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Exploring Demographic Properties and Amplification Efficiency of N and ORF-1ab Gene in Suspected COVID-19 Patients in Dhaka, Bangladesh

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ABSTRACT:

Background: In late December 2019, an outbreak of pneumonia with an unknown cause occurs in Wuhan, China. Later, the causative agent was identified as SARS-CoV-2. Early laboratory diagnosis plays an important role in any outbreak by cutting the spread of infection by isolating the infected person. Nucleic acid detection via PCR remains the gold standard because no alternative scientific method for detecting SARS-CoV-2 is available.
Objective: The goal of this study was to learn more about the demographic characteristics of SARS-CoV-2-affected people and the sensitivity pattern of the N & ORF-1ab gene among them. **Methods:** A swab from the nasopharynx for nucleic acid detection was collected from 3183 SARS-CoV-2 suspected cases attending outdoors and indoors at Aichi Hospital and Japan East-West Hospital. **Results:** 649 (20.4%) were found PCR positive. Among the positive cases, the male was 478 (73.7%) and the female was 171 (26.3%), mean age was 40.35 ± 14.551 years, ranging from 4 – to 90 years. The highest prevalence of SARS-CoV-2 was identified in the 21–40 years old group (50.7%), followed by the 41–60 years old group (33.1%). The N gene was found to be 100% positive and ORF-1ab was 22.03% positive. **Conclusion:** According to the findings of this study, males and younger generations are at an elevated risk of contracting the virus, and maybe a source of infection for other family members. According to this study, the N gene has higher sensitivity and amplification efficiency than ORF-1ab.

KEY WORDS: SARS-CoV-2, COVID-19, coronavirus, N gene, ORF-1ab gene.

INTRODUCTION

On December 31, 2019, a novel coronavirus that causes pneumonia was discovered in Wuhan, China.[1] Since the first case was identified, 42,966,344 confirmed cases and 1,152,604 deaths have been reported to the World Health Organization (WHO).[2] Because the virus is highly contagious and spreads quickly in nature, WHO classified the sickness as a pandemic on March 11, 2020.[3] The first incidence was confirmed in Bangladesh on March 8, 2020. Following that, a total of 398,815 confirmed cases and 5,803 deaths were reported to WHO until October 26, 2020.[4] It was first known as 2019-nCoV, but the Coronavirus Study Group of the International Committee on Taxonomy of Viruses (ICTV) renamed it severe acute respiratory syndrome coronavirus 2 (SARS-Cov-2), and the condition caused by it was named COVID-19 by the WHO.[5,6]

The coronavirus SARS-CoV-2 is the seventh coronavirus to infect humans.[6] Human coronavirus (HCoV)-229E, HCoV-NL63, HCoV-HKU1, HCoVOC43, Middle East respiratory syndrome coronavirus (MERS-CoV), and severe acute respiratory syndrome coronavirus were the previous six coronaviruses (SARS-CoV).[7] MERS-CoV, SARS-CoV, and SARS-CoV-2 are three beta coronaviruses that are more pathogenic and cause more severe respiratory tract sickness, as well as affect other systems such as the cardiovascular and renal systems.[8,9]

SARS-CoV-2 is a virus that is enveloped. The nucleic acid is a positive sense, nonsegmented, and ss RNA. Among RNA viruses, it has a genomic size of about 30 kb, which is quite large. This virus displays a high frequency of mutation

(deletion) & recombination which is unusual for an RNA virus with a non-segmented genome. With the complete genome sequencing, it was discovered that SARS-CoV-2 shares the most homology with Bat SL-CoVZC45 and Bat SL-CoVZXC21 coronaviruses, but differs from SARS and shares 79.7% similarities.[10] A 5' untranslated region (UTR), a conserved replicase domain (ORF-1ab), four genes S, E, M, and N that encode structural proteins spike, envelope, membrane, and nucleocapsid proteins, a 3' UTR, as well as other unidentified nonstructural ORFs make up the genome.[8,11] SARS-CoV-2 has highly conserved ORF-1ab, E, and N genes. The ORF-1ab-located RNA-dependent RNA polymerase (RdRp; nsp12) plays a critical function in RNA synthesis and is prone to frequent mutation and recombination.[9,12,13] As a result, the conserved domains (ORF-1ab, E, and N genes) are frequently selected as standard target genes for molecular detection of SARS-CoV-2. ARS-CoV-2 has a 3% case fatality rate, compared to 10% and 34.5 percent for SARS-CoV and MERS-CoV, respectively. However, the fast-increasing number of cases is a reason for concern.[14,15] The management of any emerging infectious illness requires timely and accurate laboratory examination of samples from cases under investigation. Because there are presently no effective antiviral medicines or vaccines against SARS-CoV-2, early diagnosis, isolation, and supportive care are essential for the prevention of COVID-19. Molecular methods have been used to identify any pathogens for several years successfully. Although, due to negative results in certain suspected cases of the disease based on their clinical presentation and exposure history, the sensitivity and reliability of polymerase chain reaction (PCR) have been questioned. The gold standard for diagnosing SARS-CoV-2 is currently reverse transcription-polymerase chain reaction (RT-PCR) detection of viral nucleic acid. In the same sample, the sensitivity patterns of the N and ORF-1ab genes are varied. The sensitivity pattern of the N gene was found to be roughly 10 times greater than that of the ORF-1ab gene, according to Chu *et al.*[16] As a result, the current research studied the demographic features and sensitivity pattern of the N and ORF-1ab genes in SARS-CoV-2 infected cases.

MATERIALS AND METHODS

This cross-sectional study was done from June 2020 to August 2020. Samples were collected from all suspected cases of COVID-19 patients attending the outdoor & indoor at Aichi Hospital, Uttara, and Japan East-West Medical College Hospital, Turag, Dhaka. A total of 3183 suspected cases of COVID-19 patients were enrolled in this study after taking consent.[17] The nucleic acid of the SARS-CoV-2 virus was detected from nasopharyngeal swabs collected in viral transport media. The work was carried out in Aichi Hospital's PCR laboratory.

Nucleic acid detection: Sansure Biotech Novel Coronavirus (2019-nCoV) Nucleic Acid Diagnostic Kit was used to detect ORF-1ab and N genes of SARS-CoV-2 using rRT-PCR (real-time reverse transcription polymerase chain reaction). The viral RNA was firstly extracted using the releasing reagent that came with the PCR kit. A 200 µL specimen was pipetted into a 1.5 ml Eppendorf tube, centrifuged for 5 minutes at 12,000 rpm, and the supernatant fluid was carefully discarded, leaving the precipitation at the bottom. After that, 50 µL of Sample Release Reagent was added to each tube and vortexed for 5 seconds. Then, in a PCR reaction tube, 30 µL of PCR-Mastermix was mixed with 20 µL of processed material, and amplification was carried out. All the procedures were conducted inside the Biosafety cabinet II. A typical S-shape amplification curve or Ct ≤ 40 of the N and/or ORF-1ab genes were considered positive. If there was no characteristic S-shape amplification curve or Ct ≤ 40 of N and/or ORF-1ab gene and Ct value ≤ 40 or no amplification curve for internal control, the test was considered negative. If there was no characteristic S-shape amplification curve or Ct > 40 of N, ORF-1ab, and the internal control gene, the result was deemed invalid. The data was analyzed using SPSS. A descriptive analysis of all relevant variables was done by using proportion, central tendency, and dispersion.

RESULTS

In this study, a total of 3183 suspected COVID-19 cases were included. There were 2415 (75.9%) males and 768 (24.1%) females. Their mean age at study entry was 36.94 ± 14.015 years (mean ± SD), ranging from 2 – 96 years (Table I).

Table I: Distribution of study population according to their sex and age.

Study population			
Sex (n= 3183) No. (%)		Age (year)	
Male	**Female**	**Mean ± SD**	**Age range**
2415 (75.9)	768 (24.1)	36.94 ± 14.015	2 - 96

Among the cases, 649 (20.4%) were positive for SARS-CoV-2 and 2534 (79.6%) were negative for SARS-CoV-2 (Figure 1).

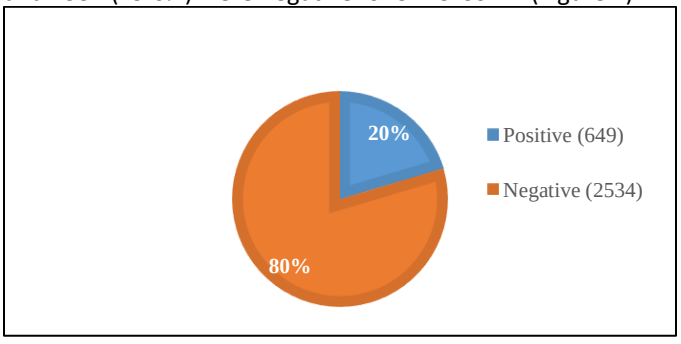

Figure 1: Distribution of study population according to their SARS-CoV-2 rRT-PCR result (n = 3183)

There had been 478 (73.7%) males and 171 (26.3%) females among the positive cases. Their mean age at study entry was

40.35 ± 14.551 years (mean ± SD), ranging from 4 – 90 years (Table II).

Table II: Distribution of study population positive for SARS-CoV-2 according to their sex and age.

Study population			
Sex (n= 649) No. (%)		Age (year)	
Male	Female	Mean ± SD	Age range
478 (73.7)	171 (26.3)	40.35 ± 14.55	4 - 90

The highest frequency of SARS-CoV-2 positivity was found in the 21 to 40 year age group (50.7%), followed by 41 to 60 years (33.1%), 61 to 80 years (9.4%), ≤20 years (5.8%) and ≥81 years (0.9%). (Figure 2).

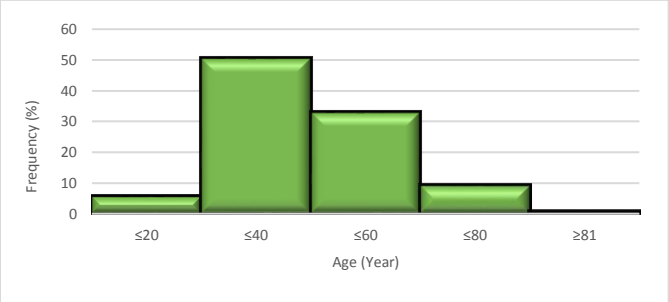

Figure 2: Covid-19 infection rates in various age categories among SARS-CoV-2 positive individuals (n= 649).

The frequency of positivity for SARS-CoV-2 was high in July among the 3 months (Figure 3).

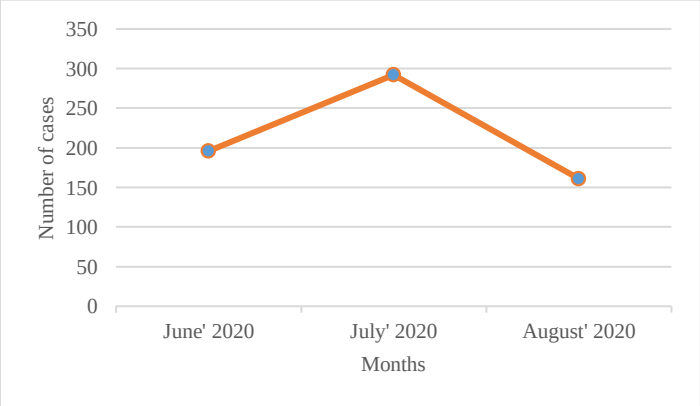

Figure 3: Month-by-month distribution of SARS-CoV-2 positive patients (n= 649).

SARS-CoV-2's N gene was found in all cases positive for SARS-CoV-2, while ORF-1ab was found in 143 (22.03%) patients. (Table III).

Table III: N and ORF-1ab gene frequency in SARS-CoV-2 positive patients (n= 649).

Gene	Positive No. (%)	Negative No. (%)
N gene	649 (100%)	00 (0%)
ORF-1ab	143 (22.03%)	506 (77.9%)

The mean Ct value of the N and ORF-1ab genes was 27.84 4.61 and 37.00 3.35, respectively, among the cases positive for both genes. Among them, the lowest and highest CT values of N were 17.59 and 39.69, respectively. The lowest and highest Ct values of ORF-1ab were 21.26 and 43.06, respectively. (Table IV).

Table IV: The mean Ct value of the N and ORF-1ab genes in cases that tested positive for both genes (n = 143).

Gene	Lowest Ct value	Highest Ct value	Mean ± SD
N	17.59	39.69	27.84 ± 4.61
ORF-1ab	21.26	43.06	37.00 ± 3.35

DISCUSSION

SARS-CoV-2, a novel coronavirus, was discovered in Wuhan, China, in late 2019. Since then the virus gradually spread all over the world. As the definite treatment and vaccine against SARS-CoV-2 are not still available, an early and definite laboratory diagnosis is necessary so that the spreading of infection can be reduced by isolating the infected person.

In this study, we found 649 (20.4%) cases positive for SARS-CoV-2 among 3183 suspected cases. This finding is almost identical to Biswas *et al.*'s as well as our national statistics.[18] But in another study done in Bangladesh, the rate of positivity is higher than in our study.[19] This finding is also much lower than the study done by Rui *et al.*[20] In this study, most of the suspected cases were males (75.9%) and also SARS-CoV-2 positive cases (73.7%). This finding is very much closer to the study done by Akram *et al.* (71%), Italy (60%), and the United States (63%), but more than the other studies done in Bangladesh and China.[18,19,21-25] Male predominance is concerning because male predominance has been linked to an increased risk of mortality.[26]

SARS-CoV-2 positive individuals had a mean age of 40.35 ± 14.551 years, while suspected cases had a mean age of 36.94 ± 14.015 years. The lowest and highest age was found to be positive for SARS-CoV-2 was 4 years and 90 years, respectively. This observation is similar to those of others.[18,19,27] The majority of positive cases (50.7%) were detected in the age category of 21 to 40 years, followed by 41 to 60 years (33.1%). This finding is in agreement with the findings of other studies.[18,19,27,28] But in studies in China and Italy, the frequency of positivity was found to be high in the age group of 41 to 70 years.[21,22,24,27] Most affected cases within the age group 21 to 40 may be due to the youth age group in Bangladesh is more

than elderly people.[29] This study included data only for 3 months, June to August. Among 3 months the highest cases with positive PCR test was found in July.

The N and ORF-1ab genes of SARS-CoV-2 were detected in this study. In all of the positive cases, the N gene was found. But the positivity of ORF-1ab is only 22.03%. Some other researchers also found almost similar findings. In their research, Chu *et al.* discovered that the N gene assay is about 10 times more sensitive than the ORF-1ab, while Liu *et al.* discovered that the ORF-1ab positivity rate was about 62.5 %.[16, 30] The mean Ct value of the N and ORF-1ab genes was 27.84 ± 4.61 and 37.00 ± 3.35, respectively, among the cases positive for both genes. In most cases, the N gene's Ct value is lower than that of the ORF-1ab gene in the same sample. It signifies that the N gene has a higher sensitivity and amplification efficiency than ORF-1ab. It is comparable to the findings of Liu *et al*. study's[30]. It could be because samples containing infected cells express subgenomic mRNA, resulting in more N gene copies, are being tested for SARS-CoV-2.[31] Again the low positivity of SARS-CoV-2 in this study may be related directly to the quality of the collected sample. As a result, other genes must be detected to confirm clinically suspected cases. Clinical parameters, additional laboratory findings, comorbidities, and mortality status were not included in this study. As the most positive cases found within the youth group, another study including those parameters is needed. As this is qualitative PCR, we cannot detect the viral load. However, research into the relationship between viral load and affected people's morbidity and mortality is required.

Conclusions

Emerging and reemerging infectious illnesses are easily transferred across the world as a result of global migration. In any outbreak, it is very much important to know the causative agent and source of infection. In this case, laboratory testing is quite important. Until and unless additional diagnostic procedures for SARS-CoV-2 are approved, rRT-PCR remains the gold standard. The low positive result of SARS-CoV-2 and the variation of sensitivity and amplification efficiency in this study raise the need to test other samples, i.e., urine and stool, for clinically suspected cases because the virus is shaded for a significantly longer time than respiratory samples to avoid a false-negative result. As the most positivity was found among the male and young generation, it is an alarming situation because they are the most mobilized person in the family. So further study is required to know whether these young people play the role of the source of infection to their other family members.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES:

1. World Health Organization (WHO). Coronavirus. Geneva: WHO; 2020. Available from: https://www.who.int/health-topics/coronavirus.
2. WHO Coronavirus Disease (COVID-19). WHO; 2020. Available from: https://covid19.who.int.
3. World Health Organization (WHO). Coronavirus. Geneva: WHO; 2020. Available from: WHO | World Health Organization
4. World Health Organization (WHO). Coronavirus. Geneva: WHO; 2020. Available from: https://covid19.who.int/region/searo/country/bd
5. Lu R, Zhao X, Li J, Nilu P, Yang B, Wu H, et al. Genomic characterization and epidemiology of 2019 novel coronavirus: implications of virus origins and receptor binding. Lancet. 2020; 395: 565-74. https://doi.org/10.1016/S0140-6736(20)30251-8
6. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, et al. A novel coronavirus from patients with pneumonia in China, 2019. The New England Journal of Medicine. 2020; 382: 727-33. https://doi.org/10.1056/NEJMoa2001017 PMid:31978945 PMCid:PMC7092803
7. Dandan Li, Jiawei Z, Jinming Li. Primer design for quantitative real-time PCR for the emerging Coronavirus SARS-CoV-2. Theranostics. 2020; 10: 7150-62. https://doi.org/10.7150/thno.47649 PMid:32641984 PMCid:PMC7330846
8. Cui J, Li F, Shi ZL. Origin and evolution of pathogenic coronaviruses. Nature Reviews Microbiology. 2019; 17: 181-92. https://doi.org/10.1038/s41579-018-0118-9 PMid:30531947 PMCid:PMC7097006
9. Su S, Wong G, Shi W, Liu J, Lai ACK, Zhou J, et al. Epidemiology, genetic recombination, and pathogenesis of coronaviruses. Trends in Microbiology. 2016; 24: 490-502. https://doi.org/10.1016/j.tim.2016.03.003 PMid:27012512 PMCid:PMC712551
10. Chen L, Liu W, Zhang Q, Xu K, Ye G, Wu W et al. RNA based mNGS approach identifies a novel human coronavirus from two individual pneumonia cases in 2019 Wuhan outbreak. Emerging Microbes and Infections. 2020; 9: 313-9. https://doi.org/10.1080/22221751.2020.1725399 PMid:32020836 PMCid:PMC7033720
11. Shi ZL, Guo D, Rottier PJ. Coronavirus: epidemiology, genome replication and the interactions with their hosts. Virologica Sinica. 2016; 3: 1-2. https://doi.org/10.1007/s12250-016-3746-0 PMid:26908210 PMCid:PMC7091033
12. Shannon A, Le NTT, Selisko B, Eydoux C, Alvarez K, Guillemot JC, et al. Remdesivir and SARS-CoV-2: Structural requirements at both nsp12 RdRp and nsp14 Exonuclease active-sites. Antiviral Research. 2020; 178. https://doi.org/10.1016/j.antiviral.2020.104793 PMid:32283108 PMCid:PMC7151495
13. Elfiky AA. Ribavirin, Remdesivir, Sofosbuvir, Galidesivir, and Tenofovir against SARS-CoV-2 RNA dependent RNA polymerase (RdRp): A molecular docking study. Life Sciences. 2020; 253: 117592. https://doi.org/10.1016/j.lfs.2020.117592 PMid:32222463 PMCid:PMC7102646

14. Otto MA. Wuhan virus: What clinicians need to know. Medscape Medical News. 2020, (cited 2020 Jan 27). Available from: https://www.medscape.com/viewarticle/924268.
15. Changtai W, Zhongping L, Zixiang C, Xin H, Xu M, He T and Zhang Z. The establishment of reference sequence forSARS-CoV-2 and variation analysis. Journal of Medical Virology. 2020; 92: 667-74. https://doi.org/10.1002/jmv.25762 PMid:32167180 PMCid:PMC7228400
16. Chu DKW, Pan Y, Cheng SMS, Hui KPY, Krishnan P, Liu Y, et al. Molecular diagnosis of a novel coronavirus (2019-nCoV) causing an outbreak of pneumonia. Clinical Chemistry. 2020; 66: 549-55. https://doi.org/10.1093/clinchem/hvaa029 PMid:32031583 PMCid:PMC7108203
17. National Guidelines on Clinical Management of Coronavirus Disease 2019 (Covid-19). DGHS. 2020. Available from: https://hsd.gov.bd/sites/default/files/files/hsd.portal.gov.bd/go_ultimate/d5b4c82f_749a_4462_bbe3_60e8d33875ec/COVID_Guideline_V4.30.3.2020.pdf.
18. Biswas S, Azad KAK, Hoque MM, Mowla SGM, Banu SS, Sharmin R and Shamzuzzaman SM. Real Time-PCR (RT-PCR) positivity for SARS-CoV-2 in suspected COVID-19 patients presented in a tertiary care hospital. Journal of Bangladesh College of Physicians and Surgeons. 2020; 38: 67-70. https://doi.org/10.3329/jbcps.v38i0.47439
19. Akram A, Yeasmin M, Khan THMEU, Monir MBB, Tarana MN, Arina S, et al. Demographic Characteristics of 1082 Positive Cases of COVID-19 Patients: Experience at National Referral Laboratory of Bangladesh. Bangladesh Journal of Infectious Disease. 2020; 7: S22-6. https://doi.org/10.3329/bjid.v7i00.50158
20. Rui Liu, Huan Han, fang Liu, Zhihua, Lv, Kailang Wu, et al. Positive rate of RT-PCR detection of SARS COV-2 infection in 4880 cases from one hospital in Wuhan, China from Jan to Feb 2020. Clinica Chimica Acta. 2020; 505: 172-5. https://doi.org/10.1016/j.cca.2020.03.009 PMid:32156607 PMCid:PMC7094385
21. Li Q, Guan X, Wu P, Wang X, Zhou L, Tong Y, et al. Early transmission dynamics in Wuhan, China of Novel Coronavirus- infected pneumonia. The New England Journal of Medicine. 2020; 382: 1199-207. https://doi.org/10.1056/NEJMoa2001316 PMid:31995857 PMCid:PMC7121484
22. Ai T, Yang Z, Hou H, Zhan C, Chen C, Lv W, et al. Correlation of Chest CT and RT-PCR Testing in Coronavirus Disease 2019 (COVID-19) in China: A report of 1014 cases. Radiology. 2020; 296: E32-E40. https://doi.org/10.1148/radiol.2020200642 PMid:32101510 PMCid:PMC7233399
23. Zhang JJ, Dong X, Cao YY, Yuan YD, Yang YB, Yan YQ, Akdis CA and Gao YD. Clinical Characteristics of 140 patients infected by SARS COV-2 in Wuhan, China. Allergy. 2020; 75: 1730-41. https://doi.org/10.1111/all.14238 PMid:32077115
24. Grasselli G, Zangrillo A, Zanella A, Antonelli M, Cabrini L, Castelli A, et al. Baseline characteristics and outcomes of 1591 patients infected with SARS-CoV-2 admitted to ICUs of the Lombardy Region, Italy. Jama. 2020; 323: 1574-81. https://doi.org/10.1001/jama.2020.5394 PMid:32250385 PMCid:PMC7136855
25. Bhatraju PK, Ghassemieh BJ, Nichols M, Kim R, Jerome KR, Nalla AK, et al. Covid-19 in critically ill patients in the Seattle region-case series. New England Journal of Medicine. 2020; 382: 2012-22. https://doi.org/10.1056/NEJMoa2004500 PMid:32227758 PMCid:PMC7143164
26. Li X, Xu S, Yu M, Wang K, Tao Y, Zhou Y, et al. Risk factors for severity and mortality in adult COVID-19 inpatients in Wuhan. 2020; 146(1): 110-118. https://doi.org/10.1016/j.jaci.2020.04.006 PMid:32294485 PMCid:PMC7152876
27. Guan WJ, Ni ZY, Hu Y, Liang WH, Ou CQ, He JX, et al., Clinical characteristics of coronavirus disease 2019 in China. New England Journal of The Journal of Allergy and Clinical Immunology medicine. 2020; 382: 1708-20. https://doi.org/10.1056/NEJMoa2002032 PMid:32109013 PMCid:PMC7092819
28. 54% Covid-19 cases in 18-44 age group, 51% deaths among those over 60 years: Health ministry. Available at: https://timesofindia.indiatimes.com/india/54-covid-19-cases-in-18-44-age-group-51-deaths-among-those-over-60-years-health-ministry/articleshow/77883146.cms.
29. Population monograph of Bangladesh. 2015. Available at: http://203.112.218.65:8008/WebTestApplication/userfiles/Image/PopMonographs/Volume-9_Age-Sex.pdf.
30. Liu Y, Wang Y, Wang X, Xiao Y, Chen L, Guo L, et al. Development of two TaqMan real-time reverse transcription-PCR assays for the detection of severe acute respiratory syndrome coronavirus-2. Biosafety and Health. 2020; BSHEAL-00064: 1-6. https://doi.org/10.1016/j.bsheal.2020.07.009 PMid:32838286 PMCid:PMC7375968
31. Simons FA, Vennema H, Rofina JE, Pol JM, Horzinek M, Rottier P and Egberink HF. A mRNA PCR for the diagnosis of feline infectious peritonitis. Journal of Virological Methods. 2005; 124: 111-6. https://doi.org/10.1016/j.jviromet.2004.11.012 PMid:15664058 PMCid:PMC7112896

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