Gargle and mouthwash can replace nasopharyngeal swab sampling when concentrated with a new method for diagnosis of COVID-19

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Abstract

Diagnosis of COVID19 is based on reverse transcription-polymerase chain reaction (RT-PCR) testing of nasopharyngeal swab (NPS) samples. As NPS sampling is a discomforting invasive procedure, studies were conducted to investigate the performance of saliva and mouthwash (MW) samples in RT-PCR. There is limited data about MW samples. We have developed a new method for concentrating gargle and mouthwash (GMW) samples to be used in RT-PCR. In our study, we aimed to investigate the performance of concentration of GMW samples in detection of SARS-CoV-2. A paired sample of NPS and GMW samples were collected from patients in 11 centers in Turkey. MW samples were concentrated using MyMagiCon-RW100® (Bio-T, Istanbul, Turkey). NPS, GMW and concentrated GMW samples were tested by RT-PCR for the presence of SARS-CoV2 and the results were compared. The viral RNA was detected in 47.5% of NPS samples, in 28.8% of GMW before concentration and in 37.5% GMW samples after concentration. Concentration of samples increased the number of samples in which SARS-CoV2 RNA was detected by 16.6%. The RT-PCR of concentrated GMW samples yielded better results than the RT-PCR of NPS samples in two centers. Concentrated GMW sampling can be an alternative method to NPS sampling in rapid and accurate diagnosis of COVID-19.

Introduction

The pandemic of the coronavirus disease 2019 (COVID-19) which is caused by a severe acute respiratory coronavirus 2 (SARS-CoV-2) continues to infect many people worldwide. Currently, the diagnosis of COVID-19 is mainly based on reverse transcription-polymerase chain reaction (RT-PCR) testing [1,2]. The major clinical specimen for RT-PCR is nasopharyngeal swab (NPS) sampling which is an invasive method with complications like epistaxis. It requires expertized personnel and poses increased risk of viral transmission to the sampling person. Besides it requires continuous supplies of swabs and transport media. Therefore, alternative sample collection strategies which has less threat of transmission and more comfort are needed.

For detection of SARS-CoV-2 by PCR, which clinical specimen is more appropriate, is still unclear. Saliva or MW has many advantages over NPS because of easier collection of samples, having no need for experienced personnel and minimal requirement of supplies. Therefore, in various studies as a clinical sample for RT-PCR, saliva or mouth washing (MW) were investigated whether it is a reliable tool or not for COVID-19 diagnosis and monitoring when compared with gold standard samples (swab of nasopharynx or throat). Although there are numerous studies with saliva, only limited studies were found in literature with MW [3,4].

In this study we aimed to investigate the performance of a new method and tool, for concentrating liquid biological samples, which enables to use gargle and mouth-wash (GMW) samples in RT-PCR, as an alternative to NFS samples, which can be collected without the need of a health-personnel.

Materials And Methods

A paired sample of GMW and NPS were prospectively collected from 11 centers in Turkey. The patients presenting with signs/symptoms suggesting SARS-CoV-2 infection underwent a NPS and GMW collection. All the patients enrolled to the study, acknowledged understanding the aims of the study and signed a consent form prior to collection of clinical samples.

After collecting NPS samples, patients were instructed to take a few sips of regular drinking water, and then to gargle and rigorously rinse their mouth forcefully with this water for at least 10 seconds and put it back to an empty sterile collection cup. 20 ml MW was collected.

Gargle and mouthwash samples were concentrated using MyMagiCon-RW100® (Bio-T, Istanbul, Turkey) as instructed in the user guide. Briefly, 20 mL of sample was put into the tube and waited for 5 minutes for the absorbent beads to swell and absorb most of the fluid and the mixture turned in a gel-like form. The concentrated sample was collected with an automatic pipette by inserting the pipette tip in between the beads and aspirating the fluid (Figure 1).

The RT-PCR was performed by using commercial PCR kits (Bioeksen and A1 Lifesciences, İstanbul, Turkey) and the results obtained from NPS and GMW samples, before and after concentration, were compared.

Since we have observed variability in the sensitivity of detection of SARS-CoV2 by RT-PCR in concentrated GMW between study centers we have also investigated the effect of the brand of drinking water used in different centers. For this purpose, we have prepared simulated GMW samples by adding SARS-CoV2 grown in cell culture, to GMW of healthy volunteers at the same concentration and studied them by RT-PCR and compared the Ct values.

Statistical analysis

Categorical data were given in numbers and percentages and continuous variables were presented as median. Pearson Chi-square test and McNemar's test were used for the diagnostic performance of PCR tests applied to nasopharyngeal swap sample (NPS), mouthwash sample (MW) and mouthwash sample concentrated with *MyMagiCon*® (MMC-MW), a p value less than or equal to 0.05 was considered significant. Statistical analyses were performed with a software (IBM SPSS Statistics for Windows, version 23.0).

Results

A total of 1721 patients were recruited into the study and samples pairs of NPS and GMW were collected. The PCR results of NPS, GMW and concentrated GMW of 11 centers are given in Table 1 and Figure 2.

In all centers concentration of GMW samples increased the sensitivity of COVID-19 detection. It was determined that the PCR positivity between GMW and concentrated GMW, increased in 16.6 % (ranging

from 8 to 38.5 % in different centers). In two centers concentrated GMW detected more positive cases than NPS samples.

When the results of 11 centers were evaluated, the percentage of PCR positivity of NPS, GMW and concentrated GMW showed prominent variability. This variability turned out to be mainly to the brand of water used in obtaining GM samples (Figure 3). Additionally due to the inclusion of only patients with positive NPS PCR in some centers.

The viral RNA was detected in 817 (90.7%) nasopharyngeal, in 495 (55%) gargle and mouthwash before concentration and in 645 (71.6%) after concentration among the total RT-PCR positive patients.

A total of 902 patients (52.4%) tested positive for the SARS-CoV-2 virus by NPS and/or GMW and/or concentrated GMW. In 43.3% patients SARS-CoV2 RNA was positive only in NPS samples. On the other hand it was positive in 3.4% and 9.0% of the GMW and concentrated GMW samples respectively, without being positive in NPS samples.

There was a moderate agreement between PCR tests using NPS and MMC-MW and significant difference in the detection rate (McNemar's test $\kappa=0.605$, $p<0.01$). Concentration of samples increased the number of samples in which SARS-CoV-2 RNA was detected by 16.6 %. This increase varied between 8 and 38.5% among centers.

Discussion

Nasopharyngeal or throat swab are uncomfortable invasive procedures, which increase the infection risk to health care workers. Besides being time-consuming, it is labor intensive and depends on the availability of supplies such as nasal swabs and transport media. Therefore, an alternative diagnostic specimen which is noninvasive, easy to collect, cost-effective and having low COVID-19 infection risk for healthcare workers, is urgent necessity. For this purpose, saliva [5-8] and mouthwash samples [3,4,9] were used in different studies [3-9]. Although there are numerous studies with saliva, only limited studies were found with mouthwash samples [3,4].

In our study we investigated the performance of **MyMagiCon-RW100®**, a new method which enables concentrating GMW samples for increasing the sensitivity of detecting microorganism by RT-PCR. We also compared the diagnostic utility of GMW and concentrated GMW samples with gold standard sample, NPS.

Overall, concentrated GMW-based SARS-CoV-2 testing showed moderate concordance and sensitivity with nasal swab samples. However, the PCR of concentrated GMW samples yielded better results than the PCR of NPS in two centers. In all centers concentration of GMW samples increased the sensitivity of COVID-19 detection. The PCR positivity between GMW and concentrated GMW increased in 16.6 % (ranging from 8 to 38.5% in different centers).

When the results of each center examined separately, the percentage of PCR positivity of NPS, GMW and concentrated GMW showed variability. There can be several reasons for this variability. First of all, in some centers only patients with positive NPS were included. However, there can be COVID-19 patients with negative PCR results in NPS samples which was observed in centers which included all NPS samples either positive or negative by PCR. Secondly, we have shown that different brands of drinking water used across the centers, which affected the RT-PCR amplification, is another explanation for false negative results. Therefore, adding a standard drinking water, which is shown not to inhibit PCR, as a part of MyMagiCon-RW100® kit can eliminate false negative PCR results. It will be beneficial, standardization of the collection of GMW sampling technique and the mouthwash fluid, in future studies.

Biber et al [3] also investigated the utility of using mouthwash (MW) samples for the detection of SARS-CoV2. The patients either asymptomatic or with mild symptoms were included in the study. Although 84.6% (116/137) of oronasopharyngeal (ONP) swabs were positive by at least one of the genes (N, E, R), MW detected 70.8% (97/137) positivity. Unfortunately, MW samples missed samples especially NPS samples with Cq value >30. The median of Cq values of all positive ONP swabs (all three genes) was found as 30 compared to a median of 32 of positive MW. They also compared the results of different fluids (saline, distilled water, commercial mouthwash containing alcohol and commercial mouthwash without alcohol) for mouth rinsing. The result of water was similar to saline in contrast to other solutions, which had lower detection rate.

There are several studies which compared saliva to NPS and in most of them saliva sensitivity was found either comparable to that of NPS [10-16] or higher than NPS [17-19]. Although there are several studies which compared saliva with NPS, only symptomatic patients were included in most of them. There are limited study investigating the performance of saliva compared to NPS in asymptomatic individuals [19-22]. Alkhateeb et al [22] compared saliva and NPS of 33 symptomatic and 12 asymptomatic known SARS-CoV-2-positive patients. Though, saliva showed lower sensitivity (36%) in asymptomatic patients than symptomatic patients (80%), it detected infections with lower Ct values. Opposite to the results of A1, there are researches in which saliva has same or sometimes higher sensitivity for detecting asymptomatic carriers compared to NPS [21-23]. Savela et al [23] evaluated the sensitivity and sample type for detecting early infections of COVID-19 in asymptomatic individuals. They quantified SARS-CoV-2 RNA viral loads in anterior-nares nasal swabs and saliva samples which were obtained twice-daily and found that SARS-CoV-2 RNA first appears in saliva compared to nasal-swab samples. In other studies it has been demonstrated that SARS-CoV-2 RNA can appear in saliva 24–48 h prior to detection by nasopharyngeal swabs [19,24,25], and 1.5–4.5 days prior to detection by anterior nasal swabs [23]. In the study of Yokoda et al [19] high sensitivity and specificity was detected in NPS and saliva specimens of 1924 asymptomatic individuals.

One of the limitation of our study is including only symptomatic patients into the study. Overall it is estimated that 17 to 30% of SARS-CoV-2 positive individuals remain asymptomatic [25,26]. As SARS-CoV-2 can spread from individuals before symptom onset and from asymptomatic individuals, the performance of GMW sampling in asymptomatic population must be also investigated.

In conclusion, the GWM sampling is a simple, rapid, cheap and self-collection method. According to the results of this study, concentrated GMW can be an alternative sampling method to NPS in rapid and accurate diagnosis of COVID-19. Standardization of the method by using water with low ion concentration will increase the performance of the test and lower the variability of the results. Additional studies are needed including both symptomatic and asymptomatic patients for accurate evaluation of GMW and concentrated GMW sampling as a screening test for COVID-19.

Declarations

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References

1. World Health Organization. (2020). Laboratory testing for coronavirus disease 2019 (COVID-19) in suspected human cases: interim guidance, 2 March 2020. World Health Organization. <https://apps.who.int/iris/handle/10665/331329>
2. Centers for Disease Control and Prevention (2020) Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Persons for Coronavirus Disease (2019) (COVID- 19), 14 April 2020. <https://www.cdc.gov/coronavirus/2019-nCoV/lab/guidelines-clinical-specimens.html>
3. Biber A, Lev D, Mandelboim M, Lustig Y, Harmelin G, et al. (2021) The role of mouthwash sampling in SARS-CoV-2 diagnosis. *Eur J Clin Microbiol Infect Dis* 40: 2199-2206
4. Lai CK, Lui GC, Chen Z, Cheung YY, Cheng KC, et al. (2021) Comparison of self-collected mouth gargle with deep-throat saliva samples for the diagnosis of COVID-19: Mouth gargle for diagnosis of COVID-19. *J Infect* 83: 496-522
5. Caulley L, Shaw J, Corsten M, Hua N, Angel JB, et al. (2021) Salivary testing of COVID-19: evaluation of serological testing following positive salivary results. *BMC Infect Dis* 21: 410
6. Fernandes LL, Pacheco VB, Borges L, Athwal HK, de Paula Eduardo F, et al. (2020) Saliva in the Diagnosis of COVID-19: A Review and New Research Directions. *J Dent Res* 99: 1435-1443
7. Fernandes P, Ferreira F, Morais OM, Ramos CMT, Fernandes EMR, et al. (2021) Performance of saliva as a specimen to detect SARS-CoV-2. *J Clin Virol* 142: 104913
8. Plantamura J, Bousquet A, Otto MP, Bigaillon C, Legland AM, et al. (2021) Performances, feasibility and acceptability of nasopharyngeal swab, saliva and oral-self sampling swab for the detection of severe acute respiratory syndrome coronavirus 2. *Eur J Clin Microbiol Infect Dis* 40: 2191-2198

9. Michel W, Farber J, Dilas M, Heuft HG, Tammer I, et al. (2021) A combined oro-nasopharyngeal swab is more sensitive than mouthwash in detecting SARS-CoV-2 by a high-throughput PCR assay. *Infection* 49: 527-531
10. Abasiyanik MF, Flood B, Lin J, Ozcan S, Rouhani SJ, et al. (2021) Sensitive detection and quantification of SARS-CoV-2 in saliva. *Sci Rep* 11: 12425
11. Azzi L, Carcano G, Gianfagna F, Grossi P, Gasperina DD, et al. (2020) Saliva is a reliable tool to detect SARS-CoV-2. *J Infect* 81(1): e45-e50
12. Caulley L, Corsten M, Eapen L, Whelan J, Angel JB, et al. (2021) Salivary Detection of COVID-19. *Ann Intern Med* 174: 131-133
13. Hanson KE, Barker AP, Hillyard DR, Gilmore N, Barrett JW, et al. (2020) Self-Collected Anterior Nasal and Saliva Specimens versus Health Care Worker-Collected Nasopharyngeal Swabs for the Molecular Detection of SARS-CoV-2. *J Clin Microbiol* 58(11): e01824-20. doi: 10.1128/JCM.01824-20.
14. Jamal AJ, Mozafarihashjin M, Coomes E, Powis J, Li AX, et al. (2021) Sensitivity of Nasopharyngeal Swabs and Saliva for the Detection of Severe Acute Respiratory Syndrome Coronavirus 2. *Clin Infect Dis* 72: 1064-1066
15. Sakanashi D, Asai N, Nakamura A, Miyazaki N, Kawamoto Y, et al. (2021) Comparative evaluation of nasopharyngeal swab and saliva specimens for the molecular detection of SARS-CoV-2 RNA in Japanese patients with COVID-19. *J Infect Chemother* 27: 126-129
16. Vaz SN, Santana DS, Netto EM, Pedroso C, Wang WK, et al. (2020) Saliva is a reliable, non-invasive specimen for SARS-CoV-2 detection. *Braz J Infect Dis* 24: 422-427
17. Kojima N, Turner F, Slepnev V, Bacelar A, Deming L, et al. (2021). Self-Collected Oral Fluid and Nasal Swab Specimens Demonstrate Comparable Sensitivity to Clinician-Collected Nasopharyngeal Swabs for Coronavirus Disease 2019 Detection. *Clin Infect Dis* 2:73(9): e3106-e3109
<https://doi.org/10.1093/cid/ciaa1589>
18. Moreno-Contreras, J, Espinoza, M. A, Sandoval-Jaime, C, Cantú-Cuevas, M. A, et al. (2020). Saliva Sampling and Its Direct Lysis, an Excellent Option To Increase the Number of SARS-CoV-2 Diagnostic Tests in Settings with Supply Shortages. *J Clin Microbiol* 22;58(10): e01659-20.
<https://doi.org/10.1128/JCM.01659-20>
19. Wyllie AL, Fournier J, Casanovas-Massana A, Campbell M, Tokuyama M, et al. (2020) Saliva or Nasopharyngeal Swab Specimens for Detection of SARS-CoV-2. *N Engl J Med* 383: 1283-1286
20. Vogels CBF, Watkins AE, Harden CA, Brackney DE, Shafer J, et al. (2021) SalivaDirect: A simplified and flexible platform to enhance SARS-CoV-2 testing capacity. *Med (N Y)* 2: 263-80 e6 doi: 10.1016/j.medj.2020.12.010
21. Yokota I, Shane PY, Okada K, Unoki Y, Yang Y, et al. (2021) Mass Screening of Asymptomatic Persons for Severe Acute Respiratory Syndrome Coronavirus 2 Using Saliva. *Clin Infect Dis* 73: e559-e65
22. Alkhateeb KJ, Cahill MN, Ross AS, Arnold FW, Snyder JW. (2021) The reliability of saliva for the detection of SARS-CoV-2 in symptomatic and asymptomatic patients: Insights on the diagnostic

performance and utility for COVID-19 screening. Diagn Microbiol Infect Dis 101: 115450

23. Savela ES, Winnett A, Romano AE, Porter MK, Shelby N, et al. (2021) SARS-CoV-2 is detectable using sensitive RNA saliva testing days before viral load reaches detection range of low-sensitivity nasal swab tests. medRxiv 7: 2021.04.02.21254771. doi: 10.1101/2021.04.02.21254771. Preprint
24. Borghi E, Massa V, Zuccotti G, Wyllie AL. (2021) Testing Saliva to Reveal the Submerged Cases of the COVID-19 Iceberg. Front Microbiol 12: 721635
25. Pollock AM, Lancaster J. (2020) Asymptomatic transmission of covid-19 BMJ 371:m: 4851
<http://dx.doi.org/10.1136/bmj.m4851>
26. Johansson MA, Quandelacy TM, Kada S, Prasad PV, Steele M, et al. (2021) SARS-CoV-2 Transmission From People Without COVID-19 Symptoms. JAMA Netw Open 4: e2035057

Table

Table 1. Sensitivity of COVID-19 PCR in Nasopharyngeal, Mouthwash and Concentrated Mouthwash Samples of each center

Center	NPS	GMW	Concentrated GMW	Increase in sensitivity by concentration
A	55(78.6%)	30(42.9%)	57 (81.4%)	38.5%
B	76 (66.7%)	67 (58.8%)	101 (88.6%)	29.8%
C	40 (85.1%)	17 (36.2%)	27 (57.4%)	21.2%
D	40 (88.9%)	16 (35.6%)	25 (55.6%)	20.0%
E	41 (100%)	24 (58.5%)	30 (73.2%)	14.7%
F	60 (93.8%)	36 (56.3%)	45 (70.3%)	14%
G	142 (95.3%)	77 (51.7%)	97 (65.1%)	13.4%
H	60 (98.4%)	26 (42,6%)	35 (57.4%)	13.1%
I	38 (88.4%)	24 (55.8%)	28 (65.1%)	9.3%
J	43 (100%)	17 (39.5%)	21 (48.8%)	9.3%
K	222 (99.1%)	161 (71.9%)	179 (79.9%)	8%
Total	817 (90.7%)	495 (55%)	645 (71.6%)	16.6%