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Whole Saliva (Oral Bio Fluid) as a Non-Invasive Biological Marker or Specimen for Detecting the Novel Corona Virus in Covid-19 Patients: A Multicentric Study in Surat, Gujarat Population

Gunvant Parmar[1], Neeta Khandelwal[2], Stuti Jhala[3], Apexa Rana[3]

[1]Professor & Head, Department of Dentistry, Govt. Medical College Surat.
[2]Professor, Department of Microbiology, Govt. Medical College Surat
[3]Tutor, Department of Dentistry, Govt. Medical College Surat.

Corresponding Author: Gunvant Parmar

ABSTRACT

Covid-19 is a respiratory disease caused by the SARS COV-2 virus. WHO states sample collection on upper respiratory specimens, including nasopharyngeal and oropharyngeal swabs along with endotracheal aspirate and bronchoalveolar lavage, among the various sample collection methods saliva has been investigated and reported as a potential source for diagnosis. Thus we propose to evaluate the current situation to investigate the potential use of saliva sample as a noninvasive tool for diagnosis of covid-19.

Objective: We aimed to detect and estimate the potential use of saliva as a noninvasive tool for diagnostic of SARS- cov-2

Methods: A study was conducted among 200 individuals who attended the designated Covid Hospital, Surat, and Gujarat between 19/10/2020 to 1/12/2020. We collected saliva samples along with the nasal and oral swabs. Real time polymerase chain reaction was performed, and the results of the saliva samples and nasal/oral swabs were compared.

Results: A total of 200 samples were analyzed. 20%samples were tested positive in both swab and saliva and 82 % were tested negative from both the samples. From the overall samples, 11 % were tested positive from swab samples but were negative from saliva samples. However 5% of the samples were tested positive from saliva sample but were negative for swab samples. In the overall samples there was a significant difference in the results between the swab and saliva samples ($p<0.001$) the Wilcox on signed rank test between the saliva and swab results revealed a similar pattern in Surat.

Conclusions: the collection of saliva samples is non invasive and non aerosol generating with significant advantages, such as lower cost, easy to obtain, sample self collection, lower risk of contamination and there is no need of trained staff for sample collection. So it should an alternative specimen for the diagnosis of covid-19.

Key words: oral biofluid, whole saliva, biological marker, covid-19, corona virus.

INTRODUCTION

The Coronavirus Disease 2019 is a respiratory disease, considered a pandemic by WHO. It is caused by severe acute respiratory syndrome related coronavirus 2 (SARS-COV-2), so designated by the international committee of viruses (ICTV). It was initially named 2019 Novel Coronavirus. This new pathogen characterized as an enveloped virus with a single stranded RNA genome belongs to the realm riboviria, order nidovirales, suborder cornidovirineae, family coronaviridae, and genus betacoronovirus and to the species of severe acute respiratory syndrome related coronavirus. It starts binding to the host cells and through viral RNA replication process; it produces virions and releases them by exocytosis for new infections. For this, structural proteins are needed for virion

assembly and the spike glycoprotein present on the virion surface is responsible for binding to the host receptor. [1, 2]

Corona virus disease is a highly contagious infectious disease. Symptoms of the disease are non specific like fever, cough, dyspnea, fatigue etc. Some patients may also experience headache, dizziness, loss of taste and smell. And gastrointestinal symptoms like nausea, vomiting and diarrhea. [3, 4] This is very much common with other viral diseases which are seasonal thereby complicating the provisional diagnosis. Severe onset of disease may lead to acute respiratory distress syndrome and even death. Many a times, hospitalization is required. In case of severity, admission to an intensive care unit may also be needed. It is thought to spread primarily through respiratory droplets via close personal contact with an infected person. Detection of sars-cov-2 in patient specimens is the first crucial step for the guidance of treatment, effective infection control in the hospital and control of infection in the community. To determine the potential of using a salivary sample for the diagnosis of covid-19, we conducted a cross sectional study investigating the correlation of detection of SARS COV-2 in saliva samples and nasopharyngeal/throat swabs in south Gujarat population who visited the designated Covid Hospital, Surat. [3, 4, 5]

Saliva is a common and transient medium for virus transmission. Saliva droplets are generated in different sizes while breathing, talking and sneezing. Large droplets fall easily to the ground and only set up short distance transmission. However, Saliva could form aerosols and reach a distant host along with air flow in a favorable environment. [6, 7]

Viral pneumonia typically results in the production of purulent sputum. So, oropharyngeal and oral swabs are recommended for upper respiratory tract infections. (Specimen type for the SARS cov-2 diagnostic testing. [8]

However, collection of this specimen requires close contact between the health care worker and patients affected. This may increase the biosafety risk to health workers through the creation of aerosols generated by the patients during specimen collection. It also gives some discomfort to the patient while taking the swabs. Sometimes may cause bleeding and sometimes swabs cannot be collected properly if patients is not co-operative and the result may be false negative. [9, 10] Based on these issues finding a safe alternative method is crucial. One of the non invasive methods for collecting the specimens is to drool in a sterile container. Self collected saliva specimens in comparison with nasal and oral swabs will decrease the chance of exposing the health care workers. In Covid-19, Saliva as a diagnostic specimen provides a simple and more efficient tool for diagnosis of viruses during this virus outbreak. [11, 12, 13]

In this sense saliva, presents a risk of transmission to dentists and other health care workers as salivary samples may contain the virus in the process of replication. Saliva is a possible mode of transmission in the positive test case for viral culture, as it can be expelled through droplets in cough or even in cases with no respiratory symptoms. [14]

Saliva appears to be a fluid of enormous potential in health assessment, especially due to non invasive nature of its collection, which can be performed by individuals without particular training and need of major equipment. [15]

MATERIALS & METHODS

Study Design: Cross Sectional

The study protocol was reviewed and approved by the ethical clearance committee on human rights related involving human subjects of office of the Dean, Government Medical College, Majuragate, Surat.

Study population: - A study was conducted among 200 individuals who attended the designated Covid Hospital, Surat between 19/10/2020 to 1/12/2020. The purpose of this study was explained to the patients in

the language they understand and the written consents were obtained from the patients who were willing to participate in the study.

Inclusion criteria:-participants who are willing to provide the whole saliva sample along with nasopharyngeal/ or pharyngeal swabs for diagnostic purpose. History of fever, acute respiratory symptoms, travel history from an endemic area of covid within 14 days, history of contact with an individual who has confirmed to have or suspected of having Covid -19, age above 18 years.

Exclusion criteria; below 18 years, patients deprived of their liberty, adult protected under guardianship, or vulnerable persons, patients bought in a state of an emergency.

SPECIMEN PROCESSING

Saliva collection through passive drooling is a gold standard technique when collecting the oral fluid for biological testing. Our study was performed doing the same on patients who had come for screening at the designated Covid Hospital, Surat. This technique, avoids localized secretions of salivary glands providing a more consistent specimen. Also, its ease of use reduces participant burden and improves compliance for collecting the saliva. The basic armamentarium for saliva collection was obtained (figure-1). The saliva was allowed to pool in the mouth for about a minute. The patient was asked to tilt the head forward and sideways to gently guide the saliva into the sterile vials. These vials contain viral transport medium (figure-2). These samples were approximately 1-2 ml in volume. They were labelled, sealed and kept in polythene covers. The routine, nasopharyngeal swab was also collected in these patients. The salivary samples were labelled as DS (Dental Saliva) along with the designated patient number for the nasopharyngeal swab. This sample was then sent immediately to the Microbiology lab for RTPCR, for analysis and detection of ORF and N gene. This was done by the laboratory technicians of Microbiology Department in a fully automated nucleic acid extraction system as per ICMR guidelines. [16, 17, 18]

Figure-1: The basic armamentarium for saliva collection

Figure-2: Viral transport medium in polythene bag

RTPCR WORKFLOW

The detection of covid-19 virus in the specimen was performed by RTPCR amplification of the covid-19 virus ORF and N gene fragments, using ICMR approved SARS cov-2 nucleic acid diagnosis kit.

The covid-19 virus generally gathers in the throat or inside the nose of a person. The RT-PCR test diagnosis process starts with the sample collection by swab from the naso and oropharyngeal.

The cells and the nucleus are lysed by lysis buffer and N.A extraction is done. It is a combination of the residual genetic material of the individual and the RNA of the virus.

The RNA is then converted to DNA using specific enzyme via reverse transcription short fragments of DNA are also added to create a mixture.

If there is a virus in the sample then the short fragments of the DNA accord to the target divisions of the viral DNA.

The mixture is then put into an RT-PCR machine. The RTPCR machine heats and cools the mixture by cycling it through temperatures to ignite specific chemical reactions. Through this process the target sections of viral DNA get their new identical copies.

The RT-PCR covid-19 test machines carries out repeated cycles with the sample this helps in copying the target sections of the viral DNA. At the end of each cycle the number of copies of the viral DNA gets doubled. As a result about 35 billion new copies of the viral DNA sections form each strand of the virus are generated by the end of the process. The entire procedure takes upto 4 to 8 hours. [19, 20, 21]

Statistical Methods

Data were analyzed for normality and descriptive statistics were presented as a number (%) for categorical variables and mean±standard deviation.

Data was analyzed statistically using SPSS version 23 software.

T-Test is applied for quantitative data

RESULTS

A total of 200 (N=200) samples were analyzed.82% samples were showing similar results between swabs and saliva samples. {Table-1}

Table: 1 Comparison for the detection of SARS-COV-2 RTPCR between the naso/oropharyngeal swabs and saliva specimen

SR. NO.	Nasopharyngeal /oral swab		Saliva specimen		Total	Percentage
	Positive	Negative	Positive	Negative		
1	22	-	-	22	22	11%
2	10	-	10	-	10	5%
3	-	6	6	-	6	3%
4	-	164	-	164	164	82%

20% samples were tested positive in both swab and saliva. The median ct values of the ORF and n genes were 20 and 20.5 respectively in swab whereas the median ct value for ORF and N gene was 27 and 27 respectively in saliva. {Table-2}

Table: 2 both naso/oro pharyngeal swab and saliva specimen positive

Sr.No	Age	Sex	Swab result	CT value n	CT value Orf	Saliva result	CT value n	CT value Orf
10	55	1	1	21	24	1	29	30
106	65	1	1	25	27	1	29	33
127	50	2	1	33	32	1	33	34
148	51	2	1	14	11	1	27	27
152	20	1	1	25	24	1	28	27
159	65	2	1	22	21	1	24	22
164	35	1	1	20	18	1	25	27
166	32	2	1	14	13	1	26	27
171	42	1	1	17	17	1	26	27
192	56	1	1	19	19	1	27	29

Sex:- 1 for male, 2 for female

From the overall samples, 11 % were tested positive from swab samples but were negative from saliva samples. The median Ct values of the ORF an N gene was 24 and 23.5 respectively in swab samples. However 5% of the samples were tested

positive from saliva sample but were negative for swab samples. Whereas the ORF and N gene were 27.5 and 22 respectively in saliva specimen {Table-3}

Table: 3 nasal/oropharyngeal swab negative but saliva swab positive

Sr.No	Age	Sex	Swab result	Saliva result	CT value n	CT value orf
67	36	1	0	1	27	29
130	39	1	0	1	28	28
135	21	1	0	1	22	23
153	29	1	0	1	28	27
154	28	1	0	1	29	31
162	25	2	0	1	26	26

Swab/saliva result: 1 for positive,0 for negative

Table 4:- t-test paired samples

t-Test: Paired Two Sample for Means			t-Test: Paired Two Sample for Means		
	Variable 1	***Variable 2***		***Variable 1***	***Variable 2***
Mean	21	27.4	Mean	20.6	28.3
Variance	32.88889	6.488889	Variance	40.71111	11.78889
Observations	10	10	Observations	10	10
Pearson Correlation	0.71495		Pearson Correlation	0.624849	
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
df	9		df	9	
t Stat	-4.70679		t Stat	-4.85805	
P(T<=t) one-tail	0.000555		P(T<=t) one-tail	0.000449	
t Critical one-tail	1.833113		t Critical one-tail	1.833113	
P(T<=t) two-tail	0.00111		P(T<=t) two-tail	0.000898	
t Critical two-tail	2.262157		t Critical two-tail	2.262157	

There is no significant difference in the distribution of swab and saliva results. In the overall samples there was a significant difference in the results between the swab and saliva samples ($p<0.001$) the Wilcoxon signed rank test between the saliva and swab results revealed a similar result in 164 samples (82%)

The results between swab and saliva samples were similar in 164 cases (82%) and the difference in the results between the saliva and swab samples were significant ($z= -3.024$, $p=0.002$). {Table-4}

The cross tabulation of the swab and saliva samples results also revealed a significant association between the results of the swab and saliva specimens in Surat populations.

DISCUSSION

The study results showed that the value of testing saliva samples were positive for covid 19.

In our study the saliva sample was self generated and collected by drooling technique by the patients without any need of coughing. This procedure of collecting the saliva might be less aerosol generating and might reduce the risk of transmission of infection, for health care workers working in the hospital. The 4 specimen who were positive in saliva sample and negative in oral/nasal swab of these 4 samples the ct values of the orf and n genes were31 & 29 respectively. Later these patients were admitted for oxygen saturation. Therefore results of this test could represent a true infection.

The study done was prospectively collected data on patients who came at the designated covid hospital Surat who were at high risk of covid-19. Including with History of fever, acute respiratory symptoms, travel history from an endemic area of covid within 14 days, history of contact with an individual who has confirmed to have or suspected of having Covid -19, age above 18 years. In addition all patients were verified with the reference standard.

Our limitations was that our testing was focused on the individuals who came for the screening with having symptoms of fever, cough, but the spectrum of the disease also ranges from asymptomatic through upper respiratory tract infection to acute respiratory distress. Therefore the performance of the saliva test for the

detection of SARS COV-2 for asymptomatic patients remains unknown. The number of covid -19 cases in our study was limited by the decline in the number of cases in Surat Gujarat.

This is a cross sectional study, however longitudinal studies are needed to confirm the presence of corona virus in the saliva samples over a period of time

There were 2-3% of the cases were the saliva result is positive while the swab result is negative

The swab results were positive, but the saliva results were negative in 10-11% of the samples.

There was a significant difference in the CT values of the swab and saliva samples.

However with the current pandemic situation, with high risk of infection among health care workers a saliva sample can be a alternative specimen to collect for the diagnosis of covid-19 where the recourse are very limited.

CONCLUSION

Saliva can have potential applications in the context of COVID -19 by direct transmission of the virus. With the development of appropriate sample collection and processing methods saliva can be provide useful clinical information of the disease and could be potentially included in the guidelines for sample collection for the diagnosis ,disease management and control of diseases.

Saliva can be a non invasive specimen for screening SARS COV-2 suspected patients. It should be recommended as a viable alternative to oro-nasopharyngeal specimen as it may cause discomfort to patients for covid-19 testing.

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REFERENCES

1. E.Pasomsub, S.P.Watcharananan, K Boonyawat, P.Janchompoo, G.Wongtabtim, W.Suksuwan, S. Sangkanuparph, A. Phuphuakrat, et al, : saliva sample as a noninvasive specimen for the diagnosis of coronovirus disease 2019: a cross sectional study. Clinical microbiology and infection, htpps: //doi. Org/10.1016/j.cml.2020.05.001.
2. Jose.J. Ceron, Elsa Lamy, Silvia Martinez-Subiela, Pia Lopez-Jornet, Fernando Capela-Silva et.al: Use of saliva for diagnosis and monitoring the SARS cov-2 a general perspective: J Clin Med. May 2020 9(5):1491
3. Omid Fakheran, Mnadana Dehghannejad and Abbasali Khademi et.al: Saliva as diagnostic specimen for detection of Sars – cov-2 in suspected patients; a scoping review infectious diseases of poverty (2020)9:100
4. Laszlo Mark Czumbel, Szabolcs kiss, Neelli Farkas, Ivan Mandel Anita Hegyi, et al: Saliva as a candidate for covid-19 diagnostic testing: a Meta analysis. Medrix27, May 2020.
5. Khursid Z, Asiri FYI, Al Wadaani H. Human saliva: non invasive fluid for detecting novel corona virus (2019-n Cob). Int J Environ Res Public Health, 2020; 17(7):2225
6. Liu I, Wei Q. Alvarez X, Wang H, Du Y, Zhu H, et al. epithelial cells lining salivary glands ducts are early target cells of severe acute respiratory syndrome coronovirus infection in the upper respiratory tracts of rhesus macaques. J virol 2011; 85; 4025-30.
7. To KK, Tsang OT, Leung WS, Tam AR, Wu TC, Lung DC et al. temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARSACOV-2: an observational cohort study. Lancet infect Dis 2020.
8. Chan JF, Yuan S, KOK KH, To KK, Chu H, Yang J,et al. a familial cluster of pneumonia associated with the 2019 novel corona virus indicating person to person transmission; a study of a family cluster. Lancet 2020; 395; 514-23.
9. Huang C, Wang Y, Li X, Ren I, Zhao J, Hu Y, et al. clinical features of patients infected with 2019 novel corona virus in Wuhan, China. Lancet 2020; 395:497-506.

10. Guan WJ, Ni ZY, Hu Y, Liang WH, Ou CQ, He JX, et al. clinical characteristics of corona virus diseases 2019 in China, N Engl J Med 2020;382:1708-20
11. World health organization, laboratory testing for 2019 novel corona virus (2019-n Cov) in suspected human case 2020. Available from : https:// www.who.int/publications-detail/laboratory -testing-for-2019-novel-coronovirus-in-suspected-human-cases-20200117.
12. Wang Wk, Chen SY, Liu IJ, Chen YC,Chen HL, Yang CF,et al. Detection of SARS-associated corona virus in throat wash and saliva in early diagnosis. Emerg Infect Dis 2004;10:1213-9
13. Loeffeholz MJ, Tang YW. Laboratory diagnosis of emerging human corona virus infections - the state of the art. Emerg Microbes Infect.2020; 9(1):747-56.
14. Chojnowska S,Baran T, Wilinska I, Sienicka P, Cabaj –Wiater I, Knas M, Human saliva as a diagnostic material. Adv Med Sci.2018; 63(1):185-91.
15. To KK, Tsang OT, Chik-Yan Yip C, Chan KH, Wu TC, Chan JMC, et al. Consistent detection of 2019 novel corona virus in saliva. Clin Infect Dis. 2020; ciaa 149, https://doi.org/101093/cid/ciaa149.
16. To KK, Lu L, Yip CC, Poon RW, Fung AM, Cheng A, et al. Additional molecular testing of saliva specimen improves the detection of respiratory viruses. Emerg Microbes Infect.2017;6(6):e49.
17. To KK, Yip CCY, Lai CYW, Wong CKH, Ho DTY, Pang PKP, et al. Saliva as a diagnostic specimen for testing respiratory virus by a point-of-care molecular assay: a diagnostic validity study. Clin Microbiol Infect 2019,25(3):372-8.
18. Zheng S, Yu F, Fan J, Zou Q, Xie G, Yang X, et al. saliva as a diagnostic specimen for SARS- Cov-2 by a PCR based assay a diagnostic validity study 2020.
19. Adhikari SP, Meng S, Wu YJ, Mao YP, Ye RX, Wang QZ, et al. epidemiology, causes, clinical manifestations and diagnosis, prevention and control of corona virus disease (covid-19) during the early outbreak period: a scoping review. Infect Dis Poverty. 2020;9 (1) 1-12.
20. Williams E Bond K Zhang B, Putland M, Williamson DA. Saliva as a noninvasive specimen for detection of SARS –COV-2 J Clin Microbiol.2020 JCM, 00776-20 https:// jcm.asm.org/content/early/2020/04/17/JCM 00776-20,
21. Azzi L, Carcano G, Gianfagna F, Grossi P, Dalla Gasperina D, Genoni A, et al. Saliva as a reliable tool to detect SARSA-COV-2. J Inf Secur. 2020; 81(1): e 45-50

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