Effectivity Analysis of SARS-CoV-2 Nasopharyngeal Swab Rapid Testing Kits in Pakistan: A Scenario of Inadequate COVID-19 Diagnosis

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Short report

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Abstract

COVID-19 pandemic has urged the need of rapid detection of SARS-CoV-2 in limited time frame. To cope with current, COVID-19 expanding scenario, accurate diagnosis of SARS-CoV-2 should be ensured by both national and international health organizations. Sporadic marketing of SARS-CoV-2 rapid detection kits raises questions regarding quality control and assurance. To aim of this study was to examine the effectiveness of SARS-CoV-2 nasopharyngeal swab based rapid detection kits, in comparison to gold standard USFDA approved triple target real-time polymerase chain reaction. A cross-sectional study of 1500 suspected COVID-19 patients was conducted. 100 RT-PCR confirmed patients nasopharyngeal swabs were evaluated for RDT. The COVID-19/SARS-CoV-2 NSP based RDT analysis showed 78% reactivity. Among RT-PCR confirmed negative subjects, 49.3% showed false positivity. The positive predictive analysis revealed 67.82% values, while the negative predictive vales of were 64.40%. The NSP RDTs showed limited sensitivities and specificities compared to gold standard RT-PCR. Accurate surveillance of COVID-19 is dependent upon authentic and validated SARS-CoV-2 detection nation-wide, which needs to be monitored by higher authorities. This study is critical for designing adequate measures by several COVID-19 strategic organizations to prevent future viral epidemics.

Introduction

COVID-19 is caused by Severe acute respiratory syndrome (SARS) coronavirus 2 (SARS-CoV-2), the prototype virus of the family *Coronaviridae* which preferentially infects respiratory tract cells, but also affect other organs such as; brain, conjunctiva, heart, liver, lungs, kidneys and pharynx (1,2). COVID-19, ‘CO’ stands for ‘corona,’ ‘VI’ for ‘virus,’ and ‘D’ for disease. Formerly, this disease was referred to as “2019 novel coronavirus” or “2019-nCoV”. The first case of pneumonia patient with unknown cause was officially reported on December 8, 2019. Several cases of pneumonia infected by 2019-nCoV were found in Wuhan, Hubei Province. World Health Organization announced its outbreak as 6th public health emergency of international concern in January 2020, but two months later it was declared as pandemic. As a large viral family, the coronaviruses can cause major diseases such as colds, Middle East Respiratory Syndrome (MERS), and severe acute respiratory syndrome (SARS). 2019-nCoV is a kind of new coronavirus which has not been found in human before. It belongs to novel β-coronavirus with an envelope, round or oval particles and is often polymorphic, with a diameter of 60-140nm. WHO stated global number of COVID-19 positive cases have been mounted to 50,266,033 (including 21,730,622 cases from America, 13,366,839 cases from Europe, 9,697,585 in South-East Asia, 3,337,885 in Eastern Mediterranean, 1,362,566 cases in Africa, and 769,795 in Western Pacific) among which 1,254,567 deaths were reported worldwide (3).

The SARS-CoV-2 contain four major structural proteins; nucleocapsid, matrix core protein, envelop, and glycoprotein spike surface. SARS-CoV-2 utilizes angiotensin-converting enzyme 2 ACE2 receptor expressed on Alveolar type 2 progenitor (AT2) epithelial cells. The virus penetrates host cell through clathrin- and caveolae- independent endocytic pathways and *via* host cell directed network of G-protein-

coupled receptors it may activate c-Jun N-terminal Kinase (JNK) and Janus Tyrosine Kinase (JAK)-Signal Transducer and Activator of Transcription (STAT) pathways, for enhanced viral replication (4).

Those who are infected by COVID-19 often have the symptoms of fever, weakness and dry cough. Few patients have the symptoms of nasal congestion, runny nose, sore throat and diarrhea. Severe patients often suffer from dyspnea and/or hypoxemia one week after onset, and very severe patients rapidly progress to acute respiratory distress syndrome, septic shock, intractable metabolic acidosis and coagulation disorders. Notably, patients with severe or critically ill patients may have moderate to low fever or even no obvious fever. Patients with mild symptoms just have slight fever and weakness without pneumonia. Patient without symptoms have been also reported. When cultured in vitro separately, 2019-nCoV can be found in human respiratory epithelial cells in about 96 hours; when cultured in Vero E6 and Huh-7 cell lines separately, about 6 days. It is known that COVID-19 is mainly transmitted through respiratory droplets and contact. Routes of transmission such as aerosol and digestive tract have yet to be investigated further (3,4).

Rapid diagnostic tests (RDTs) are easy to use, cheaper and safe to use, but there are several potential concerns regarding validation and accurate performance of these diagnostic assays (5). Previously, in comparison to gold-standard PCR positive patients, we showed the sensitivities of nasopharyngeal swab (52%) and saliva based (21%) SARS-CoV-2 antigen based rapid diagnostic kits in Pakistan (6). To validate further the accurate diagnosis of nasopharyngeal swab rapid diagnostic kits we further evaluated COVID-19 antigen based kits.

Materials And Methods

A cross-sectional, among 1500 COVID-19 suspected subjects, was conducted during the period of November, 2020 from capital twin cities (Islamabad and Rawalpindi) of Pakistan. After comprehensive medical examination and history of SARS-CoV-2 suspected patients, the consent was obtained by trained counselor and specimens were sent for analysis at Islamabad Diagnostic Center (Specialized Center for COVID-19), Islamabad, Pakistan. The study was approved by institutional review board of IDC Pakistan. Study investigators had professional background and capacity related to diagnostic test evaluation. The investigators had a full understanding and knowledge of specific contents of the protocol and all indicators through training. The quality control of the IDC laboratory met the requirements of quality control of clinical laboratory to ensure the standardization of experimental operating procedures. The integrity and accuracy of was verified using gold standard means.

As standard, RT-PCR confirmed positive cases were pre-selected for diagnostic kit evaluation. The samples were examined against SARS-CoV-2 *via* Real-Time PCR assay (Roche, USA) after RNA extraction through Auto pure 32 Zybio China, using standard Primerdesign. The assay included positive control template and RNA internal extraction control. USFDA approved Triple target genes (Sarbecovirus E gene, SARS-CoV-2 N gene, and SARS-CoV-2 RNA-dependent RNA polymerase) were used, along with Seegene kit (#RP10244Y Allplex™ 2019-nCoV Assay, Seegene South Korea) based RT-PCR. The detection limit was

(100) copies/ml. Positive samples with exponential growth curve and Ct value ≤ 40 were considered as SARS-CoV-2 positive patients. To examine the accuracy of SARS-CoV-2 antigen detection from NSP samples, the colloidal gold labeled SARS-CoV-2 N protein monoclonal antibody based immunochromatographic rapid diagnostic kit (Lepu Medical, China 20CG2704X) tests were performed and evaluated *via* gold-standard RT-PCR results. After collection, the samples were put in the treatment fluid, stored at 2~8℃ and tested within 24h. The samples cannot be placed at room temperature for a long period of time. Discordant results were either excluded or reconfirmed. Whole procedures were performed in accordance to standard manufacturer protocols. The sensitivity, specificity, positive predictive values, and negative predictive values were calculated using gold standard RT-PCR results.

Results

About 1500 COVID-19 suspected patients were enrolled this study and tested for SARS CoV-2 using gold standard RT-PCR based COVID-19 antigen detection kits at Islamabad Diagnostic, Center G8 Markaz, Pakistan. We selected 100 RT-PCR positive subjects for evaluation of nasopharyngeal swab based SARS-CoV-2 antigen detection kits. The kit showed zero cross reactivity against Adenovirus, Cytomegalovirus, Epstein-Barr virus, human Coronavirus OC43, human Metapneumovirus, Influenza A virus, Influenza B virus, Measels virus, Mumps virus, Mycoplasma pneumonia, Norovirus, Respiratory Syncytial virus, Rotavirus, and Varicella Zosyer virus.

The accuracy of NSP detection kit was determined by triple target USFDA approved Seegene kit. Among selected subjects, 60% were male while 40% were females. 4% of these subjects were children below the age of 18 years. Mean age was 42 (ranging 3-72). In comparison to gold standard, RT-PCR based COVID-19 detection kit, the NSP based rapid diagnostic test showed 78 % reactivity, while remaining 21% showed false negative results. Further validation, was performed to examine the false positivity among PCR negative subjects. The data revealed that 49.3% of the subjects showed false positive results. The sensitivity and specificity were 78% and 50.66%, while the positive predictive value and negative predictive vales of the assays were 67.82% and 64.40% respectively Table 1. Cycle threshold (Ct) values and lineal trend line of 100 RT-PCR positive samples taken at different days was also maintained (Data not shown).

Discussion

Previously we evaluated SARS-CoV-2 rapid diagnostic test in Pakistan and demonstrated that RDT based diagnostic kits need to be investigated before commercialization of the products (6). Despite of limited sensitivities and specificities several SARS-CoV-2 RDT based kits are being utilized by many diagnostic centers across Pakistan which needs to be re-monitored on priority basis to estimate accurate COVID-19 trends among several populations in Pakistan.

During first wave of COVID-19 in Pakistan, people vigilantly implemented standard operating procedures and successfully combat SARS-CoV-2. However, during second wave of COVID-19 in Pakistan, there were

several unknown reasons possibly one of them would be ineffective testing strategy or poor diagnostic means which bring misunderstandings during the initial screenings. Several RDT based diagnostic tests were used with no reference to gold standard RT-PCR based detection.

Since WHO recommendations indicate that SARS-CoV-2 RDTs tests should have minimum sensitivity of ≥ 80% and specificity of ≥ 97% (7). During the second wave of COVID-19 we aimed to re-investigate new COVID-19 testing RDT diagnostic kits. In continuation of our previous study (6), current study indicated that in comparison to gold standard, RT-PCR based COVID-19 detection kit, the nasal RDT showed improved 78 % sensitivity, while 21% showed false negative results. The NSP based RDTs kit showed zero cross reactivity against Human metapneumovirus, Mumps virus, Rotavirus, Adenovirus, Epstein-Barr virus, human Coronavirus OC43, Mycoplasma pneumonia Varicella Zosyer virus, Respiratory Syncytial virus, Cytomegalovirus, Measels virus, Influenza viruses and Norovirus.

Nasopharyngeal and oropharyngeal swabs of 82 RT-PCR confirmed patients revealed sensitivity of 93.9% via SARS-CoV-2 antigen test kits (8). Similar study from China, reported 68% sensitivity in 208 RT-PCR confirmed nasopharyngeal swab samples (9). While 83.43% sensitivity was reported by SARS-CoV-2 RDT kits in Kuwait (10). Recently, from Pakistan nasal and saliva based RDTs revealed sensitivities of 52% and 21% respectively (6). The current study revealed that RT-PCR analysis from study enrolled subjects (during second COVID-19 wave) revealed that prevalence rate of COVID-19 during the period of November in Islamabad/Rawalpindi region of Pakistan was 6.67%, which is 22 times higher than COVID-19 prevalence during October 2020 (6).

Conclusion

Current study is more reliable, as same nasal samples were used for comparison of RT-PCR and RDTs, without possible distribution error from other specimen. The kit manufacturer analysis showed 90% sensitivity of nasal RDTs among Chinese population. However possibly due to different epidemiological conditions or interracial factors as compare to China, the findings of present study are not consistent with Pakistani population, which warrants further in-depth investigations on RDT in Pakistan. The rapid tests results in current study were not satisfactory, and combination-test algorithm should be employed for accurate diagnosis of SARS-CoV-2 during second COVID-19 wave in Pakistan.

Declarations

Ethics approval and consent to participate:

The study has been approved by ethical review board of Islamabad Diagnostic Center Pakistan, and informed patients concern was obtained.

Consent to publication:

All authors approved the submission of the manuscript for publication

Availability of data and material:

The data is available and can be used for the academic or research purposes.

Competing interests:

The authors have no conflict of interest.

Funding:

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Authors Contribution:

RU is Principal Investigator (PI) of the study. RU and US conceived the study. US wrote manuscript and analyzed the data, US is co-PI of this study. ZZP assisted in manuscript writing and AAK and ZZP assisted in data analysis, SRU, AR, ZA, and HR performed the experiments.

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List Of Abbreviations

WHO: World Health Organization

SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus 2

MERS: Middle East Respiratory Syndrome

NPS: Nasopharyngeal Swab

RDT: Rapid Diagnostic Testing

ACE2: Angiotensin-Converting Enzyme 2

AT2: Alveolar type 2 progenitor

Ang II: Angiotensin II

JNK: c-Jun N-terminal Kinase

JAK-STAT: Janus Tyrosine Kinase (JAK)-Signal Transducer and Activator of Transcription

RT-PCR: Real-Time Reverse-Transcription Polymerase Chain Reaction

Ct: Cycle Threshold

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Table

Table I. Evaluation of diagnostic accuracy of nasopharyngeal swab based SARS-CoV-2 rapid diagnostic kits in Pakistan.

Samples	Ct (Av)	Real-Time PCR	Age (Av)	NSP-RDT	Fever	Cough	Breathing problem
Male (*n*=60)	27.7	Positive	40.02 (19-72)	Reactive (*n*=48)	Positive	Positive	Positive
				N-reactive (*n*=12)			
Female (*n*=40)	28.8	Positive	44.1 (20-66)	Reactive (*n*=31) N-reactive (*n*=9)	Positive	Positive	Positive
Children (*n*=4)	30.6	Positive	9.75 (3-17)	Reactive (*n*=4) N-reactive (*n*=0)	Positive	Positive	Negative
Total (*n*=100)	29	Positive	42 (3-72)	Sensitivity (78%) Specificity (50.66%) Positive predictive value (67.82%) Negative predictive value (64.40%)	Positive	Positive	Positive