Evaluation of using distal part of endotracheal tube samples for SARS-COV-2 diagnosis by RT-PCR

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ABSTRACT

Objective: The reverse transcription-polymerase chain reaction (RT-PCR) analyses method is the most important diagnostic method in the diagnosis of SARS-CoV-2 virus infection. In this research, we aimed to investigate the positivity of SARS-CoV-2 by RT-PCR from distal part of the endotracheal tube (DPET) samples, which have not been investigated in any study yet.

Materials and Methods: A total of 48 patients with a diagnosis of COVID-19 hospitalized in the intensive care unit receiving mechanical ventilation and whose conditions resulted in death or extubation were included in the study. The distal 6 cm part of the orotracheal intubation tube was removed from the patient (including the cuff). DPET samples were mixed with viral transport medium and vortexed; then, it was centrifuged at 4500g for 4 minutes. RNA isolation was performed by taking 400 µl from the supernatant and then SARS-CoV-2 RT-PCR was studied.

Results: In 15 patients (31.25 %) the swab samples were PCR positive, 42 patients (87.5 %) had positive computed tomography finding and 48 patients (100 %) had positive clinical findings. Among the patients whose oropharynx (OP)/nasopharynx (NP) combined swab sample was positive for RT-PCR, the rate of RT-PCR positivity detected in DPET samples was 26.7%. While OP/NP combined swab sample was negative, DPET RT-PCR positivity rate was found to be 9.09%.

Conclusions: Patients with positive DPET RT-PCR are detected when the swab is negative. These findings suggest that DPET can be used as a good lower respiratory sample without the risk of particle spread and transmission to healthcare personnel.

Keywords: COVID-19; mechanical ventilation; endotracheal intubation tube; intubation

INTRODUCTION

The new coronavirus disease (COVID-19) caused by the SARS-CoV-2 virus emerged in Wuhan, China, in December 2019 and was identified as a pandemic by the World Health Organization (1). Diagnosis of COVID-19 is based on clinical symptoms, investigation of the viral genome with Reverse-transcription polymerase chain reaction (RT-PCR), chest X-ray or computed tomography (CT) scan, and lastly serological blood tests (2). RT-PCR analysis is the best and most used method for qualitative and quantitative diagnosis of viruses, including coronaviruses (3). Although RT-PCR analysis is usually performed from nasopharynx (NP) swab, oropharynx (OP) swab, combined NF and OF swab, it has been stated that it can be used for RT-PCR from other samples such as sputum, bronchoalveolar lavage, saliva, nasal washing, aspiration fluids and tissue biopsies (4,5). Well taken lower respiratory tract samples are preferred so as to promote the success of the method shown in the article.

It has been shown that approximately 80% of the patients have mild illness, 20% require hospitalization, and also approximately 5% of them need intensive care (6). Patients appear relatively stable at first, but can rapidly deteriorates with severe hypoxia. The basic table noticed in these cases is acute respiratory distress (ARDS) (7).
When high-flow oxygen therapy and non-invasive mechanical ventilation treatment are insufficient, patients are oxygenated by using invasive mechanical ventilation. As in particular bronchoalveolar lavage or bronchial washing fluid is recommended for COVID-19 patients who are followed up with orotracheal endotracheal intubation in invasive mechanical ventilation. It is generally not preferred because of the spread around and the risk of infection for healthcare personnel (8).

There are many factors affecting the sensitivity of RT-PCR. Analytical factors such as the existence of an inhibitory substance in the sample fluid, the utilization of inaccurate amounts of the components used, errors in the sampling technique, inability to adjust temperature parameters, and finally, mutations affecting the binding site of the primer (9,10) as well as preanalytical factors. Therefore, while clinical and CT findings support COVID-19 in many patients, RT-PCR negative results can be encountered (11,12). Due to the difficulties and the risks in collecting lower respiratory tract samples, these types of samples are used less frequently in diagnosis.

In this study, it is aimed to investigate the positivity of SARS-CoV-2 by RT-PCR from distal part of endotracheal tube (DPET) samples, which have not been used in any study before, in order to indicate the situation in the lower respiratory tract in intubated patients.

**MATERIAL and METHODS**

**Study group**

The study was conducted by obtaining a prospective (71522473 / 050.01.04 / 283) approval from Sakarya University Faculty of Medicine ethics committee. A total of 48 patients hospitalized in the intensive care unit of Sakarya University Medical Faculty Training and Research Hospital with the diagnosis of COVID-19 who died as being connected to an invasive mechanical ventilator or who were extubated were involved in the study. The data of the patients were obtained from the hospital information management system used by Sakarya University Medical Faculty Training and Research Hospital (Karmed, Kardelen Software, Mersin, Republic of Turkey). These patients were hospitalized in the intensive care unit, and their clinical findings, radiological findings, or SARS-CoV-2 RT-PCR positivity were evaluated. The data of the patients who accepted to participate in the study (age, hospitalization days, nasopharynx/oropharynx swab SARS-CoV-2 RT-PCR, CT findings, drug therapy for COVID-19 and comorbidities etc.) were recorded regularly. Patients with CT findings consistent with suspected viral pneumonias and patients with clinical findings such as fever, cough and shortness of breath that could not be explained by another condition were included in the study.

**Sample collection**

A 6 cm in length sample was taken from the lower end of the orotracheal intubation tube (including the cuff) from patients who were died or extubated during intensive care treatments. All samples taken were placed in a sterile container and sent to the laboratory in accordance with the cold chain rules with a triple transport system by following the infection prevention and control procedures.

Nucleic acid isolation and RT-PCR for SARS-CoV-2

After the samples were accepted in the microbiology laboratory, the samples were taken to the 3rd level biosafety negative pressure room. DPET samples were mixed with the viral transport medium and kept for a while, vortexed and then centrifuged at 4500g for 4 minutes. 400 µl of the supernatant was loaded onto the BioRobot EZ1 (Qiagen, Germany) device and 60 µl of elution was taken. Total nucleic acid isolation was performed with EZ1 Virus Mini Kit v2.0 (Qiagen, Germany) in line with the recommendations of the company. For RT-PCR study, a mixture of 10 µl master mix, 2 µl primer, 8 µl template RNA was constituted with the genesig Real-Time PCR COVID-19 (Primer Design, UK) kit and a total reaction volume of 20 µl. PCR temperatures and times were as follows: reverse transcription for 10 minutes at 55° C, enzyme activation for 3 minutes at 95° C, 10 seconds at 95° C, 60 seconds at 60° C for a total of 50 cycles of denaturation, binding, and elongation. Curves with a Cycle Threshold (CT) value lower than 45 and observed sigmoidal at the end of the reaction were interpreted as positive for SARS-CoV-2 RNA.

**Statistical analysis**

Statistical analyses were done by using SPSS for Windows software (ver. 22.0; SPSS Inc., Chicago, IL, USA). Descriptive analysis of the variables was expressed as mean ± standard deviation (SD) or as numbers (n) and percent (%). The chi-square test and for small samples the Fisher’s exact test was applied to compare qualitative data. A p-value of <0.05 was considered statistically significant.

**RESULTS**

While 6 (12.5%) of 48 patients who were intubated in the intensive care unit were still alive, 42 (87.5%) of them died. Twenty-one of these patients (43.75%) were female, 27 (56.25%) were male. In 15 patients (31.25%) OP/NP combined swab RT-PCR results were positive, 42 patients (87.5%) had positive CT findings, and 48 patients (100%) had positive clinical findings. The average age of deceased patients was 69.93 ± 14.5, the average age of the survived patients was 58.5 ± 19.70, while the average age of all patients was 68.5 ± 15.5. DPET. The mean day of intubation for all patients was 17.8 days. This number was 6.8 days in DPET positive patients and 13.9 days in negative patients. The demographic data of the patients included in the study are presented in Table 1.

PCR results obtained from swab and DPET samples are presented in Table 2. In terms of SARS-CoV 2 RT-PCR results, no significant difference was found between the OP / NP combined swab and DPET samples of the patients (p> 0.05) (Table 2).
DISCUSSION

The most commonly used method in the diagnosis of COVID-19 infection is to show the presence of the virus in NF and OF swab samples by RT-PCR (12). However, at the time the samples taken, aerosol is released into the environment and poses a high risk for the healthcare personnel who are in close contact with the patient.

Although the use of lower respiratory tract samples for diagnosis increases the sensitivity, the sampling procedure is riskier and because it is an invasive procedure, much less lower respiratory tract samples are used in diagnosis. In this study, RT-PCR results of DPET samples representing lower respiratory tract samples and OP/NP combined swab samples were compared in the diagnosis of COVID-19 infection.

It was examined whether or not DPET samples can be used as an effective diagnostic method for the patients receiving mechanical ventilation in intensive care. COVID-19 shows its clinical signs with upper respiratory tract symptoms such as fever, dry cough, and dyspnea (13). Although CT findings may vary according to age, underlying disease, immune status, and stage of the disease, it can be used as a strong recommendation for diagnosis (14). RT-PCR analysis is the most robust and widely used method for qualitative and quantitative diagnosis of viruses, including coronaviruses (3).

Oxygen mask, high flow oxygen therapy, non-invasive mechanical ventilation and invasive mechanical ventilation therapy are generally used in the treatment of patients who need oxygen. Especially after the development of ARDS due to SARS-CoV 2, the need for oxygen increases further, and when non-invasive oxygen therapy is insufficient, oxygenation is exerted to be provided by invasive mechanical ventilation. Orotracheal intubation tube is usually used to connect the patient to a mechanical ventilator. The distal part of the intubation tube (including the cuff) was used to obtain the lower respiratory tract sample, because of the fact that the end of the intubation tube is in the trachea and is in constant contact with the secretions in the lung in the trachea above the carina.

Among the patients whose OP/NP combined swab sample was RT-PCR positive, the rate of RT-PCR positivity detected in DPET samples was 26.7%, while the DPET RT-PCR negative rate was 73.3%. It was thought that the lower rate of DPET RT-PCR was due to the DPET sample taken after the patients were hospitalized for a long time. It was observed that DPET samples were taken after an average of 6 days from the patients who were DPET RT-PCR positive as the swab was RT-PCR positive, and DPET samples were taken after an average of 17.6 days from the patients who were negative for DPET RT-PCR while the swab was RT-PCR positive.

<table>
<thead>
<tr>
<th>Sample type</th>
<th>DPET, n (%)</th>
<th>Total, n (%)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smear, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>4 (26.7)</td>
<td>11 (73.3)</td>
<td>15 (31.25)</td>
</tr>
<tr>
<td>Negative</td>
<td>3 (9.1)</td>
<td>30 (90.9)</td>
<td>33 (68.75)</td>
</tr>
<tr>
<td>Total</td>
<td>7 (14.6)</td>
<td>41 (85.4)</td>
<td>48 (100)</td>
</tr>
</tbody>
</table>

DPET: Distal part of entubation tube, n: Number of patients, %: Percentiles.
Reliable evidence has shown that the SARS-CoV-2 incubation period is approximately six days (interquartile range, 2-11 days) (15). Similarly, we found that the average time of sampling of DPET RT-PCR positive patients was six days.

While OP/NP combined swab sample was negative, DPET RT-PCR positivity rate was 9.09%. Although combined swab RT-PCRs were negative, DPET RT-PCR was positive in 3 patients, suggesting that DPET could be used as a sample from the lower respiratory tract. Two of the three patients were deceased, and one of them has been exubated with healing. The average number of days of hospitalization for these three patients was eight days.

Although RT-PCR is used as the key standard in diagnosis, finding inaccurate negative and positive results are a substantial problem to be taken into consideration. It has been reported that many suspected cases considered to be typical COVID-19 with clinical and CT findings could not be diagnosed (12). Therefore, RT-PCR is not used as the only criterion to exclude disease. Even though diagnostic errors can always be experienced, it becomes even more important in infectious epidemics such as COVID-19. While false positive or false negative results are a threat to the health of the individual, they can also cause errors and restrictions in the emergency plans and measures created by the national and international authorities to control the epidemic

In particular, reporting a false negative result to a person infected with SARS-CoV 2 causes disruption of isolation and restrictive measures, transmission to the community, and especially insufficient detection of households who are thought to be potentially infected and must be screened. In laboratory studies, not using suitable materials for sampling, inadequate sample collection, insufficient sample volume and quality, inaccurate transportation methods and storage problems come to the forefront in the preanalytic phase.

According to current diagnostic criteria, viral nucleic acid tests play a vital role in determining hospitalization and isolation. However, CT may be a more reliable, practical, and rapid method to diagnose and assess COVID-19 in some cases, especially in the area affected by the epidemic. CT has been a guide in the diagnosis, especially in patients whose clinic is compatible with COVID-19 but whose PCR is negative. In a study conducted in China, it was reported that 59% of 1014 patients had swab RT-PCR positivity, while 88% of them had positive CT findings (11). Recent studies have shown that the sensitivity of chest CT in COVID-19 patients with false-negative PCR results is 98% (16,17). In another study, in 36 patients with COVID-19 pneumonia, the CT sensitivity was 97.2%, while the RT-PCR sensitivity was shown as 83.3%, and it was stated that RT-PCR might initially give false results (18). While different PCR positivity rates were encountered in studies conducted with different methodologies, false negativity was emphasized in all studies. In our study, the positivity rate of CT findings among all patients was 87.5%. While the swab was RT-PCR negative, we found the rate of CT findings being positive to be 84.8%. In all patients, the swab PCR positivity rate was 31.25% (15/48 patients).

In the study conducted by Liu et al., 38.2% of 4880 patient samples were found to have RT-PCR positivity, while the positivity rate was 38.25% in nasal and pharyngeal samples, 49.12% in sputum samples and 80% in bronchoalveolar fluid samples (19). This study shows us that the sample taken at the bronchoalveolar level has a higher RT-PCR positivity rate. It is not preferred in patients with COVID-19 pneumonia because of the high particle release in samples taken under bronchoscopy for bronchoalveolar lavage. In our study, while the OP / NP combined swab RT-PCR positivity rates were similar, the DPET RT-PCR positivity rate, which we used as a lower respiratory tract sample, was 14.6% among all patients (7/48 patients), and 26.7% in patients with swab RT-PCR positive (4/15 patients) required us to look for the differences in sampling time. Tao Ai et al. showed the meantime to become negative is 6.9 days after RT-PCR positivity developed (11). The most important data appeared in the study was that there were 3 patients who were DPET RT-PCR positive while the swab was RT-PCR negative. While the average hospitalization days of these patients were eight days, the average hospitalization days of DPET RT-PCR negative patients was 12.4 days. As a result, in this study, it was investigated whether DPET samples can be used for the first time in the diagnosis of COVID-19. DPET RT-PCR positivity rate was found to be high especially in patients with short hospitalization days. Patients with positive DPET RT-PCR were detected when the swab was negative. These findings suggest that DPET can be used as a good lower respiratory sample without the risk of particle spread and transmission to healthcare personnel. However, more comprehensive studies are needed on this subject.

This study was planned to evaluate DPET as a diagnostic sample. However, our DPET RT-PCR positivity rate remained relatively low due to the long hospitalization period of intensive care patients and the very low rates of cure and extubation of COVID pneumonia. This study was conducted in the early part of the epidemic and at that time, changing the tube of the intubated patient was not part of the protocol in patient management. For this reason, we could not compare OP/NP swab and DPET by taking samples from all patients on the same day. If we were doing this study with our current patient management information, we could evaluate the effectiveness of DPET in diagnosis by taking samples from each patient on the days we determined beforehand.

CONCLUSION

In this study, it is investigated whether DPET samples can be used in the diagnosis of COVID-19 for the first time. DPET RT-PCR positivity rate is found to be high especially in the patients with short hospitalization days. As in our study, we know that there is a group of patients who are clinically compatible with COVID-19 but with negative swab PCR results. We think that DPET can be used to represent the lower respiratory tract, especially in this patient group. These findings suggest that DPET can be used as a good lower respiratory sample without the risk of particle spread and transmission to healthcare personnel. In addition, these results we obtained made us think that DPET could be used as a postmortem diagnostic tool even if it is not in routine diagnosis.
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Ethical approval: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by Local Ethical Committee.

REFERENCES


