ORIGINAL RESEARCH

Evaluating Saliva And Gargle Lavage As A Cost-Effective Option To Throat And Nasal Swabs For Sars-Covid-2-Rna Stability And Diagnosis

[1]Dr. Pooja Pawaria, [2]Dr. Pallavi Gupta, [3]Dr. Prerna Kataria, [4]Dr. Abhishek Sinha

[1]Senior Resident, Department of Clinical Pathology, Pt. B. D. Sharma PGIMS, Rohtak, Haryana, India
[2]Assistant Professor, Department of ENT, Indian Naval Hospital Ship, Kalyani, Visakhapatnam, Andhra Pradesh, India
[3]Professor, Department of Periodontology and Implantology, D J College of Dental Sciences and Research, Modinagar, Uttar Pradesh, India
[4]Assistant Professor and Head, Department of Dentistry, Patna Medical College and Hospital, Patna, Bihar, India

Corresponding author
Dr. Abhishek Sinha,
Assistant Professor and Head, Department of Dentistry, Patna Medical College and Hospital, Patna, Bihar, India

ABSTRACT
Background: Currently, RT-PCR (reverse transcription-polymerase chain reaction) using nose and throat swab (NTS) is the most effective and widely used technique for conclusive diagnosis of COVID-19. Due to the lack of available gargle liquid, alternative sampling techniques such gargle lavage have only had limited application and effectiveness.
Aims: The goal of the current investigation was to determine the SARS-CoV-2 RNA stability at 4°C in normal saline used as a transport medium and gargle solution. The agreement between saliva/gargle liquid, nose swabs, and throat swabs in identifying SARS-CoV-2 was also evaluated in the current investigation.
Methods: In Paired samples of saliva, gargles, and NTS were collected from 30 individuals whose real-time RT-PCR (RT-PCR) positive diagnosis for COVID-19 had been verified. The collected gargle lavage samples were split into two aliquots for analysis to determine the stability of SARS-CoV-2 RNA in normal saline. One aliquot was processed 24 to 30 hours after being stored at 4 degrees Celsius, while the other was treated with regular saliva and an NTS sample in 4-6 hours. Statistical analysis was used to determine how well the cycle threshold (Ct) values for the two aliquots agreed.
Results: 13.33% (n=4) of participants with negative NTS and 6.66% (n=2) of subjects with positive NTS had negative saliva samples. 73.33% (n=22) of the participants with positive NTS and 6.66% (n=2) of the subjects with negative NTS had positive saliva samples.
There were 3.33% (n=1) negative samples for NTS positive and 16.66% (n=5) negative samples for NTS in the comparison of gargle lavage samples processed after 24–30 hours. For NTS positive samples, there were 80% (n=24) gargle lavage positive samples, however for NTS negative samples, there were none. There were a total of 83.33% (n=25) positive samples from the gargle lavage and 16.66% (n=5) negative samples. There were 3.33% (n=1) negative samples that were positive for NTS and 13.33% (n=4) positive results for NTS for gargle lavage samples that were processed right away.
Conclusions: According to the current investigation, SARS-CoV-2 RNA is stable in gargle samples that have been kept in normal saline for around 24–30 hours.
To identify SARS-CoV-2 RNA using RT-PCR, suitable and affordable collection techniques include saliva and gargle lavage. These techniques are also accepted, affordable, and straightforward ways to collect samples, lowering costs and the effort placed on healthcare workers related to sample collection.
Keywords: COVID-19, gargle lavage, nasal swab, throat swab, saliva, SARS-CoV-2.

INTRODUCTION
Nearly 170 million instances of COVID-19 were documented worldwide, with India being the most severely impacted nation. A comprehensive approach helped bring the epidemic under control. It is crucial to identify and quarantine individuals who have active COVID-19 illness in order to stop its spread, which can only be done with precise detection and testing. Acceptable sample collecting techniques and steady test availability are key components of effective and

accurate testing.[1] Combination NTS (nose and throat swabs) is the most dependable, accurate and frequent sample collection method for COVID-19-affected individuals for RT-PCR. VTM (viral transmission medium), flocked swabs, protective clothing, and qualified healthcare workers are needed for proper sampling of nose and throat swabs. Saliva and gargling is another example approach that may be used as an alternate standard and has more benefits than nasal and throat swabs. In case of nose and throat swabs, a small body of research suggests using gargle and saliva to identify SARS-CoV-2.[2] However, little information is available on the viral RNA stability in saliva and gargle lavage samples for COVID-19 cases. The RNA stability in these samples had a significant impact on the acceptability of the sample collection techniques and the RNA in the samples that were collected. The stability of these samples is required since processing and delivery of the samples take longer than expected.[3] In order to determine and evaluate the SARS-CoV-2 RNA stability at 4°C in normal saline as a transport medium and gargle liquid, the current investigation was carried out. The agreement between saliva/gargle liquid, nose swabs, and throat swabs in identifying SARS-CoV-2 was also evaluated in the current investigation.

METHOD AND MATERIAL

The goal of the current investigation was to determine the SARS-CoV-2 RNA stability at 4°C in normal saline used as a transport medium and gargle solution. The agreement between saliva/gargle liquid, nose swabs, and throat swabs in identifying SARS-CoV-2 was also evaluated in the current investigation. The participants who had been accepted to the Institute with a COVID-19 made up the research population. All subjects gave their verbal and written informed permission after being given a thorough explanation of the study's design. 30 participants from both sexes who had been diagnosed with COVID-19 on RT-PCR and were hospitalized to the hospital's isolation wards within two days (48 hours) after the diagnosis were included in the research. Subjects who were unable to follow directions, which could not gargle and those who were under the age of 18 were excluded from the research. Saliva was collected first, then nasal and throat swabs, and the gargle lavage was done last. Trained healthcare professionals used nylon-flocked swabs to collect throat samples from the tonsillar region and posterior pharyngeal wall, and they took nasal swabs from both nostrils at the middle turbinate levels. The swabs were immediately put into the sterile tube with the VTM (viral transport medium) and sealed after being collected. Saliva samples were taken directly from the patients. In order to collect 2 ml of saliva, subjects spit into the sterile container at varied intervals. Each individual received a jar containing 5 ml of saline for gargle lavage. All of the containers were made outside of the isolation wards to prevent contamination and dissemination. After being

instructed to gargle for about 20 seconds, each subject was instructed to spit into the same container. The samples were sealed in the container after collection and transported in accordance with the guidelines and practices. RNA stability was tested in two aliquots of normal saline gargle samples, one of which was processed with regular saliva and an NTS sample within 4-6 hours and the other after being stored at 4°C for 24–30 hours. Statistical analysis was used to determine how well the cycle threshold (Ct) values for the two aliquots agreed. The samples were then processed and subjected to rRT-PCR with incubation with buffer and were incubated for sample liquefaction. A second PCR test was conducted on samples that were inconclusive in order to evaluate the suitability of the sample collection. Using SPSS software version 21 (Chicago, IL, USA) and one-way ANOVA and t-test for results formulation, the gathered data were statistically evaluated. The information was presented as percentages, numbers, means, and standard deviations. The significance threshold was held at 0.05.

RESULTS

The goal of the current investigation was to determine the SARS-CoV-2 RNA stability at 4°C in normal saline used as a transport medium and gargle solution. The agreement between saliva/gargle liquid, nose swabs, and throat swabs in identifying SARS-CoV-2 was also evaluated in the current investigation. The study included a total of 30 subjects from both genders with a confirmed diagnosis of COVID-19 on RT-PCR and was admitted to the isolation wards of the hospital within 2 days (48 hours) following diagnosis of COVID-19. The demographic characteristics of the study subjects are listed in Table 1. It was seen that mean age in asymptomatic, symptomatic, and total study subjects were 31.5 ± 11.8, 44.4 ± 16.6, and 40.4 ± 16.4 years respectively. In asymptomatic subjects, there were 80% (n=8) males and 20% (n=2) females. SARS-CoV-2 was detected in Gargle lavage (24-30 hrs) sample, Gargle lavage immediate sample, saliva sample, and NTS sample in 70% (n=7) asymptomatic subjects where no comorbidity was seen in any subject. In 20 symptomatic subjects, there were equal males and females with 50% (n=10) subjects and comorbidity in 25% (n=5) subjects. As demonstrated in Table 1, positive was seen in 85% (n=17), 90% (n=18), 80% (n=16), and 85% (n=17) of symptomatic participants in the Gargle lavage (24–30 hours) sample, Gargle lavage immediate sample, saliva sample, and NTS sample, respectively. Comparing the positivity in the nasal throat sample and saliva samples, it was discovered that 13.33% (n=4) of participants with negative NTS and 6.66% (n=2) of those with positive NTS had negative saliva samples. 73.33% (n=22) of the participants with positive NTS and 6.66% (n=2) of the subjects with negative NTS had positive saliva samples. Comparatively, in the present investigation,

there were 205 (n=6) NTS negative samples and 20% (n=6) saliva negative samples, compared to 80% (n=24) NTS positive samples in table 2. There were 3.33% (n=1) negative samples for NTS positive and 16.66% (n=5) negative samples for NTS in the comparison of gargle lavage samples processed after 24–30 hours. For NTS positive samples, there were 80% (n=24) gargle lavage positive samples, however for NTS negative samples, there were none. There

were a total of 83.33% (n=25) positive samples from the gargle lavage and 16.66% (n=5) negative samples. There were 3.33% (n=1) negative samples that were positive for NTS and 13.33% (n=4) positive results for NTS for gargle lavage samples that were processed right away. According to Table 3, no sample tested negative for NTS, and 83.33% (n=25) of the positive gargle lavage samples were.

Characteristics		Asymptomatic % (n=10)	Symptomatic % (n=20)	Total % (n=30)
Mean age (years)		31.5±11.8	44.4±16.6	40.4±16.4
Total		10	20	100 (30)
Gender	Males	80 (8)	10 (50)	18 (60)
	Females	20 (2)	10 (50)	12 (40)
Samples	Gargle lavage (24-30 hrs)	70 (7)	17 (85)	24 (80)
	Gargle lavage (immediately)	70 (7)	18 (90)	25 (83.33)
	Saliva positive	70 (7)	16 (80)	24 (80)
	NTS positive	70 (7)	17 (85)	24 (80)
Comorbidities		0	5 (25)	5 (16.66)

Table 1: demographic characteristics of the study subjects

Parameters	Variables	NTS		
		Positive n[%]	Negative n[%]	Total n[%]
Saliva	Negative	2 (6.66)	4 (13.33)	6 (20)
	Positive	22 (73.33)	2 (6.66)	24 (80)
Gargle lavage (24-30 hrs)	Negative	1 (3.33)	5 (16.66)	6 (20)
	Positive	24 (80)	0	24 (80)
	Total	25 (83.33)	5 (16.66)	30 (100)
Gargle lavage (immediately)	Negative	1 (3.33)	4 (13.33)	5 (16.66)
	Positive	25 (83.33)	0	25 (83.33)
	Total	26 (86.66)	4 (13.33)	30 (100)

Table 2: Effectiveness of nasal throat swabs, saliva, and gargle lavage samples for finding SARS-CoV-2 in study participants.

DISCUSSION

The goal of the current investigation was to determine the SARS-CoV-2 RNA stability at 4°C in normal saline used as a transport medium and gargle solution. The agreement between saliva/gargle liquid, nose swabs, and throat swabs in identifying SARS-CoV-2 was also evaluated in the current investigation. 30 participants from both sexes who had been diagnosed with COVID-19 on RT-PCR and were hospitalized to the hospital's isolation wards within two days (48 hours) after the diagnosis were included in the research. The average age of the asymptomatic, symptomatic, and overall study participants was found to be 31.5 11.8, 44.4 16.6, and 40.4 16.4 years, respectively. There were 80% (n=8) men and 20% (n=2) females among the asymptomatic participants. 70% (n=7) of asymptomatic participants with no signs of comorbidities had SARS-CoV-2 found in Gargle lavage (24–30 hours) sample, Gargle lavage immediate sample, saliva sample, and NTS sample. Equal numbers of men and women (n = 10) and comorbidities (n = 5) were present in the 20 symptomatic participants. Positive results were seen in 85% (n=17), 90% (n=18), 80% (n=16), and 85%

(n=17) of the symptomatic participants in the Gargle lavage (24–30 hours) sample, Gargle lavage immediate sample, saliva sample, and NTS sample, respectively. These demographics were similar to those in the studies conducted by Arora A et al[4] in 2021 and Saito M et al[5] in 2020, which evaluated people who had the same characteristics as the subjects of the current investigation. In the current investigation, saliva and nasal throat samples were also examined for positivity; negative saliva samples were found in 6.66% (n = 2) of participants with positive NTS and 13.33% (n = 4) of subjects with negative NTS. 73.33% (n=22) of the participants with positive NTS and 6.66% (n=2) of the subjects with negative NTS had positive saliva samples. In contrast to the 20% (n=6) negative and 80% (n=24) positive saliva samples, there were 205 (n=6) negative and 80% (n=24) positive NTS samples in the current investigation. These findings were in line with those of Druce J et al[6] in 2012 and van Doremalen N et al[7] in 2020, who found a comparable level of positive in saliva and NTS samples tested for COVID-19.

There were 3.33% (n=1) negative samples for NTS positive and 16.66% (n=5) negative samples for NTS

in the comparison of gargle lavage samples processed after 24–30 hours. For NTS positive samples, there were 80% (n=24) gargle lavage positive samples, however for NTS negative samples, there were none. There were a total of 83.33% (n=25) positive samples from the gargle lavage and 16.66% (n=5) negative samples. There were 3.33% (n=1) negative samples that were positive for NTS and 13.33% (n=4) positive results for NTS for gargle lavage samples that were processed right away. Only one sample tested negative for NTS, making up 83.33% (n=25) of the positive gargle lavage samples. These findings concurred with those of Guo WL et al.[8] and Mittal A et al,[9] who found that gargle samples exhibited a comparable level of positive to NTS swabs as reported in the current investigation.

CONCLUSION

Within its constraints, the current study comes to the conclusion that the RNA stability in the normal saline sample is equivalent to that of other transport media, suggesting that it would be a good replacement for the current medium. According to the findings of the current investigation, SARS-CoV-2 RNA is stable in gargle samples kept in normal saline for around 24–30 hours. To identify SARS-CoV-2 RNA using RT-PCR, suitable and affordable collection techniques include saliva and gargle lavage. These techniques are also accepted, affordable, and straightforward ways to collect samples, lowering costs and the effort placed on healthcare workers related to sample collection. The present study did, however, have certain drawbacks, such as a limited sample size and regional biases. Therefore, more longitudinal research with a bigger sample size will be necessary to draw a firm conclusion.

REFERENCES

1. World Health Organization. Laboratory testing for coronavirus disease 2019 (COVID-19) in suspected human cases: interim guidance, 2 March 2020. Available from: https://apps.who.int/iris/handle/10665/331329, assessed on July 2, 2020.
2. Arora A, Mundhe BA, Patidar H, Dhumale AJ. A Retrospective and descriptive study of clinical profile of Laboratory confirmed COVID -19 patients. Turkish Journal of Physiotherapy and Rehabilitation; 2021, 32:3:26519-23.
3. World Health Organization. WHO Coronavirus Disease (COVID-19) Dashboard. Available from: https://covid19. who. int, accessed on May 30, 2021.
4. Malecki M, Lüsebrink J, Teves S, Wendel AF. Pharynx gargle samples are suitable for SARS-CoV-2 diagnostic use and save personal protective equipment and swabs. Infect Control Hosp Epidemiol 2020;2:248-9.
5. Saito M, Adachi E, Yamayoshi S, Koga M, Iwatsuki-Horimoto K, Kawaoka Y, et al. Gargle lavage as a safe and sensitive alternative to swab samples to diagnose COVID-19: A case report in Japan. Clin Infect Dis 2020;71:893-4.
6. Druce J, Garcia K, Tran T, Papadakis G, Birch C. Evaluation of swabs, transport media, and specimen transport conditions for optimal detection of viruses by PCR. J Clin Microbiol 2012;50:1064-5.
7. van Doremalen N, Bushmaker T, Morris DH, Holbrook MG, Gamble A, Williamson BN, et al. Aerosol and surface stability of SARS-CoV-2 as compared with SARS-CoV-1. N Engl J Med 2020;382:1564-7.
8. Guo WL, Jiang Q, Ye F, Li SQ, Hong C, Chen LY, et al. Effect of throat washings on detection of 2019 novel coronavirus. Clin Infect Dis 2020;71:1980-1.
9. Mittal A, Gupta A, Kumar S, Surjit M, Singh B, Soneja M, et al. Gargle lavage as a viable alternative to swab for detection of SARS-CoV-2. Indian J Med Res 2020;77-81