Original Research Article

Assessing the role of saliva and gargle lavage as a cost-effective alternative to the throat and nasal swabs for diagnosing Covid-19

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Abstract

Background: Presently, the most reliable and common method for definitive diagnosis of COVID-19 is the use of nasal swabs and throat swabs (NTS) by RT-PCR (reverse transcription-polymerase chain reaction). Limited use and efficacy of other sampling methods like gargle lavage have been seen clinically owing to the non-availability of gargle liquid. **Aims:** The present study was conducted to assess and evaluate the SARS-CoV-2 RNA stability at 4° C in the normal saline as a transport medium and gargle liquid. The present study also assessed the agreement of saliva/gargle liquid and nasal swabs and throat swabs in detecting SARS-CoV-2. **Methods:** In 30 subjects who had confirmed positive real-time RT-PCR (RT-PCR) positive diagnosis for COVID-19, paired samples of saliva, gargles, and NTS were acquired. For detection of SARS-CoV-2 RNA stability in the normal saline, the collected gargle lavage samples were divided into two aliquots where one sample was processed after 24-30 hours after storing at 4° C, whereas, another sample was processed with routine saliva and NTS sample within 4-6 hours. The agreement was assessed between cycle threshold (Ct) values for the two aliquots using statistical analysis. **Results:** Negative saliva samples were seen in 6.66% (n=2) subjects with positive NTS and 13.33% (n=4) subjects with negative NTS. A positive saliva sample was seen in 73.33% (n=22) subjects with positive NTS and 6.66% (n=2) subjects with negative NTS. Concerning the comparison of gargle lavage samples processed after 24-30 hours, there was a 3.33% (n=1) negative sample for NTS positive and 16.66% (n=5) for NTS negative. There were 80% (n=24) gargle lavage positive samples for NTS positive and no positive sample for NTS negative. There was total 83.33% (n=25) gargle lavage positive samples and 16.66% (n=5) gargle lavage samples negative samples. For gargle lavage samples processed immediately, there were 3.33% (n=1) negative samples that were positive for NTS and 13.33% (n=4) samples that were positive for NTS. **Conclusions:** The present study concludes that SARS-CoV-2 RNA remains stable in the gargle samples stored in the normal saline for nearly 24-30 hours. Saliva and gargle lavage serves as acceptable and cost-effective sampling methods for detecting SARS-CoV-2 RNA using RT-PCR. These methods are also acceptable, inexpensive, and simplified methods of collecting samples reducing expenses and workload on the healthcare professionals concerning the sample collection.

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

Introduction

COVID-19 was declared a pandemic in 2020 by WHO and is caused by SARS-CoV-2. COVID-19 was reported in more than 150 countries across the globe with nearly 170 million cases with India being the most affected country. With multidisciplinary involvement, the pandemic was bought under control. To limit its spread, it is vital to detect and isolate subjects with active COVID-19 disease which is possible only by accurate detection and testing. Effective and accurate testing relies largely on acceptable sample collection methods and reliable test availability[1].

The most reliable, accurate, and common sample collection of COVID-19 affected subjects for RT-PCR is combined NTS (nasal and throat swabs). Proper sampling of nasal and throat swabs requires VTM (viral transport medium), flocked wabs, protective gears, and trained healthcare professionals. Another sample method serving as alternative criteria is saliva and gargle that can have more advantages in comparison to nasal and throat swabs. Few literature data suggest the use of saliva and gargle as an alternative to nasal and throat swabs in detecting SARS-CoV-2. However, scarce data focus on viral RNA stability on gargle lavage and saliva samples for COVID-19 specimens[2].

Acceptability of the sample collection methods and RNA in the collected samples was largely affected by the RNA stability in these samples. Considering the delay in processing and transport of the samples, the stability of these samples is necessary[3]. Hence, the present study was conducted to assess and evaluate the SARS-CoV-2 RNA stability at 4° C in the normal saline as a transport medium and gargle liquid. The present study also assessed the agreement of saliva/gargle liquid and nasal swabs and throat swabs in detecting SARS-CoV-2.

Material and methods

The present study was conducted to assess and evaluate the SARS-CoV-2 RNA stability at 4° C in the normal saline as a transport medium and gargle liquid. The present study also assessed the agreement of saliva/gargle liquid and nasal swabs and throat swabs in detecting SARS-CoV-2. The study population was comprised of the subjects with COVID-19 admitted to the Institute. After explaining the detailed study design, informed consent was taken from all the subjects in both written and verbal form.

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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 exclusion criteria for the study were subjects who were not able to follow the instructions, who could not gargle, and the subjects who were younger than 18 years.

Nasal and throat swabs were collected first followed by the collection of saliva and the gargle lavage at the last. Throat samples were collected from the tonsillar area and posterior pharyngeal wall with nylon-flocked swabs by trained healthcare professionals and the nasal swabs were collected from both the nostrils at the middle turbinate levels. Following the collection of the swabs, they were placed into the sterile tube immediately in the VTM (viral transport medium) and were sealed. Samples from the saliva were collected by the subjects themselves. Subjects spitted saliva various times in the sterile container where 2ml saliva was collected.

For gargle lavage, a container having 5 ml saline was given to all the subjects. To avoid contamination and spread, all the containers were prepared outside the isolation wards. All the subjects were asked to gargle for nearly 20 seconds and they spitted back in the same container. Following collection, the samples were sealed in the container and were transported following the instructions and protocols.

RNA stability was assessed in the gargle samples of normal saline which were divided into two aliquots where one sample was processed after 24-30 hours after storing at 4° C, whereas, another sample was processed with routine saliva and NTS sample within 4-6 hours. The agreement was assessed between cycle threshold (Ct) values for the two aliquots using statistical analysis. The samples were then processed and subjected to rRT-PCR with incubation with buffer and were incubated for sample liquefaction. In inconclusive samples, an additional PCR test was done to assess the adequacy of the collected samples.

The collected data were subjected to the statistical evaluation using SPSS software version 21 (Chicago, IL, USA) and one-way ANOVA and t-test for results formulation. The data were expressed in percentage and number, and mean and standard deviation. The level of significance was kept at $p<0.05$.

Results

The present study was conducted to assess and evaluate the SARS-CoV-2 RNA stability at 4° C in the normal saline as a transport medium and gargle liquid. The present study also assessed the agreement of saliva/gargle liquid and nasal swabs and throat swabs in detecting SARS-CoV-2. 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.

Table 1: demographic characteristics of the study subjects

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 number (n)		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)

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. In symptomatic subjects, in Gargle lavage (24-30 hrs) sample, Gargle lavage immediate sample, saliva sample, and NTS sample, positivity was seen in 85% (n=17), 90% (n=18), 80% (n=16), and 85% (n=17) subjects respectively as shown in Table 1.

On comparing the positivity in the nasal throat sample and saliva samples, negative saliva samples were seen in 6.66% (n=2) subjects with positive NTS and 13.33% (n=4) subjects with negative NTS. A positive saliva sample was seen in 73.33% (n=22) subjects with positive NTS and 6.66% (n=2) subjects with negative NTS. There were 20% (n=6) saliva negative and 80% (n=24) saliva positive samples, whereas, there was a total of 80% (n=24) NTS positive and 205 (n=6) NTS negative samples in the present study as depicted in table 2.

Table 2: Comparison of nasal throat swabs to saliva and gargle lavage samples in detecting SARS-CoV-2 in the study subjects

Parameters	Variables	NTS		
		Positive	**Negative**	**Total**
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)

Concerning the comparison of gargle lavage samples processed after 24-30 hours, there was a 3.33% (n=1) negative sample for NTS positive and 16.66% (n=5) for NTS negative. There were 80% (n=24) gargle lavage positive samples for NTS positive and no positive sample for NTS negative. There was total 83.33% (n=25) gargle lavage positive samples and 16.66% (n=5) gargle lavage samples negative samples. For gargle lavage samples processed immediately, there were 3.33% (n=1) negative samples that were positive for NTS and 13.33% (n=4) samples that were positive for NTS. There were 83.33% (n=25) positive gargle lavage samples that were positive for NTS and no sample was negative for NTS as shown in Table 3.

Discussion

The present study was conducted to assess and evaluate the SARS-CoV-2 RNA stability at 4° C in the normal saline as a transport medium and gargle liquid. The present study also assessed the agreement of saliva/gargle liquid and nasal swabs and throat swabs in detecting SARS-CoV-2. 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.

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. In symptomatic subjects, in Gargle lavage (24-30 hrs) sample, Gargle lavage immediate sample, saliva sample, and NTS sample, positivity was seen in 85% (n=17), 90% (n=18), 80% (n=16), and 85% (n=17) subjects respectively. These demographics were comparable to the studies of Arora A et al[4] in 2021 and Saito M et al[5] in 2020 where authors assessed subjects of similar characteristics as in the present study.

The present study also compared the positivity in the nasal throat sample and saliva samples, negative saliva samples were seen in 6.66% (n=2) subjects with positive NTS and 13.33% (n=4) subjects with negative NTS. A positive saliva sample was seen in 73.33% (n=22) subjects with positive NTS and 6.66% (n=2) subjects with negative NTS. There were 20% (n=6) saliva negative and 80% (n=24) saliva positive samples, whereas, there was a total of 80% (n=24) NTS positive and 205 (n=6) NTS negative samples in the present study. These results were consistent with the results of Druce J et al[6] in 2012 and van Doremalen N et al[7] in 2020 where authors showed similar positivity in saliva and NTS samples assessed for COVID-19.

Concerning the comparison of gargle lavage samples processed after 24-30 hours, there was a 3.33% (n=1) negative sample for NTS positive and 16.66% (n=5) for NTS negative. There were 80% (n=24) gargle lavage positive samples for NTS positive and no positive sample for NTS negative. There was total 83.33% (n=25) gargle lavage positive samples and 16.66% (n=5) gargle lavage samples negative samples. For gargle lavage samples processed immediately, there were 3.33% (n=1) negative samples that were positive for NTS and 13.33% (n=4) samples that were positive for NTS. There were 83.33% (n=25) positive gargle lavage samples that were positive for NTS and no sample was negative for NTS. These results were in agreement with the findings of Guo WL et al[8] in 2020 and Mittal A et al[9] in 2020 where gargle samples showed similar positivity to NTS swabs as reported in the present study.

Conclusion

Within its limitations, the present study concludes that RNA stability in the normal saline sample is comparable to other transport media which could act as a suitable alternative to the existing medium. The present study concludes that SARS-CoV-2 RNA remains stable in the gargle samples stored in the normal saline for nearly 24-30 hours. Saliva and gargle lavage serves as acceptable and cost-effective sampling methods for detecting SARS-CoV-2 RNA using RT-PCR. These methods are also acceptable, inexpensive, and simplified methods of collecting samples reducing expenses and workload on the healthcare professionals concerning the sample collection. However, the present study had a few limitations including a small sample size and geographical area biases. Hence, more longitudinal studies with a larger sample size will help reach a definitive conclusion.

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