The Effect of Correlation of Laboratory-Developed Test and Initial Symptoms and False Negatives in RT-PCR Strategies for COVID-19 Patients with Beta Variants

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Abstract

Background Reverse transcription-polymerase chain reaction (RT-PCR) assays detect severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The number of viruses in the sample varies between patients; it depends on sample location, nasal or throat, and with time infection spreads. Previous studies showed that the viral load of coronavirus disease 2019 (COVID-19) infection is the peak just before symptoms onset. Furthermore, positive and negative results depend on test site, sampling, and timing method; RT-PCR can be 1 to 30% false-negative result.

Methods and Materials Within this study, we took RT-PCR test from COVID-19 positive patients who already had the confirmation of the disease either by lung...
Introduction

Since the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is an RNA virus, it is very mutable, so numerous variants of SARS-CoV-2 are spreading worldwide. To prevent the spread of disease, countries all over the world have adopted social-distancing policies. This has limited the movement of people, disrupted their daily activities, and instituted work-from-home strategy for all employment sectors. The coronavirus disease 2019 (COVID-19) climate has devastatingly impacted global education as well. In COVID-19, patient’s lung is the first organ that is infected first and then it spreads in many organs such as the heart, liver, and kidneys. Before spreading viral pneumonia, SARS-CoV-2 can be measured in saliva, blood, sputum, and urine even though high viral loads of SARS-CoV-2 RNA are being found in salivary gland and saliva, potentially illustrating the importance of this biofluid for testing the disease in asymptomatic condition. If the immune system can defend against COVID-19 at first, pneumonia will not be developed. Although there are no specific symptoms in COVID-19 patients, there has been frequent symptoms such as fever, cough, diarrhea, and fatigue, and diagnosis of COVID-19 relies on the detection of viral ribonucleic acid (RNA) sequences by using reverse transcription-polymerase chain reaction (RT-PCR).

Sufficient viruses on the test site, sampling methods, and timing can result in a positive test. Previous research showed that in COVID-19 infection, viral loads rise just before onset of symptoms and at symptom; furthermore, false-negative results from respiratory samples for SARS-CoV-2 ranged from 1 to 30%.

The World Health Organization suggests that COVID-19 patients who have long symptoms with negative RT-PCR test, should undergo repeat testing (including the sampling of the lower respiratory tract) with continued infection prevention measures. Even though RT-PCR has the outcome of false-negative results, it is still the most conventional method for testing because saliva collection is quite comfortable for patients as well as being easy, cheap, and noninvasive with minimal equipment required. It should also minimize the nosocomial transmission of 2019-nCoV to healthcare workers and saliva has shown true potential as an ideal noninvasive diagnostic specimen, with a high degree of sensitivity and specificity for the detection of the SARS-COV-2 virus. This study aimed to determine the relationship between the false-negative result and the laboratory-developed test and initial symptoms in COVID-19 patients and their individual and additive power to predict in-hospital patients.

Materials and Methods

This Ethics Committee-approved prospective observational study includes the Masih Daneshvari Hospital. During the COVID-19 pandemic peak, this center was the first COVID-19 dedicated hospital in Iran.

Trial Registration

This research was supported by the Masih Daneshvari Hospital and Shahid Beheshti Medical Sciences, and the Shahid Beheshti Medical Sciences approved the protocol of this study with ethical code IR.SMBU.NRITTLD.1400.008.

Study Population

The study was conducted on 49 patients aged between 32 and 77 years old with an average of 53.24 years old referred to Masih Daneshvari Hospital. The illness of these patients was confirmed according to the symptoms such as dyspnea, diagnostic criteria, including laboratory tests (complete blood count, erythrocyte sedimentation rate, C-reactive protein, D-dimer, ...), radiopacity in radiographs, and computed tomography (CT) scans detection of the lungs. The study was explained to all the patients, and they agreed to take the RT-
PCR test, both nasopharyngeal and oropharyngeal swabs. We repeated the test once again for negative tests, and if the result came back negative, we included them as negative in the result. Of 49 patients, 32.3% with COVID-19 had negative RT-PCR.

**Exclusion Criteria of the Study Population**

Patients were admitted to the intensive care unit with shortness of breath (>30 breaths/min), oxygen saturation of 90 at rest with partial oxygen pressure, and nasal oxygenation (5–6 L/min) per fraction of inspired oxygen (FiO2) less than 300 mm Hg. Patients with weakened immune systems, cough, patients with sepsis (Sequential Organ Failure Assessment (SOFA) score 2 or higher), shortness of breath, significant comorbidities, and fever (obstructive pulmonary disease, chronic kidney disease, chronic COVID-19 infection, diabetes, and congestive heart failure) were also admitted to the intensive care unit. The presence of hypoxemia, hypercapnic acidosis, despite the introduction of nasal oxygen at a high flow rate (oxygen flow rate ≥ 40%, and FiO2 ≥ 60%) or severe shortness of breath with increased work (frequency) of breathing are all indicators of mechanical ventilation (a set of assistive products, intercostal recession or nasal enlargement and set of expiratory muscles). Patients with adult respiratory distress syndrome requiring mechanical respiration were assigned a lung-protective ventilation mode.

**PCR Analysis and PCR Ct Values**

Viral RNA extraction was performed using a high pure viral nucleic acid kit (Roche, Switzerland) following the manufacturer’s protocol. PCR was performed using the extracted nucleic acid and a real-time COVID-19 commercial kit (Pish-taz, Iran). Briefly, a reaction included 15 mL of 9 U of the enzyme, 1 mL of primer & probe mix (RdRp/N/IC), 5 mL water, and 5 mL of extracted RNA. Then, PCR was carried out with a reverse transcription step at 50 °C for 20 minutes, cDNA initial denaturation at 95 °C for 3 minutes, and finally 45 cycles of 10 seconds at 94°C and 40 seconds at 55°C. Light Cycler 96 (LC96) PCR machine (Roche, Germany) was used to perform the amplification and to evaluate Ct (cycle threshold) values. Ct values less than or equal to 29 were considered strong positive reactions, Ct values of 30 to 37 indicated positive reactions, and Ct values of 38 to 40 represented weak reactions indicative of minimal amounts of target nucleic acid.

**Result**

Descriptive statistics used statistical indicators such as frequency, mean, and standard deviation. In inferential statistics, the default normality of the Kolmogorov–Smirnov test was checked and confirmed due to the need to use parametric tests to analyze the data. Then, the Pearson correlation test was used to examine the relationship between the variables, and multiple linear regression was used to predict after the Durbin Watson test and data alignment through SPSS software, version 25. Initially, the normality of the data was confirmed by the Kolmogorov–Smirnov test, and the conditions of Pearson correlation analysis were observed. Findings show that out of 49 patients, 32.3%, despite having COVID-19 disease, had a negative RT-PCR test; their age was between 32 and 77 years with an average of 53.24. Out of 49 patients 25 patients were male and 24 female. The results as shown in Table 1 are descriptive statistics from the center-orientation index and the dispersion index, which include the mean and standard deviation of research variables. Also, in inferential statistics, there has been a positive and significant relationship between weight (r = 0.253) and CT at the time of hospitalization of COVID-19 patients and a negative and significant relationship between O2 saturation without oxygen therapy (r = −0.296) and CT at the time of hospitalization of COVID-19 disease as Table 2 shows the results of the study showed multiple regression assumptions.

According to the value obtained from the multiple correlation coefficient, the model can predict 67.7% of the disease. Among the variables, weight had a negative and

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Mean, standard deviation, and correlation coefficients of research variables in patients with COVID-19 ((p &lt; 0.05))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variable</td>
<td>Half-value</td>
</tr>
<tr>
<td>CT upon arrival</td>
<td>32.31</td>
</tr>
<tr>
<td>Age</td>
<td>53.24</td>
</tr>
<tr>
<td>Weight</td>
<td>83.02</td>
</tr>
<tr>
<td>Onset of symptoms</td>
<td>9.08</td>
</tr>
<tr>
<td>LDH</td>
<td>608.06</td>
</tr>
<tr>
<td>CRP</td>
<td>36.45</td>
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<tr>
<td>LYM</td>
<td>17.28</td>
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<tr>
<td>O2 without oxygen</td>
<td>94.41</td>
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</table>

Abbreviations: COVID-19, coronavirus disease 2019; CRP, C-reactive protein; CT, computed tomography; LDH, lactate dehydrogenase; LYM, —.
significant relationship, and O2 saturation without respiratory support had a negative and significant relationship with COVID-19 disease, and due to the beta value, the share of O2 saturation without respiratory support is more than weight.

**Discussion**

Our study included 32.2% of patients with COVID-19 symptoms and pulmonary involvement in CT-scan, had two negative PCR tests reported, taken from both oropharynx and nasopharynx swaps. Furthermore, the variant of the virus we worked on was the B.1.351 (beta variant) species.

For nasopharyngeal swaps, positive rates have been reported moderate, while oropharyngeal swaps have shown a low positive rate due to the systematic review and meta-analysis done by Bwire et al.\(^8\)

We considered factors such as age, sex, weight, and CT scan upon arrival, lactate dehydrogenase, C-reactive protein, Lymphocyte count, LYM, O2 sat without oxygen, and symptom onset. Based on our findings, the patient’s weight was inversely related to the CT value; patients with high body mass index (BMI) had a poor prognosis.

Also, in the case of O2 saturation, higher O2 saturation, the RT-PCR test was less likely to be false negative.

The systematic review by Ingrid Arevalo-Rodriguez et al reported a false-negative rate in the RT-PCR test with an average of 0.11 rate and emphasized retesting because nearly 54% of patients whose clinical signs were suspected of having COVID-19 had a negative RT-PCR test in the first trial.\(^13\)

There are various factors involved in the negative and positive results of the RT-PCR test. According to the systematic review by Sue Mallett, the time of onset of symptoms and the sample transport tube are the most critical factors that affect the test result.\(^14\)

In analytical considerations by Rahbari et al, which examined possible errors in the detection of COVID-19, preanalytical errors were considered as the main factor in detecting coronaviruses, such as equipment, location, and sampling time.\(^15\)

In a study by Zhou et al, it has been said that the use of high-sensitivity kits is effective in reducing false-negative rates. Based on this, it is recommended to evaluate the ability of detection kits before routine usage. Moreover, continuous amplification can increase the detection rate of low viral load specimens. As a result, it can significantly reduce the false-negative rate of SARS-CoV-2.\(^16\)

Due to the importance of this disease, the negative RT-PCR cannot be easily ignored. According to the retrospective cohort study by Xiao et al, in addition to CT scan, in patients with a negative RT-PCR test, it was suggested to measure the levels of CD3, CD4, CD8, CD19, immunoglobulin M, C3 complement, and C4 complement for better screening.\(^17\)

In addition to RT-PCR testing, various diagnostic methods have been studied in a systematic review by Mistry et al. Variable sensitivity was also reported in their study. However, this device could be a new form of the COVID-19 test.\(^18\)

A systematic review and meta-analysis by Subali and Wiyono discuss that the reverse transcriptase loop-mediated isothermal amplification is more sensitive and specific compared to RT-PCR.\(^19\) Furthermore, droplet digital PCR (ddPCR) has been studied by Sun et al and ddPCR points to the distinctive clinical detection of SARS-CoV-2 to minimize random errors when compared to RT-PCR, suggesting that it could be a powerful addition to existing standard RT-PCR.\(^20\) However, the RT-PCR test is still preferable to other tests, supplemented with other methods to achieve the best and most accurate results.\(^8,9,21\)

Lung involvement is not necessarily correlated to the result of real-time RT-PCR; in a study, Xingzhi Xie et al found chest CT findings in negative RT-PCR. A typical diagnostic finding, ground-glass opacity, was viral pneumonia due to (COVID-19) infection (chest CT for typical COVID-19).\(^22\)

RT-PCR tests are sensitive and specific, and false-positive rarely occurs, but insufficient quantities of the virus in the sample can result in a false negative.\(^7\)

**Conclusions**

Our study is one of the first studies to describe and build a model for patients who initially confirmed negative for COVID-19, but were subsequently retested and confirmed positive for COVID-19. We show that a pragmatic model can be built to predict which patients should be retested for COVID-19, and those 49 patients are sufficient number of

<table>
<thead>
<tr>
<th>Predictive variables</th>
<th>$r$</th>
<th>$F$</th>
<th>$DF$</th>
<th>$SE$</th>
<th>$p$-Value</th>
<th>Impact coefficient</th>
<th>$p$-Value</th>
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<tbody>
<tr>
<td>Gender</td>
<td>0.527</td>
<td>0.677</td>
<td>1.66</td>
<td>8</td>
<td>5.92</td>
<td>0.031</td>
<td>1.17</td>
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<td>Onset of symptoms</td>
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<td>0.028</td>
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<tr>
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<td>0.972</td>
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<td>O2 sat without oxygen</td>
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<td>0.471</td>
</tr>
</tbody>
</table>

Abbreviations: CRP, C-reactive protein; LDH, lactate dehydrogenase; LYM, —; SE, standard error.
patients to be screened. Among the variables, weight had a negative and significant relationship, and O2 saturation without respiratory support had a negative and significant relationship with COVID-19 disease. Due to the beta value, the share of O2 saturation without oxygen therapy is more than weight at a BMI of more than 23 kg/m². We found an increase in the risk of severe COVID-19 leading to positive tests and hospitalization. More research and studies are being conducted to assess the cost-effectiveness of the retesting method in clinical practice, as well as its usefulness.

Authors’ Contributions
SGH helped in conceptualization, methodology, supervision, project administration, and writing—review & editing. SAN was involved in conceptualization, data curation, methodology, and supervision. MD contributed to conceptualization, formal analysis, methodology, project administration, and writing—review & editing. MKG and GhM were involved in investigation, validation, and writing—original draft. NK and EA helped in investigation and writing—original draft. AE contributed to investigation, validation, and data curation

Availability of Data and Materials
The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Declaration of Interests
No grant has been reported during the conduct of the study. Personal contributions have been reported during the conduct of the study. All authors declare no competing interests.

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