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Evaluation of the Immunochromatography Assay's Diagnostic Performance for Quickly Detecting the Presence of COVID-19 Antigen in Patients with Positive PCR Results

Angham Abdulrazzaq Breesam [1], Mohammed Yawoz Nooruldeen [2]

1. Msc. Microbiology, Kirkuk Directorate of Health/ Iraq. Email: angbreesam@ntu.edu.iq

2.Northern Technical University/ College of Health and Medical Techniques/ Kirkuk/ Medical Laboratory Techniques Department/ Kirkuk/ Iraq. Email: mohammedyawoz@ntu.edu.iq.

Corresponding author: Angham Abdulrazzaq Breesam, M.sc. Microbiology, Kirkuk Directorate of Health/ Iraq. Email: angbreesam@ntu.edu.iq.

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Abstract: Severe acute respiratory syndrome coronavirus 2 (SAR-CoV-2) is the novel infectious disease agent that causes COVID-19. The laboratory confirmation of COVID-19 is based on nucleic acid-based virus genome sequencing and real-time PCR and serological methods, rapid antigenic testing. The aim of the study was to evaluate the diagnostic sensitivity of the immunochromatography method by comparing it with PCR in covid-19 diagnosis. The study involved 116 samples, 90 samples included covid-19 patients and 26 samples included healthy, as a control group. Two nasopharyngeal swabs (NP) were taken, one for an antigenic test cassette and the other swab for re-confirmation of the infection by a real-time RT-PCR. The result of this study showed that there was a significant difference (P value = 0.0001) between RT PCR and rapid Ag tests in patients and healthy groups. The low sensitivity recorded for rapid antigen detection kits was 69% and 70%, respectively, compared with the 100% sensitivity of PCR. This study demonstrated an elevated positive rate of rapid Ag positive during the period (8–14 days) of symptoms onset. This study concluded that the rapid antigen test is a helpful tool for detecting the presence of COVID-19 infection, but it may not be the best alternative due to its low sensitivity.

KEYWORDS: COVID-19, Rapid antigen test, RT-PCR.

Introduction

Coronavirus disease 2019 (COVID-19) causes respiratory tract disease which may lead to serious progressive pneumonia and multi-organ failure, also death of severely infected people. The World Health Organization (WHO) has classified the ongoing outbreak as a pandemic after the first case was reported in Wuhan, China, in December 2019 [1]. There are about two dozen distinct species, which have been classified into four genera (alpha, beta, gamma, and delta) based on antigenic cross-reactivity and genetic makeup. Strains harmful to humans and other mammals are only found in the alpha-and beta coronavirus genera [2].The SARS-CoV 2 genome codes for a polyprotein (ORF1ab) involved in viral RNA transcription and replication, four structural proteins: E for envelope; M for membrane; N for nucleocapsid, which is required for viral synthesis, and the S protein for spike, which allows the virus to enter and infect the host cell

[3,4,5,6]. The COVID-19 infection primarily affects the respiratory system and is characterized by a cough, fever, and occasionally pneumonia as well as shortness of breath. Patients may also experience other clinical manifestations in the heart, gastrointestinal tract, and central nervous system [7]. The virus can be detected in upper airway secretions such as tracheal aspirates, nasopharynx swab or sputum, bronchoalveolar lavage, blood, urine, and stool [8,9]. COVID-19 is diagnosed primarily through epidemiological history, clinical signs, and additional tests such as nucleic acid detection, CT scan, immune identification technology, enzyme-linked immunosorbent assay (ELISA), and blood culture [10]. The relationship of some biochemical parameters with COVID-19 infection in Mosul City was done by across sectional study in cohort of 240 (116 males and 124 females) individuals with positivity COVID-19 and healthy112 control. It revealed that the majority of the COVID-19 patients showed increased levels of serum ferritin, LDH and D-Dimer, while having a reverse effect with serum GOT and GPT that showed normal value in patients with COVID-19 compared with healthy control [11].

RT-PCR is used to detection a genetic targets of virus in respiratory samples. They are considered the current gold standard for SARS-CoV-2 microbiological identification in respiratory samples, but they take a long time to perform and process, need equipment and highly skilled laboratory staff, and are expensive [12,13]. Antigen detection tests (ADT) are rapid diagnostic methods based on lateral immunochromatography, already in use for other respiratory viruses (e.g., influenza virus, Respiratory Syncytial Virus (RSV). Due to the low viral load in its acute phase, ADT's diagnostic capacity in these other viral processes is limited [14]. It is a fast, low-cost test that can be performed by a healthcare professional without intensive training and specialized equipment and the principle of it is based on the movement of a liquid sample [15]. This study aimed to evaluate the diagnostic sensitivity of the immunochromatography method by comparing it with PCR in COVID-19 diagnosis.

Material and method

The study was conducted at AL- shifaa hospital between (December /2021 and February 2022), and included 116 participants. The two nasopharyngeal (NP) swabs were taken from the two groups. The first swabs were taken from suspected COVID-19 patients; this swabs were then put in viral transport media (VTM). Viral RNA was extracted from 200 µL of nasopharyngeal swabs by using an RNA extraction kit (Kogenebiotech, Korea) in accordance with the manufacturer's guidelines. For all samples, RT-PCR was performed for N and ORF1ab genes using the one-step RT-PCR kit (Aehealth, UK). The second swab was obtained from the same patients who had positive PCR results, and it was then placed in an extraction buffer tube for detection of the SARS-CoV-2 Nucleocapsid Protein antigens using Panbio™ COVID-19 AG Rapid Test cassette (Abbott, Germany). After 15 min, the results were interpreted as a negative result was indicated by the presence of only the control line (C) and no test line (T) within the result window and a positive result was indicated by the presence of the test line (T) and the control line (C) within the result window.

Statistical Analysis

The Statistical analysis was performed with Qi square, and the p value =0.05 [16].

Result

The samples from 116 participants in this study were underwent to polymerase chain reaction (PCR) and rapid antigen tests. Our results demonstrated a significant difference (P value = 0.0001) between RT PCR qualitative results and rapid Ag tests in both patients and healthy control groups when Qi square was performed with a positivity rate (n = 90, 77.59%) for PCR and (n = 62, 53.45%) for rapid Ag, Table 1.

Table 1: Number and percentage of positivity of RT-PCR in Comparison with rapid antigen Test using Qi square.

Comparison of PCR with Rapid Ag	Positive		Negative		Total	P value
	No.	%	No.	%		
PCR	90	77.59	26	22.41	116	0.0001
Rapid Ag	62	53.45	54	46.55	116	

Table 2 illustrates the number of rapid Ag test result between patients and healthy control with peak rate of positive result in patients (n=62, 68.80%) out of 90 compared to 0% in control group. While, only (n=28, 31.10%) were negative despite their positive PCR result and 26 (100%) of the control participants were negative.

The sensitivity of rapid Ag tests in COVID-19 patients and healthy control groups was at a rate of (69%) when compared with the standard RT PCR test.

Table 2: Number and Percentage of Infection with COVID-19 patients and Control Group.

COVID-19 TESTS	Patients		Control	
	No.	%	No.	%
Rapid test Ag+	62	68.80%	0	0%
Rapid test Ag-	28	31.10%	26	100%
Total	90	99.90%	26	116
Rapid Ag Sensitivity	69%			

The data demonstrated in Table 3 exhibits the positivity of rapid Ag test according to days of symptoms appearance were 15.5% of the patients gave positive results in the 1st week of

infection and most of them 38.88% were positive during 8-14 days of infection whereas only 14.44% were positive after day 15 of infection.

Table 3: Number and Percentage of Symptoms onset in Patients of COVID-19 in Days According to RAT Test.

Rapid Ag	Time from symptom onset					
	0-7 days		8-14 days		more days	
	No.	%	No.	%	No.	%
Rapid Ag+	14	15.5	35	38.88	13	14.44
Rapid Ag-	4	4.44	14	15.55	10	11.11
Total	18	19.94	49	54.43	23	25.55
	90					

Figure (1) showed no significant difference in the percentage of rapid Ag between smokers and non-smokers with (p value=0.8912) when Qi square was used.

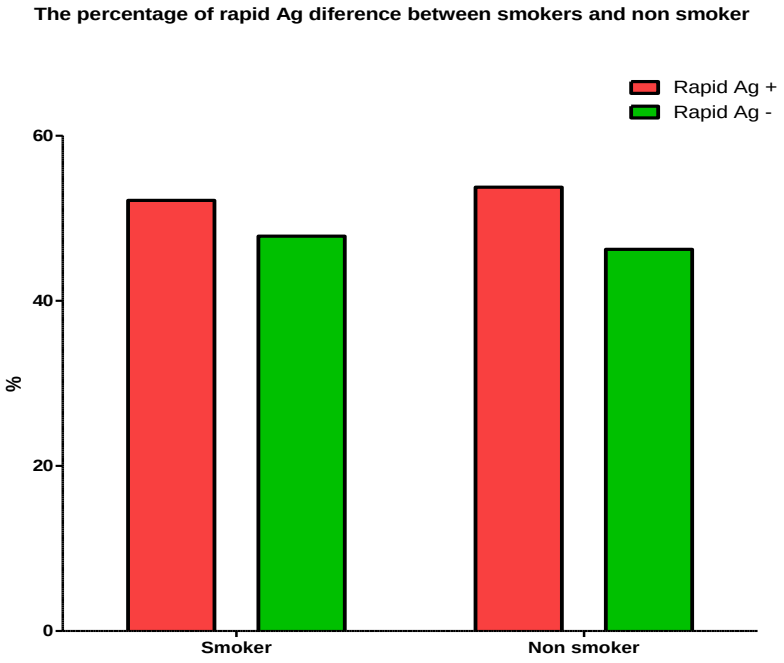

Figure 1: The percentage of rapid Ag difference between smokers and non-smokers.

The table (4) includes 90 patients with COVID-19 that were confirmed with RT RCR, among whom 44 females (48.89%) and 46 males (51.11%), also 26 subjects of healthy control, 14 females (53.85%) and 12 males (46.15%).

Table 4: The number of positive COVID-19 patients and healthy control according to gender distribution.

Parameters		Patients with COVID-19		Healthy controls	
		No.	%	No.	%
Gender		44	48.89%	14	53.85%
	Female	46	51.11%	12	46.15%
		90	100%	26	100%

Discussion

The research involved a total of 116 participants, all individuals were screened and tested with COVID-19 RT PCR commercial kits as well as rapid antigen detection kit. The rapid Ag detection test for COVID-19 is a simple rapid method and require no skills for practice and generate result as such it consider a method of choice in the lack of skills and expertise as well as it require no equipment and expert technicians as in the case of RT PCR to run the machine and to interpret the data produced by the device [17,18]. Our data revealed that there was significant difference between RT PCR and rapid Ag tests in patients and healthy control groups, this due to the fact that RT PCR is a gold standard method for diagnosis of COVID-19 and is superior to other methods [19], this result is in conflict with Becker *et al*,(2020) who recorded low RT PCR positivity rate compare to saliva rapid Ag test for SARS Cov2 [20]. The RT PCR results found in this research were 100% positive for those patients with symptoms or hospitalized with 100% sensitivity and were negative in all the control group, this data is in conflict with other studies [20,21] that recorded low sensitivity of RT PCR in detection of the virus in clinical swabs extracted from pharyngeal cavity. The discrepancies in our result and other research could be attributed to the fact of bias selection of the patients as most of them were hospitalized due to the complications of SARS-Cov-2 and the fact that they were previously screened by RT PCR and were positive.

In this study the sensitivity recorded for rapid antigen detection kit utilizing nasopharyngeal swabs were 69%, this result is in parallel with Becker *et al*., 2020 and Möckel *et al*., 2021 who reported a close sensitivity (69.2) to our result [20,22]. However, our data differs from Azzi *et al*., 2020 and Chaimayo *et al*., 2020 who recorded increased sensitivity of the rapid Ag test for COVID-19 in comparison to RT PCR [23,24,25]. The conflicting results suggest different aspects to consider including the date of sample extracted from each donor as well as the sensitivity of commercial kit used to detect the infection in addition to the protocol of sample collection, these factors could play crucial role in generating true positive or true negative results hence, influencing the sensitivity and specificity of the data.

This study demonstrated when patients were grouped according to the onset of the disease and testing time, there was an elevated positive rate of rapid Ag positive during the period (8-14 days) of symptoms onset. These findings disagree with other studies [24,26] that reported elevated positivity rate of rapid Ag test in the early phase of infection nearly in the 1st week of symptoms onset. These finding could be due to the fact that viral load is elevated during the early phases of infection [25]. The more viral particle recovered in the specimen the more the likelihood of obtaining positive test result [27]. This could increase the positivity of rapid antigen compared to samples with low viral load. Also, the time of sample collection as well as the technique of extracting the specimen could play an important role in generating false negative result. Other study recorded increase sensitivity of rapid Ag test in a proportional manner and this reflect the importance of viral load recovered in the collection procedure [27].

On the other hand, the data generated from rapid Ag test is not confident enough to judge the patients of having the infection or being asymptomatic hence, the need for superior method such as RT PCR to confirm such result is vital and can give a true indication of the epidemiology status in community [25].

The rapid Ag test is mostly based on the detection of viral protein S1 and N for both SARS like and SARS Cov2 infection [28], thus the positive result may not exclude corona like infection as the antibodies could be generated for both virions. In addition, the protocol of RT PCR is based on detection of vial genome that presented in the viral RNA after the amplification process which yield millions of vial copy that lead to high sensitivity of the RT PCR. Whereas, in the case of rapid Ag the principle is relied on detecting viral antigen in the tested sample via Antibody to vial protein found in the test cassette, this may result in low sensitivity and specificity and the less reliability of rapid Ag in comparison with RT PCR [29,30,31]. However, rapid Ag test relatively sensitive, specific and easy to handle by even non skilled laboratory usurers and labor intensive technique to screen suspected individuals [32].

Conclusion

In conclusion, the rapid antigen test is a helpful tool for detecting the presence of COVID-19 infection, but it may not be the best alternative due to its low sensitivity and lack of confirmation.

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