to its noninvasive and reliability.

Keywords. Throat washings; Oropharyngeal Washings; 2019 novel coronavirus; diagnostics; COVID-19.
A new pandemic infectious disease caused by 2019 novel coronavirus (2019-nCoV, also called SARS-CoV-2) is raging worldwide and becomes the biggest global health threat [1]. As of 31st March 2020, a total of 787125 laboratory-confirmed cases have been reported all over the world. Therefore, rapid differentiation and accurate identification of 2019-nCoV are crucial to plan timely and appropriate measures for public safety.

Nasopharyngeal (NP) and oropharyngeal (OP) swabs have been recommended for the detection of 2019-nCoV by WHO. Researchers have proven that NP swab specimen was superior to the OP swab specimen for the examination of 2019-nCoV [2]. However, NP swabs are not the optimal specimen for many reasons. Firstly, healthcare workers are at a great risk of infection due to the production of aerosol during the sampling. Secondly, the quality of NP swabs is inconsistent between collections, which may lead to the occurrence of false-negative results. Thirdly, patient may experience discomfort, including pain, itchy and bleeding, during the sampling. The biggest advantage of sputum specimen is its noninvasive characteristics, but only 28% of patients with COVID-19 have sputum symptom [3].

Throat washing is a non-invasive and easy-access sample, which can remarkably reduce the chance of exposing healthcare workers to 2019-nCoV. More epithelial cells, which can enhance the positive detection rate of 2019-nCoV, are acquired by forcing the cricopharyngeal muscle to oscillate over posterior pharyngeal wall with sterile normal saline. In view of the aforementioned advantages, throat washings have been utilized for the screening of 2019-nCoV in this study. The comparisons of efficacy between throat washings and NP swabs were also performed in this paper.

Methods

Ethical approval

The study was performed in accordance with the principles of the Declaration of Helsinki and approved by ethics committee of the First Affiliated Hospital of Guangzhou Medical University (approval number:
2020-46). Written informed consent was waived in light of emerging infectious diseases. Data were analyzed and interpreted by the authors.

Subjects

11 subjects with laboratory-confirmed COVID-19 were included in this study from March 23th to March 31st, 2020 in the First Affiliated Hospital of Guangzhou Medical University. According to the Chinese management guideline for COVID-19 (version 7.0) [4], all the patients enrolled in this study met the diagnosis criteria of COVID-19.

Acquisitions of specimen

Throat washing was harvested by asking patient to oscillate over posterior pharyngeal wall with 20 ml sterile normal saline. After 5-10 seconds, spit out normal saline from their throat to a sterile container.

NP swabs were sampled as described previously [2].

Nucleic Acid Extraction and Real-time Reverse Transcription–Quantitative Polymerase Chain Reaction (RT-PCR) for 2019-nCoV

Clinical specimens were tested with the common RT-PCR assay kits which have been purchased from the companies. These kits were certified by the Chinese government. However, the product information, especially the detection sequence of SARS-CoV-2, could not be fully obtained on account of the protection of trade secrets. Yet it is known that the detection target of RT-PCR focuses on NP and ORF1ab gene of SARS-CoV-2, and a positive result requires both gene tests to be positive. The detection operation is in accordance with the instructions of the products.

Statistical analysis

Categorical variables were expressed as frequencies and evaluated by Chi-squared test. SPSS software version 22.0 (SPSS Inc., Chicago, Illinois, USA) was used for statistical analysis. A two-sided P value less than 0.05 was considered to be statistically significant.
Results

11 laboratory-confirmed COVID-19 participants were enrolled in this study, including 6 remain hospitalized subjects and 5 discharged subjects. Among them, there were 9 males and 2 females, aged from 26 to 83 years. 24 paired throat washings and NP swabs, including 5 in discharged patients and 19 in hospitalized, were performed. Samples were collected at a median of 53 days after symptom onset (range: 48-57 days). Among them, 14 paired throat washings and NP swabs were both negative for the detection of 2019-nCoV. However, the other 5 paired samples got inconsistent results, of which the throat washing specimen showed positive and the NP swabs presented negative results. Using the Chi-squared test, we identified positive testing rate of throat washing was much higher than that of NP swabs ($P=0.031$) (Table1).

Discussion

Throat washings have been firstly introduced as the specimen for the detection of 2019-nCoV in this study. 24 series of paired throat washings and NP swabs specimens were evaluated, and we found throat washing specimen was significantly superior to the NP swab specimen for its higher positive detection rate of 2019-nCoV nucleic acid.

Variations in the detection of virus nucleic acid with different samples have been confirmed [5-6]. It is well recognized that NP swabs were more sensitive than OP swabs for the inspection of 2019-nCoV nucleic acid (89% VS 54%, $P < 0.001$) [2]. In spite of the high positive detection rate, the risk of exposure to 2019-nCoV did not improve by using NP swabs samples. Moreover, as we mentioned before, the application of NP swab was limited for its own disadvantages. Sputum was a useful non-invasive method for the detection of 2019-nCoV, but still confined to the occurrence of sputum. Induced-sputum was identified as an unsafe inappropriate specimen for the production of aerosol [7]. Therefore, there is an urgent need for a novel optimal sampling plan.
Saliva specimen can be easily obtained by noninvasive techniques and its application could remarkably minimize the risk of 2019-nCoV transmission [8]. Researchers also found descending trend of 2019-nCoV viral load by using saliva specimens, indicating the important role of saliva in early detection [9]. Another study has also interpreted the potential role of saliva for the detection of 2019-nCoV [10]. However, the efficiency comparison between saliva specimen and NP swabs, whose validity and reliability has been certified in previous studies, has not yet been described.

Throat washing is a promising candidate for 2019-nCoV screening due to its safety and reliability. Its utility and efficacy in 2019-nCoV detection have been well described in this study. The number of laboratory-confirmed COVID-19 cases is increasing day by day with a shortage of medical healthcare workers. Throat washing may play a potential role to reduce the workload of sampling.

There are limitations in our study. Sample size is small for the decrease of COVID-19 cases in China. The decline of 2019-nCoV viral load in patients enrolled in this study, whose sampling time was long after symptom onset, may have contributed to the low overall positive detection rate of 2019-nCoV.
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Declaration of interest

The authors have declared no conflicts of interest and alone are responsible for the content and the writing of the manuscript.

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Table 1. The comparisons of efficacy for 2019-nCoV detection between throat washings and NP swabs

| Throat washings | NP swabs | Total |
|-----------------|----------|-------|
|                 | Positive |Negative|
| Positive        | 1        | 6     | 7     |
| Negative        | 0        | 17    | 17    |
| Total           | 1        | 23    | 24    |