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Research Article

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Comparison of Positivity Rates of COVID-19 Rapid Antigen Tests (RAT) and GeneXpert During COVID-19 Pandemic in a Tertiary Care Hospital, Mumbai, Maharashtra

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ABSTRACT

The advent of the coronavirus disease 2019 (COVID-19) in India caused a new range of challenges in diagnosing the virus & containing its community spread. Various point-of-care tests have been introduced for rapid diagnosis. Although rapid antigen tests (RAT) are the most commonly used, the false-negative rates are high. The purpose of this study was to compare the positivity rate of a molecular diagnostic test such as GeneXpert in RAT negative cases of COVID-19 during COVID pandemic. A retrospective observational analysis conducted on a total of 13633 symptomatic patients' samples were tested for RAT & GeneXpert of which 1508 were positive (11.06%). Out of 20526 symptomatic patients tested for GeneXpert, 3811 were positive (18.56%) and out of 12125 RAT negatives, 214 patients tested GeneXpert positive (1.8%). The main conclusions of this study were that negative rapid antigen tests should always be confirmed with a molecular diagnostic test such as GeneXpert or RT-PCR for the diagnosis of COVID-19 in symptomatic patients. Proper methods of sample collection must be ensured as improper methods might lead to increase in the number of false-negative results on point-of-care &; screening tests.

Key Words: *COVID-19, GeneXpert, RAT*

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INTRODUCTION

The pandemic of corona virus disease 2019 (COVID-19), prompt interventions in terms of early detection and clinical management along with isolation of positive cases is of utmost importance. This helps to limit not only the spread of severe acute respiratory syndrome caused by corona virus 2 (SARS-CoV-2) infections but also the morbidity and mortality associated with it. As a response to the growing SARS-CoV-2 pandemic and reagents shortages for rapid molecular systems, multiple rapid point-of-care tests (POCTs) were added to the diagnostic pipeline for COVID-19[1]. Rapid testing of symptomatic individuals remains an important diagnostic challenge during the current coronavirus disease 2019 (COVID-19) pandemic[2]. Reverse transcription-PCR (RT-PCR) is considered the gold standard for accurate diagnosis of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) with a theoretical turnaround time (TAT) of only a few hours[3]. However, the real TAT can easily be more than 24 h due to limited laboratory capacity, high sample volumes, and recurrent worldwide shortages of reagents and consumables. While Ag-RDTs are substantially less sensitive than RT-PCR, they could offer the possibility of rapid, inexpensive, and early detection of most infectious COVID-19 cases. An evaluation of the potential economic benefit of a repeated screening program with Ag-RDT showed that the fiscal, macroeconomic, and health benefits far exceed its cost[4].

In order to simultaneously investigate analytical [RT-PCR versus Rapid Antigen Test (RAT)] and sampling procedures (single NP swab vs naso & oropharyngeal swab in same VTM), we conducted a retrospective observational analysis in hospitalized patients with the aim to –

1. To study the positivity of COVID-19 by Rapid Antigen Test (RAT)
2. To study the positivity of COVID-19 by GeneXpert (CBNAAT)
3. To compare Rapid Antigen Test & GeneXpert

4. To estimate the percentage GeneXpert positivity in RAT negative samples

MATERIALS AND METHODS

This study is a retrospective observational analysis done on symptomatic OPD & IPD patients from September 2020 to June 2022 after obtaining approval from institutional ethical committee. Proper collection of specimens is a vital step in the laboratory diagnosis of infectious diseases. Improper collection of specimens may lead to false negative test results. Nasopharyngeal swabs for Rapid Antigen test (RAT) & nasopharyngeal along with oropharyngeal swabs in the same viral transport medium (VTM) for GeneXpert (CBNAAT) were collected of patients presenting with COVID-19 symptoms.

All samples were collected & handled by universal safety precautions and transported to COVID-19 laboratory in cold chain. Various RAT kits which were commercially available during different phases of the pandemic waves, approved by Indian Council of Medical Research (ICMR) were used for testing namely, SD Biosensor Standard Q COVID-19 antigen kit, Meril Ag test kit, SENSIT Ag Ubio Biotech test kit, Immuno Quick Ag test kit, Oscar Ag test kit, LabCare Diagnostics Ag test kit, Alpine Ag test kit, Angcard COVID-19 Rapid Antigen Test Kit & PATHKIT Simple COVID-19 Antigen detection kit. These kits detect the Nucleocapsid (N) protein of SARS CoV-2 virus. The NAAT kit used in the study was Cepheid GeneXpert Xpress SARS CoV2 kit which detects the envelope (E) gene & the Nucleocapsid (N2) gene of SARS CoV2 virus. Samples were collected and inserted into a pre-filled buffer tube for antigen extraction for 10-15 seconds. The swab was then squeezed and mixed with a buffer. After adequate mixing, one to two drops were added to the sample wells of the RAT cassette.

Results were noted after 15-20 minutes. The appearance of both control and test lines denoted a valid positive result, while the appearance of only the control line denoted a valid negative result. No appearance of control line was considered as invalid & test was re-run.

A positive RAT on a symptomatic patient was considered as confirmed positive as per ICMR protocol. On obtaining a negative RAT from a symptomatic patient, a repeat naso& oropharyngeal swab was collected immediately and a CBNAAT test was performed on the freshly collected repeat sample and the results were noted as per manufacturer's guidelines. CT value of below 35 was considered as positive result.

DATA MANAGEMENT

Clinical & epidemiological data of patients was taken and filled in the prescribed ICMR COVID-19 format. Patients found positive either by an RAT or by NAAT were designated as COVID-19 positive. RAT negative patients were confirmed by repeat testing by CBNAAT. Patients found negative by NAAT were classified as COVID-19 negative. The first wave of COVID-19 lasted from August 24, 2020 to February 28, 2021, while the second wave lasted from March 1, 2021 to April 30, 2021.The collected data were entered into Microsoft Excel 2020 and was analysed using SPSS 27.0 TRIAL version. The confidentiality of patient-level data was maintained throughout the study period.

RESULTS

Of the 20526 symptomatic cases subjected to CBNAAT testing, 3811 (13.08%) were found to be positive and of the 13633 symptomatic patients tested by RAT, 1508 (11.06%) were found to be positive and were not tested further according to the ICMR guidelines (Table 1). The remaining 12125 symptomatic RAT negatives were subjected to further confirmatory testing by CBNAAT and 214 (1.8%) cases were found to be positive and the remaining were confirm negative (Table 2). Also, on further observation & analysis of CT values obtained by CBNAAT of symptomatic RAT negative cases, around 34.11% patients with low CT values (<25) were also missed by a RAT. Whereas, around 65.89% patients having CT values between 25-35 and above were missed by RAT. A CT value wise break down of percentage positivity amongst RAT negative cases is described in Table 3.

Table 1 – Comparison of positivity rates of RAT & CBNAAT.

Kit Used	**No. of patients**	**% Positivity (Positive)**	**Negative**
RAT	13633	13.08% (1508)	12125
CBNAAT	20526	11.06% (3811)	16715

P- value: 0.001* significant

Table 2 – Percentage positivity rate of CBNAAT amongst RAT negative cases.

Patients tested by RAT	**RAT Negative**	**CBNAAT Positive amongst RAT Negatives**	**% Positivity**

13633	12125	214/12125	1.8 %

Table 3 – CT value wise distribution of RAT negative but CBNAAT positive cases

CT value	**No. of patients**	**Percentage**
15-20	40/214	18.69%
21-25	33/214	15.42%
26-30	53/214	24.77%
31 or above	88/214	41.12%

P- value: 0.38 not-significant

DISCUSSION

In the present study, the percentage positivity of RAT was found to be 1508/13633 (11.06%). In a study by *Hada et al.,* of the 2,096 patients tested, 297 (14.2%) were found to be positive by RAT[5].In another study done by *Brihn et al.,* 58 of 1732 (3%) patients tested positive for COVID-19 by rapid antigen testing[6].In one study done by *Mak et al.,* to evaluate the performance characteristics of RAT, 51/160 cases were found to be positive with percentage positivity being 31.81%[7].

This study showed a GeneXpert CBNAAT percentage positivity of 3811/20526 (18.56%). In a study conducted by *Magray et al.* in 2022, out of 50918 cases subjected to RTPCR, 4650 cases were detected COVID-19 positive with percentage positivity of 9.13%[8]. In another study done in student population by *Ferte et al,* RTPCR done on 692 symptomatic cases revealed 63.50% (440/692) positivity [9]. Also, Jacobsen et al. in 2021 demonstrated that amongst the 4811 paired conclusive test results from the RT-PCR and antigen tests, 221 cases were RT-PCR positive with 4.6% as percentage positivity [10].

Also, it was observed that out of the 12125 patients that tested negative on screening by RAT, when subjected to repeat confirmatory testing by CBNAAT, 214 (1.8%) cases were detected as COVID positive. In a retrospective study done by *Munne et al.,* RT-PCR data of 412 RAT negative symptomatic cases, 139 (33.7%) were positive by RT-PCR[11].

In another study done by *Hada et al.,* in a total of 2168 patients tested, the percentage positivity rate of the RT-PCR tests among the antigen-negative samples was 4.34% in the first wave of the pandemic whereas it was 8.08% in the second wave[5].

In the present study, among the 41.12% cases which were missed on antigen testing, the discrepancy can be attributed to the low viral load in the patients (CT value >30). A study done by *Sadeghi et al* concluded that the lower CT values were associated with higher viral load resulting in more viral spread, severe illness and death[12].A similar study done by *Aranha et al* concluded that individuals with high CT value(more than or equal to 31) could clear the viral RNA in a shorter duration as compared to patients with lower CT value of 25 or less[13].

As per the literature, the percentage of false negative SARS-CoV-2 result is high in RAT[14]. In our study, the date of onset of symptoms was not known in many cases as it was not a mandatory field in the ICMR specimen referral form. However, the date of RAT and collection of the sample for RT-PCR was the same. Hence comparisons of these are possible. Confirmation of antigen negative tested individuals by RT-PCR especially for symptomatic or high-risk groups is critical for prompt isolation and clinical management. Our results suggest need for focused and intensive (multi-modality) testing in groups at high risk for SARS-CoV-2 infection. A confirmatory NAAT testing should take place immediately after an initial negative RAT for timely diagnosis of COVID-19 infection in symptomatic & high-risk groups[15]. Also, proper sample collection is the most important step in the laboratory diagnosis of COVID-19 infection as improper sample collection might lead to increase in the number of false-negative results on point-of-care & screening tests.

LIMITATIONS

1. False positive cases were not identified or discussed in this study.
2. Same Antigen Detection kit could not be used throughout the duration of this study due to dependency on the subject to availability of ICMR approved kits during various phases of pandemic.
3. Cross-reactions with other endemic corona viruses & respiratory viruses were not assessed.

4. No comparison of viral load with CT values.

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