Comparison of Rapid Antigen Detection Test with Reverse Transcription Polymerase Chain Reaction in Highly Suspected COVID-19 Patients

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ABSTRACT

Objective: To compare Rapid Antigen Test (RAT) with Reverse Transcription Polymerase Chain Reaction (RT-PCR) in highly suspected COVID-19 patients and to determine its diagnostic parameters.

Study Design: Hospital-based, descriptive/observational study.

Place and Duration of the Study: Department of Medicine/COVID Complex, Medical Teaching Institution/Lady Reading Hospital, Peshawar, Khyber Pakhtunkhwa, Pakistan, from October 2021 to April 2022.

Methodology: A total of 300 highly suspected cases of COVID-19 of either gender admitted in the COVID Complex of the hospital, were included. Data from the patients, including RAT and RT-PCR for COVID-19, were collected retrospectively. RT-PCR was used as the reference test and compared with RAT. Diagnostic statistics of RAT, with their respective 95% confidence intervals were calculated for RAT in diagnosing COVID-19, with significance at $p \leq 0.05$.

Results: Among the 300 patients, 137 (45.7%) were males and 163 (54.3%) were females. The mean age was 56.80 ± 13.72 years. On screening, 138 (46%) patients tested positive and 162 (54%) were negative by RAT; whereas 213 (71%) tested positive and 87 (29%) were negative on RT-PCR. The sensitivity and specificity of RAT were 54.5% (95% CI: 47.52%-61.28%) and 74.7% (95% CI: 64.25%-83.42%), respectively. Positive predicted value was 84.1% (95% CI: 78.26%-88.53%) and negative predictive value was 40.1% (95% CI: 35.63%-44.79%). The positive likelihood ratio was 2.15 (95% CI: 1.47-3.15). The negative likelihood-ratio was 0.61(95% CI: 0.50-0.74). The overall accuracy was 60.33% (95% CI: 54.55%-65.91%).

Conclusion: There was a low sensitivity and specificity of the RAT for COVID-19, with an overall accuracy of 60.33%, compared with RT-PCR.

Key Words: *COVID-19, Rapid Antigen Test, Sensitivity, Specificity, RT-PCR.*

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INTRODUCTION

Since the first reported case in December 2019, the rapidly emerging severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) pandemic has been causing public health challenges worldwide.[1] Infection with *SARS-CoV-2* can be asymptomatic, and it may cause mild symptoms of fever and cough or severe viral pneumonia. In severe cases, some patients may develop acute respiratory distress syndrome (ARDS) and even result in death, with an average mortality rate of 6% (range 1–14.4%).[1,2]

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Since the symptoms of COVID-19 infection are non-specific and are also present in other viral diseases like influenza; therefore an early diagnosis of the disease is important to isolate and treat the cases.[3] The current WHO-recommended gold standard test for diagnosis of COVID-19 infection is the detection of nucleic acid by Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) of nasopharyngeal secretions.[4] RT-PCR is, however, time-consuming and an expensive test, since it requires specialised laboratories and qualified, trained staff. Therefore, there is a high demand for alternative assays such as antigen detection tests, which can detect the presence of the virus in respiratory samples, and is relatively quick, cheap, and easy to perform.[5,6]

Rapid Antigen Tests (RATs) can be performed quickly and serve as point-of-care testing.[7] These tests can be helpful to overcome the overwhelmed diagnostic laboratories and global RT-PCR reagent shortages.[8] As the viral load, measured by RNA copies, peaks near symptom onset and contagiousness begin even earlier than that, RATs may have the highest sensitivity in the most contagious individuals.[9-11]

There are wide variations in the reported sensitivity of RAT; the manufacturers' claimed sensitivity is often higher than those of independent assessments.[12] The World Health Organization's RAT target product profile aims at sensitivities above or equal to 80% and specificities above or equal to 97%.[13,14] So, this study was designed to compare the RAT with RT-PCR for highly suspected COVID-19 patients to know the overall diagnostic accuracy like sensitivity, specificity, predictive values, and likelihood-ratios of the RAT. The findings of this study will help in the early diagnosis of COVID-19 for the early institution of treatment strategies and exclusion of other non-COVID cases, which ultimately will help in the reduction of the burden on COVID-specific wards and high dependency units (HDUs) in hospitals.

METHODOLOGY

This retrospective study was conducted in the Department of Medicine/COVID Complex (COVID-19 specific wards and high dependency units), Lady Reading Hospital (LRH), Peshawar, from October 2021 to April 2022. Data of 300 patients were included retrospectively by non-probability consecutive sampling technique, keeping 80% sensitivity of Antigen based rapid detection test (RTD) in diagnosing COVID-19;[13,14] 95% confidence interval, and 5% margin of error, using WHO calculator.

All highly suspected COVID-19 patients admitted to the COVID Complex of LRH Peshawar, aged 18 years and above of either gender were included in the study. Non-consenting COVID-19 patients; patients with pneumonia secondary to bacteria and other viruses like H1N1; and severely immune-compromised patients, due to any reason, were excluded.

Patients with acute respiratory symptoms (fever, cough, shortness of breath) and radiological findings of infiltrates on chest radiographs were considered highly suspected cases of COVID-19. RAT was a rapid diagnostic tool for the detection of COVID-19 antigen in nasopharyngeal swabs obtained from the highly suspected cases of COVID-19. RT-PCR was the diagnostic tool for the detection of COVID-19 viral RNA, based on nucleic acid amplification test (NAAT) by PCR assay in nasopharyngeal swabs obtained from the highly suspected COVID-19 patients.

After approval from the Hospital Ethical Review Board, data of 300 highly suspected COVID-19 patients were retrieved from the Hospital Information Management System (HMIS) stored in the hospital's computer system. Nasopharyngeal swabs obtained from all the patients for the RAT which was performed on the Abbot COVID-19 Ag-rapid test device and conventional RT-PCR performed by the Genrui Biotech Inc, China. Complete blood count, blood glucose level, urea, creatinine, liver biochemistry, inflammatory markers (Ferritin, D-dimers, and lactate dehydrogenase), and arterial blood gases were analysed, and chest radiographs were taken. Demographic and clinical details of the patients, and results of the RAT and RT-PCR for COVID-19 were noted.

Data were entered and analysed using IBM® SPSS® version 23. Mean ± standard deviations were calculated for the numerical data and frequency/percentages were used for the categorical data. Diagnostic statistics of RAT like sensitivity, specificity, positive and negative predictive values (PPV, NPV), likelihood-ratios (LRs), and test accuracy, with their respective 95% confidence intervals (95% CIs), were calculated for Antigen-based rapid detection test (RAT) in diagnosing COVID-19. RT-PCR was used as a reference test and compared with RAT. The level of significance was $p \leq 0.05$. The results are presented in Tables I and II.

Table I: RAT and RT-PCR test results for COVID-19 patients (n=300).

	RT-PCR* positive	RT-PCR negative	Total
RAT* positive	116(84%)	22(16%)	138(46%)
RAT negative	97(60%)	65(40%)	162(54%)
Total	213(71%)	87(29%)	300(100%)

*Chi-square p-value: χ^2 (df1) =21.163, p=0.000. *RAT: Rapid Antigen Test. *RT-PCR: Reverse Transcription-Polymerase Chain Reaction.*

Table II: Diagnostic statistic parameters of the Rapid Antigen Test (RAT).

Statistic Parameters	Value	95% Confidence Interval
Sensitivity	54.46%	47.52% - 61.28%
Specificity	74.71%	64.25% - 83.42%
Positive predictive value (PPV)	84.06%	78.26% - 88.53%
Negative predictive value (NPV)	40.12%	35.63% - 44.79%
Positive likelihood ratio (LR+)	2.15	1.47 - 3.15
Negative likelihood ratio (LR-)	0.61	0.50 - 0.74
Accuracy	60.33%	54.55% - 65.91%

RESULTS

Among 300 patients, 137(45.7%) were males and 167(54.3%) were females. The age of the patients ranged from 16 to 100 years, with a mean age of 56.80 ± 13.72 years. Forty (13.3%) patients were young, under 40 years of age; 163 (54.4%) were in middle age, 41-60 years, and 97 (32.3%) were elderly patients. Duration of symptoms ranged from 2 to 120 days with a mean duration of 13.30 ± 12.66 days. Of the 300 patients, 138 (46%) tested positive for RAT, whereas 213 (71%) were positive for RT-PCR, as shown in Table I.

An analysis of diagnostic statistics of the RAT results, as shown in Table II, revealed a sensitivity of 54.46% (95% CI:47.52% - 61.28%) and specificity of 74.71% (95% CI: 64.25% - 83.42%); PPV of 84.06% (95% CI: 78.26% - 88.53%) and positive likelihood- ratio of 2.15 (95% CI: 1.47 - 3.15) and accuracy of 60.33% (95% CI: 54.55% - 65.91%).

DISCUSSION

Although RT-PCR for COVID-19 is the most reliable, gold standard test for diagnosis of COVID-19; however, it is an expensive and time-consuming test which requires specialised laboratories/techniques, and trained staff. RAT is an alternate, cheap, and easy--to-perform test for the rapid diagnosis and isolation of COVID-19 patients. However, for a test to be reliable, WHO has set an Ag-RAT target product which should have sensitivity above or equal to 80% and specificity above or equal to 97%.[13,14] In this study, the sensitivity of the COVID-19 Ag rapid test device was 54.46%, and the specificity was 74.71%. This is far below the standard set by the WHO and other studies performed locally and internationally. Larik *et al*. in a study in Quetta, Pakistan, found a sensitivity and specificity of 80% and 74%, respectively.[15] However, in another

study, Saeed *et al*.[16] found a sensitivity of 52% in Islamabad/Rawalpindi, Pakistan, similar to the findings in this study. Similarly, in another study conducted in Uttar Pradesh, India, Pandey *et al*. found a sensitivity of 53.6% and specificity of 97.35%, PPV of 81.1%, and NPV of 90.7%.[17] On the other hand, higher sensitivity and specificity of 94.6% and 99.5% respectively were found by Hussan *et al*. in Haripur, Pakistan.[18] Pena *et al*, in Chile,[19] found a sensitivity of 69.86% and specificity of 99.61%, PPV of 94.44% and NPV of 97.21%, and accuracy of 97.04% using *SARS-CoV-2* RAT SD Biosensor, Inc. Republic of Korea. Another study from Chile revealed the sensitivity of two kits of 62% and 85%, respectively, with 100% specificity.[20]

Contrary to this study, a study conducted in Germany showed a sensitivity of 78·3%, specificity of 99·5%, PPV of 93.9%, and NPV of 97·8%.[21] Similarly, another study from Los Angeles, California demonstrated a sensitivity of 72.1% and specificity of 98.7% in the symptomatic population.[22] Berger *et al*. from Geneva, Switzerland reported the sensitivity and specificity of two different RAT kits as 85.5%, 100%, and 89%, 99.7%, respectively.[23]

Evidently, there are wide variations in the reported sensitivity, specificity, and other diagnostic parameters of the rapid antigen tests. In this study, lower sensitivity and specificity of RAT were found as compared to the other studies in Pakistan and abroad. The possible explanation for this discrepancy is that most of the patients in this study were referred from the other peripheral hospitals of the province where they remained admitted for quite a few days; which also explains the duration of symptoms of 2 to 120 days with a mean period of 13.30 ± 12.66 days. The viral load is highest during the first week of illness and thereafter declines steadily over the next weeks.[6,9-11] RAT sensitivity is, therefore, highest during the first week of symptoms and decreases thereafter, while RT-PCR continues to detect the virus even with a low viral load.[9-11,24] Sometimes, RT-PCR detects the presence of viral RNA including dead virus and viral fragments in the late presenters, which might not be correlated with the transmission.[7] This phenomenon is reflected in the results where RAT was positive in 138 (46%) patients but the RT-PCR was positive in 213 (71%) patients. Secondly, the samples for RAT and RT-PCR were not taken at the same time; the RAT was performed at the presentation and the PCR was performed a day or two after admission in the COVID Complex. This might have resulted in technical discrepancies in the samples taken. Differences in results with other studies could also be explained based on differences in the sample sizes, techniques, variations of the test kits and their validation, variations in the technique between different laboratories, *etc.*

A remarkable number of patients presented with the typical clinical and radiological features of COVID-19 had negative results of RT-PCR and/or RAT. They were managed as COVID on clinical grounds. RT-PCR, though the gold standard and most accurate test, does not always give 100 percent certainty; this may occur at the beginning and end of the infection.[21] A negative result does not rule out the possibility of COVID-19. The negative result may be due to a variety of factors like the poor quality of the collected specimen, improper timing and inappropriate handling of the specimen collection, and viral mutations.[4] The yield of antigen-based rapid tests is high in case of high viral load which occurs early in the course of COVID-19.[8,10,19] The RAT has an important role in the screening, early detection, and isolation of COVID-19 patients and in controlling the pandemic using contact tracing, especially in the low-income-countries lacking RT-PCR facilities, and mass-screening at jam-packed areas like air/sea ports, train and bus stations, borders, and festivals/religious congregations.[25] Viral load is the most important determining factor of RAT sensitivity. Other factors include the anatomical site of specimen collection and storage.[24]

RAT tests have lower sensitivity as compared to RT-PCR; however, they can be used in the community areas outside of the laboratory. A negative RAT result in symptomatic patients should be confirmed with RT-PCR. A false-negative RAT result might lead to delayed diagnosis, patient isolation, and treatment; and hence failure in control and prevention of COVID-19 infection.

This study was limited by relatively small sample size, descriptive study design, late presentation of some patients, and timing variation in sampling for RAT and RT-PCR.

CONCLUSION

This study showed low sensitivity and specificity of the RAT for COVID-19, with an overall accuracy of 60.33%, compared with RT-PCR in patients admitted with COVID-19 disease. A negative RAT test in a highly suspected patient should always be confirmed with RT-PCR for COVID-19, and the patient must be kept in isolation at home or hospital till the PCR report.

ETHICAL APPROVAL:
This study was approved by the Institutional Review Board (IRB) of the hospital (Ref: No.336/LRH/MTI, Dated: 25.04.2022).

COMPETING INTEREST:
The authors declared no competing interest.

PATIENTS' CONSENT:
Patients' consent for inclusion in the study and using their data for publication was obtained prior to commencing this study.

AUTHORS' CONTRIBUTION:
MARA: Conception and design of the study, literature search, manuscript writing and editing; graphical abstract designing, and proofreading of the manuscript.
ZA, NI: Data collection, statistical analysis, and manuscript review/editing.
All authors accept the accountability/responsibility of the data accuracy and approved the final version of the manuscript for publication.

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