APPLICATION OF THE STANDARDIZED NASOPHARYNGEAL SWAB SAMPLING FOR THE NUCLEIC ACID DETECTION OF COVID-19

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ABSTRACT

Introduction*: The aim of the present study was to investigate a relatively convenient, safe, and sensitive sampling method in the nucleic acid detection of the 2019-novel coronavirus (2019-nCoV).*

Materials and method*: The nasopharyngeal swab samples of patients admitted to the 13 inpatient areas of the Tumor Center, Xiehe Hospital of the affiliated Tongji Medical College of Huazhong University of Science and Technology (temporarily transformed into a designated hospital for critical patients with COVID-19) from February 21 to 23, 2020 were used for the nucleic acid detection analysis of 2019-nCoV. The nasopharyngeal swab samples in the present inpatient area were obtained by a standardized sampling method.*

Results*: A total of 663 samples were collected from the tumor center with 125 positive ones. Among these samples, 33 samples were collected from the present inpatient area, and 11 cases (33%) were positive. A further 630 samples were collected from other inpatient areas, in which 114 samples (18%) were positive. The difference in the positivity between the present inpatient area and other areas was statistically significant.*

Conclusion*: The standardized nasopharyngeal swabs sampling had a high positive detection rate in the nucleic acid detection of 2019-nCoV and was safer and more convenient for medical staff and worthy of wider clinical use.*

Keywords: *A2019-nCoV, COVID-19, nasopharyngeal swab, nucleic acid detection, Wuhan, positive rate.*

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Introduction

In late December 2019, pneumonia caused by a novel coronavirus infection broke out in Wuhan, Hubei, China, and subsequently spread all over China and the world. On January 13, 2020, this virus was officially named by the World Health Organization (WHO) as the 2019 novel coronavirus (2019-nCoV) [(1)]. The disease caused by this virus became known as COVID-19. With the development of medical science and technology, molecular biotechnology is widely used in the rapid detection and accurate diagnosis of respiratory virus infection[(2)]. The nucleic acid detection of 2019-nCoV in the nasopharyngeal swab sample is the most important criterion for the diagnosis of COVID-19[(1, 3)].

During the clinical practice of the diagnosis and treatment of COVID-19 in Hubei, there is a phenomenon that the nasopharyngeal swab samples from patients by the pre job training medical staff

which it may test positive at the recheck after testing negative. In the detection of the respiratory virus by PCR, the positivity may be affected by different parts of sampling and different sampling methods (4). In order to reduce the false negativity of the nucleic acid detection and decrease the further spread of the virus caused by discharged patients who are still infectious, we summarized the standardized nasopharyngeal swab sampling method of nucleic acid detection so as to provide some reference.

Materials and methods

Clinical materials

About 130 medical staff participated in nasopharyngeal swab nucleic acid sampling. All medical staff received pre job training, including wearing and taking off protective clothing and taking nasopharyngeal swab nucleic acid sampling. A total of 663 nasopharyngeal swab samples of inpatients from the tumor center from February 21 to 23, 2020, were collected in the present study. The nasopharyngeal swab samples in the present inpatient area were obtained by the standardized sampling method. This study was conducted with approval from the Ethics Committee of Fujian Medical University Union Hospital. This study was conducted in accordance with the declaration of Helsinki. Written informed consent was obtained from all participants.

Sampling methods

The samples for nucleic detection from other inpatient areas were sampled by the conventional nasopharynx swab or oropharynx swab sampling method. In the present inpatient area, the "eight steps" nasopharynx swab sampling method for the nucleic acid detection summarized by clinical practice was adopted, and the details were as follows:

• Medical staff should wear protective clothing together with a facial screen, generally with level 3 protection.

• The medical staff should communicate with the patients to explain the purpose, significance, and general process of the operation, and obtain the cooperation of the patients.

• Select a well-ventilated environment, where the patient sits down with the body and head against the wall and leans back about 30 degrees. The patient's head is turned slightly to the left, and a facial mask is worn.

• The medical staff should stand on the right side of the patient. After preparing the nasopharynx swab, the patient is asked to pull down the mask to expose the nose while the mouth remains covered.

• The operation staff should stabilize the patient's head with the left hand and hold the nasopharynx swab with the right hand. The nasopharynx swab should be inserted with rotation from the patient's ipsilateral anterior nostril. The nasopharynx swab should be inserted along the direction of the nose bottom, which is perpendicular to the face, then go backward, inward and downward, along the junction of the nose tip and the nasal septum into the nasal cavity. This should continue until there is a sense of touching the wall, indicating that the swab has reached the back wall of the nasopharynx. The depth of the nasopharynx swab inserted into the nasal cavity is from the tip of the nose to the earlobe, approximately 10-12cm in length.

• Keep the nasopharynx swab inside for approximately 15-20 seconds, and then rotate the nasopharynx swab clockwise and anticlockwise twice, each time for approximately five seconds.

• Pull out the nasopharynx swab smoothly along the direction of the nose tip, and immediately ask the patient to put on the facial mask.

• Open the sampling tube vertically, put the head end of the nasopharynx swab into the sampling tube, break the tail of the swab at the crease of the swab, and tighten the cover of the sampling tube to prevent leakage of the liquid inside the tube.

Statistic analysis

SPSS 26.0 software was used for data analysis. The χ^2 test was adopted for enumeration data. $P < 0.05$ was considered statistically significant.

Results

A total of 663 samples were collected from the tumor center, with 125 positive ones. Of these 663 samples, 33 were collected from the present inpatient area, and 11 cases (33%) were positive. The remaining 630 samples were collected from other inpatient areas, and 114 samples (18%) were positive. As shown in Table 1, the positivity in the samples obtained by the standardized nasopharynx swab was higher than that in samples obtained by the conventional nasopharynx swab, and the difference was statistically significant ($X^2 = 4.759$, $P = 0.039$). After the tumor center was temporarily transformed into a designated hospital for patients with critical COVID-19, from February 15, all the assisting

medical teams were stationed during the same period to take over the wards, and the municipal command center randomly arranged patients to be admitted in different wards.

	Positive (Positive rate)	Negative	Total
Tumor center	11(33%)	22	33
Other inpatient areas	114(18%)	516	630

Table 1: Comparison of the results of virus nucleic acid detection by different sampling methods between the present inpatient area and other inpatient areas in the center. *X^2=4.759, P=0.039.*

Discussion

COVID-19 is a public health emergency that threatens the world[5]. The prevalence and proportion of this serious disease are high in the elderly and those with pre-existing conditions(6,7). COVID-19 is highly infectious, with the main route of transmission being through respiratory droplets and close contact. It is possible to transmit the disease through aerosols when exposed to high concentration aerosols for a long time in a relatively closed environment[8]). The population is generally susceptible to COVID-19. Therefore, timely and accurate diagnosis of the disease is essential to control its outbreak and ensure the safety of people's lives.

Real-time fluorescence RT-PCR of the 2019-nCoV nucleic acid detection is an important basis for the diagnosis, treatment, and discharge evaluation of COVID-19 because it provides the pathogenic evidence, which is the gold standard for the diagnosis of infectious diseases[9]. Nucleic acid detection is an unequivocal diagnostic method.

The reasons are as follows:

• There exists a window phase in the detection of pathogen antibodies, which appears in the later stage, lagging behind the detection of the nucleic acid.

• The sensitivity of nucleic acid detection is higher than other laboratory methods.

• The positive nucleic acid test occurs earlier than the changes in features of the lung CT, and there is a lack of significant changes in the lung CT in asymptomatic patients.

• If the quantitative detection of nucleic acid could be carried out, the degree of virus infection could be dynamically monitored, and the curative effect could be evaluated. 2019-nCoV can be detected in respiratory secretions, blood, feces, urine, and other body fluids. Clinically, the respiratory secretion samples are commonly used, such as the bronchoalveolar lavage fluid (BALF), sputum from the deep site, nasopharynx swabs, and oropharynx swabs. According to the positivity in nucleic acid detection, the rank is as follows: BALF > sputum from the deep site > nasopharynx swab > oropharynx swab. Because the BALF and sputum from the deep site belong to the samples of the lower respiratory tract, clinically, it is difficult to obtain, and the exposure risk to the operators is relatively high. Therefore, the easiest and most popular method for the detection of the virus's nucleic acid is to collect the samples from the upper respiratory tract, such as oropharyngeal and nasopharynx swabs. Moreover, it is also an effective method for the rapid diagnosis of virus infection of the respiratory tract or the carrier[10,11].

The reason for the false negativity in the nucleic acid detection are as follows:

• Quality problems in sample collection and sampling (timing, location, and method).

• Quality problems in nasopharyngeal swabs and collecting tubes.

• Problems in the preservation and transportation of samples after sampling.

• Problems in the detection kit.

• Problems in the detection technology. Clinicians must try to improve the quality of sample collection and sampling and improve the positivity in the nucleic acid tests under the condition of self-protection and safety.

We believed that the sensitivity of viral nucleic acid detection in the nasopharynx swabs was higher than that in the oropharynx swabs for the following reasons:

• The nylon flocking swab used in the nasopharynx swab was better than the cotton swab used in the oropharynx swab. Daley et al.,[12] showed that the efficiency of microbial transferring from the object surface by the nylon flocking swabs was 20%-60% higher than that of other swabs. Dalmaso et al.,[13] suggested that the villi on the nylon flocking swabs could increase the surface area of the swab contacting with the evidence, maximize the transfer of cells to the swab surface, and fully release the adhered cells during the DNA extraction, so as to obtain a higher concentration of template DNA. However, some studies[14] suggested that there was no significant difference between the two swabs.

• The viral load at the nasopharynx was higher than that at the oropharynx, so the positivity was higher in samples from the nasopharynx. Recently, the relationship between the viral load and the

duration of COVID-19 was analyzed in 17 patients with COVID-19 by the Guangdong Disease Control Center and Prevention, and the team at the Sun Yat-sen University. It was found that a high viral load could be detected in the upper respiratory tract soon after the onset of disease in patients with COVID-19. The viral load at the nasopharynx was higher than that at the oropharynx after sampling by the nasopharynx swab and the oropharynx swab with the same cotton swab[15]. Another study in Sweden also found that even when swabs made of the same material were used, the positivity of the nasopharynx swabs was almost 19 times higher than that of the oropharynx swabs. Therefore, even if the swab material was not considered, the nasopharynx swab was recommended for the respiratory virus sampling[16].

• When collecting the oropharynx swabs sample, the medical staff should face the patient's mouth directly. Considering that 2019-nCoV infection is through droplets and airborne contaminants, the sampling process is often short and cursory, and it is difficult to collect enough specimens, resulting in false negatives. However, in the case of the nasopharynx swab sampling, the medical staff might be adjacent to the patient, and the probability of direct contact with the mouth and face is low; thus, the sample might be collected more carefully.

The medical staff should take self-protection measures before the practice of nasopharynx swab sampling, generally taking level 3 protection measures. The hands should be washed first, and staff should add protective equipment in the following order.

■ Wear a disposable round hat with all hair wrapped inside.

■ Wear the N95 mask correctly. The mask edge should be close to the face to make sure there is no air leakage during testing.

■ Wear protective clothing.

■ Wear protective goggles and keep hands away from the face.

■ Wear shoe covers.

■ Wear rubber gloves that cover the sleeves completely.

■ Wear the isolation clothing and the second gloves.

■ Wear an all-round respiratory protector or a disposable facial screen with the facial screen being vertical and covering the whole face. The nasopharyngeal swab test tube should be inspected first to see whether the package is intact, whether it is within the use-by period, and whether there is any leakage of the culture medium. Before the inspection, disinfect the hands with hand sanitizer, open the outer packing bag, and take out the test tube and swab. Check the patient's identity and paste the test barcode on the test tube. During the inspection, the test tube should be held in one hand and kept upright to prevent the culture liquid from leaking out. The swab should be held in the other hand and prevented from touching other places and becoming contaminated. After the inspection, the identity of the patient should be checked again. If there are no problems, the test tube shall be packed and put into a double-layer yellow medical waste bag. After the waste bag is sterilized, it should be put in the specimen storage place in the ward and transported for further analysis within the specified time. Once the sampling is completed, the hands should be disinfected with hand sanitizer, before taking the next patient's nasopharynx swab sample.

The operation precautions are as follows:

• The medical staff shall take good protective procedures to ensure the integrity of the protective equipment. If there is damage or splashing of the secretion, the protective equipment shall be replaced in time.

• The afferent nerve of the sneeze reflex is the trigeminal nerve. The receptors are mainly located in the lateral wall of the nasal mucosa, the front of the middle and lower turbinate, and the middle of the nasal septum. During the sampling, touching the above sensitive areas should be avoided and care taken when obtaining the swab to prevent severe reactions such as sneezing. If there is a possibility of flying sputum or droplets, the staff should take measures to avoid these.

• During the operation, the patient should sit with their head and body against the wall and lean back about 30 degrees. The patient's head should slightly turn to the left while the medical staff stands to the right of the patient. The operator should use their left hand to hold the patient's head in a fixed, still, position, to facilitate the operation and avoid spray exposure as much as possible.

• The patient should wear a facial mask. Only when the medical staff is ready for nasopharynx swab sampling can the mask be pulled down to expose the nose but still cover the mouth. The patient should be asked to put on the facial mask immediately after the operation.

• Generally, the nasopharynx swabs should enter from the same side (right side) of the patient's

nasal cavity. In cases where the right nasal cavity is narrow due to the deviation of the nasal septum to the right and the swelling of the right nasal cavity, the nasopharynx swab can enter from the left nasal cavity.

• The nasopharynx swab should be inserted deep enough to enter the nasal cavity and stay in the nasopharynx for a set time. The swab should then be rotated around to transfer as many microorganisms on the surface of the nasopharynx as possible and improve the positivity of the detection.

The present study suggested that standardized nasopharynx swabs should be used to detect the viral nucleic acids in the clinical practice in order to reduce misdiagnoses, better evaluate the progression of the disease, and reduce the possible exposure of medical staff to the virus. If the nucleic acid can be detected in other humoral fluid, it may favor the diagnosis of patients with COVID-19 and the monitoring of discharged patients.

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