



Gargle lavage as a viable alternative to swab for detection of SARS-CoV-2

Ankit Mittal¹, Ankesh Gupta¹, Shiv Kumar³, Milan Surjit³, Binit Singh¹, Manish Soneja¹, Kapil Dev Soni², Adil Rashid Khan¹, Komal Singh¹, Shivdas Naik¹, Arvind Kumar¹, Richa Aggarwal², Neeraj Nischal¹, Sanjeev Sinha¹, Anjan Trikha² & Naveet Wig¹

Departments of ¹Medicine & ²Anaesthesia & Critical Care Medicine, All India Institute of Medical Sciences, New Delhi & ³Vaccine & Infectious Disease Research Centre, Translational Health Science and Technology Institute, Faridabad, Haryana, India

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Background & objectives: Nasopharyngeal and oropharyngeal swab (NPS and OPS) collection is widely accepted as the preferred method for obtaining respiratory samples. However, it has certain disadvantages which may be overcome by gargling. The primary objective of this study was to assess agreement between gargle lavage and swab as an appropriate respiratory sample for the detection of SARS-CoV-2. The secondary objective was to assess the patient acceptability of the two sampling methods.

Methods: It was a cross-sectional study done at a tertiary care hospital in New Delhi, India, on 50 confirmed COVID-19 patients. Paired swab (NPS and OPS) and gargle samples were taken within 72 h of their diagnosis. Samples were processed by reverse transcription-polymerase chain reaction (RT-PCR) for detection of SARS-CoV-2. Post-sample collection, a 10-point scale was administered to assess the level of discomfort with either of the collection methods.

Results: All gargle samples were positive and comparable to their corresponding swab samples irrespective of the symptoms and duration of illness. The cycle threshold (C_t) values for gargle samples were slightly higher but comparable to those of swabs. Bland-Altman plot showed good agreement between the two methods. Majority (72%) of the patients reported moderate-to-severe discomfort with swab collection in comparison to 24 per cent reporting only mild discomfort with gargle collection.

Interpretation & conclusions: Our preliminary results show that the gargle lavage may be a viable alternative to swabs for sample collection for the detection of SARS-CoV-2. Adoption of gargle lavage for sample collection will have a significant impact as it will enable easy self-collection, relieve healthcare workers and also lead to substantial cost savings by reducing the need for swabs and personal protective equipment.

Key words COVID-19 - gargle lavage - nasopharyngeal swab - oropharyngeal swab - SARS-CoV-2

The pandemic caused by SARS-CoV-2 has already led to more than 20 million cases and more than 700,000 deaths¹. The global alliance against the COVID-19 pandemic has mostly focussed on finding a cure, developing a vaccine or using better diagnostic tests. However, a crucial factor that has been missed out is to reassess the traditional practices, one of which is methods for the collection of respiratory samples. Among the various sample collection methods that are currently approved [nasal/nasopharyngeal/throat swabs, sputum, nasopharyngeal aspirate, bronchoalveolar lavage (BAL), *etc.*] swabs are the most commonly employed method. Swab collection has several drawbacks also as it requires training, exposes the healthcare workers (HCWs) to the virus-containing aerosols, has poor patient acceptability and is resource intensive. An alternative sample collection method that could overcome most of these limitations without compromising the yield of the test is the need of the hour. One such method is the collection of gargle lavage. Although the use of gargle specimens is not new, at present, there is little published information on the suitability of gargle specimens to diagnose SARS-CoV-2 infection. This study was, therefore, conducted to assess the performance of gargle lavage in comparison to nasopharyngeal and oropharyngeal swabs (NPS and OPS) for the detection of SARS-CoV-2.

Material & Methods

This was a cross-sectional study conducted at the All India Institute of Medical Sciences (AIIMS), New Delhi, India, over a period of one month (May-June 2020). Ethical clearance was obtained from the Institutional Ethics Committee (IECPG: 193/20.05.2020).

The primary objective was to evaluate the agreement between gargle lavage and swabs (NPS and OPS) as a method for collection of respiratory samples for the detection of SARS-CoV-2. The secondary objective was to measure the level of discomfort/acceptance of patients for both the sampling methods. After taking individual's written informed consent, paired swab and gargle lavage samples were collected from 50 consecutive reverse transcription-polymerase chain reaction (RT-PCR)-confirmed patients with SARS-CoV-2 infection within 72 h of diagnosis by a trained healthcare professional. All patients were admitted as per the existing national and hospital policies at the time of this study. Children (age <18 yr) and patients (n=7) who did not consent or who were

unable to perform gargle/follow instructions were excluded (visibly breathless, critically ill and patients with altered sensorium). Post-sample collection, a numerical rating scale² was administered to assess the level of discomfort perceived by the patients during sample collection by both the methods. Patients ranked their pain/discomfort on a scale of 0 to 10, with a score of 0 implying no discomfort and 10 implying severe discomfort.

Sample collection: (i) NPS collection: The patient's head was tilted back to an angle of 70°. A flexible swab (flocked with medical-grade nylon microfibres) was inserted through the nares parallel to the palate (not upwards) until resistance was encountered or the distance was equivalent to that from the ear to the nostril of the patient. The swab was gently rubbed and rolled and then left in place for several seconds to absorb secretions before removing. (ii) OPS collection: Swab was inserted into the posterior pharynx and tonsillar areas. It was rubbed over both the tonsillar pillars and posterior oropharynx. Touching the tongue, teeth and gums was avoided. After collection, both the swabs were put into a single tube containing 2 ml normal saline and were secured with a screw cap. (iii) Gargle lavage collection: The participants were provided with pre-filled screw-capped containers (containing 8-10 ml normal saline) and asked to perform gargle for 15-20 sec and spit back into the container. The collection vials were prepared outside the COVID ward using sterile normal saline to avoid any contamination. These were opened inside the ward only at the time of sample collection.

Laboratory methods: Viral RNA was isolated using QIAamp viral RNA mini kit, following the manufacturer's instructions (Qiagen, Hilden, Germany). Briefly, 140 µl of swab (NPS and OPS) sample or gargle lavage was mixed with 560 µl AVL buffer containing one per cent carrier RNA (5.6 µl), and viral RNA was purified through different steps; 5 µl of RNA was subjected to RT-PCR analysis using the primers and probes recommended by the CDC, USA. SOLIScript 1-step Probe Kit (Solis BioDyne, Newmarket Scientific, UK) was used to perform the RT-PCR, with TaqMan reagents to detect the target sequence. The results were given as qualitative results (positive or negative) and cycle threshold (C_t) values. Only when all controls exhibited the expected performance and both 2019-nCoV marker (N1 and N2) C_t growth curves crossed the threshold line within

40.00 cycles ($<40.00 C_t$), the test was considered positive³.

Statistical analysis: Categorical variables were represented by counts and percentages, whereas quantitative variables were represented by mean \pm standard deviation (SD). Agreement between the two modalities was assessed by Bland-Altman (BA) analysis. A scatter plot was constructed in which the differences between the paired measurements (C_t values) were plotted on Y-axis and average of the measures (C_t values) of the two methods on X-axis. The mean difference (bias) in values obtained with the two methods was represented by a central horizontal line on the plot. The SD of differences between paired measurements was used to construct horizontal lines above and below the central horizontal line to represent the upper and lower limits of agreement (mean bias \pm 1.96 SD). The data were analyzed using IBM SPSS Statistics for Windows, version 25 (IBM Corp., Armonk, NY, USA).

Results

The mean age of the study population ($n=50$) was 45.08 ± 12.78 yr; 60 per cent were male and 22 per cent were asymptomatic at the time of study. The demographic characteristics of the patients are summarized in the Table. Although patients were included within 72 h of confirmed diagnosis for this study, there was a wide variation in the duration of illness among symptomatic cases (Table).

Table. Demographics and self-reported clinical symptoms

Total number of patients	50
Age (mean \pm SD), yr	45.08 \pm 12.78
Male (%)	30 (60)
Asymptomatic (%)	11 (22)
Symptomatic (%)	
Duration of illness up to 7 days	30 (76.9)
Duration of illness >7 days	9 (23.1)
Frequency of commonly reported symptoms (%)	
Fever	28 (71.8)
Sore throat	12 (30.8)
Cough	17 (43.6)
Shortness of breath	15 (38.47)
Myalgia	8 (20.5)

Paired samples (NPS with OPS and gargle lavage) were collected from the 50 patients and analyzed by RT-PCR for SARS-CoV-2 as described. All gargle samples were positive and comparable to their corresponding swab samples. The mean C_t values obtained for each marker (N1 and N2) for every swab and gargle pair is plotted as a difference plot (Figure A and B) using the BA analysis. Although the C_t values from the gargle were slightly higher as compared to those of swab samples (bias -2.295 for target N1 and -2.528 for target N2), the C_t growth curves crossed the threshold line within 40 cycles. Patients with a duration of illness for more than seven days ($n=9$) had a higher mean C_t value compared with patients with illness duration up to seven days ($n=30$). There were no discrepant results in the analyzed samples despite variation in the symptoms and duration of illness.

In all, 28 per cent (14/50) reported mild discomfort (score 1-3), 48 per cent (24/50) complained of moderate discomfort (score 4-6) and 24 per cent (12/50) reported severe discomfort (score 7-10) with swab collection. On the other hand, only 24 per cent (12/50) reported mild discomfort (mainly attributable to the salty taste), whereas 76 per cent (38/50) complained of no discomfort with gargle collection. The median discomfort score was 5.5 for swab collection versus 0 for gargle collection.

Discussion

Currently, various sample collection methods are approved such as OPS, NPS and nasopharyngeal aspirates for upper respiratory tract specimens as well as sputum, tracheal aspirate and BAL fluid for lower respiratory tract specimen⁴. Both OPS and NPS have certain limitations, while gargle is an easy-to-perform procedure, can be performed by the patients themselves without much training and may have better patient acceptability. The adoption of gargle for sample collection will translate to substantial cost savings as it would cut down not only the need for swabs and personal protective equipment (PPE) but also the need to develop and maintain special infrastructure for swab collection. This was demonstrated in a study conducted in Germany where the authors utilized this method for testing of HCWs for COVID-19. They tested 924 HCWs using gargle and consequently saved 225 PPEs and 1000 swabs⁵.

The current practice of collection of swabs requires trained professionals who get exposed to the virus-containing aerosols and remain at high-

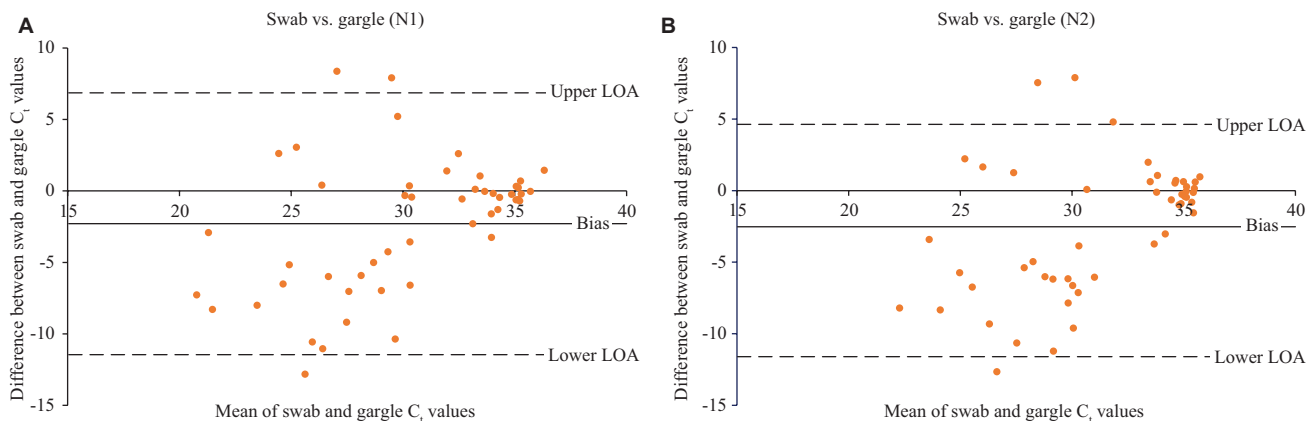


Figure. Difference plot of C_t values of swab (NPS and OPS) versus gargle samples in sample pairs for N1 and N2 targets (**A** and **B**, respectively). Gargle samples are comparable to the paired swab samples as a method for respiratory sample collection. However, C_t values of the gargle samples were higher than that of the swab, as indicated by the line of mean difference (bias of -2.295 for target N1 and -2.528 for target N2). The dotted lines represent upper and lower limits of agreement (LOA).

risk of acquiring the infection. Though Tu *et al*⁶ have suggested that self-collected nasal and middle turbinate swabs may be clinically acceptable with sensitivities above 90 per cent, the lower bound of the confidence interval was <90 per cent. Gargle samples, on the other hand, did not miss any of the cases in this study. Gargle can be self-collected at home and submitted at designated collection centres, thus overcoming most of the difficulties surrounding sample collection by swabbing.

Bennett *et al*⁷ compared throat swab to gargle samples for the detection of respiratory pathogens and demonstrated that gargle samples were more sensitive than throat swab, and the overall diagnostic yield was higher in the gargle samples. Similarly, Saito *et al*⁸ showed higher viral load of SARS-CoV-2 in gargle sample as compared to swab. In our study, the C_t values were slightly higher in the gargle samples. This may be explained by low viral load in pharyngeal samples as compared to nasal samples and the possible effect of dilution as gargle was collected with 10 ml normal saline, whereas swabs were put in vials with 2 ml normal saline after collection⁹.

Another major advantage of gargle is its better acceptability. While the majority complained of moderate-to-severe discomfort with swabs, only a small proportion of patients reported mild discomfort with gargling. A similar study evaluated patients' perception of nasopharyngeal aspirate collection and reported that 26 per cent found the procedure very uncomfortable and 34 per cent found it more uncomfortable than blood collection by venepuncture¹⁰.

A disadvantage of gargling could be the generation of infectious aerosols. Whether the risk of aerosol generation was similar to swab collection (commonly leads to coughing and sneezing) or higher was not clear. To minimize the risk of transmission due to aerosols and to maximize the benefits of this method of collection, it would be best to employ it for home collection. Furthermore, it cannot be used in patients who are critically ill as well as in young children/patients (altered sensorium, *etc.*), who may not be able to follow instructions/perform gargle.

The major limitation of this study was its cross-sectional design and it was performed only on a limited number of positive cases. In addition, it would be necessary to evaluate the performance using different viral RNA isolation platforms.

In conclusion, the study highlights the usefulness of gargle lavage as an appropriate respiratory sample collection method. It is a viable alternative to conventional swab collection with several distinct advantages and will have significant clinical and public health impacts in terms of better acceptability, easy self-collection, sparing of HCWs and cost-effectiveness.

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Conflicts of Interest: None.

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For correspondence: Dr Naveet Wig, Department of Medicine, 3rd Floor, Teaching Block, All India Institute of Medical Sciences, New Delhi 110 029, India
e-mail: naveetwig@gmail.com