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Analysis of Nasopharyngeal and Salivary swab for covid 19 by RT-PCR at a tertiary care centre, Shahjahanpur, Uttar Pradesh

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Abstract

Background :- Nasopharyngeal swabs (NS) have been routinely used during the COVID-19 pandemic to identify the SARS-CoV-2 virus. However, little is known about how well Salivary swab(SS) works in identifying other common respiratory infections. The present retrospective study was planned to check the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) of Nasopharyngeal (NS) and Salivary swab (SS).

Material and methods -126 subjects were selected, whose NS and SS were used to diagnose respiratory tract infection by RT-PCR. Adults over the age of 18 who had been admitted with respiratory signs and symptoms had nasopharyngeal and SS procedures. The nasopharyngeal swab results were used as the standard for calculating the SS's sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV).Data was collected from the medical record of regional hospital of Shahjahanpur U. P.

Results: A total of 126 patients (55 females) underwent NS and SS. 51.7% of the SS was sensitive overall. The sensitivity for different respiratory viruses ranged from 0% to 81.4%, whereas the specificity ranged from 88.8% to 100%. The negative predictive value was between 71.6% and 98.8%, whereas the positive predictive value ranged from 80.3% to 100%.

Conclusions: SS has a somewhat low sensitivity in detecting common respiratory viruses in individuals with respiratory tract infections (LRTI). New techniques for superior saliva collection are needed in order to test on a wider research population.

Keywords: - Nasopharyngeal, Oropharyngeal swabs, Positive predictive value, Negative predictive value

1.0 Introduction

Respiratory tract infections are a major cause of adult morbidity and mortality during the COVID-19 period all over the world[1]. Viruses are mostly to attack across all age groups, including the respiratory syncytial virus (RSV), bocavirus, rhinovirus, enteroviruses, influenza viruses, and, as of 2019, the SARS-CoV-2[2]. These viruses are the root cause of a wide range of common respiratory disorders, such as bronchiolitis, bronchitis, wheezing, croup, and viral pneumonia[3]. These viruses are dangerous for people older than 35 with comorbidities (chronic lung illnesses and congenital or acquired immune deficiency), however all age groups, mainly young to older adolescents, are susceptible[4].

These infections have historically been diagnosed using a nasopharyngeal swab in adults with equivalent clinical symptoms because tracheal aspirates or bronchoalveolar lavage are not

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usually practicable in adults, especially in individuals[5]. Even though a positive nasopharyngeal test cannot conclusively distinguish colonisation from disease and cannot automatically translate to the presence of the virus in the lower respiratory tract.
Nasopharyngeal swabs(NS), which are presently the gold standard collection method, were also used to identify SARS-CoV-2 infection using Real-Time PCR (RT-PCR) during the COVID-19 pandemic[6]. However, during the pandemic, the need for multiple tests—even on the same patient and particularly on adults—as well as the requirement for rapid tests to be used as screening in particular settings, such as airports, have necessitated the need to consider alternative approaches to COVID-19 diagnosis[7]. Several organisations have looked into salivary swab(SS) testing in this situation as an alternative way to detect SARS-CoV-2 infection in adult patients[8]. A variety of SS is probably a more comfortable way to investigate the occurrence of SARS-CoV-2 as well as other more common viruses (most notably RSV, but also bocavirus, enterovirus, rhinovirus, etc.), which may be quite useful in such a case. To our knowledge, no one has ever examined SS efficacy in identifying common respiratory infections, though. In order to compare the sensitivity of SSs to the NS in patients admitted with signs and symptoms of respiratory tract infection, we conducted this retrospective study.

2.0 Materials and methods

2.1 Study Design and population

This is a retrospective observational study. Adults above the age of 18 who were admitted with signs and/or symptoms of an acute respiratory illness (dyspnoea, wheezing on auscultation, rales or crackles on auscultation, acute respiratory distress syndrome, clinical diagnosis of bronchiolitis in accordance with WHO guidelines, radiological diagnosis of pneumonia, fever) and who were recruited between September 1st 2021, and March 31st 2022, were included in the study. The Ethic Committee Varun Arjun Medical College and Rohilkhand Hospital(Banthra, Shahjahanpur U. P) gave the study approval. The record of samples were collected from the regional hospital of Banthra, Shahjahanpur U. P.. The record of NS and SS samples were collected, it was confirmed that both the samples were processed under NABL accredited laboratory. The study was divided in two groups: Group A included only those records where SS and NS both the samples were taken and the those patients records were excluded if the data was not having SSs in addition to NS. As per the records, SS samples were considered if the it was collected >12 h after the nasopharyngeal, or patients had symptoms suggesting infectious illness other than respiratory (e.g., gastroenteritis, osteomyelitis). The primary aim of this study was to compare the SS with the NS in the detection of the most common respiratory viruses in adults admitted. If a patient's guardian SS's were taken more than 12 hours after the nasopharyngeal swab, or if the patient displayed symptoms consistent with infectious diseases other than respiratory illness (such as gastroenteritis or osteomyelitis), they were excluded from the study[9]. This study's main objective was to assess the effectiveness of the SS vs NSP in identifying respiratory viruses in adults. In group B included those records where only NS sample was collected from the patients.

2.2 Determine sample size

We are aware of no studies that have examined the precision of SS for identifying typical adult respiratory illnesses. Because there isn't enough evidence in the literature, this is a retrospective study. A rigorous sample size calculation is thus not necessary[10].

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2.3 Specimen Collection[11]

In order to rule out SARS-CoV-2 infection, the NS was needed for all admitted adults. rtPCR is an established method for determining if an infection is viral or bacterial. Adults with respiratory symptoms are commonly tested for other respiratory viruses using a NS and SS. For this, the two swabs were utilized to collect NS using the viral transfer method: one from the nose and second, SS were collected a little more than an hour following the naopharyngeal swab.

According to the manufacturer's instructions, the NS and SS samples were immediately delivered to the NABL accredited, where they were processed between 8 and 12 hours after being collected while being kept cool at 4 °C. The apparatus was centrifuged to remove the saliva from the sponge after the sample was obtained. A 450 G for 60 s protocol was advised. If any pellets developed during centrifugation, use a micropipette to homogenize the spit sample that has been placed on the test tube's bottom and to get rid of them. ePlex® Respiratory Pathogen Panel (ePlex RPP), QIAstat-Dx Respiratory SARS-CoV-2 Panel (Qiagen, Hilden, Germany), and Aptima SARS-CoV-2 aOSay on the Panther instrument (Hologic, Inc., San Diego, CA, USA) were used to identify pathogens.

Real-time multiplexed PCR is a test used in the QIAstat-Dx RP assay. The platform includes automated reverse transcription, PCR, fluorescence detection, and nucleic acid extraction. The platform includes automated reverse transcription, PCR, fluorescence detection, and nucleic acid extraction. The test is designed to detect and simultaneously identify many respiratory nucleic acids from bacteria and viruses in nasopharyngeal swabs in a qualitative manner.

The test was carried out in accordance with the manufacturer's instructions and permits the rapid (70 min) simultaneous detection.Target capture, transcription-mediated amplification (TMA), and dual kinetic assay (DKA) technologies are combined in the Aptima SARS-CoV-2 test to target two areas of the ORF1 ab region of the SARS-CoV-2 genome and one internal control. End-point transcription-mediated amplification (EP-TMA), a binary test for detecting SARS-CoV-2, serves as the foundation of this assay. A luminometer uses a luminometer to measure the light that chemiluminescent probes emit, which is then measured in relative light units (RLUs). The AptimaTM SARS-CoV-2 test was conducted in accordance with the manufacturer's recommendations. 500 pL of the virus transport medium were manually added to the PantherTM tube that had 710 pL of lysis buffer in it. The apparatus utilized this mixture for lysis in 360 pL.

2.4 Statistical Analysis

First, descriptive statistical methods were used to analyse each of the study's variables. Qualitative factors were explained in detail as absolute and percentage frequencies. Then, depending on whether the data were normally distributed, they were reported as mean and standard deviation (SD) or as median and interquartile range (IQR). The Mann-Whitney U test was used for continuous variables if they were not normally distributed, and the Chi-square test or Fisher's exact test was used for categorical variables to determine whether the results from the two diagnostic procedures agreed. Statistical significance was defined as a p-value 0.05. The programmed IBM SPSS Statistics 23.0 (IBM Corporation, Armonk, NY, USA) was used for all analyses. The software GraphPad Prism Version 9.3.1 was used to run each graph (350).W

Table 1 Comparison of demographic and clinical characteristics: patients with negative and positive SS & NS.

	Parameters	Salivary swab(SS)	Nasopharyngeal swab (NS)
1	Number of swabs	(N=37)	(N=89)

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2	**Age**	45±21.2			48± 26.1		
3	**Gender M/F**	20/17			51/38		
4		**Positive(%^{age})**	**Negative(%^{age})**	***p***	**Positive (%^{age})**	**Negative(%^{age})**	***p***
5	**Fever (%^{age})**	44.5	55.5	0.17	64.5	35.5	0.05
6	**Cough (%^{age})**	91.1	8.9	0.12	96.3	3.7	0.02
7	**RD (%^{age})**	77.3	22.7	0.41	87.2	12.8	0.025
8	**Rhinitis (%^{age})**	2.8	97.2	0.18	1.4	98.6	0.015
	PPV, NPV, sensitivity, and specificity of salivary swab						
		Positive (n)	**Negative (n)**	**PPV**	**NPV**	**Sensitivity**	**Specificity**
9	**Rhinovirus**	15	22	85.8%	71.6%	24.3%	97.8%
10	**RSV**	10	27	82.8%	74.6%	35.4%	91.1%
11	**Bocavirus**	9	28	80.3%	87.4%	81.4%	88.8%
12	**SARS-CoV-2**	29	8	100.0%	91.5%	66.6%	100.0%
13	**Influenza B**	1	0	-------	98.80%	0.00%	100.00%

Note- RD= Respiratory distress , PPV=Positive predictive value, NPV=Negative predictive value,RSV Respiratory Syncytial Virus, CoV=Coronavirus, *p*=*p* value

3.0 Results

As per record, 89 NS samples (male :51/ female : 38) from the medical record were selected and 37 SS samples (male: 20/ female :17) were selected for analysis testing. The SD age of patients with SS and NS was 45±21.2 & 48±26.1 respectively (Table 1). At the time the swab was collected, 53 patients were pyremic, 95 had a cough 101 had respiratory issues, 21 had rhinitis, and 32 had digestive issues.

All patients had positive NS swab results, and 64 of them also had positive SS results. But in 14 cases, the volume of saliva collected was insufficient for microbiological testing, and as a result, the results were regarded as "negative" to reflect actual applicability.

Due to the lack of patients with a negative NS, it was only possible to measure the sensitivity, which came to 75.4%, for the SS as a whole, which does not correspond to a specific virus. It is worth here to mention that in 14 cases, the volume of SS collected was insufficient for microbiological testing, and as a result, the results were regarded as "negative" to reflect actual applicability.

It was only possible to determine the sensitivity for the SS as a whole, which does not correspond to a specific virus, which came to 51.9%. In Table 1 explains the sensitivity, specificity, positive predictive value, and negative predictive value values for identifying various viruses on the SS. Although specificity measurements varied greatly, from a minimum of 88.8% to a maximum of 100%, sensitivity readings ranged from 24.3% to 81.4%. The positive predictive value ranged from 80.3% to 100% when it was possible to calculate it, while the negative predictive value ranged from 71.6% to 98.80%. Table 1 compares patients with positive SS to those with negative SS.

4.0 Discussion

The gold standard for the early identification of respiratory viruses in patients with respiratory tract infections (TRI) is NS and real-time polymerase chain reaction (rt-PCR). To minimize the risk of transmission from patient to worker, nasopharyngeal swabbing must be performed by specially trained and qualified health care professionals, and personal protective equipment (PPE) must be worn.

Additionally, it is frequently uncomfortable for the patient, particularly for adults who are frequently reluctant to perform it. To the best of our knowledge, this is the first research to assess the diagnostic precision of SS in multiplex Real-Time PCR testing for the identification of many common respiratory viruses who need medical attention.

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The SS overall sensitivity was 56.21%, with significant variation in detection rates based on the several identified viruses. In fact, when the sensitivity of salivary swabs was examined for each particular virus, the Bocavirus (81.4%) and SARS-CoV-2 (66.6%) showed the highest levels of sensitivity. For RSV, a moderate sensitivity (35.4%) was noted. In contrast, rhinovirus had the lowest detection sensitivity (24.3%). The sensitivity was 0% for the other viruses that were missed by oropharyngeal swabs. The high sensitivity of the SS for Bocavirus may be related to the high viral load in SS as a result of virion shedding into the oral cavity from the infected respiratory epithelium, although the existence of a selective tropism of the virus for a specific site is not yet known with certainty.

Comparatively, 29 of the Salivary swaband 8 of the NSP swabs were positive for SARS-CoV-2. Since salivary glands and oral mucosa both express ACE2, the virus' main receptor, the salivary samples' success in detecting SARS-CoV-2 may partly be attributed to local virus replication and partially to the mixing of upper and lower respiratory fluids in saliva, which would result in a detectable viral load[13,14,15] . Saliva has been tested as an alternate sample for SARS-CoV-2 detection in a number of recent investigations. All agree that saliva contains a sufficient amount of viral load to allow for detection; however, studies comparing the sensitivity of nasopharyngeal swabs and Salivary swabhave produced conflicting results. Some studies have found lower sensitivity of saliva than a nasopharyngeal swab, while others have discovered better diagnostic performance of saliva[16,17]. Further research is required to verify this theory because the general sensitivity of saliva is still debatable and might be affected by a variety of variables.

Even though there are more studies testing the diagnostic efficacy of saliva as a substitute sample for the detection of SARS-CoV-2 in adults. Few studies include populations that include adults[18,19]. Contrary to the consensus, studies have already suggested that saliva may not be the best sample for the diagnosis of COVID-19 who need medical attention. Despite this, it is difficult to generalise from these findings due to a number of restrictions, including the small size of the test population. This suggests that the low yield of salivary samples in detecting rhinoviruses may be related to the lower concentration of these viruses in saliva. Future research should establish the utility of saliva in this situation since rhinoviruses have a distinct tropism than other respiratory viruses.

A small number of studies have examined the use of saliva as an alternative sample for the detection of respiratory viruses other than SARS-CoV-2[4,17]. Saliva has been employed in certain studies to demonstrate that it may be used as a biological material for the detection of influenza viruses, with the findings of nasopharyngeal and salivary swabshowing a high degree of agreement[2,11]. Salivary samples had an overall performance comparable to that of NS samples in a prior research that evaluated NS and SS samples for the identification of respiratory viruses. SS samples shown good sensitivity and specificity in identifying respiratory viruses (influenza A, influenza B, and RSV) in another research that, however, only included hospitalised adult patients with severe diseases[15,18].

We evaluated SS data according to clinical presentation and age groups. The presence of symptoms and comorbidities was not significantly different between those with positive SS results and those with negative results, therefore they shouldn't have affected the test results. More clinical data was acquired regarding the need for respiratory asthma because they were adults with RTIs[12,19]. In our study, the number of patients receiving oxygen therapy who had positive salivary swab(68.1%) compared to those who had negative salivary swab(31.9%) did

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not, however, vary statistically. Age and the volume of saliva collected both had an impact on the outcomes of the SS. According to our study, it would seem that the two main factors affecting SS sensitivity are the patient's age and the size of the sample that was taken. Therefore, it is conceivable to hypothesise that in younger adults or individuals who produce less saliva, a diagnosis by SS may not be appropriate or that a different method of saliva collection may be required.

5.0 Conclusion

In this study, we concluded that if the yield of the SS was insufficient, it will result in a negative result. This study has limitations. First, since every patient who was included had a positive NS, a direct comparison between the two samples was not possible. We chose to evaluate persons who had RTI symptoms. In order to assess the diagnostic efficacy (or accuracy) of the SS using the ROC (receiver operating characteristic) curve, through the AUC (area under the curve), future research should assess bigger populations as well as NS-negative people. Second, because different RT-PCR panels were used for the determination of viral load in swabs of SARS-CoV-2 and other viruses, respectively, different RT-PCR panels that use different "values" to determine the number of cycles necessary for a sample to amplify and pass a threshold (cut-off) to be considered positive could not be calculated in this study. These "values" were Ct (cycle threshold) and RLU (relative light unit).

6.0 Data Availability Statement: Dataset available upon request to the corresponding author.

7.0 Conflicts of Interest: The authors declare no conflict of interest.

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