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DETECTION OF SARS-COV-2 NUCLEIC ACID BY RT-PCR IN SYMPTOMATIC AND ASYMPTOMATIC PATIENTS IN DHAKA, BANGLADESH

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is responsible for the underlying etiology of COVID-19, a contagious respiratory disease. Proper diagnosis is required to prevent infection from spreading among persons and communities. Precise viral detection is a prerequisite to control the COVID-19 pandemic. Thus, the objective of this study is the detection of SARS-CoV-2 nucleic acid by Real-Time Reverse Transcription-Polymerase Chain Reaction (Real-Time RT-PCR) method of the suspected symptomatic and asymptomatic individuals in Dhaka, Bangladesh. This research was performed in different local hospitals in Dhaka, Bangladesh from June 2021 to January 2022. Samples from suspected individuals were collected following WHO guidelines. Then the samples were placed into a Viral Transport Medium (3 ml sterile VTM). After vortexing, Sansure Biotech Inc. virus nucleic acid RT-PCR test kits were used for diagnosis. Real-Time RT-PCR method was used to identify the positive and negative results of the samples. A total number of 416 COVID-19-suspected individuals were enrolled in this study; 297 (71%) tested real-time RT-PCR-positive, and 119 (29%) were negative. The test results of patients with COVID-19 symptoms were collected and analyzed. Among the positive samples, the positive rate of male patients was higher than female patients. Between age groups, 20 – 59 has shown the highest positivity rate and the lowest was observed below age group 10. Age distribution and clinical characteristics were compared with the neighboring country's COVID-19 outbreak survey and found to be quite similar. Real-time RT-PCR for SARS-CoV-2 remains the gold standard identification method to verify COVID-19 cases unless and until the World Health Organization (WHO) recommends any alternative approach. Early detection at the primary level of SARS-CoV-2 may aid the clinicians in treatment of COVID-19. All the necessary guidelines need to be followed even after the post-COVID-19 situation.

Keywords: COVID-19, Real Time RT-PCR, SARS-CoV-2, Symptomatic, Asymptomatic, Dhaka, Bangladesh

INTRODUCTION

As of December 2019, numerous cases of suspected viral origin have been found in Wuhan City, Hubei Province, China (1). Initially, this viral infection was known as the novel coronavirus (2019-nCoV disease) but was later renamed coronavirus disease 2019 (COVID-19) due to the WHO global agreement (2). Severe Acute Respiratory Syndrome, SARS-CoV-2 is the causative agent of COVID-19. Coronaviruses, belonging to the coronavirus family, are enveloped single-stranded viruses and are zoonotic in nature (3, 4). The rapid transmission rate and coexisting mortality rate make SARS-CoV-2 a greater threat than other previously developed coronaviruses, including SARS-CoV and MARS-CoV. The virus can become even more terrifying when asymptomatic patients spread it to infected people (5). Since March 8, 2020, when the first COVID-19 case was reported in Bangladesh, the nation has seen multiple waves of infection, resulting in over 20 million cases and 29,498 deaths to date (6, 7). A total of 141,801 people is contaminated with the virus and 1783 deaths in the country as of 29 June 2020. Bangladesh implemented a nationwide lockdown as part of its pandemic response to stop the spread of the virus (8, 9). Bangladesh's economy faced difficulties as a result of the epidemic, especially in its crucial ready-made garment (RMG) sector, which found it difficult to strike a balance between worker safety and productivity. Other than this economic sector, the pandemic situation also disturbed the mental health of the students in Bangladesh. The long-time home quarantine period caused disturbance and deterioration in students' study habits and performance at work, which eventually resulted in the growth of stress and dysfunctional learning behaviors (10). As the impact of COVID-19 was a lot, it was necessary to detect the disease among the suspected patients so that proper treatment could be given to reduce the risk.

The diagnosis mostly depends on clinical side effects and signs and consequent examinations (11). However, there's expanding proof that numerous patients with COVID-19 are asymptomatic or have fewer side effects to be recognized. The detailed predominance of asymptomatic patients extended from 1% to 19.2%

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(12, 13). Tragically, asymptomatic cases can transmit the infection to others, subsequently acting as a silent harbor of the contamination (12). In the past reports have appeared that this infection was confined to asymptomatic people, and the contaminations had been transmitted from asymptomatic patients (14). As there are troubles in screening for asymptomatic contaminations, it is troublesome for national anticipation and control of this scourge unless suitably tended to. Before that, it is fundamental to know the detailed picture and contrasts of both symptomatic and asymptomatic COVID-19 patients' characteristics and clinical appearances (if present). As there is restricted comparative information on the people with symptomatic and asymptomatic COVID-19, this study was conducted to assess the SARS-CoV-2 nucleic corrosive location rate in Dhaka, Bangladesh, in both symptomatic and asymptomatic individuals using RT-PCR. This information would offer assistance set successful preventive, and control methodologies against this serious public well-being threat.

MATERIALS AND METHODS

Study area and duration: This study was conducted in different local hospitals in Dhaka, Bangladesh. The data were recorded between June 2021 and January 2022.

Sample collection, storage, and transportation: Nasopharyngeal and oropharyngeal swabs from the suspected individuals were collected by following the WHO guidelines (15) from 416 patients, with a mean age of 6-102. The Novel Coronavirus (2019-nCoV) Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing) is intended for qualitatively detecting nucleic acid from SARS-CoV-2 in respiratory specimens. Collection was conducted carefully to avoid possible contamination in collection, storage, and transportation. Each specimen was treated with the appropriate care because it was considered to be infectious. Collection swabs had an aluminum or plastic shaft and a synthetic tip, like nylon or Dacron. Cotton swabs with wooden shafts are not advised, and calcium alginate swabs are not acceptable. After sample collection, swabs were stored in the Sample Storage Reagent immediately. When using the Sample Storage Reagent provided by the manufacturer, direct lysis was possible using the Sample Release Reagent RNA fast-releasing technology provided in this kit.

Storage and delivery of specimens: Specimens were immediately processed or those specimens to be tested within 24 hours were stored at 4°C. On the other hand, specimens that could not be tested within 24 hours were stored at -70°C or below (in the absence of -70°C storage conditions, specimens can be stored at -20°C for 10 days, nucleic acid can be stored at -20±5°C for 15 days). Multiple freeze/thaw cycles were avoided during the overall procedure. Specimen transportation was carried out in a sealed foam box with ice by maintaining the temperature between 2-8°C.

Fast and simplified sample extraction method: For clinical specimens preserved in Sample Storage Reagent, sample processing utilized the Sample Release Reagent RNA fast-releasing technology provided in the kit. 200 µL of the specimen was taken into a 1.5 mL EP tube by pipetting, centrifuged at 12,000 rpm for 5 min, and then the supernatant fluid was carefully discarded. Removing the precipitation in the bottom was avoided. 50 µL sample Release Reagent was added into each tube followed by vortexing for 5 seconds. This lysed sample was eligible to be directly added to the rRT-PCR reaction.

Preparation of reagents: Prepare the 2019-nCoV-PCR MasterMix (26 µL 2019-nCoV-PCRMix +4 µL 2019-nCoV PCR-Enzyme Mix) based on the total number of specimens, 2019-nCoV-PCR-Positive Control and 2019-nCoV-PCR-Negative Control and mix thoroughly. The remaining reagent must be stored at -20°C immediately.

Processing and loading of specimens: 30 µL of 2019-nCoV-PCR Master Mix was added into each well. Wells were covered and transferred to the sample processing area. The reagent mix filled well-received 20 µL of the extracted RNA, which was added in the following order: 2019-nCoV-PCR-Negative Control, patient specimen(s), and 2019-nCoV-PCR-Positive Control. Each well was covered, centrifuged at 2000 rpm for 10 seconds, and placed into Applied Biosystems ABI 7500 real-time RT-PCR system and the exact location of controls and each specimen were recorded.

RT PCR reaction conditions and parameters: According to the protocol of the kit, PCR tubes were placed into the PCR instrument (Life Technologies QuantStudioTM 5) to perform the test. The PCR process takes 50–60 min to complete different cycles such as reverse transcription, denaturation, annelation, extension, and finally, to give the final results (Table 1).

Table 1: Polymerase reaction protocol (QuantStudioTM 5) polymerase reaction cycle including temperature and time range.

No.	Steps	Temperature	Time	Cycle No.
1	Reverse transcription	50°C	30 min.	1
2	cDNA pre-denaturation	95°C	1 min.	1
3	Denaturation	95°C	15 sec.	45
4	Annealing, extension and fluorescence collection	60°C	30 sec.	1

RESULTS AND DISCUSSION

A total of 416 COVID-19-suspected individuals were enrolled in this study; 297 (71%) tested real-time RT-PCR-positive, and 119 (29%) were negative (Figure 1a). The test results of patients with COVID-19 symptoms were collected and analyzed. The age distribution and clinical characteristics were compared with the neighboring country's COVID-19 outbreak survey. Among the positive samples, the positivity rate of male patients was higher than female patients (Figure 1b) between the age group, 30-39 showed the highest positivity rate and the lowest rate was observed below age group 10 (Figure2). Among the positive cases, the common symptoms were fever 174 (31%), cough 148 (26%), headache 37 (7%), loss of taste and smell 15 (3%), sore throat 56 (10%) and muscle pain 51 (9%). Several patients also had the complication with, chest pain 17 (3%), weakness 8 (1%), shortness of breath 19 (3%), unknown 24 (4%), none 15 (3%) (Figure 3).

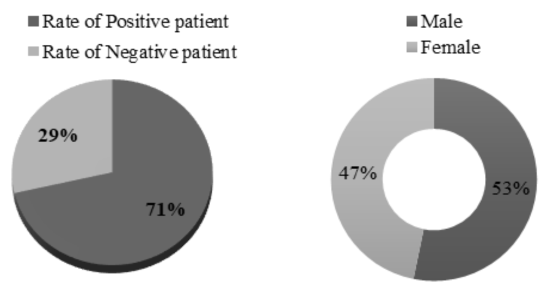

Figure 1 a): Rate of positive and negative patients. Total sample = 416; Positive sample = 297; Negative sample = 119. Figure 1 b) Positive rate among female and male patients; Female = 139 (47%) whereas male 158 (53%); Positive rate among males is higher than females.

Distribution of COVID Patients by Age

In our study, among the various age groups, the cohort aged 30-39 years had the highest infection rate, and the age group less than 5-19 years had the lowest infection rate (Figure 2). Infections were also observed in those over 80 years old. The same range was given by Mowla et al. (2020) (16). All age groups are at risk of contracting COVID-19, but older people are at much higher risk of developing serious illness due to physiological changes resulting from aging and underlying health conditions (17). With a median age of 27.6 years in Bangladesh, young and middle-aged Bangladeshis are predominantly working-class and are more likely to be forced to leave their homes for work and are therefore at risk of acquiring infection (18). Our results are consistent with earlier reports (19, 20), resulting in lower prevalence among younger age groups, which is supported by several studies. Another study (21), from Bangladesh found strong associations of COVID-19 risk factors with age outcomes. This is consistent with our research results. All of these results point to practical measures to secure, limit, and slow the progression of SARS-CoV-2 transmission in vulnerable populations.

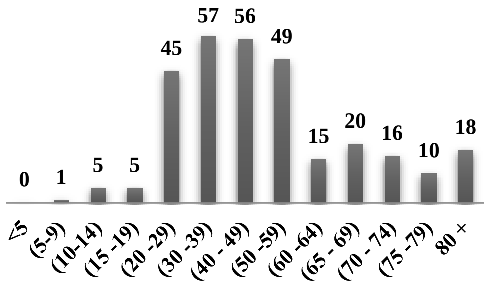

Figure 2: Age distribution of all COVID-19-positive patients from different locations in Dhaka.

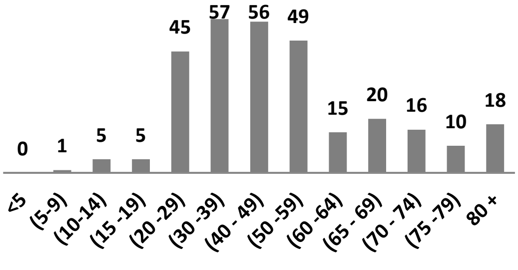

Figure 3: Age Distribution of all COVID-19-positive patients.

Distribution of COVID-19 Patients by Sex

In the current study, we found that the positive rate was higher in males than in female patients. 139 females (47%) were found positive as opposed to 158 male patients (53%). This phenomenon may be because in our nation men ought to go to catastrophe regions more than women for work and other activities.

According to Zhu et al., (2020) (22), reducing a woman's susceptibility to viral infection may benefit from the preservation of her X chromosome and gender, so social or cultural changes may be made early in an outbreak. Men are most likely to be exposed to the virus for several reasons. Current infection results indicate that men are more susceptible to COVID-19 than women. Gender differences in infection rates can be attributed to various physiological parameters, including weak viral receptor expression, metabolic differences, and behavioral differences between males and females (23). Relevant results from Cleveland Clinics in Ohio and Florida showed that a man had an increased risk of becoming positive for COVID-19 (24).

Clinical characteristics of COVID-positive patients

In our study, we recorded some clinical characteristics of COVID patients. In 174 patients' fever was recorded as the highest clinical characteristic. The second most common symptom was cough which was recorded in 148 cases. The sore throat was the third most common symptom observed in 56 cases. Some of the patients also experienced muscle pain (51 cases), headache (37 cases), loss of taste & smell (15 cases), chest pain (17 cases), shortness of breath (19 cases), and weakness (8 cases). 15 patients were diagnosed as asymptomatic and 24 patients' symptoms were unknown.

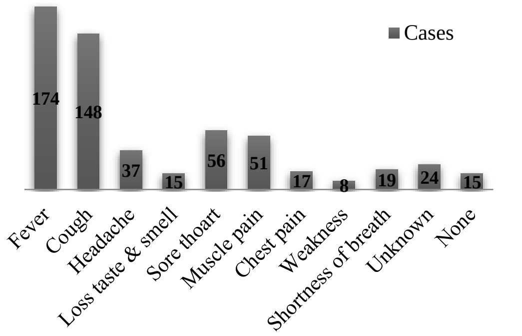

Figure 4: Clinical characteristics of all COVID-19-positive patients collected from different locations in Dhaka.

In contrast, a study conducted in Pakistan discovered that 165 patients (85.05%) and 168 patients (86.60%) had coughs. Of the patients, 71 (36.60%) reported feeling tired, and 24.74% reported having trouble breathing. 10.31% of cases showed signs of digestive distress, while 6.70 percent of cases had taste and smell loss. Eight individuals, or 4.12%, on the other hand, had no symptoms (25). Over 40% of participants in an Indian study who presented with fever and cough had dyspnea, cough, and fever as their main symptoms. There have also been reports of headache, sore throat, exhaustion, anosmia, nasal congestion, gastrointestinal problems, and chest pain. 2.7% of individuals did not exhibit any symptoms (26, 27). In a Chinese study, out of 7736 hospitalized patients, 43.8% had a fever at the time of admission, and 88.7% got sick while in the hospital. In 67.8% of patients, a cough was reported. The prevalence of diarrhea (3.8%) and nausea or vomiting (5.0%) was lower. Additionally, this study found that at least one comorbidity affected 23.7% of the general population (20). According to a study conducted in Nigeria, the main symptoms are exhaustion and dyspnea, followed by fever and cough (28).

Analysis of the results

The test results were determined based on the cycle threshold (Ct) value. According to the instructions of the Sansure PCR kit, the FAM and ROX channel both detects a typical s-type amplification curve and the CY5 channel has an amplification curve. An s-type amplification curve with Ct ≤ 40 means a positive result, and Ct > 40 means a negative result. If the FAM, ROX and CY5 channel does not detect a typical s-type amplification curve (No Ct), or Ct > 40, it means that the sample concentration is too low or there is an inhibitory reaction of interfering substances, the test result of the sample is invalid, the reason should be found and excluded, and the sample should be re-sampled for repeated experiments. If the amplification curve is between 35 and 38, the result should be re-checked. Only if the results remain compatible can a result be treated as positive. Normally CT value of 35 is acceptable in different laboratories in our country. The ROX (N) gene amplification curve in a COVID-19 PCR assay displays a distinctive sigmoid pattern, which indicates the presence of viral RNA. The target gene is amplified in the log phase, which causes the fluorescence to rise sharply at the beginning of the curve. Eventually, the fluorescence signal reaches a plateau where it stabilizes.

An estimation of the viral load is provided by the cycle threshold (Ct) value, which is found where the curve intersects the threshold line. Lower Ct values are indicative of larger viral loads. In a similar vein, a strong rise in fluorescence is usually seen on the FAM (ORF 1ab gene) amplification curve, indicating successful target gene amplification. A favorable outcome for SARS-CoV-2 is confirmed by the existence of a distinct exponential development phase that is followed by a plateau (Figure 4, 5).

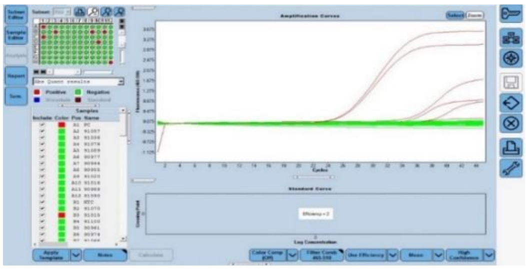

Figure 5: FAM (ORF 1ab gene) amplification of 2 Positive Control and 4 COVID-19 Positive Patients Samples.

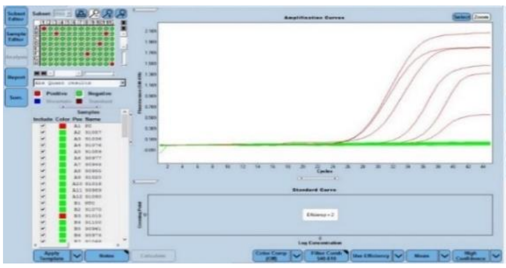

Figure 6: ROX (N gene) amplification of 2 Positive Control and 4 COVID-19 Positive Patients Samples.

Statistical analyses

Patients with and without COVID-19 symptoms were evaluated and analyzed using the qualitative data presentation. To assess the diagnostic performance, samples were obtained using aseptic methods, and the outcomes were examined using a PCR kit. The detection effectiveness of the PCR kit was assessed by computing its sensitivity, specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV), and kappa value, as well as their respective 95% confidence intervals (CI). Sensitivity measures how many genuine positive instances the test accurately identified, demonstrating its capacity to find the virus in affected people. Specificity indicates how well the test excludes non-infected people by measuring the percentage of true negative cases that are correctly recognized. The likelihood that a positive test result accurately identifies the presence of an infection is represented by PPV, whereas the probability that a negative result accurately shows the absence of an infection is represented by NPV. The degree of agreement between test results and the actual infection status after controlling for change is measured by the kappa value. Favorable PPV and NPV values imply reliable predictions of infection status, while high sensitivity and specificity point to strong diagnostic performance. The diagnostic accuracy of the test is confirmed by a high kappa value, which shows strong concordance between the test results and the real infection status.

Explanation of detection result:

Conclusion	Amplification curve characteristics
2019-nCoV Positive	A typical S-shape amplification curve is detected at FAM and/or ROX channel and the amplification curve is detected at CY5 channel, Ct < 40.
2019-nCoV Negative	There is no typical S-shape amplification curve (No Ct) or Ct > 40 detected at FAM and ROX channel, and the amplification curve, which is detected at CY5 channel, Ct >40.

CONCLUSION

With a total of 297 confirmed cases out of 416 people examined, our study concludes that the SARS-CoV-2 pandemic has had a substantial impact on Bangladesh. The study adhered strictly to WHO protocols for sample collection, storage, and transportation, assuring biosafety and accurate and dependable identification of SARS-CoV-2 in the patients. According to results from prior studies, fever and cough were the most common symptoms, but different regions showed varying patterns of symptom presentation. Since the working population is more likely to be exposed to the virus, targeted interventions are necessary, as evidenced by the higher positivity rate among the 20–59 age range. The clinical characteristics are compared with research from Pakistan, India, China, and Europe to highlight the variety of COVID-19 presentations and the significance of contextualizing symptom data locally. Longitudinal studies to track the development of symptoms and the effects of new variations should be the main focus of future research. Furthermore, investigating low-cost diagnostic techniques and expanding accessibility to RT-PCR and other testing technologies will be essential for controlling and averting future outbreaks. The incorporation of innovative diagnostic techniques, such as testing based on nanomaterials, may improve the capacity for detection and assist continuous pandemic management initiatives.

CONFLICTS OF INTERESTS

The authors have declared that no competing interests exist.

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ETHICS APPROVAL

Ethical approval has been taken from the Stamford University Bangladesh Research Ethics Committee (SUBREC).

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