Research article

SARS-CoV-2 molecular identification and clinical data analysis of associated risk factors from a COVID-19 testing laboratory of a coastal region in Bangladesh

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ABSTRACT

Background and aim: Outbreak of COVID-19 seems to have exacerbated across the globe, including Bangladesh. Scientific literature on the clinical data record of COVID-19 patients in Bangladesh is inadequate. Our study analyzes the clinical data of COVID-19 positive patients based on molecular identification and risk factor correlated with three variables (age, sex, residence) and COVID-19 prevalence in the four districts of Chattogram Division (Noakhali, Feni, Lakshmipur and Chandpur) with an aim to understand the trajectory of this pandemic in Chattogram, Southern Bangladesh.
Methods: A cross-sectional study is conducted in the context of RT-PCR-based COVID-19 positive 5,589 individuals diagnosed with SARS-CoV-2 infection from the COVID-19 testing laboratory, Abdul Malek Ukil Medical College, Noakhali-3800, Bangladesh. For molecular confirmation of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), standard diagnostic protocols through real-time reverse transcriptase-polymerase chain reaction (RT-qPCR) were conducted. Different patient demographics were analyzed using SPSS version 22 for exploring the relationship of three factors – age, sex, and residence with a cumulative number of COVID-19 positive cases and prevalence of COVID-19 in four districts in Chattogram division. The data was recorded between May to July, 2020.
Results: Among the three parameters, the present study revealed that 20–40 cohort had the highest incidence of infection rate (51.80%, n = 2895) among the different age groups. Among the infected individuals, 56.8% (n = 3177) were male and 43.2% (n = 2412) were female, denoting males being the most susceptible to this disease. Urban residents (52.7%, n = 2948) were more vulnerable to SARS-CoV-2 infection than those residing in rural areas (47.3%, n = 2641). The prevalence of COVID-19 positive cases among the four districts was recorded highest in the Noakhali district with 36.8% (n = 2057), followed by the Feni, Lakshmipur and Chandpur districts with 25.9% (n = 1448), 20.8% (n = 1163) and 16.5% (n = 921), respectively.
Conclusions: This study presents a statistical correlation of certain factors linked to Bangladesh with confirmed COVID-19 patients, which will enable health practitioners and policy makers to take proactive steps to control and mitigate disease transmission.

1. Introduction

A number of pneumonia cases of suspected viral origin were found in Wuhan city, Hubei, China by December 2019 [1]. This disease was identified first as novel coronavirus (2019-nCoV Disease) which was later renamed as the Corona virus disease 2019 (COVID-19) by WHO under a global agreement [2]. Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) has been responsible for the underlying pathogenesis of COVID-19, a contagious disease of the respiratory system. Coronaviruses are enveloped single-stranded virus, zoonotic in nature belonging to *Coronaviridae* family [3]. In addition to other substantial coronavirus properties, the rapid transmissibility rate and the comorbidity mortality

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rate have made SARS-CoV-2 more deadly than other coronaviruses formerly evolved, including SARS-CoV and MERS-CoV. While infected people can spread SARS-CoV-2 by respiratory droplets, spread from non-symptomatic patients makes the virus even more terrifying [4].

Although Wuhan city was the onset of the virus in China, it is wreaking havoc all over the world. Recent studies indicate that the clinical range of COVID-19 can vary across the globe between various ethnicities and geographical locations [5]. Bangladesh announced the first COVID-19 confirmed case three days earlier than the WHO's global announcement of COVID 19 as 'Pandemic' on 11 March 2020 [6]. While certain precautions were initially taken, such as wearing a mask, keeping social distances, preventing gatherings were strictly enforced, but now that the prevalence of this disease has been diminishing for some time, they have led to a decrease in restrictions [7]. But now the situation is becoming more challenging due to unconsciousness and a second wave strike [8]. The unforeseeable implications and complexities concerning public safety, and uncertainty about COVID-19, social distances, isolation and quarantine, as well as economic impacts, may also activate individuals' psychological facilitators. Effects on people (e.g., uncertainty, anxiety, isolation, social pressure) and communities (e.g., educational institutions and occupational suspensions, economic fallout, and insufficient or disorderly response to healthcare issues) may trigger a broad range of psychological outcomes, including emotional responses and the emergence of psychological illness in extreme situations [9]. Depression, anxiety or stress may accompany everyday circumstances involving sensory stimulations which may disturb daily tasks, substantially impair the processing of everyday life and reduce involvement in different life situations [10]. A recent study showed, for the first time, that in Bangladesh, suicidal behavior was identified during COVID-19 was 6.1% of n = 206 individuals [11]. Affective temperaments can be regarded as a significant suicide risk indicator [12]. Individuals that have been substantially impaired by the COVID-19 pandemic may encounter consistent difficulties in the processing of sensory stimuli that have been significantly correlated with higher depression, impulsivity, and anxiety [10].

Assessment of the pandemic condition on the basis of various variables is essential to understand the existence, severity, geographical pattern of spread, possible risk factors, susceptibility and risk levels, and hotspots of transmission. As Bangladesh is a densely populated country with 1265 people per square km having 60% people between 15-64 ages with a male female sex ratio of 1.04 [13], it is necessary to gain a precise understanding of transmission dynamics of COVID-19 in different geographic regions in the country.

However, Chattogram (formerly known as Chittagong) is the largest division of Bangladesh, located in its southern part, where the largest sea port is situated next to the city [14]. The flatland regions of this division are more densely populated than the hilly and remote areas. Noakhali, Feni, Lakshmipur and Chandpur districts are known as flatland according to geographical position [14]. However, both areas have distinct demographic, socio-economic and cultural characteristics. In addition, differences in these traits have a major role on the epidemiology of SARS CoV-2 by impacting the population. For economic and commercial reasons, various groups of citizens from various places come together in this division owing to their regional location and socio-economic background and are engaged in unrestricted movements. Thus, this location is considered as a highly infectious zone for contact transmission of SARS-CoV-2 [15]. The first three cases of COVID-19 were detected in Chattogram on 23 March 2020. According to the WHO report, November 2020, the Chattogram Division had the 2nd highest infection rate of COVID-19 in Bangladesh, with a linear rise in reported cases in all 11 (eleven) districts of the Chattogram Division [16].

In the light of all the evidence, including the geographical location, demography and population characteristics of the Chattogram division, it is necessary to classify the infectious trends and the various factors relevant to the outbreak of COVID-19. This study aims to perform an epidemiological analysis on COVID-19 positive cases focusing on molecular detection and risk factor to investigate the specific parameters (age, sex and residence) responsible for aggravating the COVID-19 cases and comparative analysis of the prevalence of COVID-19 among the four districts of Chattogram (Noakhali, Feni, Lakshmipur and Chandpur) as a pioneering approach for the epidemiological characterization of COVID-19 individuals tested in Abdul Malek Ukil Medical College Noakhali, Chattogram division, Bangladesh, the results of which will aid in further decision making in terms of containing and managing the spread of the disease as well as deploying prevention methods and to understand the trajectory of this pandemic in four districts of Chattogram, Southern Bangladesh.

2. Methods

2.1. Study area

The current study was conducted in the COVID-19 diagnostic laboratory at the Department of Microbiology of Abdul Malek Ukil Medical College, Noakhali-3800, Chattogram division, Bangladesh. However, samples from four districts of Chattogram were included in the present study. Apart from Noakhali, samples from Feni, Lakshmipur and Chandpur districts were also tested at the COVID-19 diagnostic laboratory of Abdul Malek Ukil Medical College.

2.2. Study sample size and period

In this study, a total of 5,589 individuals with confirmed COVID-19 positive cases tested at the COVID-19 diagnostic laboratory of Abdul Malek Ukil Medical College, Noakhali-3800, Bangladesh, were included as the target population in this study. The data was recorded between May to July 2020.

2.3. Sample collection

The authorities of reported health complexes collected nasopharyngeal and oropharyngeal swabs from the suspected individuals by following the WHO guidelines [17], where two separate swab samples were preserved in normal saline containing collection tubes. Immediately after collection of samples, proper cold chain was preserved and sent to the PCR lab at the Department of Microbiology of Abdul Malek Ukil Medical College, Noakhali-3800, Chattogram division, Bangladesh which is specialized as the COVID-19 diagnostic laboratory and then the samples were processed via real-time RT-PCR, a laboratory technique for *in-vitro* qualitative detection of COVID-19, according to the WHO interim guidance [17]. Among the positive cases of SARS-CoV-2, patient demographic and clinical statistics have been compiled by experienced physicians and individual patient data have also been submitted to the laboratory for more information of epidemiologic studies.

2.4. RNA extraction and PCR reaction

In compliance with the manufactory instructions given in the kit, Viral RNA was extracted from the patient's derived specimens using a SanSure Biotech Sample Release reagent (SanSure Biotech, China), RNA fast-releasing technology. 200 μL of the sample was pipetted into a 1.5 mL EP (Eppendorf) tube, centrifuged at 12,000 rpm for 5 min, and then the supernatant fluid was carefully discarded, preventing accumulation in the rim. A sample reagent of 50 μL was then added to each tube and vortexed for 5 s. The lysed sample was added directly to the rRT-PCR reaction. According to kit manual, 20 μL lysed sample was added in 0.2 mL PCR tube as template followed by adding 30 μL master mix. Here, master mix constitutes 26 μL PCR mix [Premiers (4.62%), Probes (1.15%), dNTPs(3.85%), $MgCl_2$(0.77%), Rnasin (0.48%), PCR buffer (89.13%)] and 4 μL Enzyme mix [RT Enzyme (62.5%), Taq Enzyme (37.5%)]. The final PCR reaction volume is 50 μL.

2.5. RT-qPCR analysis

A real-time RT-PCR method detected the presence of SARS-CoV-2 in the respiratory specimens. RNAs were analyzed for SARS-CoV-2 identification by RT-PCR (Quant Studio 5, Thermo Fisher Scientific) using the Sansure RT-PCR kit (Sansure Biotech Inc., China). As described in the product manual, technical methods and interpretations of the findings have been evaluated. PCR qualitative identification of the N gene (ROX channel) and ORF1ab region (FAM channel) of SARS-CoV-2 was identified with Novel Coronavirus (2019-nCoV) Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing) from SanSure biotech. The human RNA gene (CY5 channel) had been used as an internal control to regulate inhibition of PCR. Real-time RT-PCR was run on Quant Studio 5, Applied Bio-Systems by Thermo Fisher Scientific PCR system. After RT-qPCR, the result was analyzed using Quant Studio design and analysis software version 1.5.1. One positive control and one negative control were both carried out as quality monitoring steps to verify the detecting process.

2.6. Data analysis

All field derived data, including sex, age, and residence were recorded into a Microsoft Excel 2010 spreadsheet and sorted for a better presentation of outcomes. The sheet was cleaned, assembled and imported into the statistical software for social science (SPSS) 22 (SPSS Inc, Chicago, IL, USA) for final analysis. Clinical data were expressed as mean, median, mode, SD and variation in SPSS version 22. Pearson Chi-square test was performed to find out the association of the binary result of SARS-CoV-2 with the patient's factors. P values of less than or equal to 0.05 (two-sided) were considered statistically significant. Descriptive statistics such as frequency and percentage w used. Tables 1, 2, and 3 were used to describe the sample characteristics.

2.7. Ethical approval and informed consent

Prior to sample collection, verbal permission from every suspected individual was taken, and subsequently two swab samples were collected with minimum discomfort of patients to validate COVID-19. Then, patient demographics and clinical statistics were accumulated among the positive cases of SARS-CoV-2. Finally, consent from the Principal of Abdul Malek Ukil Medical College was taken to conduct the current study.

3. Results

The prevalence of circulating SARS-CoV-2 positive cases in different age groups among the 5,589 COVID-19 positive individuals tested in the COVID-19 diagnostic laboratory of Abdul Malek Ukil Medical College, Noakhali, Chattogram division, Bangladesh, was 51.80% (n = 2895) and 23.03% (n = 1287) in the age groups of (21–40) years and (41–60) years, respectively, which were higher than other age groups (Figure 1 and Table 4). Overall mean age was 36.42 and standard deviation of age was 16.593 (Table 1).

In case of gender, the prevalence was 56.8% (n = 3177) in males and 43.2% (n = 2412) in females showing males had an increase of 13.6% positive case than females (Figure 2).

Between the urban and rural areas, the patient demographic showed distinct consequences for the positive cases of COVID-19. Covid-19 is extremely infectious, so population density has a significant influence on its dissemination. Among the significant variables, people living in urban area are at more risk of having SARS-CoV-2 infection, showing 52.7% (n = 2948) of prevalence than the rural area population with 47.3% (n = 2641) prevalence of SARS-CoV-2 infection (Figure 3).

The prevalence of circulating SARS-CoV-2 positive cases in four districts of Chattogram division among the 5,589 COVID-19 positive individuals tested in the COVID-19 diagnostic laboratory of Abdul Malek Ukil Medical College was 36.8% (n = 2057) recorded in Noakhali,

denoting the highest prevalence of COVID-19 positive individuals among the four districts considered in this study, followed by Feni, Lakshmipur and Chandpur with 25.9% (n = 1448), 20.8% (n = 1163), 16.5% (n = 921) prevalence, respectively (Table 5 and Figure 4).

4. Discussion

In the current study, we investigated 5,589 COVID-19 positive patients diagnosed through RT-PCR in Abdul Malek Ukil Medical College, Noakhali-3800, Chattogram division, Bangladesh. Our study has revealed that among the different age groups, the 20–40 cohort showed the highest prone to infection rate (51.80%), followed by 41–60 age groups (23.03%) and the least infection rate (3.63%) is recorded to the individuals who were above 80 age groups. Same range was denoted by Mowla et al., (2020) [18]. The distribution of age of patients from this study reveals that middle-aged people are as likely to be infected as adults and more attractive to the virus. However, some studies suggest that their death risk is low as they function as carriers [6, 7]. While all age ranges are at risk of contracting COVID-19, older individuals are at significant risk of experiencing serious illness as a result of physiological changes arising from aging and possible underlying health conditions [19]. Bangladesh has a median age of 27.6 years, and the young and middle-aged in Bangladesh come mostly under the working class and are more likely to be forced to leave home for jobs and thus have a higher risk of being infected [20]. Our findings are also consistent with early reports [21, 22] resulting that younger people are less likely to be affected, supported by several studies. Another study [23] in Bangladesh has established a strong association with the outcome of COVID-19 risk factor of age, which is in harmony with our research findings. All these results indicate realistic measures to secure and limit transmission and to suppress progression of SARS-CoV-2 in vulnerable populations.

Our study also reveals that the prevalence of COVID-19 was 56.8% (n = 3177) in males and 43.2% (n = 2412) in females showing males had an increase of 13.6% positive case than females, which is in support with the outcomes of several studies including Wuhan, Italy, Oman and USA in which men are mostly amenable to COVID-19 [24, 25, 26, 27]. According to Zhu et al., (2020) men could be most likely at the onset of the outbreak to be exposed to the virus, for social or cultural reasons, because of the fact that women's reduction in susceptibility to viral infections may benefit from the conservation of X chromosomes and sex hormones that are of significant importance to immunity [28]. The current findings, as far as infectivity is concerned, indicates that men are more vulnerable than women to COVID-19. Additional findings indicate that men have a higher prevalence, including 56.3%, 58.1% and 67% respectively in New York and China [1, 28, 29]. The sexual variability in infection rates may be due to disparities in physiological parameters such as the expression of weak virus receptors, metabolic distinctions and different action habits in males and females. Related results from both Cleveland Clinics in Ohio and Florida indicated that males were at higher risk of being positive for COVID-19 [29]. Although most studies have reported higher prevalence in males, some studies have also shown roughly similar sex distributions [24, 30].

The research unveils the geographical difference of SARS-CoV-2 infection rates in urban and rural areas in four districts (Noakhali, Feni, Lakshmipur, Chandpur) of the Chattogram division from which samples were obtained and COVID-19 was diagnosed at Abdul Malek

Table 1. Crosstab value of age in COVID-19 positive individuals.

Variables	Age
Mean	36.42
Median	35
Mode	30
Std. deviation	16.593
Variance	275.325

Table 2. Crosstab between Residence and result.

Chi square Tests	Value	df	Asymptotic Significance (2 sided)
Pearson Chi-square	4571.813[a]	44	.000
Likelihood Ratio	4583.804	44	.000
N of Valid cases	5589		

Table 3. Crosstab between residence and referred institution.

Chi square Tests	Value	df	Asymptotic Significance (2 sided)
Pearson Chi-square	6330.328[a]	294	.000
Likelihood Ratio	6598.723	294	.000
N of Valid cases	5589		
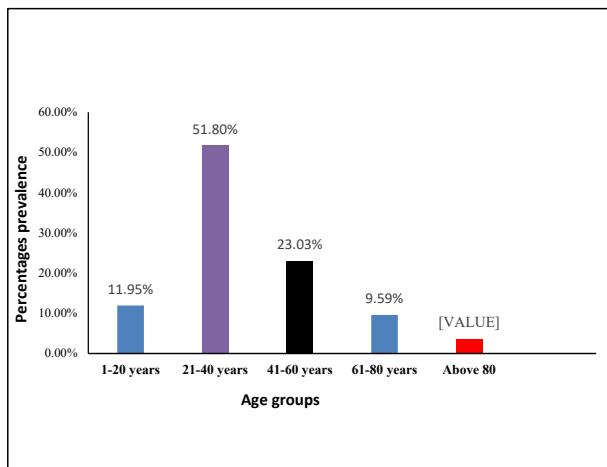
Figure 1. Prevalence of COVID-19 in different age groups.

Figure 2. Prevalence of COVID-19 by sex.

Ukil Medical College, Noakhali-3800, Chattogram, Bangladesh. The urban regions had a 52.7% prevalence of SARS-CoV-2, which was 5.4% higher than the rural regions. This distinction may result from livelihood, distinct cultures, that differ from the way of the lifestyle [31]. Owing to the lower population density of the environment, the COVID-19 lifestyle impact is comparatively low in rural areas. Several studies have established same kind of outcome among the infected individuals living in urban and rural areas which is in harmony with our findings [32, 33, 34].

The study also unveils the geographical variation of the SARS-CoV-2 infection rates in four districts (Noakhali, Feni, Lakshmipur and Chandpur) in the Chattogram division, Southern Bangladesh. In our study, the prevalence of COVID-19 positive cases among the four districts was recorded highest in the Noakhali district with 36.8% (n = 2057), followed by the Feni, Lakshmipur and Chandpur districts with 25.9%
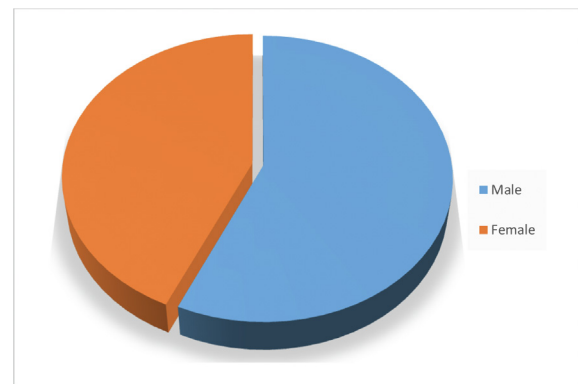
(n = 1448), 20.8% (n = 1163) and 16.5% (n = 921), respectively. The prevalence of COVID-19 in the Noakhali district is almost 2.23% higher than the prevalence in the Chandpur district. Analysis of the pandemic situation on the basis of various variables is essential to understand the existence, severity, regional pattern of spread, possible risk factors, susceptibility and risk levels, and hotspots of infection. Previous studies have shown that environmental, socio-economic and demographic factors are favorably correlated with the occurrence of COVID-19 [35].

SARS-CoV-2 currently reflects a worldwide pandemic since the SARS and MERS epidemic [36]. For the monitoring and preventive strategies of the disease, the prediction of epidemiological patterns is indispensable. After three confirmed cases of COVID-19 first diagnosed in Bangladesh, the government has placed in place protective measures to combat the spread of the deadly COVID-19 strain that has rattled the globe. As the virus is extremely infectious, quick diagnosis is the best way to isolate infected individuals [6, 7, 20]. COVID-19 deaths in Bangladesh, warned by the experts that in view of the growing number of death cases a visible

Table 4. Comparative prevalence of COVID-19 in accordance with the frequency and percentage distribution of different age groups, sex and residence of the COVID-19 positive individuals.

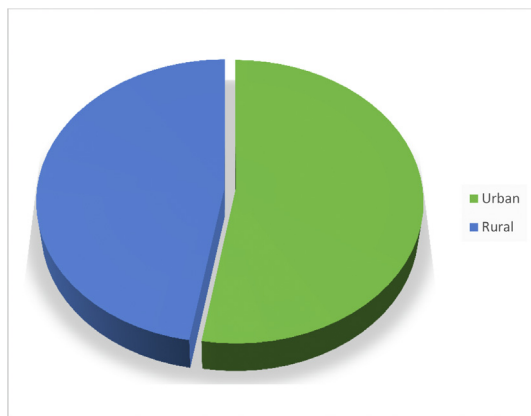| Category	Variables	Frequency (n)	Percentage (%)
Age	1–20 years	668	11.95
	21–40 years	2895	51.80
	41–60 years	1287	23.03
	61–80 years	536	9.59
	Above 80	203	3.63
Sex	Male	3177	56.8
	Female	2412	43.2
Residence	Urban	2948	52.7
	Rural	2641	47.3

Figure 3. Prevalence of COVID-19 by residence.

Table 5. Comparative prevalence of COVID-19 in accordance with the frequency and percentage distribution in four districts of Chattogram division of the COVID-19 positive individuals.

Category	Variables	Frequency (n)	Percentage (%)
Prevalence	Noakhali	2057	36.8
	Feni	1448	25.9
	Lakshmipur	1163	20.8
	Chandpur	921	16.5

scenario could be the tip of an iceberg [6]. Furthermore, in mid-July, a Bangladesh hospital owner was also detained on charges of manufacturing thousands of false negative results of COVID-19, thereby shedding light on the unregulated private sector of the country [37]. False-negative cases have significant repercussions for the containment and risk of infection of infected individuals and for the mitigation of COVID-19.

The COVID-19 crisis is expected to be a long-term phase and just having the correct facts and exercising the prescribed health alerts can help fight against this pandemic. A recent study on knowledge, attitudes and fear of COVID-19 during the rapid increase in Bangladesh reported a high prevalence of self-isolation, positive preventive health behaviors linked to COVID-19, and moderate to high levels of fear among Bangladeshi people [38]. In our view, the current research will shed light on the epidemiological dimensions during the crisis era of COVID-19. Although the research shows facts that correlate to those that were identified globally, such findings need further study and surveillance in aspect of Bangladesh's different geographical dimensions.
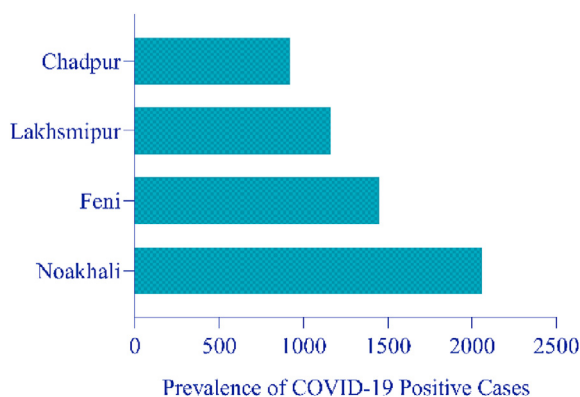

Figure 4. Prevalence of COVID-19 among four districts in Chattogram, Bangladesh.

5. Limitations

This study has some limitations, including the lack of demographic characteristics of patients and it only focuses on confirmed cases excluding negative cases of COVID-19. As we examined the samples of COVID-19 positive individuals diagnosed only at Abdul Malek Ukil Medical College, Noakhali, considering three parameters (Age, Sex, and Residence) for a view of COVID-19 epidemiology, therefore, our findings could not be generalized in the context of Chattogram division, Bangladesh. Another limitation emerges from the focus solely on the three scrutinized variables. Therefore, potentially relevant variables, like education, religiosity, and economic disparity have not been addressed. No clinical aspects (symptomatic or, asymptomatic) were included. The possible aspect of false-negative cases was excluded in the study. A long-term, well-designed epidemiological research would therefore be needed to better understand the dynamics of the pandemic and to relate signs and symptoms, genetic background and history of the patient with COVID-19 infection in Bangladesh.

6. Conclusions

This study analyzes the clinical data report collected from COVID-19 testing laboratory of Abdul Malek Ukil Medical College, Noakhali-3800, Chattogram division, Southern Bangladesh. Our findings reflect that male, middle aged individuals and people living in urban regions are prone to COVID-19 attack. Among four districts (Noakhali, Feni, Lakshmipur and Chandpur) in Chattogram division, Noakhali reported higher prevalence of COVID-19 positive cases than that of others. Since we have very little idea about this virus, this analysis can give a precise understanding of transmission dynamics of circulating virus and undertaking better control measures against COVID-19 outbreak throughout the country, including various districts of Chattogram division. The principal message of this study is that an unequal risk on the most vulnerable people in society is exerting on the interaction of the virus causing COVID-19 and its geographic environment, suggesting that better care resources may need to be allocated to communities to combat COVID-19.

Declarations

Additional information

No additional information is available for this paper.

Acknowledgements

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