ORIGINAL RESEARCH

A study on laboratory findings of Sars Cov 2 viral RNA parameters in Saliva and Respiratory Specimens in COVID 19 Patients

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ABSTRACT

Background: Reverse Transcription Polymerase Chain Reaction of respiratory samples is the diagnostic test for detecting the SARS CoV 2 infection. The nasopharyngeal and oropharyngeal swab was considered to be the best specimen for the diagnosis of the COVID 19. But the invasiveness of the procedure can reduce the likelihood of the patient permission to retest and can pose a significant risk for the treating healthcare workers. Hence the present study focusses on the viral shedding rate in the saliva, nasopharyngeal and oropharyngeal sites to propose some practical suggestions regarding the efficacy of the samples in the detection of SARS CoV 2. **Methods:**The samples were collected from the patients with or without symptoms after 5 to 7 days of their positivity. Three respiratory samples such as Saliva, Nasopharyngeal swab and Oropharyngeal swab were taken separately at the same time. The detection of SARS-CoV-2 in the specimens was performed by RT-PCR amplification of the SARS-CoV-2 RdRP and N gene fragments, using a Labgun Exofast RTPCR kit. The detection of human RNase P gene was included in the kit as a control. The test was performed in the QuantStudio™ 5 Real-Time PCR System. The result was interpreted as positive when the cycle threshold (Ct) values of both target genes were less than 30 cycles.**Result& Discussion:**In this study, we proved saliva as an acceptable non-invasive alternative source for the diagnosis and viral load monitoring of SARS-CoV-2 in a large cohort of patients. Saliva exhibited comparable sensitivity and strong agreement to the current COVID-19 diagnosis standard by using respiratory tract specimens. In summary, our study showed that saliva might serve as a promising substitutable choice to the current COVID- 19 diagnosis standard by using respiratory tract specimens with comparable performance.

Key words:RT-PCR, Saliva, Swab.

INTRODUCTION

In December 2019, China reported the outbreak of novel corona virus which caused the severe acute respiratory syndrome. This novel corona virus was then named as SARS Co V 2 because of its close relationship to the SARS CoV. The disease caused by the virus is named as covid 19. The human-to-human transmission of SARS CoV2 is through droplets while coughing, sneezing and contact with respiratory secretions infected with the virus. Some of the studies suggested that there is fecal transmission that has gastrointestinal symptoms like diarrhea. In addition, the virus has been detected in the saliva of the covid patients. The clinical symptoms include sore throat, fever, myalgia, and cough, shortness of breath, chest pain, headache and anosmia.

Most of the cases have mild or no symptoms but the patients with comorbid conditions like DM, obesity, HT or any cardiac diseases present with the severe symptoms. Pneumonia represents the most important clinical manifestation of COVID-19 infection and is the primary determinant of prognosis in severely ill patients. There is a remarkable heterogeneity in the individual course and severity of the disease. Therefore, pulmonary clearance of the virus is of particular interest[1]. An exaggerated response or reduced immune-dependent viral clearance in some patients may aggravate the pulmonary manifestation[2]. Individual differences in viral tropism, viral shedding load, duration of viral shedding and viral tissue

distribution may playaroletherein[3].

With the previous experience with the other similar respiratory virus, WHO suggested the real time Reverse Transcription Polymerase Chain Reaction of respiratory samples is the diagnostic test for detecting the SARS CoV2 infection. The nasopharyngeal and oropharyngeal swab was considered to be the best specimen for the diagnosis of the covid 19. But the invasiveness of the procedure can reduce the likelihood of the patient permission to retest and can pose a significant risk for the treating healthcare workers. Hence the present study focusses on the viral shedding rate in the saliva, nasopharyngeal and oropharyngeal sites to proposesome practical suggestions regarding the efficacy of the samples in the detection of SARS CoV2. To reduce the discomfort to the patients, the saliva can be considered as a promising tool to detect the covid virus. However, before suggesting the saliva as a means of sample collection it is essential to prove the presence of virus in this fluid. The aim of this study is to analyse the respiratory samples and salivary samples from patients already diagnosed with COVID 19 and compare the efficacy of the specimen in detection of the SARS CoV2.

MATERIALS AND METHODS

The retrospective study was carried out in the blood This study was approved by the Institutional Ethics Committee. Patients who got admitted in the covid ward are taken in the study. The samples were collected from the patients with or without symptoms after 5 to 7 days of their positivity. Three respiratory samples such as Saliva, Nasopharyngeal swab and Oropharyngeal swab were taken separately at the same time.

Collection of Saliva

Saliva were self-collected by the patients and spit into a VTM. Patients were instructed to repeatedly spit until approximately 1ml of sample was obtained, thus avoid in gmucous secretions from oropharynx or lower respiratory tract. the sample is properly labelled.

Collection of Nasopharyngeal swab:

The lab technicians wore proper PPE including FFP2(N95) mask, disposable cap, goggles, gown, apron, latex gloves and shoe covers. The patients were given brief explanation about the procedure and consent is obtained. The patient is made to sit comfortably with head rest on the chair's head support to avoid a reflex backwards movement of the head during the swab. The swab is inserted into the nostril and progress the swab through the nasal cavity until a resistance is felt which indicates the posterior wall of the nasopharynx. The swab is rubbed and rolled gently for 2 – 3 times so that it absorbs the secretions. Then the swab is slowly removed while continuing rotates it. The swab is placed in the VTM and properly labeled.

Collection of Oropharyngeal Swab

The second swab is inserted into the posterior pharynx and tonsil larareas. The swab is rubbed over the posterior pharynx and tonsilar areas avoiding touching the tongue, teeth, and gums. The swab is placed in the VTM and properly labeled. Samples were transported to PCR lab immediately for nucleic acid extraction. RNA was isolated from fresh samples here PCR testing was performed on the same day.

Proper requisition for the test is filled up with patient details such as age, sex, co- morbidities with special attention tohypertension, diabetes and obesity. Viral RNA was extracted from 200 micro lit of the samples within 26 minutes using a fully automated nucleic acid extraction system, according to the manufacturer's instructions.

RT-PCR workflow

The detection of SARS-CoV-2 in the specimens was performed by RT-PCR amplification of the SARS-CoV-2 RdRP and N gene fragments, using a Labgun Exofast RTPCR kit. The detection of human RNase Pgene was included in the kit as a control. The test was performed in the Quant Studio™ 5 Real-Time PCR System. The result was interpreted as positive when the cycle threshold (Ct) values of both target genes were less than 30cycles. The Retesting of samples were done when the internal control was not detected and if the result is in conclusive.

Statistical Analysis

The data were analysed as percentage and mean for categorical variables and continuous variables respectively. The diagnostic performance was assessed with Sensitivity, specificity, positive predictive value, negative predictive value and a 95%CI. The kappa Value is estimated for the agreement between the saliva RT-PCR and nasopharyngeal and oropharyngeal swab RT-PCR results. All statistical analyses were performed using SPSS software.

RESULT

A total number of 200 patients were included in the study, 120 males and 80 females. Age values ranged from 12 to 75 and 47% of the patients were in the age group of 50 – 75. All the 200 patients were diagnosed to have covid positive through RTPCR and admitted in the covid ward in the Chengalpattu medical college. In this study the samples like NPS, OPS and saliva were taken from the 200 covid positive patients 5 -7 days apart from the first sample. The samples taken from three sites such as NPS, OPS and saliva were subjected to RTPCR and the results were compared with the previous results to evaluate the viral shedding pattern in the respiratory samples.

Among the study population 69% were symptomatic and the most predominant symptom was found to be fever followed by cough. About 31% patients had co

morbid conditions and the commonest among them was DM followed by HT.

Table 1: RT PCR results after one week

S.no	Saliva	NPS	OPS
Positive	108	118	96
Negative	92	82	104

Out of 200 samples there were 118 NPS specimens that were positive, and 108 saliva samples were positive and 96 OPS specimen were positive. In the 200 paired samples only 66 NPS samples had corresponding positive saliva specimens with the positive predictive value of 61.1%. The kappa value denoting the measure of agreement is low (0.046). Among the total NPS positive samples, 52 corresponding samples were detected negative in saliva and 42 saliva positive samples had come NPS negative. Thus, the sensitivity and specificity is 55.93% and 48.78% respectively.

Table 2: Comparison between Saliva and nasopharyngeal swab

Saliva	NPS		Total	Saliva vs NPS	
	Positive	Negative		Sens	55.93
Positive	66	42	108	Spec	48.78
Negative	52	40	92	PPV	61.11
Total	118	82	200	NPV	43.48

We next compared Saliva sample and oropharyngeal swab among which only 70 OPS samples had corresponding positive saliva specimens with the positive predictive value of 64.81%. The kappa value denoting the measure of agreement is fair (0.362). Among the total OPS positive samples, 26 corresponding samples were detected negative in saliva and 38 saliva positive samples had come OPS negative. Thus the sensitivity and specificity is 72.92 % and 63.46 % respectively.

Table 3: Comparison between saliva and oropharyngeal swab

Saliva	OPS		Total	Saliva vs OPS	
	Positive	Negative		Sens	72.92
Positive	70	38	108	Spec	63.46
Negative	26	66	92	PPV	64.81
Total	96	104	200	NPV	71.74

We also compared nasopharyngeal and oropharyngeal swabs 70 OPS samples had corresponding positive saliva specimens with the positive predictive value of 59.32%. The kappa value denoting the measure of agreement is fair (0.265). Among the total OPS positive samples, 26 corresponding samples were detected negative in saliva and 48 saliva positive samples had come OPS negative. Thus the sensitivity and specificity is 72.92% and % respectively.

Table 4: Comparison between oropharyngeal and nasopharyngeal swab

NPS	OPS		Total	NPSvs OPS	
	Positive	Negative		Sens	72.92
Positive	70	48	118	Spec	53.85
Negative	26	56	82	PPV	59.32
Total	96	104	200	NPV	68.29

The average NPS cycle threshold (Ct) value was 16.1 cycles which was significantly lower than those for saliva specimens was 17.9 cycles and a mean difference in CT value for NPS and saliva specimens was 1.8. this means that the viral load in NPS samples is higher than the saliva samples.

Table 5: Ct values of different specimen after 5-7 days

Ct value	Saliva	NPS	OPS
<10	6	6	1
11to20	26	37	32
21to30	22	16	15
Total	54	59	48

Out of 62 asymptomatic individuals the repeat test with saliva showed 61% as positive while the NPS detected only 51% as positive and the mean Ct value for the salivary samples was 19.35.

In addition, with the aim to illustrate the viral RNA shedding pattern in saliva and predict its correlation with illness severity in patients with COVID-19, saliva specimens were serially collected from all patients, with 170 (85%) individuals classified as mild cases and 30 (15%) classified as severe cases. Our results proved that RNA levels in saliva peaked soon in one week after symptom onset, ranging from around 104 to 108 copies per mL during this time, then steadily declined. 20% (40/200) patients had a viral shedding period longer than 14 days in saliva. The prolonged presence of SARS-CoV-2 viral RNA in saliva samples was not associated with disease severity in our study. We further analyzed the correlation between viral loads in saliva and severity of illness according to the day after disease onset at the time of sampling. The mean viral load of severe cases showed no significant difference from those of corresponding mild cases for all the indicated periods. The viral RNA clearance patterns in saliva samples were also observed similarly in mild and severe COVID-19 patients.

DISCUSSION

This study was carried out to find the viral shedding pattern in respiratory samples like NPS and saliva. At present the Real Time reverse transcription Polymerase Chain Reaction(rRTPCR) on nasopharyngeal and oropharyngeal swab was considered as gold standard for the qualitative detection of SARS-CoV-2 infection. However, the nasopharyngeal and oropharyngeal swab collection is an invasive procedure and has a risk of spreading infection to the health care worker who collects the sample. To reduce the risk the saliva can be collected which can be performed by the patients itself. Several studies have stated that saliva represents a reliable source for the diagnosis of SARS-CoV-2.

In the present study 108 samples out of 200 were positive in saliva samples and 40samples were positive with saliva while the NPS samples proved negative results on the same day. The above results support the theory that saliva is a consistent tool to be used in COVID-19 diagnosis through the RT-PCR procedure. This may give a clue to the clinicians in the managing the discharges because the patients who have showed negative in the NPS could be contagious through their saliva which is a serious danger for the family and the whole society. The Ct value is also almost similar to the Ct value of the NPS samples which shows the ability to detect the virus in an effective manner. Hence, the present study shows that saliva is not only a biological fluid that could be used for qualitative detection of SARS-CoV-2, but it may be a beneficial tool to follow the course of the illness and a tool for the discharge[4].

Out of 62 asymptomatic individuals the repeat test with saliva showed 61% as positive while the NPS detected only 51% as positive and the mean Ct value for the salivary samples was 19.35. Asymptomatic patients signify an crucial issue to be addressed by the Public Health department against COVID-19, but currently there is not any promising procedure that can be used for a mass screening. Therefore we can consider saliva as a reliable biological fluid to detect SARS CoV2 as a diagnostic rapid test for a screening program and it can also be easily performed by non-healthcare professionals[5,6].

At present, the “gold” standard to detect SARS-CoV-2 infection is byreal-time reverse-transcription–polymerase-chainreaction(RT-PCR) in respiratory tract specimens, mainly nasopharyngeal(NP) and oropharyngeal(OP) swabs. However, the collection of these specimens is a relatively invasive procedure, which causes severe discomfort. In particular, the close contact involved in swab collection might put healthcare workers at higher risk for viral transmission. Saliva specimens, in contrast, can be easily self-collected by patients. Findings of previous studies have demonstrated successful detection of SARS-CoV-2 RNA in saliva, proving it as an appealing non-invasive alternative to NP or OP swabs for the diagnosis and viral load monitoring of SARS-CoV-2[7-9]. However, the clinical usefulness of saliva specimens for diagnosing COVID-19 has yet to be thoroughly evaluated due to the small sample size. Besides, the viral load dynamics in saliva samples and the relationship between viral load and disease severity are also unknown. Here, we compared the detection sensitivity of paired respiratory tract and saliva specimens in diagnosing COVID-19.

In this study, we proved saliva as an acceptable non-invasive alternative source for the diagnosis and viral load monitoring of SARS-CoV-2 in a large cohort of patients. Saliva exhibited comparable sensitivity and strong agreement to the current COVID-19 diagnosis standard by using respiratory tract specimens.

CONCLUSION

In summary, our study showed that saliva might serve as a promising substitutable choice to the current COVID-19 diagnosis standard by using respiratory tract specimens with comparable performance.

The limitations in the study are the population analysed in this study is composed of patients who got admitted after the test done outside which may influence the test quality starting from the collection and processing the sample. The Ct value is known for the repeat test only which could not be compared with the previous test and the trend could not be analysed.

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