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EVALUATION OF RAPID ANTIGEN TEST PERFORMANCE WITH CLINICAL REFERENCE STANDARD rRT-PCR TEST IN SYMPTOMATIC COVID-19 PATIENTS AT A TERTIARY CARE HOSPITAL, HYDERABAD.

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ABSTRACT

Background: Covid-19 pandemic emerged as a major public health emergency affecting healthcare services all over the world. Many manufacturers have developed different diagnostic kits for early diagnosis and treatment. But there is paucity of performance data on different diagnostic tests hence there is need for evaluation of rapid antigen test (RAT) performance with the clinical reference standard real-time reverse transcription-polymerase chain reaction (rRT-PCR) test. **Methods**: The Standard Q Covid-19 antigen test kit (SD Biosensor, Inc., Gurugram) performance was compared with the real-time RT-PCR test kit (ICMR-NIV 2019-nCoV Assay) for detection of SARS-CoV-2. The nasopharyngeal specimens from 1255 symptomatic suspected Covid-19 patients attending Osmania General Hospital, Hyderabad during Sept 2020--Feb 2021 were subjected for both the diagnostic tests. **Results**: Out of 1255 cases, 285 (22.7%) were positive, and 970 (77.2%) were negative for SARS-CoV-2. Out of 285 positive cases, 68 (23.8%) were rapid antigen test positive. The overall sensitivity of RAT was 23.8% and specificity was 100%. The sensitivity of RAT decreased as the duration of symptoms on testing date increased and specificity remained constant. Comparatively good sensitivity (67.9%) was seen when subjected to RAT in less than 3 days of duration of illness. **Conclusions**: To conclude, rapid antigen test sensitivity decreased with the increase in duration of illness and true positives reflected mean cycle threshold value of <20 whereas false negatives showed mean cycle threshold >25 on rRT-PCR test indicating that the variation in viral load reflects the rapid antigen test sensitivity.

KEYWORDS: COVID-19, SARS-CoV-2, Rapid antigen test, rRT-PCR, cycle threshold value.

INTRODUCTION

The Covid-19 pandemic emerged as a major public health emergency affecting healthcare services all over the world. WHO reported more than 110 million confirmed cases of Covid-19 (SARS-CoV-2) infection and more than 2 million deaths globally with India contributing to 11 million patients as on 9th March 2021 [1]. The real-time reverse transcription-polymerase chain reaction (rRT-PCR) received ICMR approval as directed for emergency use authorization (EUA) by the Centre for Disease Control and Prevention (CDC) for the detection of SARS-CoV-2 nucleic acid from the respiratory specimens[2]. Since it requires a biosafety level 2/3 setup and trained technicians to run the test, many manufacturers have developed rapid diagnostic kits and devices to facilitate point of care testing which helps in early diagnosis of SARS CoV-2. In the developing countries, diagnostic testing is limited to symptomatic individuals due to limited number of laboratories, limited access to molecular tests, high cost burden and other resource constraints[3]. In this study, we evaluate Rapid antigen test performance with clinical reference standard rRT-PCR test in suspected symptomatic Covid-19 patients at a tertiary care hospital, Hyderabad.

MATERIAL & METHODS

A prospective, cross-sectional study was conducted at Osmania general hospital, Hyderabad, India for a period of 6 months (Sep 2020-Feb2021). Patients and their contacts with clinical features of Covid-19 of both gender, all age groups and who gave consent to participate were included. Asymptomatic patients, Comatosed/ Unconscious patients and patients not willing to participate in the study were excluded. The study was approved by the OMC Ethics Committee and informed consent was obtained from each patient. Nasopharyngeal specimens (NPS) from patients fitting inclusion criteria were collected by following strict infection control practices. The collected swabs were subjected to both Rapid antigen test (RAT) and real time

reverse transcription polymerase chain reaction (rRT-PCR) tests. The specimens detected as negative on rapid antigen test were further tested by rRT-PCR to rule out the infection as per ICMR guidelines. Also, specimens detected as positive by rapid antigen test were reconfirmed by rRT-PCR test for analysis of CT values, although a positive test should be considered as a true positive and does not need retesting.

Rapid antigen detection test: The Standard Q Covid-19 antigen test kit (SD Biosensor, Inc., Gurugram) has a sterile swab, viral extraction tubes with buffer, nozzle cap and test device. The NP swab was directly inserted in the extraction buffer and stirred into the buffer 5-6 times before squeezing. A nozzle cap was placed tightly onto this buffer tube and 3 drops of the extracted specimen were put onto the specimen well of the test device. The test results were read after 15-30 minutes as per the manufacturer's instructions.

Real time reverse transcription polymerase chain reaction method: RNA was extracted from the samples, using the MagMAX Viral Nucleic acid Isolation Kit (Thermo Fisher Scientific, USA). A commercial rRT-PCR kit (ICMR-NIV 2019-nCoV Assay Kit V 3.1), was used to detect the E, ORF1ab, RdRP gene targets of SARS-CoV-2 along with one housekeeping gene β-actin as an internal quality control for confirming the adequacy of the sample, RNA extraction and rRT-PCR procedure in HIMEDIA real-time PCR instrument. The result was interpreted as positive or negative by considering cycle threshold cut off value ≤ 35 as per the manufacturer's instructions

***Statistical analysis*:** Diagnostic performance such as sensitivity and specificity of rapid antigen test and subgroup analysis of demographic data, duration of illness on day of testing, clinical findings, laboratory rRT-PCR cycle threshold values was done by using Microsoft Excel software.

RESULTS

Among 1255 participants, the median age was 35.2±11.3 yr with gender ratio of 2.17 (Males (n)=860 and females (n) = 395). Out of 1255 cases, 285 (22.7%) were positive, and 970 (77.2%) were negative for SARS-CoV-2. Out of 285 positive cases, 68 (23.8%) were rapid antigen test positive (True positives) and 217 (76.1%) were rapid antigen test negative (False negatives). There were no cases of False positives (Chart 1).

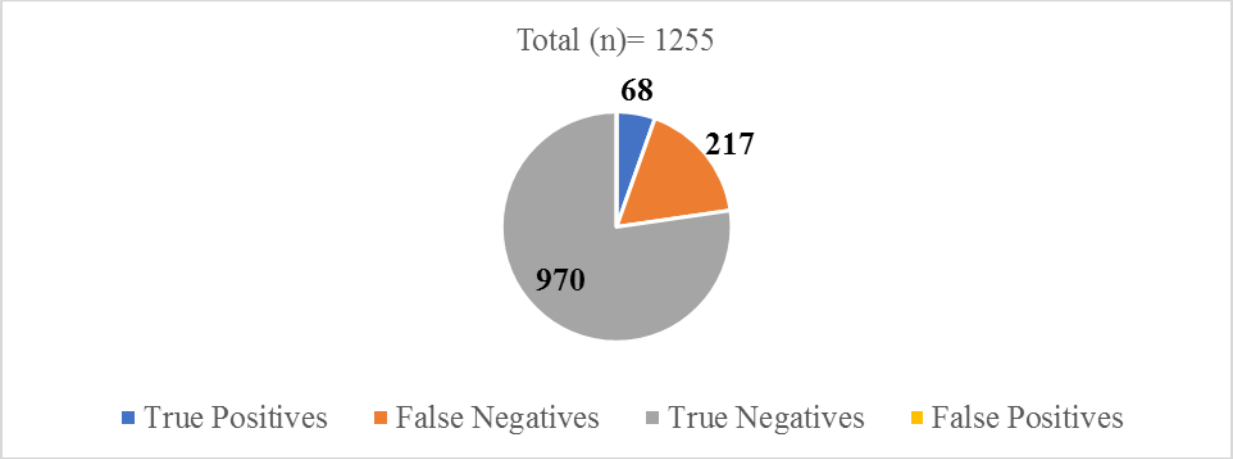

Chart 1: Distribution of total participants based on RAT and rRT-PCR results.

Among the 285 confirmed positive cases, males (n=197) were in preponderance with females (n=88) with the range of 0-14 days duration from onset of first symptom on testing day. The most common age group affected was between 18-59 yrs accounting for 75% (n=214). The clinical features were fever (90.5%) followed by cough (82.5%), dyspnea (72.2%), myalgias (70.1%) and anosmia/ageusia (35.4%). The comorbidities like hypertension, diabetes mellitus, chronic lung diseases, coronary artery diseases were observed in 52.6% (n=150) (Table 1).

Table 1: Demographic, Clinical, laboratory findings of confirmed Covid-19 cases.

Characteristics		Total PCR Positives	True positives (RAT +ve, rRT-PCR+ve)	False Negatives (RAT-ve, rRT-PCR+ve)
Gender	Males	197	45	152
	Females	88	23	65
Age	0-17yrs	4	0	4
	18-59yrs	214	54	160
	≥ 60yrs	67	14	53
Duration of	≤ 3 days	81	55	26

Symptoms onset[a]	4-6days	55	12	43
	≥ 7 days	149	1	148
Clinical features	Fever	258	60	198
	Cough	243	57	186
	Dyspnea	206	56	150
	Myalgias	200	66	134
	Anosmia/ Ageusia	101	49	52
Comorbidities	Present (HTN,DM,Malignancy,CLD,CAD)[b]	150	33	117
	Absent	135	35	100
CT Values	Envelope gene (Mean)	23.02	17.45	28.6
	ORF1ab gene (Mean)	23.6	18.2	29.0
	RdRP gene (Mean)	24.1	18.9	28.7

a: at time of sample collection ; b: Hypertension(HTN), Diabetes Mellitus (DM), Chronic lung diseases (CLD), Coronary artery disease (CAD) ; RAT; Rapid antigen test; rRT-PCR: real-time reverse transcription-polymerase chain reaction.

On analysis of CT values, True positives detected by rapid antigen tests showed mean CT values of <20 on rRT-PCR test whereas False negatives which were detected as negative by rapid antigen test showed mean CT values of > 25 on rRT-PCR test (Table 1).

Table 2: Sensitivity and Specificity of RAT test in comparison with clinical reference standard rRT-PCR test.

	Duration of first symptom onset at time of testing											
	≤ 3 days				4-6 days				≥ 7 days			
	Total P	TP	FN	TN	Total P	TP	FN	TN	Total P	TP	FN	TN
	81	55	26	499	55	12	43	375	149	1	148	96
RAT Sensitivity	67.9%				22%				0.6%			
RAT Specificity	100%				100%				100%			

RAT: Rapid antigen test; Total P : Total Positives ; TP; True positives; FN: False negatives ; TN: True negatives.

It was observed that the overall sensitivity of RAT was 23.8% and specificity was 100%. The sensitivity of RAT decreased as the duration of symptoms on testing date increased and specificity remained constant. Comparatively good sensitivity (67.9%) was seen when subjected to RAT in less than 3 days of duration of illness.(Table 2).

DISCUSSION

In the present study, out of 285 confirmed positives cases, males (69.1%) were predominantly affected majorly affecting adults (98.5%) and in patients with comorbidities (52.6%) which is in consistent with Banerjee J et al study[3]. The reason can be that the young people and non-comorbid patients have good protective immune response to eliminate the virus. The clinical features of infected patients were predominantly fever (90.5%) followed by cough (85.2%) which is in consistent with Gupta et al study[2]. The clinical features were non- specific as they can also be found in other respiratory virus infections. In the present study, sensitivity of rapid antigen test decreased as the duration of illness on testing day increased and specificity remained unchanged. Sensitivity of 67.9% was observed in less than 3 days of duration of illness. The pre-print study by the manufacturer of the kit reported an overall sensitivity of 84.38 % and specificity of 100 % in nasopharyngeal swabs.[4] Studies like Gupta et al[2] showed rapid diagnostic test with sensitivity 81.8% and specificity 99.6 %. Whereas A.Scohy et al[5] reported, rapid Covid-19 Ag Respi-strip sensitivity 30.2% and specificity100%. Even Banerjee et al study[3] reported rapid antigen detection test sensitivity 55.76% and specificity 97.43% which was correlated with the findings evaluated by ICMR. The reason for varying sensitivity depends on setting of patients tested, clinical manifestation, batch/lot of rapid kits reagents used, duration of illness on testing day, viral load, sample quality, level of extracted antigen, technical errors during sample collection/handling and processing reflects the interpretation of results.[5,6] Although Rapid antigen testing has its own advantages of less turn over time, cost effectiveness, safe due to viral inactivation, no requirement of biosafety 2/3 level laboratories set up, lesser technical expertise, but negative results cannot rule out SARS-CoV-2 infection and need to be confirmed by rRT-PCR test.[2] Nevertheless, it can be used as mass screening procedure for immediate isolation of infected individuals in outbreak management.

In the present study, the true positives detected by rapid antigen tests showed mean CT value <20 indicating high viral load and false negatives showed mean CT value >25 on rRT-PCR test which indicate decline in viral load. Even, A.Scohy et al[5] explained that the rapid

antigen detection test can detect SARS-CoV-2 true positives with high sensitivity in nasopharyngeal specimens with high viral load equivalent at least to 1.7×10^5 copies/mL (Ct < 25), but the sensitivity declines substantially when the viral load decreases (Ct > 30) equivalent to 9.4×10^3 copies/mL, which is often seen with the COVID -19 infected patients. In the present study, rapid antigen test sensitivity declined as the days of onset of symptoms on testing day increased, indicating high viral load in <3 days duration of illness can be detected as true positives. This is in consistent with explanation by Zou L et al[7] stating that SARS-CoV-2 high viral load in upper respiratory specimens was detected soon after the symptom onset and thus increasing the chances of positive antigen detection in early phase[6]. Even L Porte et al[8] showed rapid antigen based tests with high sensitivity and specificity in respiratory samples during first week of COVID-19 clinical presentation in patients.

In the present study, false negatives detected on rapid antigen testing were confirmed as positives on rRT-PCR testing implying that rRT-PCR has higher sensitivity, specificity and accuracy even at low viral loads. Corman et al[9] also states that rRT-PCR test is considered as reference standard due to its highest sensitivity for detection of different SARS-CoV-2-specific gene targets with the limit of detection (LOD) as 0.91-3.1 copies/ml. According to Rattan and Ahmad et al,[10] SARS‑CoV‑2 can be detected up to 20 days after onset of symptoms by rRT-PCR test. But on other hand, rRT-PCR has its own disadvantages of long turn over time, requirement of specialized laboratory setup with BSL-2/3 setup, expensive equipment, skilled personnel, insufficient availability of PCR reagent kits, lack of PCR infrastructure at laboratories in resource poor settings, presence of detectable level of SARS-CoV-2 in the sample collected, unable to detect the prior infections in recovered asymptomatic patients thus reflecting hinderance in implication of control measures.[3,11] False negative results by rRT-PCR can be due to inappropriate specimen collection, presence of PCR inhibitors and mutations or polymorphisms in the primer and probe binding sites, cross contamination.[3] Also, the negative and weak positive RT-PCR results detected in later course of disease can be due to subgenomic RNA, slow degradation of inactivated RNA even in infection resolved patients, discrepancies among diagnostic PCRs detecting in different targets of the SARS-CoV-2.[10,12]

Limitations of this study: Participants recruited were only symptomatic patients and their contacts. Asymptomatic patients were excluded. The 100% rapid antigen test specificity was not evaluated further for cross reactivity with other SARS CoV viruses in clinical setup.

Strengths of this study: The nasopharyngeal swabs were subjected for both rapid antigen test and rRT-PCR test in all the symptomatic patients and their symptomatic contacts. There was no time lag between index and reference standard test processing. All the positive and negative rapid antigen test results were verified by reference standard rRT-PCR test.

CONCLUSION

To conclude, rapid antigen test sensitivity decreased with the increase in duration of illness on day of testing and true positives reflected mean cycle threshold value of <20 whereas false negatives showed mean cycle threshold >25 on rRT-PCR test indicating that the variation in viral load reflects the rapid antigen test sensitivity. There was excellent specificity but overall sensitivity of rapid antigen test was 23.8% with comparable good sensitivity in less than 3 days of duration of illness.There is need for further studies to evaluate the diagnostic performance data of different COVID-19 detection tests to help in decision making of choosing right diagnostic test at right time and situation for early implementation of infection control practices.

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