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SALIVA AS A FEASIBLE OPTION TO THROAT AND NASAL SWABS FOR SARS-COVID-2-RNA STABILITY AND DIAGNOSIS

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ABSTRACT

Background: Currently, the most effective and extensively used method for definitive COVID-19 diagnosis is RT-PCR (reverse transcription-polymerase chain reaction) using a nose and throat swab (NTS). Due to a scarcity of accessible gargle liquid, other sampling procedures such as gargle lavage have had limited use and efficacy.

Aim: The current study aimed to assess the stability of SARS-CoV-2 RNA at 4°C in normal saline, which was utilized as a transport medium and gargle solution. The current study additionally assessed the agreement of saliva/gargle fluids, nose swabs, and throat swabs in diagnosing SARS-CoV-2.

Methods: Paired saliva, gargle, and NTS samples were taken from 30 people who had been diagnosed with COVID-19 using real-time RT-PCR (RT-PCR). The obtained gargle lavage samples were divided into two aliquots to test the stability of SARS-CoV-2 RNA in normal saline. One aliquot was processed 24 to 30 hours after being kept at 4°C, whereas the other was treated with ordinary saliva and an NTS sample in 4-6 hours. Statistical analysis was utilized to examine how well the cycle threshold (Ct) values from the two aliquots agreed.

Results: 13.33% (n=4) of those with negative NTS and 6.66% (n=2) of those with positive NTS had negative saliva samples. 73.33% (n=22) of the patients with positive NTS and 6.66% (n=2) of the respondents with negative NTS had positive saliva tests. In comparing gargle lavage samples processed after 24-30 hours, there were 3.33% (n=1) negative samples for NTS positive and 16.66% (n=5) negative samples for NTS. For NTS positive samples, there were 80% (n=24) gargle lavage positive samples, but no NTS negative samples. **Conclusions**: The current study found that SARS-CoV-2 RNA is stable in gargle samples stored in normal saline for approximately 24-30 hours.

Conclusion: These procedures are also widely recognized, inexpensive, and simple ways to collect samples, saving expenses and reducing the burden on healthcare staff.

Keywords: COVID-19, saliva, nasal swab, throat swab, SARS-CoV-2.

INTRODUCTION

Nearly 170 million COVID-19 cases were reported worldwide, with India being the most seriously affected country. A thorough strategy contributed to the epidemic's suppression. It is critical to identify and confine persons with active COVID-19 infection in order to halt its spread, which can only be accomplished with accurate detection and testing. Acceptable sample collection practices and consistent test availability are critical components of successful and accurate testing.1

Saliva and gargle lavage are appropriate and inexpensive collection procedures for detecting SARS-CoV-2 RNA by RT-PCR. Combination NTS (nose and throat swabs) is the most reliable, accurate, and frequent sample collection approach for COVID-19-infected people for RT-PCR.VTM (viral transmission medium), flocked swabs, appropriate clothes, and skilled healthcare professionals are required for accurate sampling of nose and throat swabs.Saliva and gargling are two approaches that may be employed as an alternative standard and offer more benefits than nasal and throat swabs.For nose and throat swabs, a small amount of studies proposes utilizing gargle and saliva to detect SARS-CoV-2.2.

However, nothing is known about the viral RNA stability in saliva and gargle lavage samples in COVID-19 patients. The RNA stability in these samples had a substantial influence on the acceptability of the sample collecting strategies. The stability of these samples is necessary since processing and transportation take longer than anticipated.3 The current study evaluated SARS-CoV-2 RNA stability at 4°C using normal saline as a transport medium and gargle liquid. The current study additionally assessed the agreement of saliva/gargle fluids, nose swabs, and throat swabs in diagnosing SARS-CoV-2.

MATERIALS AND METHODS

The current study aimed to assess the stability of SARS-CoV-2 RNA at 4°C in normal saline, which was utilized as both a transport medium and gargle solution. The current study additionally assessed the agreement of saliva/gargle fluids, nose swabs, and throat swabs in diagnosing SARS-CoV-2.

The research cohort consisted of volunteers who were admitted to the Institute despite having COVID-19. All subjects provided verbal and written informed consent after being thoroughly explained the study's design. The study comprised 30 patients of both sexes who were diagnosed with COVID-19 using RT-PCR and admitted to the hospital's isolation wards within two days (48 hours) of the diagnosis. Subjects who were unable to follow orders, couldn't gargle, or were under the age of 18 were eliminated from the study. Saliva was collected first, followed by nose and throat swabs, and the gargle lavage was done last.

Trained healthcare practitioners collected throat samples from the tonsillar area and posterior pharyngeal wall with nylon-flocked swabs, as well as nasal swabs from both nostrils at the middle turbinate level. After collection, the swabs were immediately transferred to a sterile tube containing the VTM (viral transport medium) and sealed. Saliva samples were collected directly from patients. To collect 2 ml of saliva, respondents spit into a sterile container at various times. Each person got a jar with 5 mL of saline for gargle lavage. To avoid contamination and spread, all containers were constructed outside of the isolation wards.

Each individual was asked to gargle for about 20 seconds before spitting into the same container. Following collection, the samples were sealed in the container and delivered according to the rules and norms. Two aliquots of normal saline gargle samples were examined for RNA stability: one was processed with regular saliva and an NTS sample within 4-6 hours, and the other was kept at 4°C for 24-30 hours. Statistical analysis was utilized to examine how well the cycle threshold (Ct) values from the two aliquots agreed.

The samples were then processed and submitted to rRT-PCR, followed by buffer incubation and sample liquefaction. A second PCR test was performed on inconclusive samples to determine the appropriateness of the sample collection.

The collected data was statistically examined using SPSS software version 21 (Chicago, IL, USA), as well as one-way ANOVA and t-tests for result formulation. The data were presented as percentages, numbers, means, and standard deviations. The significance level was set at 0.05.

RESULTS

The current study sought to assess the stability of SARS-CoV-2 RNA at 4°C in normal saline, which served as both a transport medium and a gargle solution. The current study additionally assessed the agreement of saliva/gargle fluids, nose swabs, and throat swabs in diagnosing SARS-CoV-2.

The study comprised 30 participants of both genders who had a confirmed diagnosis of COVID-19 on RT-PCR and were hospitalized to the hospital's isolation wards within 2 days (48 hours) after the diagnosis. Table 1 shows the research individuals' demographic characteristics. The mean age of asymptomatic, symptomatic, and total study participants was 31.5±11.8, 44.4±16.6, and 40.4±16.4 years, respectively. Asymptomatic participants consisted of 80% men (n=8) and 20% females (n=2). SARS-CoV-2 was found in a Gargle lavage (24-30 hours) sample, a Gargle lavage immediate sample, a saliva sample, and an NTS sample in 70% (n=7) of asymptomatic participants with no comorbidities.

There were similar numbers of males and females among the 20 symptomatic participants, with 50% (n=10) having comorbidities and 25% (n=5) having none. As shown in Table 1, 85% (n=17), 90% (n=18), 80% (n=16), and 85% (n=17) of symptomatic patients tested positive in the Gargle lavage (24-30 hours) sample, Gargle lavage immediate sample, saliva sample, and NTS sample, respectively. When the positivity in the nasal throat sample was compared to that in the saliva samples, it was observed that 13.33% (n=4) of those with negative NTS and 6.66% (n=2) of those with positive NTS had negative saliva samples. Positive saliva samples were obtained by 73.33% (n=22) of those with positive NTS and 6.66% (n=2) of those with negative NTS. In comparison, in the current study, there

were 205 (n=6) NTS negative samples and 20% (n=6) saliva negative samples, as opposed to 80% (n=24) NTS positive samples in table 2.

In comparing gargle lavage samples processed after 24-30 hours, there were 3.33% (n=1) negative samples for NTS positive and 16.66% (n=5) negative samples for NTS. For NTS positive samples, there were 80% (n=24) gargle lavage positive samples, but no NTS negative samples. The gargle lavage yielded 83.33% (n=25) positive samples and 16.66% (n=5) negative results. Positive NTS findings were found in 3.33% (n=1) of negative samples and 13.33% (n=4) of gargle lavage samples that were processed immediately.

According to Table 3, no samples tested negative for NTS, but 83.33% (n=25) of the positive gargle lavage samples did.

DISCUSSION

The current study aimed to assess the stability of SARS-CoV-2 RNA at 4°C in normal saline, which was utilized as both a transport medium and gargle solution. The current study additionally assessed the agreement of saliva/gargle fluids, nose swabs, and throat swabs in diagnosing SARS-CoV-2. The study comprised 30 patients of both sexes who were diagnosed with COVID-19 using RT-PCR and admitted to the hospital's isolation wards within two days (48 hours) of the diagnosis. The asymptomatic, symptomatic, and overall study participants had an average age of 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) women among the asymptomatic individuals. SARS-CoV-2 was identified in 70% (n=7) of asymptomatic patients with no symptoms of comorbidities, including Gargle lavage (24-30 hours), Gargle lavage immediate, saliva, and NTS samples. The 20 symptomatic patients included an equal number of males and women (n = 10), as well as five comorbidities. Positive findings were obtained by 85% (n=17), 90% (n=18), 80% (n=16), and 85% (n=17) of the symptomatic patients in the Gargle lavage (24-30 hours) sample, Gargle lavage immediate sample, saliva sample, and NTS sample, respectively.

These demographics were comparable to those found in studies done by Arora A et al4 in 2021 and Saito M et al5 in 2020, both of which examined adults with similar characteristics to the subjects of the current study. In the current study, saliva and nasal throat samples were also tested for positivity; negative saliva samples were detected in 6.66% (n = 2) of individuals with positive NTS and 13.33% (n = 4) of people with negative NTS.

3.33% (n=22) of the patients with positive NTS and 6.66% (n=2) of the respondents with negative NTS had positive saliva tests. Compared to the 20% (n=6) negative and 80% (n=24) positive saliva samples, the current study had 205 (n=6) negative and 80% (n=24) positive NTS samples.

These findings were consistent with those of Druce J et al.6 in 2012 and van Doremalen N et al.7 in 2020, who discovered a similar level of positive in saliva and NTS samples tested for COVID 19. In comparing gargle lavage samples processed after 24-30 hours, there were 3.33% (n=1) negative samples for NTS positive and 16.66% (n=5) negative samples for NTS. For NTS positive samples, there were 80% (n=24) gargle lavage positive samples, but no NTS negative samples. The gargle lavage produced a total of 83.33% (n=25) positive samples and 16.66% (n=5) negative ones.

Positive NTS findings were found in 3.33% (n=1) of negative samples and 13.33% (n=4) of gargle lavage samples that were processed immediately. Only one sample tested negative for NTS, representing 83.33% (n=25) of the positive gargle lavage samples. These findings were consistent with those of Guo WL et al.8 and Mittal A et al.9, who discovered that gargle samples had a similar degree of positivity to NTS swabs, as described in the current study.

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.

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TABLES

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.