ORIGINAL ARTICLE

DIAGNOSTIC VALUE OF SARS-COV-2 ANTIBODY-BASED RAPID DIAGNOSTIC TEST IN PAEDIATRIC INDIVIDUALS CLINICALLY SUSPECTED OF COVID-19

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ABSTRACT

Introduction: The diagnosis of SARS-CoV-2 infection with quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) has proven challenging in low and middle-income countries, including Nepal, due to its limited availability and higher costs. As such, a simple and cost-effective rapid diagnostic test based on the detection of SARS-CoV-2 antibodies could be an alternative for the diagnosis of individuals with COVID-19.

Method: This study aimed to examine the diagnostic parameters of the SARS-CoV-2 antibody-based rapid diagnostic test (Ab-RDT) as well as the clinical and demographic characteristics of paediatric COVID-19 suspects. The pediatric individuals (<16 years) suspected of COVID-19 who had visited the hospital between January and June 2021 were subjected to the detection of the SARS-CoV-2 genome by qRT-PCR and the induction of antibodies (IgM/IgG) by the National Public Health Laboratory-approved Ab-RDT. The results of the diagnostic tests from the overall individuals and the demographic and clinical details from the individuals confirmed with COVID-19 were collected and analyzed using SPSS version 17.0, with statistical significance considered at $p<0.05$.

Results: Among the total individuals (n=773), 193(24.98%) tested qRT-PCR-positive and 144(18.63%) tested Ab-RDT-positive. When measured against the gold standard qRT-PCR, Ab-RDT exhibits sensitivity of 74.6%, specificity of 99.7%, positive predictive value of 98.6%, and negative predictive value of 92.2% ($p<0.001$). Male sex (106/193) ($p=0.023$), the presence of multiple (≥3) symptoms (73/193) and (≥2) comorbidities (26/193), and a diagnosis of pneumonia (32/193) were identified as possible risk factors for SARS-CoV-2 infection. It seems that the low sensitivity of validated Ab-RDT could only complement qRT-PCR in the detection of SARS-CoV-2 antibodies

Conclusion: It seems that the low sensitivity of validated Ab-RDT could only complement qRT-PCR in the detection of SARS-CoV-2 antibodies

Key words: ***Ab-RDT, clinical features, COVID-19, diagnostic parameters, SARS-CoV-2***

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INTRODUCTION

The need for quick and effective testing methods to identify COVID-19 patients has grown as a result of the socioeconomic and medical challenges that COVID-19 has brought about.[1, 2] Though molecular assays including, qRT-PCR for the detection of SARS-CoV-2 RNA are currently acknowledged as the gold standard,[2, 3] their reach to a large-scale diagnosis in low and middle-income countries like Nepal is constrained by their higher cost, the necessity of complex laboratory setup, and limited reagent availability.[4] Furthermore, due to variables like the type of biological sample, fluctuating viral load, and the interval between sample collection and the onset of symptoms, diagnosis by both qRT-PCR and antigen-based rapid detection kits may consequent in high prevalence of false-negative results.[5]

Therefore, besides analyzing the demographic and clinical aspects of paediatric COVID-19 individuals, this study also examined the significance of testing for acute-phase (IgM) and/or memory (IgG) antibodies in COVID-19 suspected individuals, and identify whether Ab-RDT could leverage the diagnostic challenges associated with qRT-PCR.

METHODS

Study design, area,and period

A cross-sectional study on paediatric individuals suspected of having COVID-19 (≥7 days) was carried out at the Kanti Children's Hospital, Maharajgunj, Kathmandu, Nepal Between January and June 2021. A total of 773 paediatric individuals were included in our study.

Ethical approval

This study was ethically approved by the Institutional Review Committee (IRC) (Reference number: 599) of Kanti Children's Hospital, Maharajgunj, Kathmandu, Nepal. The data were anonymized when received from the patients.

Informed consent

The study received approval from the IRC before the study. The data was collected from January and June 2021. Written informed consent was obtained from the study participants.

Data collection

Demographic details (age and gender) and clinical findings (co-morbidity(s), symptom(s), and diagnosis of COVID-associated pneumonia) for each patient were obtained from the patient information sheet and electronic medical records. Any missing or uncertain records were collated and clarified through communication with the involved healthcare providers, patients, and their families.

Sample collection and laboratory diagnosis

The study population was categorized into two groups: cases (patients diagnosed with COVID-19 by qRT-PCR or Ab-RDT) and controls (individuals not diagnosed with COVID-19 by qRT-PCR or Ab-RDT). The COVID-19 diagnostic tests were stratified according to those that detect SARS-CoV-2 nucleic acid (RNA) and SARS-CoV-2-specific immunoglobulin (IgM and/or IgG).

How to Cite

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For qRT-PCR confirmation, nasopharyngeal and oropharyngeal swab samples from each suspected individual were collected and subjected to detection of the SARS-CoV-2 genome. For Ab-RDT confirmation, venous blood samples (2 ml) were drawn from each suspected individual in a serum separating tube, and then the serum was separated by centrifugation at 3,500 rpm for 5 minutes. The serum samples were processed in the National Public Health Laboratory (NPHL) approved (US-FDA-EUA authorized) immunochromatographic rapid test kit, as per the manufacturer's instructions, within 15 minutes.

Briefly, the required test device, reagents, and specimen were allowed to equilibrate at room temperature (15~30°C). The test device was removed from its foil pouch immediately before testing and placed on a clean, flat surface. Ten microliters of the serum sample were added to the sample well "S" of the test device, which was immediately followed by the addition of one drop of the assay buffer solution to the same well. The result was interpreted after 10 minutes. Two distinct lines, each on the well "C" and the well "M" indicated the presence of IgM antibodies. Similarly, two distinct lines, one on well "C" and the other on well "G" indicated the presence of IgG antibodies. Three distinct lines, each on well "C", on well "G", and on well "M" indicated the presence of both IgG and IgM antibodies developed against the SARS-CoV-2 antigen. However, a single line on the mark "C" indicated a negative result. The absence of the distinct line in the control well indicated an invalid test. The positive control with three distinct lines on the wells "C", "M", and "G" and the negative control with one distinct line on the well "C" were placed simultaneously.

The diagnostic value of each test was reported in terms of sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV).

Statistical analysis

Data were analyzed using SPSS software, version 17.0. Descriptive statistics [mean (± SD), n, and %] were used to characterize the study variables. We used binary or multinomial logistic regression analysis, whenever applicable, to test if there were significant differences between the variables. A natural log-transformed Q-Q plot of the age distribution of individuals with covid-19 patients was plotted. A P-value of <0.05 was considered statistically significant.

RESULTS

Table 1. Demographics of the individuals with and without COVID-19

Demographics		Groups		*p-value*
		COVID-19 (n=193)	Non-COVID-19 (n=580)	
Age (in Years)	Median (Q-1 Q3)	1.33 (0.67-9)	4 (1.25-9)	0.013
Age Groups	<1 month (n=74)	30 (15.54)	44 (7.59)	0.001
	≥1 month-<6 months (n=10)	3 (1.55)	7 (1.21)	0.711
	≥6 months-<1 year (n=111)	39 (20.21)	72 (12.41)	0.007
	≥1 year-<5 years (n=264)	55 (28.5)	209 (36.03)	0.056
	≥5 years-<10 years (n=135)	20 (10.36)	115 (19.83)	0.003
	≥10 years-<16 years (n=179)	46 (23.83	133 (22.93)	0.797
Gender	Male (n=370)	106 (54.92)	264 (45.52)	0.023
	Female (n=403)	87 (45.08)	316 (54.48)	

Demographics of the individuals with and without COVID-19

A total of 773 individuals clinically initially suspected of having COVID-19 were enrolled in the study. Among the total individuals, 193 (24.96%) were diagnosed with COVID-19 through qRT-PCR. The median age (Q1-Q3) of the patients diagnosed with COVID-19 was 1.33 (0.67-9) years (p=0.013). There were 55 (28.5) (p=0.056) patients belonging to the age group ≥1 year-<5 years, 39 (20.21) (p=0.007) to the age group ≥6 months-<1 year, and 30 (15.54) (p=0.001) to the age group <1 month. There were 106 (54.92) (p=0.023) male patients with COVID-19 (Table 1).

Age of COVID-19 patients

The COVID-19 patients' ages were not normally distributed, as shown by the data points that were not aligned to the diagonal line of the Q-Q plot with a natural log transformation (Fig. 1).

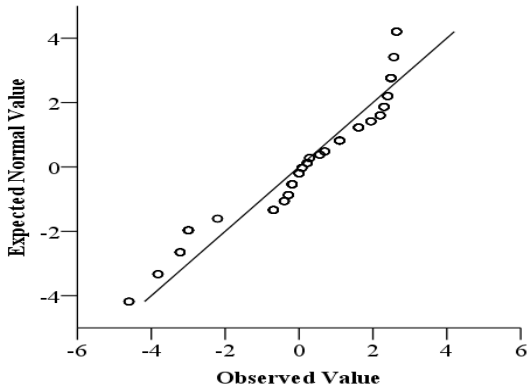

Figure 1. Natural log transformed Q-Q plot of the ages of COVID-19 patients

Clinical symptoms among COVID-19 patients

Among the COVID-19 patients (n=193), 73 (37.82%) had ≥3 symptoms, while 7 (3.63%) had no symptoms. The most common symptom was fever (n = 121, 62.69%), followed by cough (n=101, 52.33%), and headache (n=28, 14.51%). Equal incidences (n=25, 12.95%) of diarrhea and dyspnea were observed among the patients. Stridor (n=7, 3.63%) and dyskinesia (n=4, 2.07%) were infrequent among the COVID-19 patients (Table 2).

Symptoms		Number	Percent
Patients with	Asymptomatic	7	3.63
	1 symptom	41	21.24
	2 symptoms	72	37.31
	≥3 symptoms	73	37.82
Observed symptoms	Abdominal pain	8	4.15
	Anorexia	11	5.7
	Asthenia	15	7.77
	Chest Pain	14	7.25
	Cough	101	52.33
	Diarrhea	25	12.95
	Dyskinesia	4	2.07
	Dyspnea	25	12.95
	Fever	121	62.69
	Headache	28	14.51
	Myalgia	23	11.92
	Pharyngitis	14	7.25
	Rhinorroea	11	5.7
	Stridor	7	3.63
	Vomiting	19	9.84
	Others	23	11.92

Other includes ageusia (n=1), anosmia (n=3), arthralgia (n=3), bronchiectasis (n=1), crepts (n=2), dysuria (n=3), edema (n = 3), gastroenteritis (n=2), hyperhidrosis (n=1), nausea (n=2), tachypnea (n=2)

Comorbidities and diagnosis of pneumonia among COVID-19 patients

There were 51 (26.42%) COVID-19 patients with single comorbidity and 26 (13.47%) with ≥2 comorbidities. Fifty-three (27.46%) patients had hypertension, while 31 (16.06%) had diabetes, and 12 (6.22%) had other infrequent comorbidities. Among the total COVID-19 patients, 32 (16.58%) patients were diagnosed with pneumonia (Table 3).

Table 3: Comorbidities and diagnosis of pneumonia among COVID-19 patients (n=193)

Variables		Number	Percent
Patients with	Asymptomatic	7	3.63
	1 symptom	41	21.24
	2 symptoms	72	37.31
Observed symptoms	Abdominal pain	8	4.15
	Anorexia	11	5.7
	Asthenia	15	7.77
	Chest Pain	14	7.25
	Cough	101	52.33
	Diarrhea	25	12.95
	Dyskinesia	4	2.07
	Dyspnea	25	12.95
Diagnosis of pneumonia	Yes	32	16.58
	No	161	83.42

Others include benign prostatic hyperplasia (n=1), cholelithiasis (n=1), chronic kidney disease (n=1), epistasis (n=1), fibrosis (n=1), hypokalemia (n=1), hypothyroidism (n=1), IgA nephropathy (n=1), lung malignancy (n=1), paroxysmal supraventricular tachycardia (n=1), perimembranous ventricular septal defects (n=1), thrombocytopenia (n=1).

Diagnostic parameters of SARS-CoV-2 Ab-RDT compared with gold-standard qRT-PCR

Among the total individuals tested for COVID-19 (n=773), 193 (24.96%) tested positive for qRT-PCR, and 144 (18.63%) for Ab-RDT. Considering qRT-PCR as the gold standard test for the diagnosis of COVID, the Ab-RDT had 74.6% of sensitivity, 99.7% of specificity, 98.6% of PPV, and 92.2% of NPV. The sensitivities for IgM and IgG were 69.4% and 38.9%, respectively (Table 4).

Table 4. Diagnostic parameters of SARS-CoV-2 Ab-RDT compared with gold-standard qRT-PCR

Diagnostic tests	+ve Result	p-value	Over all				IgM		IgG		IgG	
			Se (%)	Sp (%)	PPV (%)	NPV (%)	Se (%)	Sp (%)	Se (%)	Sp (%)	Se (%)	Sp (%)
qRT-PCR	193	-	-	-	-	-	-	-	-	-	-	-
Ab-RDT	144	<0.001	74.6	99.7	98.6	92.2	69.4	100	38.9	99.7	33.7	100

qRT-PCR = quantitative reverse transcriptase-polymerase chain reaction, Ab-RDT = antibody based rapid diagnostic test, Se = sensitivity, Sp = specificity, PPV = positive predictive value, NPV = negative predictive value, IgM = Immunoglobulin M, IgG = Immunoglobulin G

DISCUSSION

The unbridled transmissibility of SARS-CoV-2 among people has triggered a worldwide pandemic with an enormous medical and economic toll.[6, 7] With no well-established and specific treatment for this virus, it is essential to better elucidate the effective diagnostic tools for the proper management of the infected patients.[8] Scientists and researchers throughout the world have focused on designing effective diagnostic tools for the diagnosis of patients with COVID-19.5 In this study, we discussed the prevalent clinical features presented in COVID-19 patients along with the diagnostic value of Ab-RDT in comparison to the gold standard, qRT-PCR.

The median age of COVID-19 patients in this study was 1.33 years ($p<0.05$). There are limited studies discussing the age of paediatric patients with COVID-19, and most of the published literature focuses on ages of adults with COVID-19: Chang et al.[9] (34 years), Fan et al.[10] (31.5 years), Liu et al.[11] (33.5 years). Hence, we cannot correlate the findings of paediatric age in this study to the published literature. However, the predisposition to SARS-CoV-2 infection in the children could be attributed to immature immune systems or underlying conditions, such as congenital heart disease, multisystem inflammatory syndrome (MIS-C), and other genetic conditions.[12]

This study revealed that the elevated risk for hospital admission in COVID-19 patients was independently associated with the male sex (54.92%) ($p<0.05$); the presence of multiple (≥3) symptoms at presentation (37.82%), including fever (62.69%) and cough (52.33%) as the most common symptoms; the presence of multiple (≥2) comorbidities (13.47%), including a history of hypertension (27.46%) and diabetes (16.06%); and the clinical diagnosis of pneumonia (16.58%). The increased disproportionate rate of SARS-CoV-2 infection in males in our study was attributed to differences in the immune system, genetic polymorphism, lifestyle factors including smoking, and pre-existing comorbidities.[6] The observed signs and symptoms in this study were comparable to a systematic review and meta-analysis, which showed fever (88.7%), cough (57.6%), and dyspnea (45.6%) as the most prevalent symptoms in COVID-19 patients.[13] Moreover, several studies have significantly co-related cardiovascular diseases, diabetic mellitus, and chronic lung diseases with the increased incidence of SARS-CoV-2 infection,[13,14] which is consistent with our findings of underlying diseases. Moreover, other studies have correlated poorer clinical outcomes with a greater number of comorbidities.[15,16] Similar to our findings of clinical diagnosis of pneumonia, several studies have revealed that COVID-19 patients are often clinically diagnosed with pneumonia, in 100% of all symptomatic patients and even in 50% of asymptomatic cases,[17] with bilateral pneumonia being more prevalent than unilateral (73.3% and 26.7%).[18] This may be attributed to the high expression of the angiotensin-converting enzyme (ACE2) in the lung tissue, specifically in type II epithelial cells, which are the main contributors to surfactant secretion. The reduced surfactant level in the alveoli upon viral destruction of pneumocytes causes the alveoli to collapse, which subsequently leads to pneumonia and acute respiratory distress syndrome (ARDS) in severe cases.[9]

When compared to the viral nucleic acid detection by qRT-PCR in nose/throat samples in this study, we observed excellent specificity (99.7%), PPV (98.6%), and NPV (92.2%), but poor sensitivity (74.6%) for Ab-RDT during the first week of symptom onset.

The Ab-RDT positivity in our study was lower than that published by Public Health England, which reported 93.9% sensitivity for the Abbott SARS-CoV-2 and 87.0% sensitivity for the Roche assay.[19] The lower sensitivity of Ab-RDT in this study could be due to the diagnosis of patients during the early stages of disease (7-8 days), since seroconversion in SARS-CoV-2 infection is reported to peak at

around 17–20 days.[19] A study in Germany with the IgM/IgG lateral flow immune assay showed 62.5% and 93.8% sensitivities from 5 to 9 days and 10 to 18 days of the PCR test, respectively.[20] Another study performed in patients with confirmed SARS-CoV-2 infection in China suggested 23% and 63.8% detectability of IgM and IgG by Ab-RDT test, respectively,[21] which was just opposite to our findings with higher sensitivity of Ab RDT for IgM (69.4%) compared to IgG (38.9%).

The lower sensitivity result of Ab-RDT in this study could be due to the early diagnosis of patients (within a week) and the enrollment of fewer study participants. The testing of the first and second serum samples at 7-14 days apart could have yielded higher sensitivity for the diagnostic kit.

CONCLUSION

Serological testing using Ab-RDT exhibits lower sensitivity but may be an essential supplementary tool for qRT-PCR-based diagnosis. However, in nations or regions where the healthcare system is overburdened, it can be taken into account for the diagnosis of people who are suspected of having COVID-19.

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AUTHORS CONTRIBUTION

Arun Bahadur Chand: conceptualization; investigation; writing-original draft; writing-review & editing; methodology; project administration, supervision.

Sohani Bajracharya: methodology; validation; investigation; supervision

Isha Amatya: writing-review & editing; soft-ware; investigation; supervision

Parbati Gurung: data curation; investigation; validation

Shila Shrestha: data curation; investigation; validation

Maina Dulal: methodology; software; validation

Susheela Dahal: project administration; investigation; validation

Nayanum Pokhrel: supervision; methodology; writing-review & editing

All authors have read and approved the final version of the manuscript. Arun Bahadur Chand and Ajaya Basnet has full access to all of the data in this study and takes complete responsibility for the integrity of the data and the accuracy of the data analysis.

COMPETING INTEREST

The authors declare no competing interest.