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# RT-PCR Mediated Identification of SARS-CoV-2 Patients from Particular Regions of Bangladesh and the Multi-factorial Analysis Considering Their Pre and Post Infection Health Conditions

**Kazi Mahmuda Akter[1], Tanjum Tushi[2], Sabrina Jahan Mily[3], Rashenda Aziz Mohona[4], Samin Anis[5], Adittya Kishor Chakraborty[6], Elmee Tabassum[7], Tahmid Ul Islam[2], Ozifatun Jannat Akhi[8], Israt Sultana Nishe[2], Badrun Nahar Laxy[9], Sanjida Sultana Zerin[2], Abdiqani Jama Roble[4], Md. Imran Hossain[10], Sharmin Ahmed[11] and Salauddin Al Azad[12*]**

*[1]Sir Salimullah Medical College & Mitford Hospital, University of Dhaka, Dhaka, Bangladesh.*
*[2]Department of Sociology, University of Dhaka, Dhaka, Bangladesh.*
*[3]Chittagong Medical College, University of Chittagong, Chattagram, Bangladesh.*
*[4]Department of Public Health, North South University, Dhaka, Bangladesh.*
*[5]Chattagram Maa-O-Shishu Hospital Medical College, University of Chittagong, Chattagram, Bangladesh.*
*[6]Department of Physiotherapy, State College of Health Sciences, University of Dhaka, Dhaka, Bangladesh.*
*[7]Department of Geography and Environment, University of Dhaka, Dhaka, Bangladesh.*
*[8]Department of Computer Science and Engineering, Pabna University of Science and Technology, Pabna, Bangladesh.*
*[9]Department of Economics, Eden Mohila Collage, University of Dhaka, Dhaka, Bangladesh.*
*[10]Marks Dental College, University of Dhaka, Dhaka, Bangladesh.*
*[11]Department of Medicine, Jashore Medical College, Jashore, Bangladesh.*
*[12]Fermentation Engineering Discipline, School of Biotechnology, Jiangnan University, Wuxi, Jiangsu, China.*

***Authors' contributions***

*In this research, all the authors contributed equally and cordially. Author SAA designed the methodology and supervised the project and author SA co-supervised that. Authors KMA, TT and SJM contributed their best in the data collection, curation and manuscript preparation. Author RAM competed writing the public health and nutrition related sections with the help of author AJR. The GIS section was designed and generated with the endless efforts of authors ET and OJA in collaboration. The rest of the authors helped in analyzing the non-clinical issues that can affect any SARS-CoV-2 patient's socio-economic and familial life. The manuscript has revised by each of the authors individually and they all have full consent for publication of the manuscript.*

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**Corresponding author: E-mail: abdullahsyum1992@gmail.com;*

(3) Dr. Chan Yean Yean, Universiti Sains Malaysia, Malaysia.
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## ABSTRACT

**Background:** SARS-CoV-2 is a type of coronavirus shows bizarre features with fatality rates at 34.4%. The clinical and non-clinical features of COVID-19 might be causative factors for more rapid community spreading compared to MERS and SARS-CoV-1.
**Objectives:** To study the efficacy of RT-PCR based detection of SARS-CoV-2 patients. Besides, the patients' pre and post-infection health conditions depending on selective clinical and nonclinical parameters were analyzed.
**Materials and Methods:** Clinical and non- clinical data were collected from 205 randomly selected COVID-19 patients, identified through RT-PCR from different areas of Bangladesh. The data were analyzed using statistical and analytical tools to illustrate the impact of the pandemic situation on the sufferers.
**Results:** Molecular identification of the patients through RT-PCR has been observed as the most efficient option for detecting SARS-CoV-2 patients. Patients with the smoking habit have been reported to be more prone to the COVID-19 infection; surprisingly the non-smoker female was the worse suffers. The patients aged 60-69 years were the worst sufferers with every adverse health conditions mentioned. Professionally, 28% self-employed male and 8.19% female were infected. The most infected individuals were house wife (32.029%), as next to the health care workers (22.1%). With the increased age group, a decreased number of smokers were found. Nebulization of 5% home and 12% hospital treated patients were provided, with Oxygen for 26% of hospitalized patients. After recovery, combined physical and psychological complications were observed in 19% of male and 11% female. The socioeconomic, environmental and geographical annotations exposed a clear relationship with the rate of infection as revealed from the nonclinical data analysis.
**Conclusion:** RT-PCR proved its specialty in COVID-19 detection. In addition, impacts of different clinical and nonclinical factors on the physical conditions of the nCoV patients were found significant in the research.

*Keywords: SARS-CoV-2; RT-PCR mediated identification; Bangladesh; multi-factorial analysis; pre and post infection health conditions.*

## 1. INTRODUCTION

Coronavirus is a positive-sense single-stranded RNA virus of Coronaviridae family. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a new strain of coronavirus that causes coronavirus disease 2019 (COVID-19) [1]. It was identified in January 2020 after the upsurge of a new form of pneumonia in Wuhan city of Hubei province, China, in December, 2019 [2]. Among the different prevalent COVID-19 diagnostic techniques, reverse-transcription polymerase chain reaction (RT-PCR), based on the extraction of RNA from respiratory samples particularly from nasopharyngeal swab (NPS) has been found effective and accurate [3]. Although RT-PCR is affected by several limitations such as comparatively invasive sampling, a time-consuming process, the need for specialized operators and certified laboratories; it has been recommended as gold standard by both the "European Centre for

Disease Prevention and Control" (ECDC) and the "World Health Organization" (WHO) [4]. Ascertaining hospitalization and isolation of patients, RT-PCR assay plays an essential role according to current diagnostic criteria but its inadequate stability, lack of sensitivity and relatively long processing time are harmful to the control of the pandemic situation [5].

COVID-19 has made an enormous socio-economic impact on individuals [6] and challenging socio-economic and livelihood consequences on poor communities in different places of the world [7]. The research revealed that children are less prone to COVID-19 and the mortality rate among them is significantly less than older individuals [8]. Globally the statistics of different hospitals also depict that women have less chance of being infected with respiratory system diseases including acute viral infections than men [9]. COVID-19 is severe among male smokers [10]. As expression of the angiotensin-converting-enzyme 2 receptor (ACE2R) level in human respiratory epithelial cells is increased due to tobacco smoke and SARS-CoV-2 uses a specific viral binding site on the ACE2R to enter into these cells, smokers are more prone to infection [11]. The patients who recovered from COVID-19 may experience significant health effects in the future. About 50% of coronavirus recovered patients with mild symptoms can carry the SARS-CoV-2.

Self-employed males were the main victim of COVID-19 comparing to the other professions of males [12,13]. The patients who recovered from COVID-19 may experience significant health effects in the future. About 50% of coronavirus recovered patients with mild symptoms can carry the SARS-CoV-2. The majority of COVID-19 survivors have been experiencing several health complications, including depression, fear and anxiety [14].

The enormous and multi-source data of COVID-19 have made significant challenges for pandemic mapping. GIS is a powerful tool to present data in an organized way [15] and has already been proved effective in mapping pandemics in USA, China and Israel [16].

To find out the physical, psychological and socioeconomic impact of COVID-19 in Bangladesh, this research was conducted.

## 2. MATERIALS AND METHODS

### 2.1 Isolation and Molecular Identification of SARS-COV-2

For the current experiment, data of the 205 patients, infected by COVID-19 were collected, who were selected from a number of suspected patients, diagnosed from particular locations of Bangladesh between 17$^{th}$ of July to 15$^{th}$ of August, 2020. In that case, RT-PCR based approach was followed strictly. Firstly, nasopharyngeal (NP) swab (NPS) was collected from each patient inserting a cotton swab into nostril (in <15 min). The collected specimens have proceeded to RNA extraction purposes. The purified RNA is extracted from the deactivated viral samples (in ~45 min) to commence reverse transcription process using RT-qPCR, where about an hour was taken to set each primer. Afterwards, the real time detection of the SARS-CoV-2 was observed following the threshold line at the reporting monitor within 40 cycles. In all the cases, 'Novel Coronavirus (SARS- CoV-2) Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing), Sansure Biotech Inc., China' was used. Using per kit, 45 tests were conducted with maximum accuracy.

### 2.2 Patients' Data Collection

#### 2.2.1 Clinical data

Being confirmed from the diagnosis, different clinical data have been taken from the patients, specially designed for the research purposes via direct interview and phone calls, such as- the past physiological records including obesity (O), asthma (A), diabetes (DM), hormonal (HRM), haematological (HEM), kidney diseases (K), heart conditions (HRT), arthritis (Arth), no chronic disorders (NCD) and hypertension (HTN) considering the presence of any single or multiple forms simultaneously, depending on their gender and age. At the same time, the types of treatments the patients were taking and the ultimate physical complications they were facing newly, 3 weeks after recovering from the disease, were recorded from the individual patients. In that case, cough (C), insomnia (I), anorexia (A), diarrhoea (D), loss of appetite (LA), severe weakness (SW), malaise (M), vomiting (V), stroke (S), breathlessness (B), fever (F), psychological issues (PI) and other physical and psychological issues (OPP) were considered.

### 2.2.2 Non-clinical data

Few non-clinical parameters were assessed from the patients like- their professions, economic status, education level, consciousness about their infection, environmental conditions of their surroundings, nutritional value and smoking habit.

## 2.3 Statistical Data Analysis Tools

All the data in this research paper were analyzed and graphs were generated using R Programming (Version R-4.0.2, for Mac OS X) and GraphPad Prism 8 (Linux version).

## 2.4 Geographic Information System (GIS)

The GIS data was prepared using ArcGIS Desktop 10.3 to visualize diverse impacts of SARS-CoV-2 [17].

## 3. RESULTS

Teenager male with smoking habit has been found to be more prone to COVID-19 infection than NCD. Level of NCD was decreased with the increase of age and smoking among the individuals ranged between 20 to 40 years with obesity (O), asthma (A), hypertension (HTN) and even diabetes with asthma (DM-A). Number of smokers around 40% decreased in the 40-49 years aged patients but their health complication history was diversified. Most of them had O, A, HTN, DM, Kidney problem (K), and even simultaneously DM-HTN (Fig. 1a* 'Male'). The smoking level was very low but physical complications history were high among the 50-59 years aged patients such as O, A, HTN, DM and DM-HTN in men.

Surprisingly, though there was no smoker among the patients in 60-69 years group, there were all types of complications present (Fig. 1a** 'Male'), which is very similar to the patients remained the age groups over 70 years in case of male (Fig. 1a*** 'Male'). In contrast, no female patient was smoker but the ranges of complications were comparatively higher than the men. 30-39 years old group had the DM-A, HTN, A, O and DM history (Fig. 1a* 'Female'), while 40-49 years group experienced simultaneous problems like- HTN-O and HEM-K-HRM-HRT. Mainly, DM and DM-HTN were common to the 50 year above patients. 60-69 years group patients were the worst sufferers with every adverse health conditions mentioned (Fig. 1aΨ 'Female'). The patients with more than 70 years had random complication history (Fig. 1a** 'Female').

In professional aspects, 28% self-employed (SE) male and 8.19% female were infected. 8.08% male and 5.2% female students (STD) were among the sufferers. The most infected individuals among the female were house wife (HW) and the next higher one was among the health care-related (HCR) with 32.029% and 22.1% respectively (Fig. 1b). As compared, 18.25% male and 2.1% female businessman (BNS); 9.55% unemployed (UE) male and 2.2% female; 4.34% female clinical assistant (CA) and 2.1% physiotherapist and 6.61% male police officers (P) were from the patients. Besides, few minor sectors like- teaching (T), military officer (MO) and journalist (J) were also infected (Fig. 1b).

A significant difference in drugs and treatments has been experienced between the COVID-19 patients at home and hospital. The hospitalized patients were prescribed antibiotics (AB) comparatively higher than the home isolated while, antipyretics (AP), anti-tussive (AT), anti-protozoal (APT) and vitamin (VIT) were used extensively at home patients. The hospitalized patients were given anticoagulant (ACG), minerals (M), oxygen (OXY), antiviral drugs (AVD), nebulizer (NBZ) and ICU facilities with other accessories. Anti-allergic (AA) drugs were suggested slightly higher to the home treated patients than that of the hospitalized patients.

No patient was needed oxygen during home isolation but 26% of hospitalized COVID-19 patients were required. The hospital treated patients got better medication and treatments in all aspects (Fig. 2).

The recovered patients from treatments either from home and hospitals have experienced several physical and psychological complications. Combined physical and psychological complications were noted in 19% of male patients, whereas 11% female had psychological issues; when they recovered from COVID-19. The male are the superior suffers of coughing, insomnia, asthma, diarrhea and severe weakness as compared to the female. In contrast, female was the main victim of psychological disturbance, fever, stroke and breathlessness at the post-COVID-19 period (Fig. 3).

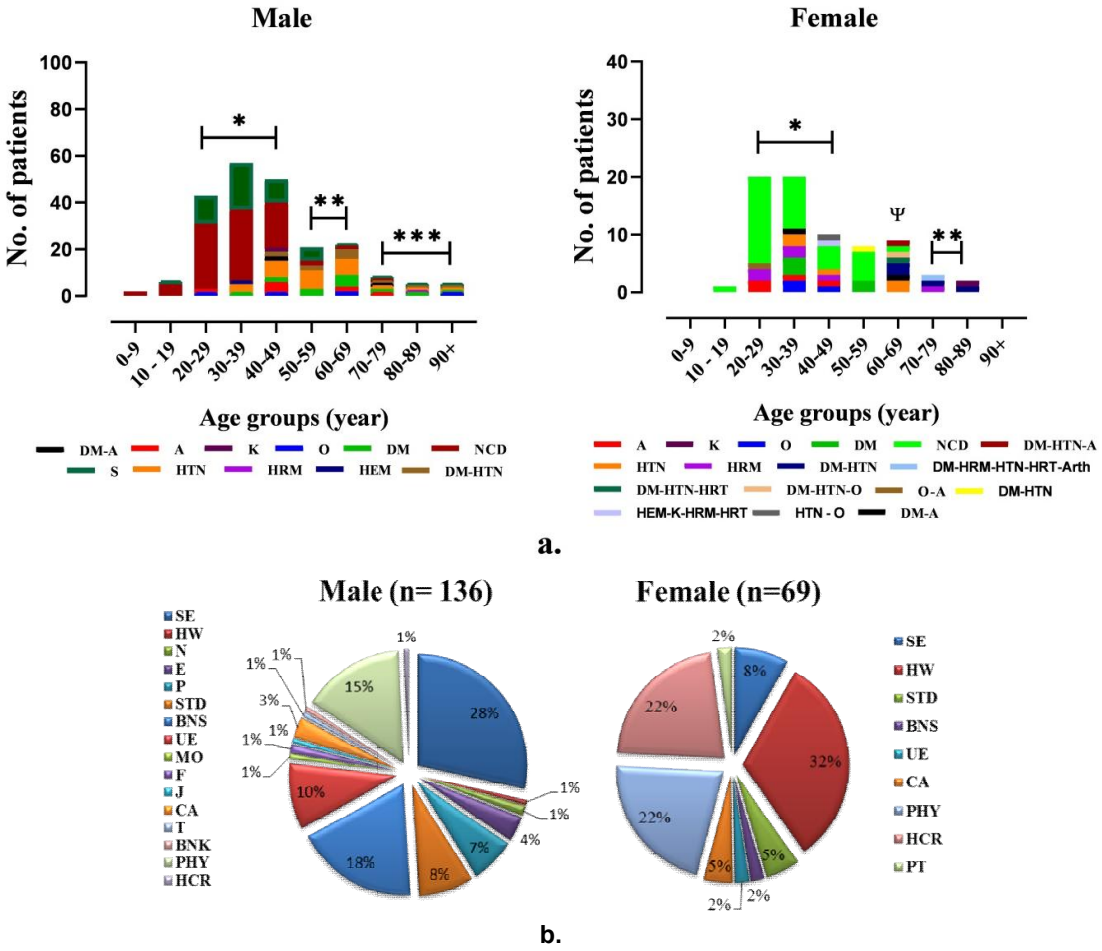


**Fig. 1. Illustration of the metadata of the health conditions of the patients, experienced previously to SARS-CoV-2 infection, considering their age groups and gender (a) and the impact of the individuals' profession to their compromisation to SARS-CoV-2 infection (b)**

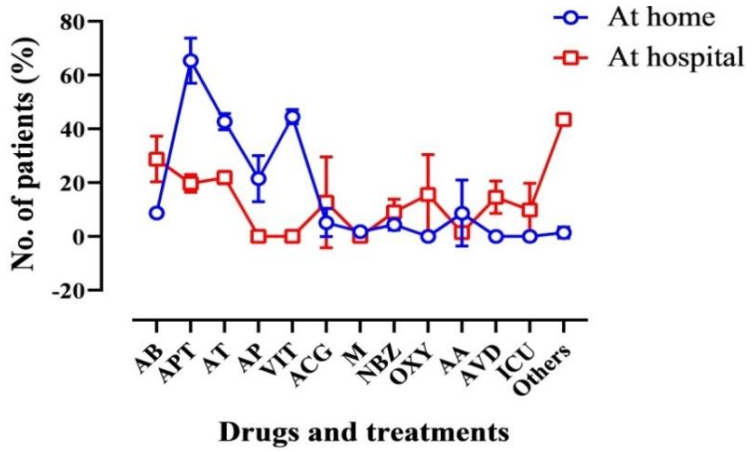


**Fig. 2. Drugs and treatments at home and hospital, taken by the patients in the present research**

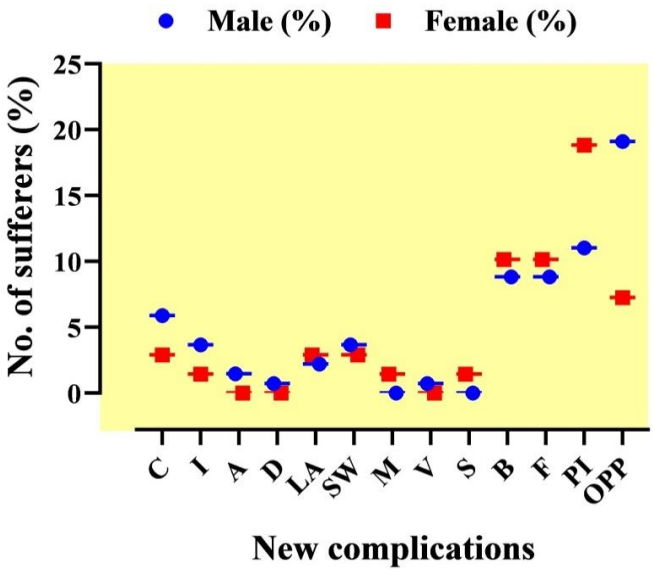


**Fig. 3. New complications faced by the patients after getting treatment**

## 4. DISCUSSION

In the present research, it has been found that RT-PCR is the gold standard for detecting SARS-COV-2 as the symptoms of the patients and positive result of RT-PCR correlates dramatically. Although RT-PCR is not a new biomedical technique [18], it is highly sensitive and specific and also integral part in the detection and control of SARS-COV-2 especially while using nasopharyngeal swab-based assay [19]. RT-PCR can also detect large number of cases in a fairly short time [20]. Real time RT-PCR was utilized in the past to detect SARS-COV but care should be taken at the time of sample collection which could result in false negative results [21]. However, in comparison to high-resolution chest computed tomography HRCT, sensitivity is slightly low but positive predictive value (PPV) is significantly high [22]. RT-PCR can also yield false negative results, if there is defective matching of probes and primers [23].

Patients with comorbidities such as obesity (O), asthma (A), hypertension (HTN), Diabetes mellitus (DM), diabetes with asthma (DM-A) and DM-HTN were found infected with COVID-19. In our study, 30-39 years old group had the DM-A, HTN, A, O and DM history (*), while 40-49 years group experienced simultaneous problems like-HTN-O and HEM-K-HRM-HRT. Predominantly, DM and DM-HTN were common to the 50 years above patients and 60-69 years group patients were the worst sufferers with every adverse health conditions mentioned (Fig. 1a) (Ψ).

Atkins et al., 2020 mentioned that in hospitalized COVID-19 infected patients' hypertension, history of fall or fragility fractures, coronary heart disease, type 2 diabetes and asthma were common comorbidities in their study [24]. Du et al., 2020 mentioned that maximal common comorbidities were hypertension, diabetes and coronary heart disease [25]. The comorbidities did not regard to be the prerequisite for symptomatic and severe COVID-19 infection without hypertension. Kidney problems (K) were found common in most of the patients of this study [26]. Some patients were found with previous history of obesity (O), Asthma (A), HTN (Hypertension), Diabetes mellitus (DM) and DM-HTN (*) simultaneously. Pei et al., 2020 mentioned in their study that, majority of their patients with COVID-19 experienced pneumonia and renal complexities, linked with higher fatality rates [27]. Acute kidney injury (AKI) was reported greater than 20% of critically sick COVID-19 patients [28]. On the contrary, in another study, acute renal impairment was exceptional and SARS-CoV-2 infection never evidents as a reason for acute renal injury or chronic renal failure [29].

In this study, male patients with age ranging from 20 to 49 years has been found with very much higher smoking and complication history (Fig. 1a). Around 60% of the infected patients are at

this age group. Most of the smoker individuals had respiratory diseases. On the other hand, no female patient was found as a smoker but the ranges of complications were much higher than the male. Patanavanich & Glantz, 2020 mentioned that smoking is a risk factor for the development of COVID-19 and smokers had higher possibility of progression to severe COVID-19 than non-smokers [30]. Similarly, Reddy et al. 2020 found that the patients with smoking history had an increased risk of severe critical COVID-19, in hospital fatality and needed mechanical ventilation more than non-smokers [31]. Lippi & Henry, 2020 mentioned that active smoking did not regard to be significantly linked with enhanced hazard of progressing towards severe disease in COVID-19 [32].

In this study, the hospitalized patients were prescribed antibiotics (AB) relatively greater than the home isolated patients. Antipyretics (AP), anti-tussive (AT), anti-protozoal (AP) and vitamins (VIT) were used thoroughly by the home patients. Similarly, all patients in Wan et al. 2020 study, were also received antibacterial therapy including antiviral therapy (interferon or Kaletra), corticosteroid and traditional Chinese medicines [33]. Hydroxychloroquine significantly ensures viral load reduction or disappearance in the patients of COVID-19 and its outcome was reinforced by antibiotics (eg. azithromycin) [34]. No treatment or therapy is found efficacious to date as mentioned by Sanders et al. 2020 [35]. Although nebulization is considered as one of the aerosol-generating medical procedures (AGMPs) that can cause fugitive emissions and potential secondary transmission of COVID-19 infection [36], in this study about 5% patients at home and 12% patients at hospital were found treated with nebulized medications. Need for mechanical ventilation may be reduced by nebulization of heparin in critically ill patients as nebulized streptokinase and heparin improve alveolar perfusion [37].

No patient at home isolation, but 26% of hospitalized COVID-19 patients of this study were given oxygen therapy. Decreased hypoxia-inducible factor 1α (HIF-1α) could hamper SARS-COV-2 viral replication due to early and proper oxygen therapy [38]. Delivery of 100% oxygen under increased atmospheric pressure up to 2.4 atm through hyperbaric oxygen therapy (HBOT) could improve the patients' outcome if administered at the earliest stages of the COVID-19 infection when arterial oxygen concentration has just been noticed reducing [39].

This research yielded that the patients, who underwent anti-coagulation therapy both at home and hospital settings, faced decreased thromboembolic complications. Male patients (n=136) showed no thromboembolic manifestations but in case of female patients (n=69) there was evidence of stroke which can be due to hyper-coagulable state. Among all the patients (n=205), there was no case of sudden death due to myocardial infarction, venous thromboembolism or pulmonary embolism. Though, research conducted by Carfora et al. (2020) showed similar findings [40], thromboprophylaxis was recommended for preventing thromboembolic events by Ribes et al. (2020) [41]. However, therapeutic and prophylactic doses of anticoagulants are still clouded and different hospitals are using different regimens amidst concurrent death of patients [42]. According to this study; while taking home medication, the percentage of vitamin intake among male and female were 43% and 46% respectively (Fig. 2) but consumption of minerals was very low among male and female patients, the percentages were 2% and 1% respectively (Fig. 2). Surprisingly, no vitamin or minerals were prescribed at the hospitals. Supplementation of Vitamin A, D, Zinc and selenium were found beneficial for both the prevention and treatment of viral infections [43]. Probiotic supplements have proved their efficacy against the respiratory viral infections [44] which are reported to be isolated from different milk derived samples [45]. In recent times, immune simulation against foreign particles has been experimented *in ovo* using different strains of *Bacillus cereus* as opportunistic pathogens [46]. Even, probiotics based opsonization is also reported *in vivo* [47], which is mediated by secondary immune response [48].

Though COVID -19 pandemic has a devastating effect on global financial security, middle income countries are suffering more than the others [49]. Ensuring the implementation of public health measures has become a great challenge not only for lower or middle income countries [50] but also for industrialist countries now. Fig. 4(e) depicted the rate of poverty in different areas of Bangladesh. In addition, this research found that self-employed males are more infected than other occupations. Among the females, housewives are mostly infected (Fig. 1b). Early Epidemiological analysis of COVID-19 in South of Iran had shown that a large number of infected patients were self-employed [12]. According to the study on the Korean workers who were at a

high risk of Covid-19 infection found that both employed and self-employed men were more infected than women [51]. Essential workers in health and social care have a higher risk of severe SARS-COV-2 infection [52].

The present research finding highlighted that the patients, who recovered from COVID-19, experienced and have been experiencing multiple neurological, physical, cognitive and emotional challenges (Fig. 3). Some key findings of this study are, 19% of male were facing combined physical and psychological complications, whereas 11% suffered psychological issues only. Life after being infected by COVID-19 is full of complications [53-55]. The prevalence of insomnia in Covid-19 positive male patients studied in the current research was similar to the research finding of many other established research findings in recent times [56,57]. On the contrary, there are chances that the quality of sleep is hampered due to the lockdown itself rather than the Covid-19 disease [58]. Emotional outbreaks including stress, depression, irritability, insomnia, fear, confusion, anger, frustration, boredom and stigma have been aggravated during quarantine due to insecurity, financial crisis, loss of family members, friends and job lessness etc psychological distress like anxiety, fearfulness, depression, stigmatization, insomnia, post-traumatic stress disorder etc (Figure 3). This finding corresponds to another study that demonstrates lock down and isolated life exacerbated the mental. In this study, it has been found that women are most vulnerable to post COVID sufferings of women notably [59,60]. On the contrary, another study analyzed few factors responsible for the tendency of the SARS-CoV-2 patients' to commit suicide [61].

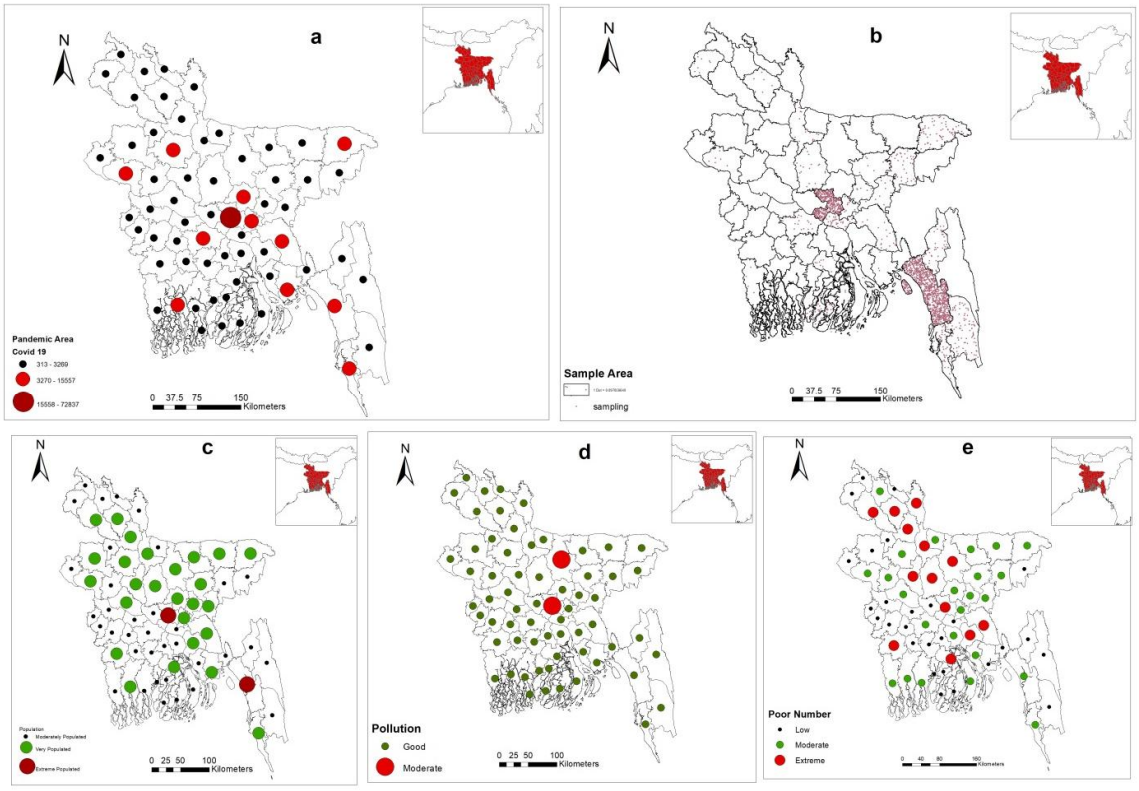


**Fig. 4. The Geographic Information System (GIS) illustrates different data like- the SARS-CoV-2 pandemic hotspots in Bangladesh (a); the sample collection and testing areas of patients in this research (b); overall pandemic frequency among the lowest to highest density populated areas (c); overall level of pollution of the infected locations in Bangladesh (d) and the economic status of the people of the pandemic areas during lockdown in all over Bangladesh (e)**

GIS is an effective way to represent spatially distributed data in an organized way. The utilization of this tool to manage diseases by public health organizations has become a necessity in line with the advancement of technology [15]. It was used in Hong Kong to analyze the dynamics of pandemic Influenza A (H1N1) in 2009 [62]. Mollalo et al., 2020 also used ArcGIS Desktop 10.7 for considering the county-level tallies of COVID-19 cases in the mainland United States [17]. In order to portrait the situation of SARS-COV-2 infection in different areas of Bangladesh. ArcGIS Desktop 10.3 has been used in this research. In Fig. 4a, the number of COVID-19 patients in Bangladesh from 17th of July to 15th of August, 2020 was depicted. The number of COVID -19 patients was higher in populated areas such as Dhaka (22%) and Chittagong (54.14%) comparing to the other areas (Fig. 4b) of Bangladesh. Stier et al., 2020 also suggested that COVID-19 was spreading faster in larger cities or populous urban areas [63].

According to Fig. 4a and 4d, it is evident that there is relationship between air pollution and COVID-19 infection. Previous studies found that ambient air pollutants were risk factors for respiratory infection by carrying microorganisms, making pathogens more invasive to humans and affecting body's immunity to make people more susceptible to pathogens [64]. Few researchers have recently described that there was a significant correlation between air-quality and COVID-19 cases [65]. On the contrary, no significant outbreaks (up to now) of COVID-19 have been observed, fortunately, in very highly populated and polluted cities in India [66]. Metropolitan areas were at much lower risk due to younger populations and better health outcomes [67].

## 5. CONCLUSION

RT-PCR proved its efficacy in detecting viral entities especially SARS-CoV-2 in the current manuscript which is very flexible and accurate. At the same time, it seems complicated to afford for the vast number of population due to the expensive operating. Besides, there are significant impacts of different clinical and nonclinical facts on the physical, psychological and social conditions of the nCoV patients depending on their pre-infection and post-infection periods. An integrative approach considering the pubic-private-partnership programs on comprehensive diagnosis and multifactorial analysis are needed to ensure the suppression of SARS-CoV-2 community transmission.

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## CONSENT

As per international standard or university standard, patients' written consent forms have been collected and preserved by the author(s).

## ETHICAL ISSUES

The Govt. of Bangladesh has made the diagnosis opened for pandemic issues with special subsidiaries and suspected individuals have been cordially coming with their personal interest and health concerns. No volunteer was used in this research for sampling purposes. An ethical approval was officially received from Jashore Medical College (JMC), with the collaboration of Research publication Guardians (RPG) as remarked with 'Govt. License No. 05-060-06021'.

## COMPETING INTERESTS

Authors have declared that there is no competing interest with the others.

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