

Prevalence of Severe Acute Respiratory Syndrome Coronavirus-2 among the Young People and Association between Diabetes, Hypertension, and Severe Acute Respiratory Syndrome Coronavirus-2

Aklima Akter¹, Nafisa Tabassum², Arifur Rahman¹

¹Department of Microbiology, Molecular Laboratory, Brahmanbaria Medical College, Brahmanbaria, ²School of Environment and Life Sciences, Independent University, Dhaka, Bangladesh

Abstract

Background: The corona virus disease-2019 (COVID-19) or corona pandemic is an outgoing pandemic caused by coronavirus 2019 (COVID-19) caused by the transmission of severe acute respiratory syndrome coronavirus-2. It was first identified in Wuhan, China, and the first COVID-19 case in Bangladesh was on March 7, 2020. A retrospective research was conducted on Brahmanbaria Medical College with COVID-19-suspected patients to understand the current situation of COVID-19 in Brahmanbaria. **Methods:** A total 752 oropharyngeal and nasopharyngeal swab samples were collected from COVID-19 suspected patients and reverse transcriptase–polymerase chain reaction test was run to identify the positive cases. **Results:** We found 28.5% ($n = 214$) of positive cases among which 22.9% ($n = 49$) were diabetes mellitus patients and 20.5% ($n = 46$) were hypertension patients too. **Conclusion:** A total of 214 (28.5%) COVID-19 positive confirmed cases among 752 COVID-19-suspected peoples were found. The most common age group of COVID-19 patients was between 31 and 40 years, which is a matter of great concern as these patients develop different postcorona syndromes such as weakness, breathlessness, and muscles pains and cannot go back to their normal daily life as before.

Keywords: Directorate general of health service, diabetes mellitus, E-gene, hypertension, RdRp gene, reverse transcriptase–polymerase chain reaction, severe acute respiratory syndrome coronavirus-2, WHO

INTRODUCTION

COVID-19 is a severe acute respiratory syndrome caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), which was first identified in Wuhan, China. This pandemic has spread rapidly in the US, Spain, Italy, and even more than 70 countries.^[1,2] SARS-CoV-2 could cause severe, even lethal pneumonia and lung failure. Within April 25, 2020, more than 2,800,000 confirmed cases and 190,000 deaths of COVID-19 have been reported worldwide.^[3] In <5 months after the first emergence of the virus in December 2019, nearly two million people in 185 countries around the globe have been identified as confirmed cases of coronavirus disease (SARS-CoV-2).^[4] Bangladesh, in southeastern Asia, confirmed the first COVID-19 case on March 7, 2020, and within April 13, 2020, the country had reported 803 cases of COVID-19 and the death toll stood at

39.^[5,6] On October 12, 2020, corona-confirmed cases were 379,738 and 5555 deaths of COVID-19 have been reported in Bangladesh, whereas globally, 37 686 296 confirmed cases of COVID-19 including 1 078 699 deaths were reported to the WHO on that same day. The USA was the world's top country which had 7,636,803 cases with 212,804 deaths due to corona, reported

Address for correspondence:

Aklima Akter,
Department of Microbiology, Molecular Laboratory, Brahmanbaria Medical
College, Brahmanbaria, Bangladesh.

E-mail: aklimabmc@gmail.com

Nafisa Tabassum,

Research assistant, School of Environment & Life Sciences,

E-mail: nafisa6020@iub.edu.bd

ORCID: 0000-0003-1077-9225

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to the WHO on October 12, 2020. As far from March 8, 2020, to October 12, 2020, according to the directorate general of health service press release, there were 379,738 COVID-19 confirmed by reverse transcriptase–polymerase chain reaction (RT-PCR) test, including 5555 related deaths (1.46%).^[7] COVID-19 or SARS CoV-2 enters into the lungs directly through the nose during breathing and multiplies in the mucosal area and cell wall of the tiny air sacs of lungs named alveoli. These viruses damage the vessels and thicken the lining of the alveoli. Finally, these sacs fill up with water and oxygen transportation is hampered [Figure 1]. No specific antiviral drugs such as remdesivir, lopinavir, ritonavir, nitazoxanide, tocilizumab, and favipiravir were found which could show 100% perfect action against this COVID-19. High mortality rate due to COVID-19 in the patients of diabetes mellitus (DM) was reported in many research reports previously.^[8] Even the use of a higher amount of inflammatory drugs causes diffusion of tissue in different parts of the body which finally leads to cytokine storm. Cytokines and excess inflammation can damage different organs of a patient's body.^[9] Our present study attempted to publish the condition of COVID-19 in our district Brahmanbaria. In addition, we found that most of the COVID-19 patients had diabetes and hypertension (HTN) so that their immune system was already weak to fight against this respiratory tract disease. Even after the recovery from deadly COVID-19 disease, we found that the infected patients suffer from postcorona syndromes such as weakness, breathlessness, and muscle pains for a long time which hamper their daily life works.

RESEARCH AREA

This research was carried out in the Department of Microbiology, PCR laboratory of Brahmanbaria Medical College, Bangladesh, over the period of August 2020

to October 2020. This laboratory is well recognized and governmentally approved only PCR laboratory in Brahmanbaria district under the Chittagong Division which has the BSL-2 facility with the minimum requirement to do RT-PCR.

Study population

A total 752 oropharyngeal (OP) and nasopharyngeal (NP) swab samples were collected from the suspected cases of COVID-19 patients and transported aseptically to the experimental laboratory by following thermostable conditions for analyzing the RT-PCR status.

Collection of samples

NP and oropharyngeal (OP) specimens were collected in viral transport medium (VTM) kit-VTM tube from the individual patient by using a sterile swab. Then, the collected samples were labeled including proper information of the patient such as name, age, sex, and other additional clinical histories (if needed).

Real-time reverse transcriptase–polymerase chain reaction method

RNA extraction method

RNA extraction is the isolation and purification of RNA from biological samples. The procedure is considered as complicated method due to the ubiquitous presence of ribonuclease enzymes in cells and tissues which can rapidly degrade RNA.^[10] In general, successful nucleic acid purification is required which can be done in four important steps. The steps are (1) effective disruption of cell or tissue, (2) denaturation of nucleoprotein complex, (3) inactivation of nucleases, for example, RNase for RNA extraction and DNase for DNA extraction, and (4) avoiding contamination.^[10,11]

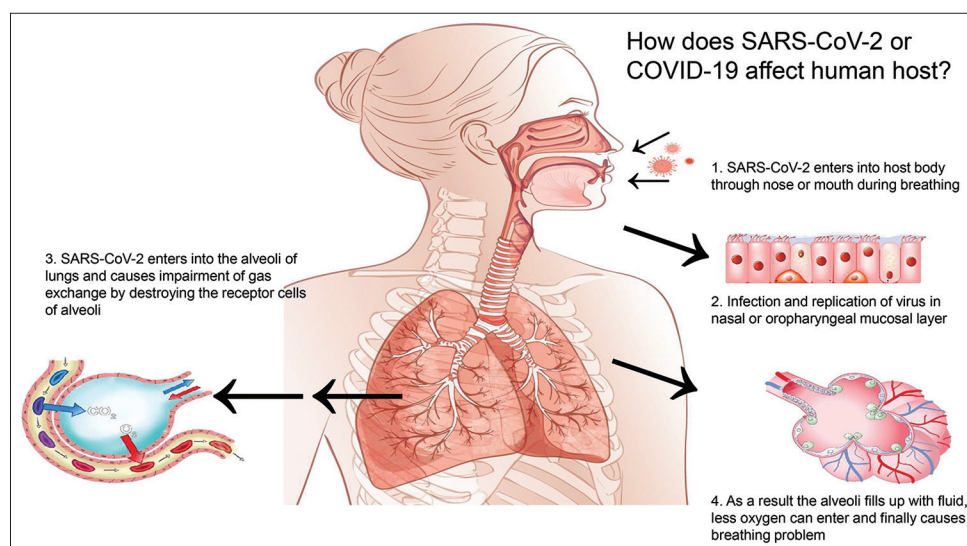


Figure 1: Coronavirus disease-2019 or severe acute respiratory syndrome coronavirus-2 affecting human host. Coronavirus disease-2019 or severe acute respiratory syndrome coronavirus-2 enters into the lungs through the nose or mouth during breathing. These viruses infect the lungs, mucosal surface by multiplying on the layer of tiny air sacs- Alveoli of the lungs. They destroy the cell wall by disrupting vessels which transfer the oxygen or carbon dioxide. As a result, it causes breathing problem, when lungs fill up with fluid, pneumonia occurs

To extract RNA, MagListo™ 5M Viral DNA/RNA Extraction Kit (BIONEER) was used according to its protocol. According to the manual, alkaline lysis method was applied with Magnetic nanobeads to efficiently minimize the isolation time. This kit can separate the viral RNA of SARS-CoV-2 from sputum, NP and OP swab samples. Lysis and precipitation, binding, washing, and elution all steps were performed as per instructions to isolate purified RNA [Figure 2].

Use of polymerase chain reaction–Kit

AccuPower SARS-CoV-2 Real-Time RT-PCR kit (BIONEER) is an *in vitro* diagnostic kit. This kit is used to diagnose COVID-19 infections which is designed to detect SARS-CoV-19(E-gene and RdRp gene) RNA from a COVID-19-suspected patient’s sample such as NP swab and OP swab through real-time polymerase reaction (PCR).^[12] Two PCR-tubes were required for each patient, one for E gene (Envelop gene) and another for RdRp gene (RNA-dependant RNA polymerase gene). According to the protocol of the kit, PCR tubes were placed into the PCR instrument (Exicycler™96, BIONEER) 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].

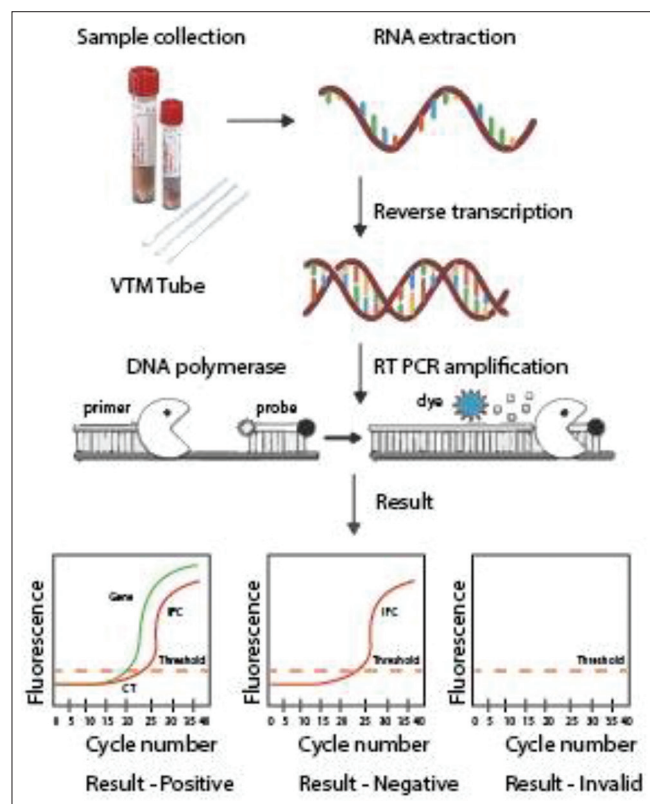


Figure 2: Sample collection, polymerase chain reaction performing and result analysis. Collected samples are used to identify the presence of coronavirus disease-2019 RNA through polymerase chain reaction test. In reverse transcriptase–polymerase chain reaction, positive cases show similar growth curve and highest peak, but in case of negative control, no similar growth curve can be observed

Reporting

After completion of PCR cycle if the Ct value of E-gene and the RdRp gene is ≤ 39 , then it is considered as positive and if the Ct value of E-gene and the RdRp gene is >39 , it is determined as negative. If internal positive control shows its Ct value within the specific range (15–35 Ct), then the whole PCR process is considered as valid.^[12]

RESULTS

A total of 752 samples of NP and OP specimens were taken in this study period from suspected patients, among which 76.7% ($n = 577$) were male and 23.3% ($n = 175$) were female [Table 2]. Among the suspected individuals, a total of 28.5% ($n = 214$) patients were COVID-19 positive according to RT-PCR test and 71.5% ($n = 528$) were negative [Table 2]. A total number of positive case patients were 214 for SARS-CoV-2. In addition, COVID-19-positive male patients were 73.8% ($n = 158$) and female COVID-19 patients were 26.2% ($n = 56$) SARS-CoV-2 positive [Table 2]. On the other hand, the total number of corona-positive case was 214, among which 49 patients had DM and 46 patients were suffering from HTN which is about 22.9% and 21.5%, respectively [Table 3]. A total of 214 (28.5%) COVID-19-positive patients were isolated from 752 COVID-19 suspected people. The most common age of affected patients was between 31 and 40 years which is about 26.6% of total positive cases [Figure 3]. About 41% to 42% of COVID-19 patients were between 41 and 60 years old.

* n = no of patients.

DISCUSSION

The first and foremost necessity to control COVID-19 outbreaks and clinical management is to collect the suspected

Table 1: Polymerase reaction protocol (exicycler™96)-polymerase reaction cycle including temperature and time range

Step	Temperature (°C)	Time	Cycle
Reverse transcription	50	30 min	1
Predenaturation	94	10 min	1
Denaturation	95	15 s	45
Annealing and extension	60	10 min	
Scan			

Table 2: Total cases of patients of different sex, results analysis, and percentage (n=752)

Patients	Total number of suspected patients, n (%)	Positive cases and percentage, n (%)	Negative cases and percentage, n (%)
Male	577 (76.7)	158 (73.8)	419 (79.36)
Female	175 (23.3)	56 (26.2)	119 (20.64)
Tovtal	752 (100)	214 (28.5)	528 (71.5)

*n=Number of patients

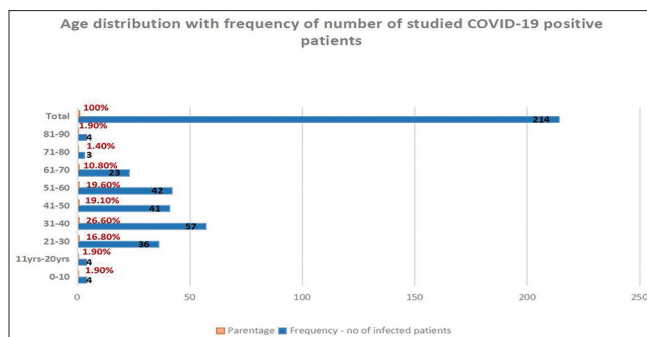


Figure 3: Age distribution with frequency of studied patients positive for coronavirus disease-2019 ($n = 218$). A total of 214 (28.5%) coronavirus disease-2019-positive patients were isolated from 752 coronavirus disease-2019-suspected people. The most common age of affected patients was between 31 and 40 years. The people of 41–60 aged are in the second position in the list of becoming infected by the coronavirus disease-2019. About 19.60% of total positive cases were 41–60 years old patients

samples and diagnose the infected patients. The rapid and proper collection of specimen from the suspected patients is a priority for clinical management and outbreak of COVID-19 control. RT-PCR helps to identify suspected cases by identifying the nucleic acid amplification of viruses. A total of 752 sample of both NP and OP specimens were taken in the study period, of which 76.7% (577) were male and 23.3% (175) were female [Table 3]. Among the suspected persons, 28.5% ($n = 214$) patients were positive for RT- polymerase chain reaction for SARS-CoV-2; 71.5% ($n = 528$) were negative. The number of infected patients in this district according to our test are quite higher in comparison with our national rate of positive cases of COVID-19 which is 21%, but consistent with the finding of the WHO, where the highest attack rate was observed from April 4, 2020, to May 4, 2020 in Dhaka district.^[13] According to our report, the total positive cases were 214 (28.5%) among 752. Most of the infected patients were men about 158 cases (73.8%) which is higher rate than female-infected patients. It might be because higher number of men are found in job sectors, transportation, and markets. In addition, among the positive cases, 49 patients had diabetics (22.9%) and 46 patients were suffering from HTN (21.5%). According to a previous report on January 2, 2020, 41 patients admitted in the hospital had been identified as having laboratory-confirmed COVID-19 infection in Wuhan, China, and most of the infected patients were men about 30 out of 41 (73%), among which eight patients had diabetics (20%) and 6 had HTN (15%).^[14] In addition, another research reported that 8.2% of cases of COVID-19 patients had diabetes and 19.7% had HTN.^[15] DM is a common endocrine disease^[16] and widely known as a chronic, low-grade inflammatory disease caused by long-term immune system imbalance, metabolic syndrome, or nutrients excess.^[17,18] Since the outbreak of COVID-19, several studies have been conducted to examine the relationship between diabetes and COVID-19.^[1,19] In recent years, it has been well recognized that diabetes and HTN are common health issues. However, the sudden emergence of

Table 3: Correlation of diabetes mellitus and hypertension with severe acute respiratory syndrome caused by coronavirus-2 positive patients ($n=214$)

COVID-19 Positive cases	Patients with other additional disease and percentage in total cases (%)
$n=214$	DM=49 (22.9)
	HTN=46 (21.5)

* n =Number of patients. DM: Diabetes mellitus, HTN: Hypertension

COVID-19 communicable disease has changed our concept toward health and disease and these patients with diabetes and HTN are at more risk.^[20] Although emerging reports have demonstrated a high prevalence of diabetes and HTN among patients with COVID-19. The prevalence of DM is anticipated to increase substantially during the next decades worldwide and is considered to be the main cause of human deaths.^[21] According to previous reports, a high prevalence of HTN was observed among COVID-19 patients in CHINA. For example, 58 of 191 Chinese patients with COVID-19 (30.4%) had HTN.^[22] Among the positive cases, most were in the 31–40 years age group that is 57 (26.6%) out of 214. This finding is consistent with other studies where 31–40 years age group is the highest population group who are COVID-19 positive.^[23]

CONCLUSION

Bangladesh is the second most affected country in south Asia, after India.^[24] Our study explains that young and middle-aged people are in more danger which is definitely an alarming news for us and this result is quite similar to the previously published articles in Asia.^[25] Diabetes and HTN are associated with an increased incidence of COVID-19. A healthy lifestyle is important for all of us, which would help us to fight against COVID-19. A high number of clinical trials have already been tested to understand the potency of different antiviral and anti-malaria drugs such as favipiravir, chloroquine, and remdesivir to cure the patients from this pandemic. Our next target should be the use of intravenous immunoglobulins as an intermediate goal as well as the invention of a proper active vaccine against this disease for the development in our research.^[26] As SARS-CoV-2 pandemic has created panic worldwide, more research is needed to evaluate the nature of the disease as well as increasing awareness is necessary to create among the population about SARS-CoV-2 infection.

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Conflicts of interest

There are no conflicts of interest.

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