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ASSESSING THE EFFECTIVENESS OF RAPID ANTIGEN TESTING IN COMPARISON TO REAL-TIME RT-PCR IN COVID-19 DIAGNOSIS

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Abstract

Background: RAT are essential to identify SARS-CoV-2-positive patients, isolate them, break the transmission chain, and contain COVID-19. However, rapid antigen tests (RATs) have low sensitivity, which can lead to missed cases. This study evaluated the effectiveness of a RAT kit with reference to the gold standard real-time RT-PCR for suspected COVID-19 patients.

Methods: We investigated 370 nasopharyngeal swabs at MTI, Khyber Teaching Hospital Peshawar, Pakistan for RAT and the real-time RT-qPCR. We evaluated the effectiveness of the RAT by determining its sensitivity, specificity, PPV (positive predictive value), NPV (negative predictive value), diagnostic accuracy and kappa statistics.

Results: The results indicated a sensitivity of 79.35% and a specificity of 98.6%. The PPV was 97.62% and the NPV was 86.89%. The accuracy between the two techniques was found to be 90.54% with a kappa coefficient of 0.800. We identified that at lower cycle threshold (CT) values, the RAT was more sensitive. However, at higher CT values, the rapid antigen test sensitivity decreased.

Conclusion: The RAT had a high specificity but its sensitivity become low with high CT values and low viral load, thus it was more likely to miss some positives cases. Our findings suggest that the rapid antigen test can be a beneficial tool in mass screening of COVID-19, particularly in settings where RT-PCR is not feasible due to resource constraints or turnaround time but it is important to be aware of its limitations.

INTRODUCTION

In late 2019, a novel human respiratory pathogen called coronavirus disease (COVID-19) emerged in Wuhan, China. This highly contagious viral illness is raised by a positive-sense RNA virus known as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (1). It had a devastating disruptions on the world's demographics causing the death of more than 6 million people worldwide, making it the most substantial global health problem after the influenza pandemic in 1918 (2). According to the WHO report 1,580,631 COVI-19 positive cases have been reported with more than 30 thousand deaths till 22 August, 2023 (3). To control the spread of this pandemic diagnosis of the people infected with SARS-CoV-2 is very important. A gold standard technique used for the identification of this viral infection is reverse transcriptase- polymerase chain reaction (RT-PCR). This is known as a reference method for identification of COVID-19 because of its high accuracy, sensitivity and efficacy. However, nucleotide-based testing of viral RNA is very expensive, laborious and time-consuming process. It requires a well-developed biosafety level (BSL-2/BSL-3) laboratory and skilled lab personnel in terms of personal and instrumentation (4). It may take hours to days to generate the report after receiving the sample. Although its sensitivity is more, but it can only be conducted in the cities having a well-established molecular virology facility and cannot be carried out in every city since these facilities are not available everywhere (5). Therefore, in order to examine the samples are transported to the centers having RT-qPCR facility which delays the test results and also increase the anxiety of the suspected COVID-19 patients (6).

Rapid antigen tests (RAT) is a rapid and cost effective method for the diagnosis of COVID-19. It does not require specific and expensive machinery have been approved for clinical purposes to improve this situation and the results are compared with that of many kinds of RT-qPCR (7). Rapid Antigen Test (RATs) can improve the diagnostic capacity during pandemic by decreasing the response time and cost for the healthcare system, especially in situation where the molecular test facility could be limited (8).

MATERIALS & METHODS

Study Population

The study was conducted on 370 random suspected patients visited the emergency triage at Khyber Teaching Hospital Peshawar - Pakistan in period from March,15, 2022 to April, 15 2022. The inclusion criteria for the study required participants to have both RAT and RT-q PCR test result. Two concurrently nasopharyngeal swabs were taken from each suspected patient. During sample collection, a pre-designed form was used to collect a complete history of the patient's clinical signs and symptoms. We conducted two tests on separate swabs: one underwent a rapid antigen test (RAT) following the manufacturer's instructions to detect SARS-CoV-2, and the other swab was subjected to SARS-CoV-2 reverse transcription polymerase chain reaction (RT-PCR) using a standard protocol. We collected and analyzed data from our Internal Hospital Information Management System (HIMS) database. The retrieved data includes patient medical record numbers (MR no), collection dates, names, ages, genders, addresses, and results from both RAT and RT-PCR.

TESTING FOR THE SARS-COV-2 VIRUS

Panbio™ COVID-19 RAT (Abbot Diagnostics Jena GmbH, Germany) kit was used to perform Rapid Antigen Test. Nasopharyngeal swab was taken from the suspected subjects and immediately processed to perform RAT using the manufacturer's guidelines. This rapid antigen test (RAT) kit operates on the rapid lateral flow immunoassay principle and is intended to provide results for SARS-CoV-2 nucleoprotein within 30 minutes. The kit includes all the necessary reagents and an instruction manual for conducting the test.

RNA EXTRACTION AND REVERSE TRANSCRIPTION POLYMERASE CHAIN REACTION (RT-PCR)

We conducted viral RNA extraction using the TANBead® Nucleic Acid Extraction Kit in conjunction with the TANBead® Nucleic Acid Extraction System, developed by (Taiwan Advanced Nanotech

Inc, Taiwan). To begin, we added 200 µl of the viral transport medium (VTM), which served as the sample, to the 96 well extraction plate. The plate also contained 500 µl of lysis buffer, 800 µl of washing buffer 1, 800 µl of magnetic beads, 800 µl of washing buffer 2, 800 µl of washing buffer 3, and 130 µl of elution buffer. The final step yielded the extracted RNA in a well containing 50 µL of elution buffer.

Reverse transcription Polymerase chain reaction was performed using the TaqPath™ COVID-19 RT-PCR Kit as per the manufacturer's instruction on QuantStudio™ 5 Real-Time PCR Instrument (Thermo Fisher Scientific, USA). This kit is designed to detect specific genes, including Nucleocapsid Protein (N), Surface Protein (S), and ORF1ab. The target genes were amplified and detected in three different channels: VIC, ABY, and FAM. An internal control (MS2) was also included in the reaction. A sample was considered positive if two or more of the target genes were detected and the MS2 control was positive. A sample was considered negative if none of the target genes were detected, but the MS2 control was positive. A sample was considered inconclusive if only one target gene was detected, regardless of the MS2 control result. A sample was considered invalid if no target genes were detected and the MS2 control was negative.

DATA ANALYSIS

The data were interpreted and assessed in Microsoft Excel to calculate percentages. Statistical analysis involved determining sensitivity, specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV), diagnostic accuracy, and the kappa coefficient, with a 95% Confidence Interval, using the SPSS software.

RESULTS

DEMOGRAPHIC CHARACTERISTICS OF THE STUDY POPULATION

This research evaluated data from 370 nasopharyngeal swabs and summarized the demographic characteristics of all patients in Table 1. Females accounted for 58.83% of the cases, while males accounted for 41.62%. Most of the samples were taken from the Emergency Triage, which made up 65.19% of the cases. The median age of the 370 cases was 37 years (range, 1-80 years), with 36.2% (134/370) presenting symptoms suggestive of COVID-19 and 63.8% (236/370) being asymptomatic. The most commonly reported symptoms were fever (36.2%), cough (34.0%), sore throat (32.8%), runny nose (32.8%), loss of smell (30.8%), body aches (24.8%), and shortness of breath (20.5%). Of the 370 cases, 44.9% (167) were vaccinated and 55.1% (203) had not received a COVID-19 vaccine. The demographic details of the study population are displayed in Table1.

Table 1: Demographic details of study population

DEMOGRAPHICS	NO	%AGE
POPULATION	370	
GENDER		
Male	154	41.6
Female	216	58.4
AGE		
1-20	32	8.6
20-40	242	65.4
40-60	62	16.8
60-80	34	9.2
Symptomatic (At least one sign or symptom)	134	36.2
Asymptomatic	236	63.8
COVID-19 VACCINATION STATUS		
Vaccinated	167	44.9
Non Vaccinated	203	55.1
CLINICAL FEATURES		
Fever	134	36.2
Cough	126	34.0
Sore Throat	122	32.8

Loss of Smell	114	30.8
Body Aches	92	24.8
Shortness of breath	76	20.5
Runny Nose	122	32.8

DIAGNOSTIC PERFORMANCE OF RAT

In order to assess the rapid antigen test's ability to distinguish the presence or absence of SARS-CoV-2, we calculated the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV), diagnostic accuracy and kappa coefficient, utilizing the RT-PCR test as the gold standard. Among a total of 370 swabs, 158 (42.7%) were determined to be positive and 212 (57.3%) negative by RT-PCR. Focusing on the 158 RT-PCR positive samples, the RAT test identified 126 (77.8%) as positive, while missing 32 (22.2%) samples and 03 (0.80%) samples tested positive by RAT and negative by RT-PCR. Among the total samples tested with both methods, 123 were true positives, 212 were true negatives, 03 were false positives, and 32 were false negatives.

The rapid antigen test revealed a sensitivity of 79.35% (123 out of 149) and a specificity of 98.6%. The PPV was 97.62%, and NPV was 86.89%. The overall diagnostic accuracy between the two techniques was 86.89%, and the kappa coefficient found to be 0.800, representing a strong agreement between the two methods.

Table 02: Representation of TP, TN, FP, FN values

TP (123)	FP (03)
FN (32)	TN (212)

Table 03: Comparison of SARS-CoV-2 diagnostic performance of RAT with gold standard Real-time RT-PCR

		Gold Standard (Real-time RT-PCR)		Total
		Positive	Negative	
Test (RAT)	Positive	123	03	126
	Negative	32	212	244
	Total	155	215	370

Performance Parameter	Percentage	95% Confidence Interval		Kappa Statistics
Sensitivity	79.35	72.31	84.98	0.800
Specificity	98.6	95.98	99.52	
PPV	97.62	93.23	99.19	
NPV	86.89	82.07	90.55	
Diagnostic Accuracy	90.54	87.13	93.12	

We also conducted a comparison of the rapid antigen tests (RAT) sensitivity with the RT-PCR test for SARS-CoV-2 at various cycle threshold (CT) values. The sensitivity of the RAT for detecting SARS-CoV-2 was determined to be 96.87% when the CT values were below 25.0. Nevertheless, the test's positive detection rate gradually decreased to 82.0% within the CT range of 25-30.0 and further declined to 52.0% when the CT values were in the range of 31-35.0. Notably, the test's positive detection rate dropped to 0% for CT values of 35.0 or higher (Table 4).

Table 4: Diagnostic performance of the evaluated rapid antigen test in RT-PCR confirmed patients

CT Value	PCR (+)	RAT (+)	Sensitivity (%age)
Overall	158	123	79.35
18-25	64	62	96.87
26-30	50	41	82.00
31-35	38	20	52.00
Above 35	6	0	0

DISCUSSION

SARS-CoV-2, which is responsible for the COVID-19 disease, initially emerged in late 2019 and rapidly spread worldwide, resulting in substantial illness and mortality (9). Despite the fact that several SARS-CoV-2 vaccines have been approved, difficulties with production, distribution, and uptake as well as the appearance of new viral variants show that herd immunity against covid-19 is still a distant prospect (10). Therefore, proper testing, contact tracing, and infectious case isolation continue to be crucial measures to stop the spread of SARS-CoV-2 and maintain the quality of the healthcare system (11).

Since the start of the SARS-CoV2 epidemic, the capacity to quickly diagnose and identify infected individuals has been essential for the management of this viral illness. The introduction of RAT tests has substantially reduced the delays in the test results, making a rapid clinical intervention and preventive measures possible however, there are still potential dangers with regard to the accuracy of the diagnostic test. Antigen-based tests have emerged as one of the most compelling choices, but there are few independent assessments of their diagnostic outcomes, therefore it's unclear whether they fit into standard diagnostic workup (12). Furthermore, this detection method may fail to detect some cases in which viral load or replication is low during the early and late stages of infection. Consequently, it remains vital to identify asymptomatic and pre-symptomatic individuals who have the potential to transmit the COVID-19 virus, particularly in its early stages, to prevent both community and hospital-acquired infections (13). Antigen-based testing increase TAT (turnaround time), which is crucial for breaking transmission chains and containing the pandemic, but it has a lower sensitivity than PCR (14).

In this current study, we assessed the diagnostic performance of Panbio™ COVID-19 RAT (Abbot Diagnostics Jena GmbH, Germany) with real-time RT-PCR analyzing different SARS-CoV-2 genes, in a cohort of 370 suspected subjects in Khyber Teaching Hospital, Peshawar. We targeted three SARS-CoV-2 genes (ORF1A, S and N genes), analyzed their CT values using Real-time PCR and compare our results with that of RAT results. The suspected subjects include 216 (58.4 %) female and 154 (41.6 %) male. Out of 370 suspected subjects 158 showed positive results for SARS-CoV-2 on Real-time PCR and 123 were true positive on RAT. The antigen test used in this study has a specificity (98.61%), sensitivity (79.35%) and accuracy (90.54%) indicating that it is very unlikely to give a false positive result, however it can give a false negative result. This result shows that the antigen test can be useful for point-of-care testing, but it should be used with caution and should be followed up with a RT-PCR test if the results are negative and the cases are symptomatic. The kit has a high PPV and NPV, making it a promising option for screening both symptomatic and asymptomatic individuals (15). The study showed some discrepancies between the real-time RT-PCR and the RAT test, 20.25% of the false-negative results were observed for high Ct values, whereas we found concordance between RAT and RT-PCR test at medium-lower Ct, reflecting the ability of the RAT to detect better at high viral load in symptomatic cases. This study was supported by the studies of Platten et al., describing that RAT give us negative result in PCR positive sample having high CT values (16). Additionally, a contradiction was observed in 3 cases that tested positive for Ag but negative for RT-PCR. Errors that may have affected the pre-analytical phase (such as sample collection) or the data collection could be a reason for this discrepancy (subjective RAT reading). The similar pattern of findings has been reported by Keaney, D et al.., in his studies in Ireland (17).

When we classified the RT-qPCR results according to the CT values, we found the CT values ranged from 18 to 38. This result shows the RAT sensitivity of almost 96.87% in positive individuals having CT values below 25. About 25.3% of the RT-PCR-positive individuals had the CT values ≥30 and showed sensitivity of 82.00% on RAT. It then quickly decreased from 84.00% to 52.0% after the CT value of 31.0 to 35.0 as shown in Table 4. We further investigated that the RAT positivity rate declined to 0% when the CT values were greater than 35.0. The specificity of the RAT was 98.61% indicating that it has a high concordance with RT-PCR when detecting negative cases. These results suggest that the low severity of the infection might have contributed in the low sensitivity of the test results. Gupta et al has reported a similar pattern in his study in New Delhi (18). This shows that we cannot rely on rapid antigen test for the asymptomatic patients having low viral load. Rapid antigen test can therefore

be used as a screening test to rule out the infection, but has a low sensitivity in contacts who are asymptomatic. At the same time, the sensitivity can increase above 90% in patients with a high viral load. Therefore, RAT is a very useful tool to prevent the spread of the disease by screening the patients with a high viral load.

Conclusion

In conclusion, this study provides valuable information on the accuracy of RAT for the diagnosis of COVID-19 at MTI, Khyber Teaching Hospital Peshawar, Pakistan. The results suggest that RAT can be used as a diagnostic tool for the rapid identification of COVID-19 cases in resource-limited settings, but it should be followed by confirmation with RT-qPCR. Further studies are required to assess the performance of RAT in different clinical settings and populations, and to identify factors that may affect its accuracy, such as the timing of testing, the type of antigen test used, and the prevalence of SARS-CoV-2 in the population.

RECOMMENDATIONS

- SARS-CoV-2 variants with mutations in different genes need to be closely monitored to assess their impact on rapid antigen tests (RATs) that use these genes as targets.
- It is crucial to evaluate the accuracy and reliability of different SARS-CoV-2 rapid antigen tests that are commercially available.

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