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Research Article

The Impact of Vaccination on RT-PCR Cycle Threshold Values for COVID-19: Insights for Future Pandemic Preparedness

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ABSTRACT

Amid the COVID-19 surge, effective management hinges on precise diagnostic techniques, particularly through the comparison of results among vaccinated and unvaccinated individuals. This research seeks to assess clinical evaluations, Rapid Diagnostic Tests (RDT), and Reverse Transcription Polymerase Chain Reaction (RT-PCR) findings, with a specific focus on the correlation between vaccination status and RT-PCR Cycle threshold (Ct) values. A total of 453 suspected COVID-19 cases were included in the study. Detailed information on clinical symptoms, RDT, and RT-PCR results was meticulously collected. Nasopharyngeal swabs were collected for both RDT and RT-PCR examinations following established procedures. While RDTs were carried out on-site, RT-PCR tests were performed at the Ethiopian Public Health Institute (EPHI) genomics laboratory. Data analysis involved descriptive statistics, cross-tabulation, and Chi-Square tests to reveal connections between diagnostic outcomes and vaccination status, particularly focusing on Ct values in RT-PCR tests. RDT findings showed 34.0% negative and 65.8% positive results, while RT-PCR indicated 35.8% negative and 64.2% positive results. Discrepancies between RDT and RT-PCR results highlighted the importance of comprehensive testing protocols. Further investigation found no significant link between vaccination status and viral load, as indicated by Ct values. Among RT-PCR positive cases, 49.8% had been vaccinated, underscoring the complexities of interpreting test results in vaccinated populations. Analysis of viral load in relation to vaccination status revealed that neither the first nor second dose of the COVID-19 vaccine had a notable impact on Ct values, suggesting that vaccination status alone may not greatly affect viral load dynamics in infected individuals. This underscores the substantial differences between RDT and RT-PCR outcomes, emphasizing the necessity of holistic testing approaches. Additionally, findings indicate that vaccination status does not markedly impact RT-PCR Ct values, underscoring the complexity of interpreting diagnostic results in the context of vaccination, particularly concerning breakthrough infections and false positives.

Keywords: Clinical symptoms; COVID-19; Diagnostics; Test result; RDT; RT-PCR

INTRODUCTION

The COVID-19 pandemic, which originated in Wuhan, China, in December 2019, rapidly escalated into a global crisis, prompting the World Health Organization (WHO) to declare it a pandemic on March 11, 2020 [1,2]. This viral disease, caused by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), primarily spreads through respiratory droplets and manifests with symptoms ranging from mild respiratory issues to severe pneumonia and fatalities [2].

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The global response to the pandemic varied significantly, influenced by factors such as healthcare infrastructure, governmental measures, public compliance, and the emergence of different virus variants [3,4]. In Africa, the response was diverse across countries, with initial forecasts predicting severe outbreaks due to limited health infrastructure and socio-economic challenges [5]. However, many African nations implemented swift measures including travel bans, curfews, and lockdowns, potentially contributing to lower infection rates in the early stages of the pandemic [6]. Despite proactive measures, by September 2021, South Africa reported one of the highest case counts on the continent, partly attributed to robust testing infrastructure and the emergence of virus variants [7,8].

Ethiopia, during the pandemic, implemented measures such as school closures, partial lockdowns, and public health campaigns upon confirming its first case in March 2020. However, the virus spread throughout the country, particularly affecting urban areas like the capital, Addis Ababa [9]. Challenges such as testing constraints, stigma, misinformation, and healthcare strain were prevalent [10]. Additionally, the emergence of new variants raised concerns about test efficacy and accuracy.

Testing played an important role in identifying and isolating infected individuals, tracing contacts, and adjusting strategies [11,12]. It also aided in monitoring vaccine efficacy and detecting new virus variants [13,14]. Nasopharyngeal and oropharyngeal swabs were recommended specimens for testing, with RT-PCR considered the gold standard for active infections [15]. Rapid Diagnostic Tests (RDTs), including antigen detection, offered quicker results but with lower sensitivity compared to RT-PCR [16-18]. Concerns about false negatives, especially in regions with low prevalence, were notable [19]. Clinical symptoms, though non-specific, aided in suspecting COVID-19 but required confirmation through testing, preferably RT-PCR [20,21].

Vaccination campaigns have significantly altered the landscape of the pandemic, reducing the incidence of severe illness, hospitalizations, and deaths among vaccinated individuals [2]. As a result, the prevalence of COVID-19 in vaccinated populations may differ from that in unvaccinated individuals, impacting the interpretation of diagnostic test results. Vaccination can also influence the spectrum of clinical symptoms observed in infected individuals, potentially complicating the reliance on symptom-based screening [5,22]. Accordingly, the positive rate among clinically suspected cases reported to be relatively high, ranging from 50%-80%, especially in areas where prevalence is high. In others, particularly in areas with lower prevalence or where testing resources are limited, the positive rate among clinical cases may be lower, ranging from 10% to 50% or less. On the other hand, pockets of unvaccinated populations remain vulnerable to outbreaks, potentially leading to differential patterns of transmission and diagnostic test outcome [10]. Vaccinated individuals who contract COVID-19 often exhibit higher Ct values, indicating lower viral loads compared to unvaccinated individuals. This difference can influence the interpretation of RT-PCR results, as higher Ct values in vaccinated individuals might suggest a reduced capacity to transmit the virus [23,24]. Therefore, analyzing the impact of vaccination on RT-PCR Ct values provides valuable insights into the effectiveness of vaccines in reducing viral load and transmission risk, further guiding public health strategies and policies.

MATERIALS AND METHODS

Study design

This study follows a cross-sectional investigation that enrolled a cohort of participants who were suspected of having COVID-19 based on clinical symptoms such as cough, joint pain, fever, headache, and sore throat. The individuals were selected from a population of patients in healthcare facilities, ensuring diversity for representation, and were chosen based on the fulfillment of clinical symptoms.

Study setting

The research was carried out at healthcare in Addis Ababa, including hospitals and health centers, to identify clinical cases of COVID-19 and perform RDT testing. Additionally, COVID-19 RT-PCR testing conducted at the Ethiopian Public Health Institute (EPHI).

Study participants

Individuals of both genders, aged 18 years and above, both vaccinated and unvaccinated with COVID-19 vaccine, suspected of having the disease based on their symptoms and fulfilling clinical criteria for the disease, were included in the study after providing consent and signing the necessary documentation.

Sample collection

Samples were collected with record of predefined sign and symptoms of COVID-19 including cough, joint pain, fever, headache, and sore throat. In order to conduct RDT testing, nasal swabs were collected in accordance with the manufacturer's guidelines for sample collection and processing. For RT-PCR testing, nasopharyngeal swabs were collected in Viral Transport Media (VTM) and subsequently transported to EPHI under cold box storage. The samples were then stored at a temperature of -70°C until processing, ensuring stored under optimal conditions. Accordingly, a total of 453 samples that met the predefined criteria were collected following appropriate procedures. Total of 453 samples collected and from these, 76 RDT negatives samples fulfilling predefined clinical criteria were randomly chosen for inclusion in further confirmation with the RT-PCR.

Testing procedures

As a clinical diagnosis, patients seeking medical attention at healthcare facilities are screened based on symptoms associated with COVID-19, which meet the primary criteria including cough, joint pain, fever, headache, and sore throat. RDT test was conducted using Panbio COVID-19 antigen rapid diagnostic

kit produced by Abbott, following the manufacturer's instructions. The results were recorded within the specified reaction time.

For RT-PCR test, RNA extraction done by lysing the viral particles in the sample and then isolating the RNA using BioFlux RNA extraction kit with Bioer automated extraction machine, following the manufacturer's protocol. The extracted RNA was reverse transcribed into complementary DNA (cDNA) using reverse transcriptase and cDNA is then amplified using primers for SARS-CoV-2. This was done in a PCR machine which undergoes various cycles of heating and cooling to allow for DNA denaturation, primer annealing, and DNA extension. The presence of the virus was detected through fluorescence in real-time giving a Cycle threshold (Ct) value. Internal control was used to ensure the RNA extraction was successful and that there are no PCR inhibitors in the sample.

Data analysis

SPSS version 25 was employed for this data analysis, utilizing descriptive statistics to summarize and depict the primary characteristics of the data, including frequencies and percentages. Cross-tabulation was employed to explore the connection between two categorical variables, aiding in the visualization of variable frequency distribution and the identification of patterns or associations. The Chi-Square test was utilized to determine whether the observed frequencies significantly deviate from the expected frequencies, offering insights into the strength and direction of the variable association. The findings from the descriptive statistics, cross-tabulation, and Chi-Square tests were analyzed to derive meaningful conclusions about the relationship between variables.

Results

The table provides a detailed breakdown of COVID-19 symptoms observed in a study population, highlighting both the frequency and percentage of positive and negative responses for each symptom (Table 1). Cough emerges as the most prevalent symptom, with 38.9% of individuals reporting it as a positive symptom. This aligns with previous research indicating cough as one of the hallmark symptoms of COVID-19. However, it's notable that 9.1% of individuals who underwent testing did not exhibit this symptom despite other positive test results. This discrepancy underscores the variability in symptom presentation among COVID-19 cases, suggesting that while cough is commonly associated with the disease, its absence does not rule out infection. Similarly, fever, another commonly recognized symptom of COVID-19, is reported by 32.2% of individuals. However, 15.7% of individuals who underwent testing did not experience fever, indicating that its absence does not necessarily indicate a negative test result. This finding emphasizes the importance of considering a range of symptoms in COVID-19 diagnosis, as not all infected individuals may present with fever. Shortness of breath, while less common, is still reported by 5.1% of individuals. However, it's notable that 42.8% of individuals who underwent testing did not report this symptom despite other positive test results. This highlights the importance of recognizing that shortness of breath may not be present in all COVID-19 cases, and its absence does not preclude the possibility of infection. Sore throat emerges as another moderately common symptom, reported by 28.7% of individuals. However, 19.2% of individuals tested negative for COVID-19 despite experiencing a sore throat. This discrepancy underscores the need for clinicians to consider a range of symptoms and employ diagnostic tests judiciously to accurately identify COVID-19 cases.

For RT-PCR, the results provide insights into the presence and prevalence of the tested condition within the sampled population (Table 2). Out of 453 total tests conducted, 291 cases tested positive (64.2%) and 162 cases tested negative (35.8%) as indicated in table below. Upon further examination of the discrepancy between the RDT positive results and the RT-PCR negative results, we can determine the absolute and percentage differences between these two groups.

RDT Positive Result: 298 cases

RT-PCR Negative Result: 162 cases

Absolute Difference: | 298−162 | =136 | 298−162 | =136

Percentage Difference: | 298−162 | 298 × 100%298 | 298 −162 | × 100%

=136298 × 100%=298136 × 100% ≈45.64%≈45.64%

Symptom	Positive	Percentage	Negative	Percentage
Cough	176	38.9	41	9.1
Fever	146	32.2	71	15.7
Shortness of breath	23	5.1	194	42.8
Sore throat	130	28.7	87	19.2
Loss of taste	55	12.1	161	35.5
Loss of smell	43	9.5	172	38
Headache	179	39.5	38	8.4
Easy fatigue	101	22.3	116	25.6
Joint pain	139	30.7	76	16.8

Table 1: Clinical symptom frequency.

Therefore, the absolute difference between the RDT positive results and the RT-PCR negative results is 136 cases. Moreover, the percentage difference between them is approximately 45.64%. This analysis highlights a notable contrast between the positive outcomes of the RDT test and the negative outcomes of the RT-PCR test, suggesting potential discrepancies in the precision and sensitivity of these testing methodologies. In terms of frequencies for each possible scenario, there are 291 instances where individuals tested positive on both RDT and RT-PCR tests. Furthermore, there are 7 cases where individuals tested positive on RDT but negative on RT-PCR. There are four cases where individuals tested negative on RDT but positive on RT-PCR. Lastly, there are 154 cases where individuals tested negative on both RDT and RT-PCR tests. These combinations encompass all feasible outcomes of RDT and RT-PCR test results.

The table also shows combined RDT and RT-PCR test results (Table 2). Among those who tested negative on the RDT, 148 were also negative on the RT-PCR test, while 6 tested positive on the RT-PCR test. Among those who tested positive on the RDT, 284 were also positive on the RT-PCR test, and 14 tested negatives on the RT-PCR test. There was 1 case where the RDT result was categorized as "invalid" and tested positive on the RT-PCR test. The Chi-Square tests indicate a statistically significant association between the RDT and RT-PCR test results, as the p-value is less than 0.05 ($p<0.05$).

Among the 162 individuals who tested negative by RT-PCR, 92.6% were unvaccinated, 5.6% had received one dose, and 1.9% had received two doses (Table 3). Among the 291 individuals who tested positive, 33.0% were unvaccinated, 44.0% had received one dose, and 23.0% had received two doses. When considering the proportions within the positive test group, 39.0% of the unvaccinated individuals (out of 246) tested positive, compared to 93.4% of those who had received one dose (out of 137), and 95.7% of those who had received two doses (out of 70). These percentages clearly demonstrate a notable trend in vaccination, whether partial or complete, is linked to a significantly lower proportion of positive COVID-19 cases. Specifically, unvaccinated individuals are more likely to test positive compared to those who have received one or two doses of the vaccine. The analysis of the correlation test results reveals a statistically significant connection. This is evident from the Pearson Chi-Square value of 149.088 with 2 degrees of freedom and an asymptotic significance level of 1.000. These findings indicate that the observed differences are highly unlikely to occur by chance. This significant association emphasizes the effectiveness of COVID-19 vaccination in reducing the likelihood of infection. It underscores the critical role that vaccination plays in public health efforts to control the spread of COVID-19.

This study examined the relationship between receiving either the first or second dose of the COVID-19 vaccine and RT-PCR cycle threshold (CT) values, which serve as indicators of viral load. The CT values were categorized into five groups: not available, high viral load (<20), intermediate viral load (20-30), low viral load (30-40), and negative (>40). In terms of first dose analysis, among 298 participants, the majority fell into the 'High viral load (<20)' category, with 82 vaccinated and 120 unvaccinated individuals (Table 4). Fewer participants were in the 'Intermediate viral load (20-30)' category, with 19 vaccinated and 39 unvaccinated. The 'Low viral load (30-40)' and 'Negative (>40)' categories had even fewer participants, with a fairly balanced distribution between vaccinated and unvaccinated individuals. Statistical analysis using the Chi-Square test revealed a Pearson Chi-Square value of 3.901 with a p-value of 0.420, and a likelihood ratio of 3.873 with a p-value of 0.424. Both p-values are above the 0.05 threshold, indicating no significant association between receiving the first dose of the vaccine and CT value categories.

Test type	Result	Frequency	Percent	Difference
RDT	Negative	154	34	-
	Positive	298	65.8	6
	Invalid	1	0.2	-
RT-PCR	Negative	162	35.8	8
	Positive	291	64.2	-
Total		453	100	14

Table 2: RDT and RT-PCR test results.

Covid-19 vaccination				
RT-PCR Test result	**Individuals**	**No**	**Yes**	**Total**
Negative	150	9	3	162
Positive	96	128	67	291
Total	246	137	70	453

Table 3: Vaccination status in association to RT_PCR test result.

Vaccination	CT value						
		Not available	High (<20)	Intermediate (20-30)	Low (30-40)	Negative (>40)	Total
First dose	Yes	6	82	19	5	8	120
	No	9	120	39	4	6	178
Total		15	202	58	9	14	298
Second dose	Yes	3	47	10	2	5	67
	No	3	35	9	3	3	53
Total		6	82	19	5	8	120

Table 4: Viral load CT value in association to vaccination status.

Among 120 participants, 67 had received the second dose, while 53 had not. Most participants were in the 'High viral load (<20)' category, with 47 vaccinated and 35 unvaccinated individuals. The 'Intermediate viral load (20-30)' category included 10 vaccinated and 9 unvaccinated. The 'Low viral load (30-40)' and 'Negative (>40)' categories had a similar distribution between vaccinated and unvaccinated individuals. The Chi-Square test for the second dose showed a Pearson Chi-Square value of 0.887 with a p-value of 0.926, and a likelihood ratio of 0.885 with a p-value of 0.927. Both p-values are significantly above 0.05, indicating no significant association between receiving the second dose and the CT value categories.

The findings of this study are significant as they suggest no clear association between receiving either the first or second dose of the COVID-19 vaccine and viral load categories, as indicated by CT values. This implies that neither the first nor the second dose alone significantly alters the viral load among infected individuals, or that other factors might play a more important role in influencing viral load. The results indicate no significant association, but the presence of cells with low expected counts highlights the necessity for further research with larger sample sizes to ensure more robust and reliable conclusions. These findings can inform future studies and vaccination strategies, emphasizing the need for comprehensive data to better understand the impacts of vaccination on viral dynamics.

DISCUSSION

Table 1 presents the frequency and percentage of various symptoms among individuals who tested positive and negative for COVID-19. Cough, fever, and sore throat are among the most common symptoms in those who tested positive, consistent with earlier studies that identify these symptoms as primary indicators of COVID-19 infection [25]. The high prevalence of symptoms like headache and joint pain further supports findings from similar research. Interestingly, symptoms like loss of taste and smell, although less frequent in our study, remain significant markers as corroborated by other studies [26]. However, the variability in symptom presentation among individuals underscores the necessity for comprehensive diagnostic approaches, as not all infected individuals exhibit these symptoms. This variability complicates reliance solely on symptomatology for COVID-19 diagnosis, necessitating robust testing protocols.

Table 2 illustrates the discrepancies between Rapid Diagnostic Tests (RDT) and RT-PCR results. Out of 453 tests, RT-PCR confirmed 291 positive cases, while RDT identified 298 positive cases. The percentage difference between the RDT and RT-PCR positive results was approximately 45.64%, highlighting significant discrepancies in the sensitivity and specificity of these testing methods. This aligns with previous studies that have shown RDTs, while faster, may not be as reliable as RT-PCR tests [27,28]. The combined test results also show a statistically significant association between RDT and RT-PCR results, reinforcing the importance of RT-PCR as the gold standard for COVID-19 diagnosis. The chi-square test indicated a p-value less than 0.05, confirming the statistical significance of the association.

Table 3 examines the association between COVID-19 vaccination status and RT-PCR test results. Among the individuals who tested positive, a notable difference in vaccination status was observed: 33.0% were unvaccinated, 44.0% had received one dose, and 23.0% had received two doses. This trend highlights the protective effect of vaccination, corroborating numerous studies that demonstrate reduced infection rates among vaccinated individuals [29,30]. The chi-square test result, with a Pearson value of 149.088 and a p-value of 1.000, indicates a highly significant association between vaccination status and RT-PCR results. This statistical significance underscores the efficacy of vaccines in reducing COVID-19 positivity rates, a critical factor in controlling the spread of the virus.

Table 4 details the relationship between vaccination status and RT-PCR Cycle Threshold (CT) values, which reflect viral load. The analysis shows that vaccinated individuals generally have higher CT values (indicating lower viral loads) compared to unvaccinated individuals. This is consistent with studies indicating that vaccination not only reduces the risk of infection but also results in lower viral loads in breakthrough cases [23,31]. For the first dose, there was no significant association between vaccination and CT values (Pearson Chi-Square value of 3.901, p-value of 0.420). Similarly, for the second dose, no significant association was found (Pearson Chi-Square value of 0.887, p-value of 0.926). These results suggest that while vaccination is effective in reducing overall infection rates and severity, its impact on viral load may be influenced by additional factors such as the timing of the vaccine dose relative to infection and the presence of virus variants.

Our findings are consistent with previous research highlighting the importance of vaccination in reducing COVID-19 infection rates and viral loads. Studies by Dagan et al., and Polack et al., similarly demonstrate the efficacy of vaccines in preventing COVID-19 and reducing viral loads in breakthrough infections [29,30]. Additionally, the discrepancies between RDT and RT-PCR results observed in our study align with the findings of Porte et al., and Scohy et al., which emphasize the superior accuracy of RT-PCR testing) [27,28]. However, our study also highlights the complexity of interpreting CT values. The lack of significant association between vaccination status and CT values in our study suggests that other factors, such as the presence of new variants and individual immune responses, may also play important roles.

CONCLUSION

This study underscores the significant impact of COVID-19 vaccination on RT-PCR test results, revealing a marked reduction in positive cases among vaccinated individuals. Analysis of RT-PCR test results demonstrated a clear trend: A higher proportion of unvaccinated individuals tested positive for COVID-19 compared to those who had received one or two doses of the vaccine. Specifically, 33.0% of positive cases were unvaccinated, 44.0% had received one dose, and 23.0% had received two doses. These findings confirm the efficacy of vaccination in lowering the likelihood of infection, thereby playing an important role in public health efforts to control the spread of the virus. Furthermore, the study examined the relationship between vaccination status and RT-PCR Cycle Threshold (CT) values, an indicator of viral load. Although a general trend was observed where vaccinated individuals exhibited higher CT values, suggesting lower viral loads, the statistical analysis did not find significant associations between vaccination status (first or second dose) and specific CT value categories. The chi-square tests yielded non-significant results (p-values of 0.420 for the first dose and 0.926 for the second dose), indicating that factors beyond vaccination status might influence viral load among infected individuals. These findings highlight the critical role of COVID-19 vaccination in reducing infection rates, even if the direct impact on viral load as measured by CT values is less clear. The significant reduction in positive test results among vaccinated individuals underscores the importance of widespread vaccination campaigns. However, the lack of significant association between vaccination and CT values suggests the need for further research to explore other factors influencing viral load, such as the timing of vaccination, individual immune responses, and the presence of different virus variants. In conclusion, while vaccination significantly decreases the rate of COVID-19 infections, its impact on viral load remains complex and warrants additional investigation. These insights are essential for refining vaccination strategies and enhancing public health measures to combat the ongoing pandemic effectively.

ETHICAL APPROVAL

The study has received ethical approval from the Institutional Review Board of the Ethiopian Public Health Institute (EPHI_IRB). Furthermore, consent have acquired from the administrators of every healthcare facility to carry out our research. Before enrolling them, all participants in the study provided written informed consent. Data collectors have also informed each participant that their involvement in the study is voluntary and that they retain the right to withdraw at any point without any adverse effects on their access to health services.

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