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RESEARCH ARTICLE

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Comparison of nasopharyngeal swab and nasopharyngeal aspiration in adults for SARS-CoV-2 identification using reverse transcription-polymerase chain reaction

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Abstract

We aimed to compare reverse transcription-polymerase chain reaction (RT-PCR) results of nasopharyngeal aspiration (NA) and nasopharyngeal swab (NS) samples in the diagnosis of coronavirus disease 2019. NS was obtained with a dacron swab and NA was performed by aspiration cannula. The sampling was performed by an otolaryngologist to ensure standardized correct sampling from the nasopharynx. RT-PCR was performed for the detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The level of agreement between the result of NA and NS samples for each patient was analyzed. The Ct values were compared. Thirty-three patients were enrolled in the study with a mean age of 56.3 years. Thirteen subjects resulted negative with both NS and NA; 20 subjects resulted positive with NA and 18 subjects resulted positive with NS. The mean values of Ct for NA samples and NS samples were 24.6 ± 5.9 and 24 ± 6.7, respectively. There was no statistical difference between Ct values of NA and NS samples ($p = 0.48$). RT-PCR for SARS-Cov2 performed with NA sample and NS sample showed a strong correlation regarding the positivity/negativity and the Ct values.

KEYWORDS

nasopharyngeal aspiration, nasopharyngeal swab, reverse transcription real-time polymerase chain reaction, SARS-CoV-2

1 | INTRODUCTION

Coronavirus disease 2019 (COVID-19) has become a pandemic in over a year, and the reverse transcription-polymerase chain reaction (RT-PCR) assay remains the most widely used testing method to detect severe acute respiratory syndrome coronavirus 2 (SARS-Cov-2).[1,2] However, obtaining a positive test is not always possible, despite the presence of clinical and radiological findings consistent with COVID-19 in hospitalized patients. This has led clinicians to collect different kinds of biological specimens.[3] The tests currently used for the direct identification of SARS-CoV-2 include specimens such as oropharyngeal and nasopharyngeal swab, nasopharyngeal aspirate, and the lower respiratory tract samples.[4–6] Upper respiratory specimens are easily obtainable and their sampling exposes the patients to less invasive procedures than the lower respiratory specimens. In contrast, collection of the lower respiratory samples necessitates a skilled operator and is also uncomfortable for the patients. Instead, collection of a nasopharyngeal swab and throat swab is simple and safe for routine diagnosis and monitoring of the SARS-CoV-2.[4]

A negative test result does not rule out the infection.[7] SARS-CoV-2 testing failure may result from preanalytic errors such as sampling error, deficiencies in transporting of the sample, and potentially limited RNA found in the samples. Testing of specimens from multiple sites may aid in reducing false-negative results.[8] In this

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study, we aimed to investigate the factor of sampling error caused due to insufficient material sampling. Nasopharyngeal sampling with aspiration provides a greater amount of secretion to be obtained so it is expected to yield more accurate results than the swab. Moving from this idea, we tested the patients with nasopharyngeal aspiration (NA) at the same time as with a nasopharyngeal swab (NS) to compare the viral load in specimens obtained by two techniques and to assess its effect on the test results.

2 | METHODS

This study was conducted in a single tertiary referral center. It is approved by the Koç University Ethical Committee, and informed consent for nasopharyngeal sampling was obtained from all patients. All procedures were performed in compliance with relevant laws and institutional guidelines and in accordance with the ethical standards of the Declaration of Helsinki. Due to the higher risk of infection due to aerosolization, sampling with NA was performed only in hospitalized adult patients in the ward under a negative pressure ventilation system. Pediatric patients are not included in the study due to their higher vulnerability to invasive procedures, and there were no other specific exclusion criteria. The patients were tested by sampling via both NS and NA at the same time. NS was obtained with a sterile Dacron swab introduced through the nostril, and it was rotated several times when the tip of the swab touched the posterior nasopharyngeal wall. The NA was performed by aspiration cannula no:8 introduced through the nostril and the aspirator was switched on for 2 s when the cannula reached the posterior nasopharyngeal wall by giving attention to keep the secretion in the first 10 cm of the cannula. The shaft of the swab was broken, and likewise, the tip of the cannula was cut and each was transported in 3 ml of viral transport medium separately. The sampling procedure was performed by an otolaryngologist to ensure standardized correct sampling from the nasopharynx.

For the detection of SARS-CoV-2, extraction and PCR assays were performed in the hospital's laboratory. The EZ1 Mini Kit (Qiagen) was used with the EZ1 Advanced XL and Rotor-Gene (Qiagen) instrument. Nucleic acid extraction was performed using the Qiagen EZ1 Mini Kit beginning from 200 µl of both NS and NA samples. The second assay, QIA prep& amp Viral RNA Kit adapted to Rotor-Gene (Qiagen) instrument, is a qualitative one-step RT PCR. In the PCR assay, 5 µl of extracted material for each sample (NS and NA) and 5 µl of master mix, a total 10 µl volume was used. The cycle threshold values (Ct) were interpreted as inversely proportional to the viral load level present in the sample. For samples analyzed with a QIA prep& amp Viral RNA kit, a Ct value less than or equal to 35 was considered positive; Ct value more than 35 was considered negative.

The statistical analysis of the data was performed with SPSS 26.0 for Windows (IBM). The level of agreement between the result of NA and NS samples for each patient is analyzed using Cohen's kappa (κ). The CT values were evaluated with the Shapiro–Wilk t-test of normality, and the independent-samples t-test was used. The sample size was chosen according to power analysis based on a significance level of 0.05. In a test for agreement between two raters using the κ statistic, a sample size of 28 subjects achieves 81% power to detect a true κ value of 0.80 in a test of H0: $\kappa = 0.40$ versus H1: κ^1 0.40 when there are three categories with frequencies equal to 0.20, 0.30, and 0.50.

3 | RESULTS

Thirty-three patients were enrolled in the study, of whom 15 were female and 18 were male. The mean age of the patients was 56.3 years (range, 31–79 years). The mean time from the symptom onset to the sampling time was 6, 15 days (range 1–12 days, median 6). Thirteen subjects resulted negative with both NS and NA; 20 subjects resulted positive with NA and 18 subjects resulted positive with NS. There were two subjects, who resulted positive with NA but negative with NS. One of these two subjects was tested on the eighth day of symptoms and the other on the 12th day of symptoms. The results showed very good strength of agreement between NA and NS regarding positivity and negativity, as the κ value was 0.87. The mean value of Ct for NA samples was 24.6 ± 5.9 and the mean value of Ct for NS samples was 24 ± 6.7. There was no statistical difference between Ct values of NA and NS samples ($p = 0.48$), so sampling with nasopharyngeal swab was found to be as efficient as nasopharyngeal aspiration.

4 | DISCUSSION

It is crucial to diagnose COVID-19 accurately by the correct method of testing to manage treatment, reduce spread of the disease, and protect healthcare professionals. There is as yet no gold standard for the diagnosis of SARS-Cov-2 infection, and RT-PCR remains the most widely used testing method.[9] The nasopharynx, as an accessible site through the nose, is the most widely used sampling site. The testing may result in false negatives because of preanalytic factors such as inadequate sampling or low viral load. The timing of sampling is also known to be an important factor for obtaining a positive result, as the viral load in the nasopharynx is low in the first days of the infection and also decreases after 7 days of symptom onset.[10] In the literature, there are studies that compared the PCR results of lower respiratory tract specimens (laryngotracheal aspirate/sputum) with NS. Nazerian et al.[11] reported a reduced false-negative rate with laryngotracheal specimens in patients with suspected SARS-COV-2 pneumonia despite a negative NPS. In contrast, Thwe et al.[12] reported an overall agreement of 96% between tracheal aspirate/sputum specimens and NS; interestingly, though, five discordant results were positive for NS but negative for sputum. As we aimed to investigate the efficacy of NS and a possible effect of increasing the sample material on test results, we compared the viral load and the test results obtained from NS with that of NA, which is expected to yield more biological material from the sampling site. In the literature, there are similar studies

for other respiratory viruses, which compares the efficacy of NS and NA, but not yet for SARS-CoV-2 infection in adults. The optimal sampling methods and the best sites to collect material for viral detection for the etiologic diagnosis of respiratory viral infections may differ between different viruses[13]; for example, sampling with aspiration was reported to increase the sensitivity of PCR for the respiratory syncytial virus when compared to sampling with a swab.[13,14]

In our study, the idea of getting NA was adopted with the intention of obtaining a positive result for subjects who resulted negative with NS but are clinically and radiologically consistent with COVID-19. However, in the study design, no such criteria were determined to prevent a selection bias and to obtain a heterogeneous group of subjects. The results demonstrated that RT-PCR for SARS-Cov2 performed with NA sample and NS sample showed a strong correlation regarding the positivity and negativity, and also the Ct values. Only two patients, who were on their eighth and 12th day of symptoms, were detected to be positive with NA but negative with NS. The Ct values were 20.71 and 23.06, respectively. It can be considered that the quantity of the biological material obtained from the nasopharynx can still affect the results in some cases, even though the sampling tool is inserted into the nasopharynx properly. NA can be applied for patients who remain negative with NS if the necessary precautions for infection control can be provided.

The participants of the study mentioned that the aspiration process caused no further discomfort than the swab, or even provided higher comfort. This is probably because of the softer and flexible structure of the cannula compared with the swab. It is less likely to obtain an insufficient amount of material with NA than that with NS. Therefore, NA may be preferred for the second sampling, especially in patients who are difficult to be sampled with swab because of a septal deviation or patient incompatibility.

The limitation of the study was the small number of subjects, which was limited on purpose for prevention of aerosolization and patient discomfort due to double sampling at the same time. The sampling was done under optimal conditions, unlike the daily routine practice. It is to be noted that both sampling methods were applied to the same patient by the otolaryngologist. Thus, it was certainly collected with absolute precision. If we consider the fact that the sampling is generally performed by other healthcare professionals, the difference in results between NS and NA would be expected to be higher in less experienced hands.

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CONFLICT OF INTERESTS

The authors declare that there are no conflicts of interest.

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REFERENCES

1. Corman VM, Landt O, Kaiser M, et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. *Euro Surveill*. 2020;25(3):2000045.
2. Motley MP, Bennett-Guerrero E, Fries BC, Spitzer ED. Review of viral testing (polymerase chain reaction) and antibody/serology testing for severe acute respiratory syndrome-coronavirus-2 for the intensivist. *Crit Care Explor*. 2020;2(6):e0154.
3. Novazzi F, Cassaniti I, Piralla A, Di Sabatino A, Bruno R, Baldanti F, For the San Matteo Pavia COVID-19 Task Force. Detection of the SARS-CoV-2 in different biologic specimens from positive patients with COVID-19, in Northern Italy. *Pediatr Allergy Immunol*. 2020;31(suppl. 26):S72-S74.
4. Mawaddah A, Gendeh HS, Lum SG, Marina MB. Upper respiratory tract sampling in COVID-19. *Malays J Pathol*. 2020;42(1):23-35.
5. Loeffelholz MJ, Tang YW. Laboratory diagnosis of emerging human coronavirus infections - the state of the art. *Emerg Microbes Infect*. 2020;9(1):747-756.
6. Hanson KE, Caliendo AM, Arias CA, et al. Infectious Diseases Society of America Guidelines on the diagnosis of COVID-19. *Clin Infect Dis*. 2020:ciaa760.
7. Zitek T. The Appropriate use of testing for COVID-19. *West J Emerg Med*. 2020;21(3):470-472.
8. Wang W, Xu Y, Gao R, et al. Detection of SARS-CoV-2 in different types of clinical specimens. *JAMA*. 2020;323(18):1843-1844.
9. Long DR, Gombar S, Hogan CA, et al. Occurrence and timing of subsequent severe acute respiratory syndrome coronavirus 2 reverse-transcription polymerase chain reaction positivity among initially negative patients. *Clin Infect Dis*. 2021;72(2):323-326.
10. Kucirka LM, Lauer SA, Laeyendecker O, Boon D, Lessler J. Variation in false-negative rate of reverse transcriptase polymerase chain reaction-based SARS CoV-2 tests by time since exposure. *Ann Intern Med*. 2020;173(4):262-267.
11. Nazerian P, Sacco RM, Solbiati M, et al. Laryngotracheal aspiration test reduce the false negative rate in patients with suspected SARS-COV-2 pneumonia despite a negative nasopharyngeal swab. *Eur J Intern Med*. Published online June 24, 2021. https://doi.org/10.1016/j.ejim.2021.06.019
12. Thwe PM, Ren P. Analysis of sputum/tracheal aspirate and nasopharyngeal samples for SARS-CoV-2 detection by laboratory-developed test and Panther Fusion system. *Diagn Microbiol Infect Dis*. 2021;99(1):115228. https://doi.org/10.1016/j.diagmicrobio.2020.115228
13. Heikkinen T, Marttila J, Salmi AA, Ruuskanen O. Nasal swab versus nasopharyngeal aspirate for isolation of respiratory viruses. *J Clin Microbiol*. 2002;40(11):4337-4339.
14. Sung RY, Chan PK, Choi KC, et al. Comparative study of nasopharyngeal aspirate and nasal swab specimens for diagnosis of acute viral respiratory infection. *J Clin Microbiol*. 2008;46(9):3073-3076.

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