Chapter 1

Introduction

COVID-19 is an infectious disease caused by the novel coronavirus, severe acute respiratory syndrome coronavirus (SARS-CoV-2). It was first identified two years back when a cluster of pneumonia of unknown etiology appeared in Wuhan, China (Guo et al., 2020). Since then, it affected around 222 countries and territories across the globe (Worldometer COVID 19 Pandemic, 2022). So far, almost 350 million cases and over 5 million deaths have been reported. In Bangladesh, at least half a million cases have been reported up to now with more than twenty eight thousand deaths and counting (DGHS, COVID 19 dashboard, 2022).

Patients with COVID-19 have a series of clinical manifestations that range from asymptomatic carriers to severely ill individuals. Symptoms such as fatigue, cough, fever, anorexia, diarrhea, nausea, vomiting, head ache and dyspnea are commonly found (Guo et al., 2020). On CT imaging the chest is usually seen to have ground-glass opacity and bilateral patchy shadowing (Guan et al., 2020) sometimes with a rounded morphology and a peripheral lung distribution. However, some of the confirmed patients have normal CT image presentations. In laboratory examinations, infected people usually have normal or reduced white blood cell counts, along with lymphocytopenia (Guan et al., 2020). However, when disease is severe, the neutrophil count increases significantly, along with other markers such as D-dimer, blood urea and creatinine levels. Additionally, inflammatory factors such as the interleukins (IL-6, IL-10) and tumor necrosis factor-α (TNF-α) increase, indicating the immune status of patients.

Complications from COVID-19 are also commonly found. These include acute respiratory distress syndrome (ARDS), arrhythmia, shock (Wang et al., 2020), acute kidney injury, acute cardiac injury, liver dysfunction and secondary infection (Huang et al., 2020). The disease tends to progress faster in elderly people, with the median number of days from the occurrence of the first symptoms to death shorter among people aged 65 years or more (Wang et al., 2020).

The disease was previously confirmed by reverse transcription-polymerase chain reaction (RT-PCR) tests, however nowadays the more rapid genetic sequencing of SARS-CoV-2 and use PCR to amplify this sequence is used in many places (Allam et al., 2020). In case of disease prevention, vaccination at a national level is underway in Bangladesh for the development of herd immunity. Apart from this, general measures such has frequent hand washing, social distancing and using a face mask can also help in reducing disease transmission.

Although many studies have been published on the mental health sufferings due to COVID-19 (Mamun and Griffiths, 2020; Islam et al., 2020) and even the association of environmental conditions on COVID-19 outbreaks (Haque and Rahman, 2020) in this country, not much has been discussed about the clinical features or risk factors that lead to a worse prognosis of the disease. Furthermore, very few studies have been found acknowledging the role of biomarkers in the treatment and outcome of COVID-19 patients in Bangladesh. Since, biochemical tests are an important aspect of monitoring progression of the disease in hospital settings (Letelier et al., 2021), it is essential to have adequate knowledge on the alterations that will most likely be found in COVID-19 patients. Although many studies have been conducted worldwide on biochemical markers and COVID-19 (Bairwa et al., 2021; Wang et al., 2020), none have been published from data in the city of Chattogram so far. Moreover, very few research was done to identify death cause in COVID 19 patients without comorbidity. Hence, this retrospective study attempts to fill this gap in knowledge about the various biochemical markers that contribute to the prognosis of COVID-19 patients without comorbidity and tell us beforehand, which cases are most likely to survive and which are not so that clinical decisions can be made early to enhance patient outcome.

1.2 Rationale

The clinical course of COVID-19 is variable and ranges from mild symptoms to severe illness and death. Among patients who are hospitalized for the disease, those with a poor prognosis tends to develop sever viral pneumonia that requires ventilator support. Despite such supportive care, a high proportion of these patients suffer rapid deterioration with respiratory failure and death (Yang et al., 2020). Early identification of severe illness risk factors can help clinicians facilitate appropriate remedial measures and help control mortality (Liu et al., 2020). Accumulated evidence has showed that many biochemical parameters become altered in COVID-19 patients. Correlating this with disease severity and associating them with prognosis of the patient can help clinicians identify what biochemical markers to focus to predict patient outcome.

1.3 Objectives

- To identify the socio-demographic information of COVID 19 patients without comorbidity admitted in hospital.
- To identify common sign symptoms of COVID 19 patients without comorbidity and its association with disease outcome.
- To identify common laboratory findings and its association with disease outcome.

1.4 Research Question:

Is there any significant association between biochemical markers and adverse disease outcome in COVID 19 patients without comorbidity?

Chapter 2

Literature review

2.1 Background

Coronaviruses (Co-V) are a class of genetically diverse viruses that have been found in a wide range of host species, including birds and mammals. Many of them cause gastrointestinal tract as well as respiratory tract infections in humans and animals (Resta et al., 1985; Zhong et al., 2003). Even though coronaviruses were first described in 1966 by Tyrell and Bynoe, who cultivated the viruses from patients with common colds (Tyrrell and Bynoe, 1966) it came in to spotlight in 2002-2003 when clusters of 'atypical pneumonia' were first reported in Guangdong Province, China that subsequently spreading to Hong Kong. Researchers named the new atypical pneumonia Severe Acute Respiratory Syndrome (SARS) and the coronavirus was called SARS Co-V (Sun et al., 2020). About 10 years later in 2012, another atypical pneumonia first emerged in Saudi Arabia that later spread to Jordan and other parts of the world leading to a nosocomial outbreak in Seoul, South Korea, in the year 2015 (De Wit et al., 2016). This atypical pneumonia was termed Middle East Respiratory Syndrome and the virus causing this disease was named MERS-Co-V. Just four years later, a new version of SARS-Co-V emerged in Wuhan City of Hubei province, China. In December 2019, a cluster of pneumonia with unknown cause were found here and subsequent virus isolation from human patients and molecular analysis showed that the pathogen was a new coronavirus. This new coronavirus was designated severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by the Coronavirus Study Group of the International Committee on Taxonomy of Viruses (Gorbalenya et al., 2020) and the disease caused by this virus was named COVID-19 by the World Health Organization. Within a few months, this recent outbreak turned in to a public health emergency of international concern taking as many as five million lives and counting (Worldometer COVID 19 Pandemic, 2022).

2.2 Classification of Corona viruses

Corona Viruses are classified under the order *Nidovirales*, family *Coronaviridae*, and subfamily *Orthocoronavirinae* (Fig. 1). Based on genetic and antigenic criteria, Co-V have been organized into four groups: alpha coronavirus (α-CoV), beta coronavirus (β-CoV, gamma coronavirus (γ-CoV) and delta coronavirus (δ –CoV) (Van Regenmortel MH et al., 2000). Among them, α- and β-CoV are able to infect mammals, while γ- and δ-CoV tend to infect birds. Previously, six corona viruses (CoVs) have been identified as human-susceptible virus, among which α-CoVs, HCoV-229E and HCoV-NL63, and β-CoVs, HCoV-HKU1 and HCoV-OC43 with low pathogenicity, caused mild respiratory symptoms similar to a common cold, respectively. The other two known β-CoVs, SARS-CoV and MERS-CoV lead to severe and potentially fatal respiratory tract infections (Yin and Wunderink, 2018).

It was found that the genome sequence of SARS-CoV-2 shares 79.5% identity to SARS-CoV.

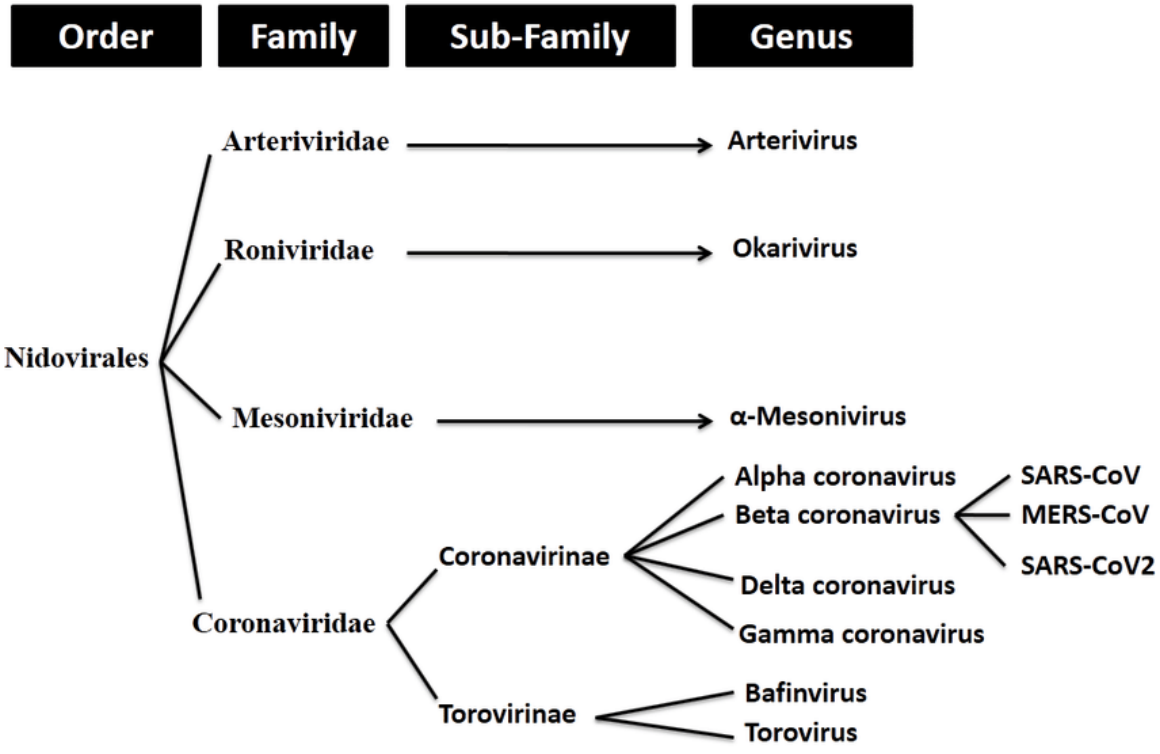

Figure 1: Coronavirus classification (Shafique et al., 2020)

2.3 Structure of corona virus

The coronaviruses are enveloped viruses with a helical nucleocapsid and a single stranded linear non-segmented positive polarity RNA. The coronaviral genome contains four major structural proteins: the spike (S), membrane (M), envelope (E) and the nucleocapsid (N) protein, all of which are encoded within the 3′ end of the genome. The S protein mediates attachment of the virus to the host cell surface receptors resulting in fusion and subsequent viral entry. The M protein is the most abundant protein and defines the shape of the viral envelope. The E protein is the smallest of the major structural proteins and participates in viral assembly and budding. The N protein is the only one that binds to the RNA genome and is also involved in viral assembly and budding (Malik, 2020). Compared with the known SARS-CoV and MERS-CoV genome, SARS-CoV-2 is closer to the SARS-like bat CoVs in terms of the whole genome sequence. Most genomic encoded proteins of SARS-CoV-2 are similar to SARS-CoVs, although certain differences exist.

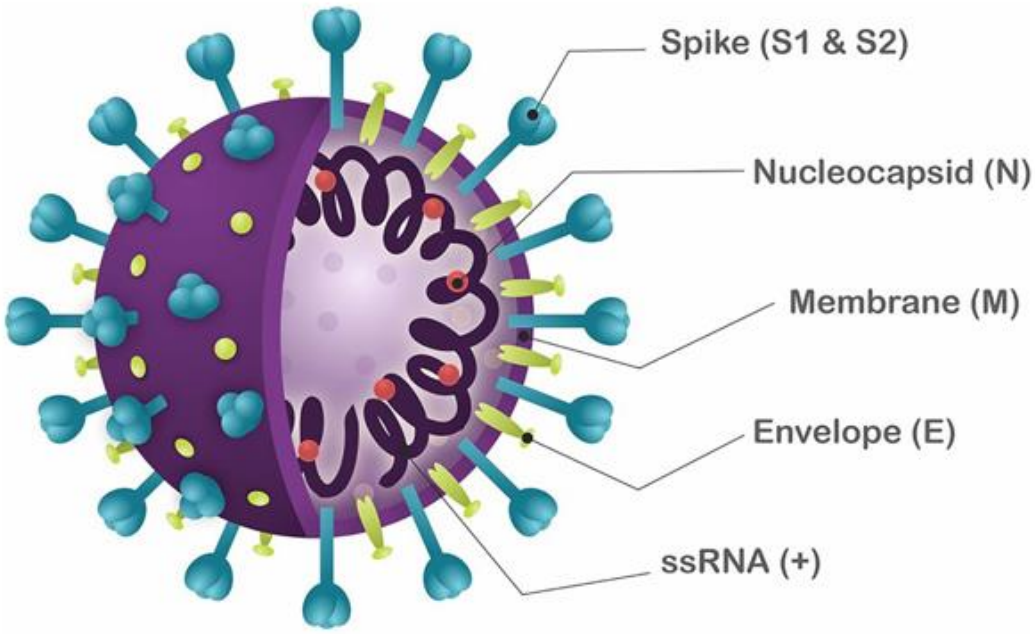

Figure 2- Structure of SARS-CoV-2 (Santos et al., 2020)

2.4 Replicative cycle

Replication of coronaviruses begin with attachment and entry. Attachment of the virus to the host cell is initiated by interactions between the **S** protein and its specific receptor. Following receptor binding, the virus enters host cell cytosol via cleavage of S protein by a protease enzyme after the viral and cellular membranes fuse together. The next step is the translation of the replicase gene from the virion genomic RNA and then translation and assembly of the viral replicase complexes. Following replication and subgenomic RNA synthesis, encapsidation occurs resulting in the formation of the mature virus. After they are assembled, the virions get transported to the cell surface in vesicles and are released by exocytosis (Malik, 2020).

2.5 Transmission and epidemiology

Based on virus genome sequencing results and evolutionary analysis, bat has been suspected as natural host of virus origin and SARS-CoV-2 might be transmitted from bats via unknown intermediate hosts to infect humans (Guo et al., 2020). Direct contact with intermediate host animals or consumption of wild animals was suspected to be the main route of SARS-CoV-2 transmission. However, the source(s) and transmission routine(s) of SARS-CoV-2 remain elusive. Human-to-human transmission of SARS-CoV-2 occurs mainly between family members, including relatives and friends who intimately contacted with patients or incubation carriers (Guo et al., 2020).

2.6 Pathogenesis and immunity

Studies in both organ cultures and human volunteers show that coronaviruses are extremely fastidious and grow only in differentiated respiratory epithelial cells. SARS-CoV-2 uses angiotensin-converting enzyme 2 (ACE2), the same receptor as SARS-CoV to infect humans (Zhou et al., 2020). Infected cells become vacuolated, show damaged cilia, and may form syncytia. Cell damage triggers the production of inflammatory mediators, which increase nasal secretion and cause local inflammation and swelling. These responses in turn stimulate sneezing, obstruct the airway, and raise the temperature of the mucosa. Although mucociliary activity is designed to clear the airways of particulate material, coronaviruses can successfully infect the superficial cells of the ciliated epithelium. Only

about one-third to one-half of infected individuals develop symptoms. Because coronavirus infections are common, many individuals have specific antibodies in their nasal secretions, and these antibodies can protect against infection. Most of these antibodies are directed against the surface projections and neutralize the infectivity of the virus. Cell-mediated immunity and allergy have been little studied, but may play a role.

2.7 Clinical findings

Major initial symptoms of COVID-19 include fever, cough, muscular soreness, and dyspnea. Some patients showed atypical symptoms, such as diarrhea and vomiting. About one fourth of the patients have at least one underlying medical condition (Wang et al., 2020). The viral infection is not age selective and can occur in any age. However, elderly male are more likely to suffer from severe illness as compared to age groups. While many cases are symptomatic, asymptomatic cases have also been documented elsewhere (Hoehl et al., 2020).

2.8 Laboratory diagnosis

Since its emergence, a wide variety of methods have been developed for the purpose of the rapid and accurate diagnosis of COVID-19. On the basis of clinical criteria alone, SARS-CoV-2 cannot be reliably distinguished from infections with other pathogens that cause similar symptoms, including influenza, seasonal Corona viruses, adenovirus, respiratory syncytial virus etc. In this context, the laboratory-based diagnosis is deemed necessary to identify cases so that proper control measures can be taken. The confirmation of a SARS-CoV-2 infection in the laboratory can be achieved by direct and indirect virology methods. While direct detection is more specific, indirect methods allow a greater opportunity for virus detection after the acute phase of the disease. In direct tests, the clinical sample is examined directly for the presence of particles, virus antigens, or viral nucleic acids, whereas indirect methods detect the serological response against the infection.

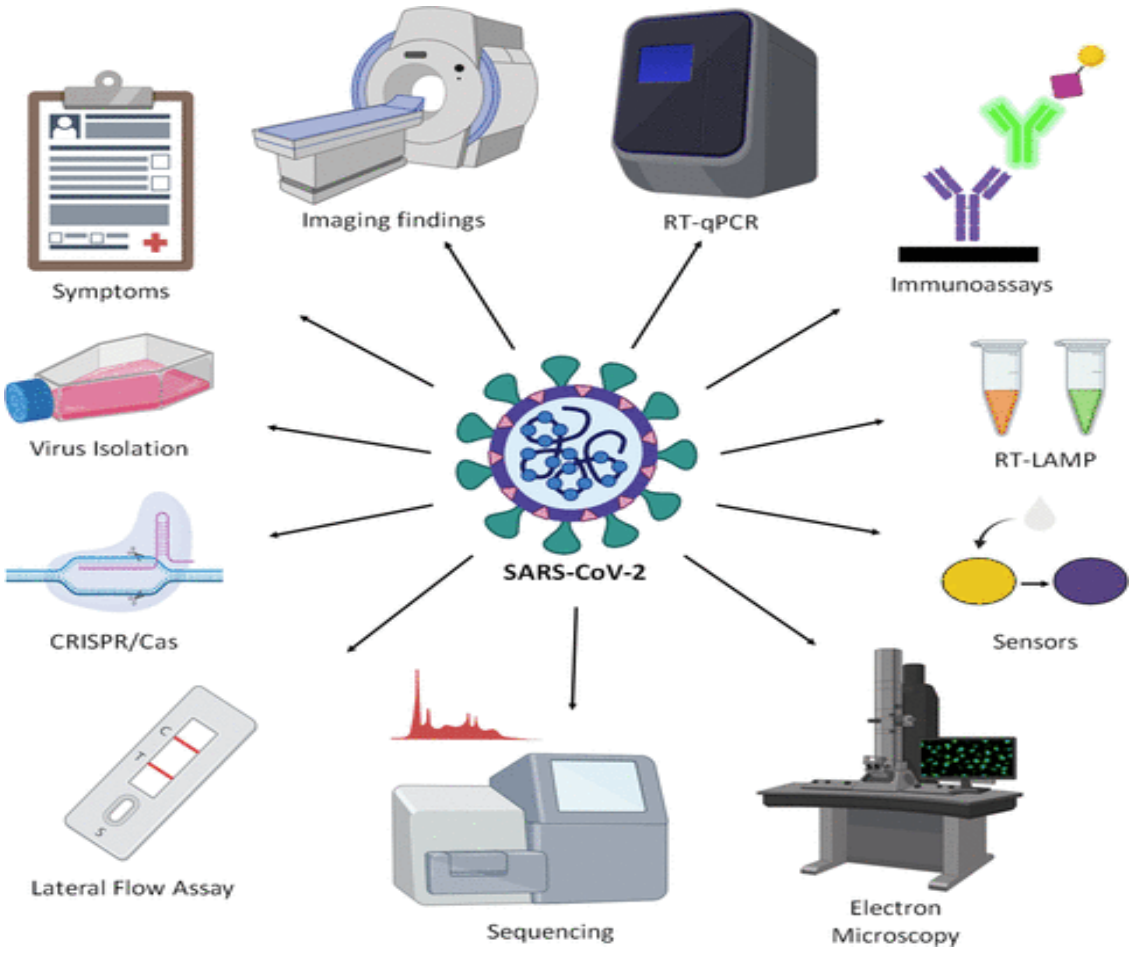

Figure 3- COVID-19 diagnostic approaches (Da Silva et al., 2020)

SARS-CoV-2 infection can be detected in a variety of clinical specimens, such as nasopharyngeal or oropharyngeal aspirates or washes, nasopharyngeal or oropharyngeal swabs, sputum, tracheal aspirates, and bronchoalveolar lavage (Wang et al., 2020). The median duration of SARS-CoV-2 shedding in respiratory samples is 24 (IRQ, 18–31) days in survivors, but shedding can last for up to 42 days (Xiao et al., 2020). In Bangladesh, detection of viral nucleic acid is the method of choice for diagnosing COVID-19. This is detected by RT-PCR, and the sensitivity of detection depends upon various factors such as specimen site, quality and storage temperature of specimen. Any faulty techniques in collection or storage may lead to inaccurate results and hence compromise diagnosis of the patient. More accurate results can be obtained from collecting specimens from lower respiratory tract and hence this site is preferred (MOHFW, National guidelines on clinical management of COVID-19, 2020).

2.9 Treatment and prevention

The treatment for COVID-19 depends on the clinical symptoms of the patients. For asymptomatic cases, isolation of the patient with supportive care is recommended. Regular hand washing, using paper towel or elbow to cough, and wearing a medical mask at all times is essential. In case of mild cases, symptomatic management and home isolation is enough. However, if patient has associated comorbidities that are controlled, they need to be carefully monitored at home using a finger pulse oximeter and danger signs should be watched out for. Mild cases with uncontrolled comorbidities require hospital admission. Such patients should receive thromboprophylaxis along with symptomatic management.

For moderate cases, symptomatic management and use of nasal cannula for O_2 therapy is given. Target SPO_2 is 94% during initial resuscitation and 90% for stable patients. For pregnant patients and patients with other organ failure target SPO_2 is 94%. Maintaining prone position for 4 -6 hours a day and use of thromboprophylaxis is necessary. Antiviral drug (Remdesivir) and steroid should be initiated. For severe cases, additionally, antibiotics need to be given. Oxygen flow needs to be escalated to maintain oxygen demand. Based on the requirement, the various devices for supply of oxygen can be used: Nasal cannula (up to 5 litre), Oxygen mask (6-10 litre) and Non-Rebreather bag with reservoir bag (10-15 litre. Demand above that needs to be supplied using a High flow nasal cannula. Other drugs such as Tocilizumab and Baricitinib are used in severe or critical COVID-19 based on patient needs (MOHFW, National guidelines on clinical management of COVID-19, 2021).

2.10 Association of hematological and biochemical markers with COVID 19 infections

While the clinical characteristics of COVID-19 are diverse, recent studies have also shown alterations in laboratory parameters among these patients. As such, these can be used as biomarkers to evaluate disease progression and categorize presenting patients as mild, severe or fatal in clinical conditions. Many laboratory parameters make it possible to assess the severity of the disease and predict the risk of evolving towards more serious afflictions such as respiratory distress syndrome, disseminated intravascular coagulation and multiple organ failure (Lippi and Plebani, 2020). Some of these are thrombocytopenia, neutrophilia, elevated liver enzymes, hypoalbuminemia, creatinine and inflammatory markers like interleukin-6 and C-reactive protein (Ramírez and Herrera, 2020). However, the main progression predictors were identified as lymphopenia, elevated D-dimers and ferritin levels while also considering LDH, troponin and CPK in the marker panel.

In the case of inflammatory response markers, COVID-19 causes an exacerbated immune reaction which provokes an inflammatory response called 'cytokine storm'. Lymphopenia and elevated pro inflammatory cytokines were reported to be frequent in severe cases of COVID-19 as compared to mild cases (Liu et al., 2020). One study by (Huang et al., 2020) showed plasma concentrations of IL2, IL7, IL10, GCSF, MCP1, IP10, MIP1A, and TNF-α to be higher in Intensive Care Unit (ICU) patients rather than patients not in ICU. Another study by Qin et al (Qin et al., 2020) showed infection related biomarkers like procalcitonin, CRP and serum ferritin to be elevated along with the inflammatory cytokines. CRP was reported to be present in higher levels in patients with disease progression as compared to people who are stable or recovering (Liu et al., 2020). The same study also showed albumin to be significantly diminished in the disease progression group. LDH that is used as a marker for lung tissue damage, is frequently abnormal in COVID-19 patients (Lippi and Plebani, 2020). However, the abnormalities are more common in patients with severe disease as compared to mild ones.

In case of cardiac markers, one meta-analysis of 28 studies found seriously ill COVID-19 patients to have increased levels of creatinine kinase-MB, troponin, myoglobin and NT-pro BNP (Li et al., 2020). Furthermore, another study by Deng et al (Deng et al., 2020)

recognized that most patients had normal levels of troponin on admission, but in about 37.5% cases levels increased during hospital stay, especially in those that died.

For hepatic markers, one study found more than 90% of patients with abnormal hepatic tests to have mild symptoms on admission. However, patients with abnormal hepatocellular or a mixed type of hepatic tests on admission were more prone to develop serious illness. Nevertheless, damage of liver due to use of medications could not be ruled out in this study (Cai et al., 2020).

Renal disease among patients with COVID-19 can present in the form of proteinuria, hematuria, or acute renal injury thus contributing to a greater mortality risk. Upon post-mortem renal histopathological examinations of patients who died from COVID-19, it was found that SARS-CoV-2 infection induced severe acute tubular necrosis and lymphocyte infiltration. The viral antigen was found in the tubules of all renal tissue samples (Diao et al., 2020). Another study showed abnormal renal parameters such as proteinuria, hematuria and leukocyturia on a routine urine test on admission among COVID-19 patients without any previous history of kidney disease (Zhou et al., 2020).

Infection with COVID-19 can cause damage to pancreatic islet cells resulting in acute diabetes. This is why amylase and lipase levels are useful for follow up purposes (Wang et al., 2020). However, a study had reported the presence of acute pancreatitis associated with this novel coronavirus among members of a family after excluding any other cause (Hadi et al., 2020). Another study displayed 17% of patients with pneumonia due to COVID-19 also showed higher values of amylase or lipase (Wang et al., 2020).

Chapter 3

Materials and methods

3.1 Description of the hospital

The data for this study was collected from four different private hospitals namely Medical Center Hospital, Max Hospital, Metropolitan Hospital and Park View Hospital, all of them are situated in the Chattogram metropolitan area. These are multidisciplinary health units. Since the emergence of the COVID-19 pandemic, these hospitals had opened a COVID-19 treatment wing with all necessary facilities. These have a well reputed RT-PCR lab for COVID-19 test and also has well equipped ICU and HDU for treating COVID cases of varying severity.

3.2 Study Design

This is a retrospective cross sectional study that was conducted in four non- government hospital of Chattogram Metropolitan City.

3.3 Study Population

A total of 103 COVID 19 patients were included in this study based on the following criteria:

i. All Patients with RT-PCR positive for COVID-19 were included in this study.
ii. All patients were free from comorbidities like Diabetes Mellitus, Hypertension, Bronchial Asthma, Chronic Obstructive Pulmonary Disease (COPD), Ischemic Heart Disease, Chronic Kidney Disease, Chronic Liver Disease, Malignancies of any aetiology, Immunological Disease such as Rheumatoid Arthritis, Systemic Lupus Erythrometosus (SLE), Vasculitis and had no history of taking immunosuppressant drugs.
iii. All patients were non-vaccinated.

3.4 Study period

Data was collected for this study over a period of one year from January 2021 to the end of December 2021.

3.5 Sampling method

Purposive sampling technique was used to collect the study sample. Patients who agreed to be enrolled in the study and who met the inclusion criteria were enrolled consecutively until the targeted sample size was completed.

3.6 Inclusion and exclusion criteria

There were some criteria for enrollment of the COVID 19 patients in this study

Inclusion criteria

i. All patients admitted at these afore mentioned hospitals during the study period with RT-PCR positive for COVID-19.
ii. All patients free of co morbidities.
iii. All patients did not receive vaccine at all.
iv. All patients who gave consent for the study enrollment.

Exclusion criteria

i. Symptomatic cases but RT-PCR negative.
ii. Patients who did not give consent.
iii. Patients with co morbid condition.
iv. Patients who received at least one dose of COVID 19 vaccination.

3.7 Ethical implications

Prior to collecting data, written permission was obtained from the director of the specified hospital. When approaching the study participants, a written informed consent was obtained from all participants or their relatives if participants were unable to respond. All collected data was kept in a secured place under lock and key. On data analysis in

SPSS version 23, all patient's names were replaced by serial numbers to protect identity. Hence, confidentiality was maintained.

3.8 Data collection tools

All data were recorded in a pre-structured questionnaire from patient's admission file, laboratory findings from patient's case record file preserved at respective hospital after consent from appropriate authorities and participants**.**

3.9 Data collection procedure

All patients meeting the inclusion criteria were approached for the study. If they had a RT-PCR result to be positive and agreed to be enrolled, they were immediately included in the study. If RT-PCR results were not available at the time of admission, then they were tested and were included only if results came back positive. All participants were followed until discharge and their lab reports on admission were collected for this study.

3.10 Data analysis

All data were initially checked for mistakes. Completed data sheets with missing or confusing responses were excluded. The data were collected, sorted out and were entered into MS Excel 2013 and exported to statistical software Statistical Package for Social Sciences (SPSS) version 23. Qualitative data was analyzed using chi-squared test. Quantitative data was analyzed using student's t- test. Multiple Regression between the factors were carried out to identify the most significant factor effecting the disease outcome. The results were presented as tables and charts. P value less than 0.05 was considered as significant.

Chapter 4

Results

4.1 Overall Socio-Demographic Characteristics of COVID 19 Patients

For this study, data on patient's age, gender, area and occupation were obtained from their record files. The results are summarized in table 1 below.

Table 1: Overall Socio Demographic Variables of COVID 19 Patients (N=103)

Variable	Category	Frequency	Percent
Age (in years)	25-34	10	9.71
	35-44	24	23.30
	45-54	25	24.27
	55-64	29	28.16
	65-74	15	14.56
Sex	Male	89	86.41
	Female	14	11.59
Area	Rural	5	4.86
	Urban	98	95.14
Occupation	Service Holder	44	42.71
	Businessman	31	30.10
	Retired	20	19.42
	House wife	8	7.77
Age (in years)	**Minimun Age**	**Maximum Age**	**Mean Age**
	25	73	50.23

The table 1 shows the ages of the study subjects based on age groups. The most common age group was 55 to 64 years age group that made up 28.16% of the total sample. This was followed by the 45-54 years age group (24.27%), 35-44 years age group (23.3%) and finally the 65-74 years age group (14.56%). The least number of cases were in the 25-34 years age group making up (9.71%) of the entire sample. The minimum age of the respondents was 25 years and maximum age was 73 years. The mean age was 50.23 years.

On looking at the gender of the study subjects, majority of them were male (86.41%) with only a few females (11.59%).

Table 1 also shows that the area of the study subjects from where they belong, majority of them were urban dwellers (95.14%) with only a few from rural area (4.86%).

Finally, we observed the occupations of the study subjects. The occupation was broadly categorized in to housewives, job holders and businessmen. The most common occupation was service holders (42.71%), followed by businessmen (30.1%), and then retired persons (19.42%). Since women were a minority in this study, housewife (7.77%) was the least common occupation. Among the study group, most of the elderly population was among the retired group. No significant association was found between any of the occupations and patient outcome.

4.2 Overall Clinical Symptoms of COVID 19 Patients

The most common symptom observed among the study subjects was Fever (100%) followed by Cough (94.2%), Headache (62.1%), Dyspnea (55.3%), Anosmia (47.6%) and Diarrhea (11.7%) respectively.

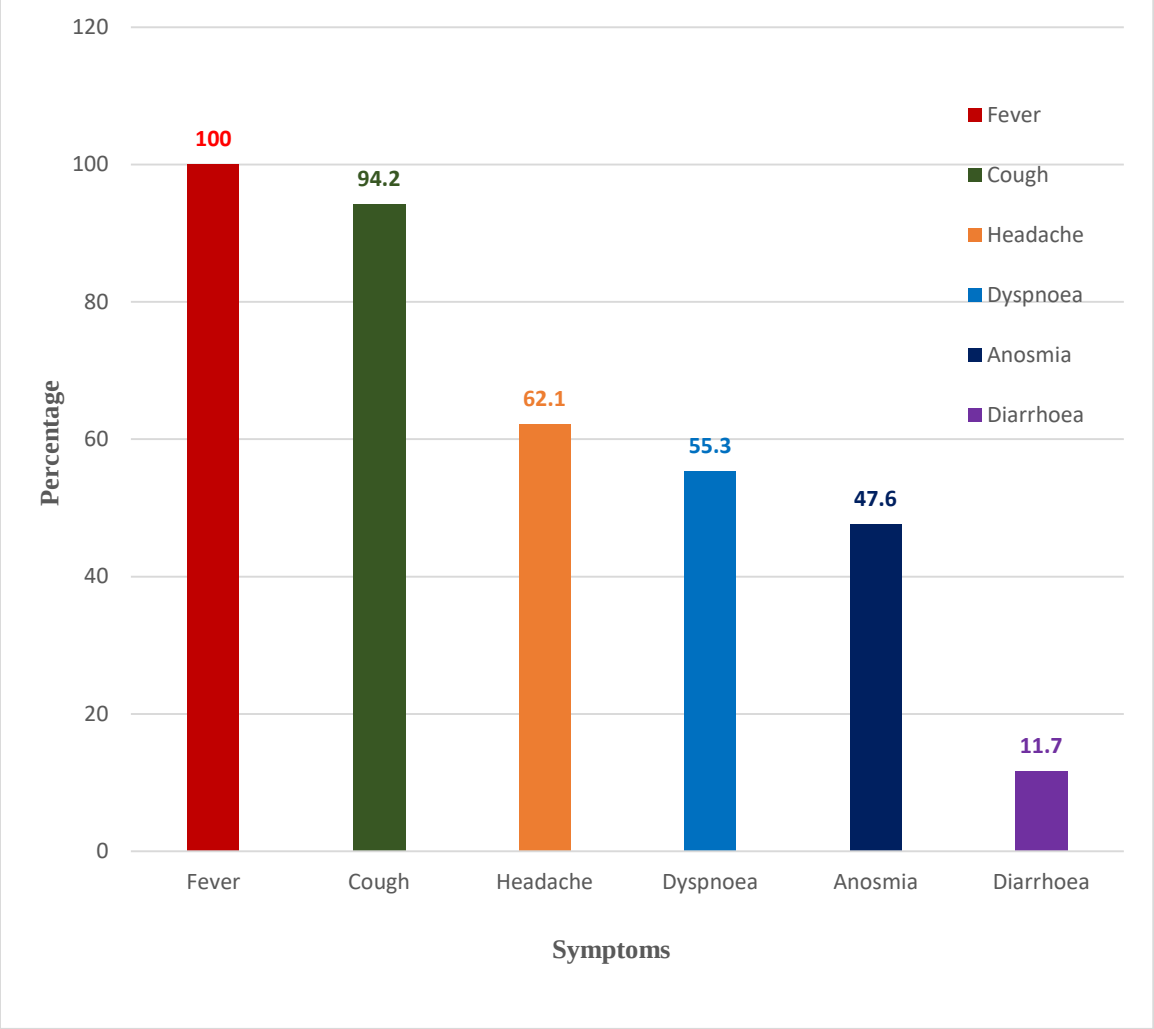

Figure 4: Percentage of Symptoms of COVID 19 Patients

4.3 Overall Laboratory Parameters of COVID 19 Patients

Table 2: Overall Laboratory Parameters of COVID 19 Patients

Lab Parameters	N	Minimum	Maximum	Mean±SD
CRP (mg/dl)	103	20	654	137.68±135.01
Hemoglobin (gm/dl)	102	7.1	14.2	10.97±1.36
ESR(mm in 1st hour)	102	22	67	46.42±9.53
WBC ($\times 10^9$/L)	103	2.4	13.28	6.48±1.34
Neutrophil (%)	103	40	91.2	76.00±7.56
Lymphocyte (%)	103	5.5	42	17.85±6.26
Platelet ($\times 10^9$/L)	103	120	353	178.62±43.96
PT (Seconds)	73	13	18	14.81±0.94
Ferritin (mcg/L)	101	42	1529	444.50±266.89
D-Dimer	103	0.05	5.2	0.99±0.87
Pro-calcitonin (ng/ml)	91	0.020	0.500	0.06±0.05
RBS (mg/dl)	102	5.0	25	9.80±3.61
SGPT (U/L)	103	12	120	44.28±12.98
SGOT (U/L)	87	22	87	51.89±11.59
Creatinine (mg/dl)	103	0.60	7.8	1.18±0.76
NT-pro BNP (pg/ml)	50	57	1275	353.10±306.36
High sensitive Trop I (ng/L)	50	0	785	40.32±116.45
IL-6 (pg/ml)	4	3.2	68	32.55±33.35
Na+ (mmol/L)	103	118	145	134.12±4.33
K+ (mmol/L)	103	2.8	4.8	3.61±0.45
Cl^- (mmol/L)	103	90	106	98.20±3.23
$HCO3^-$ (mmol/L)	103	20	32	23.50±1.51

Table 2 describes the overall laboratory parameters of the COVID 19 patients. It shows the frequency of the tests done along with their individual minimum and maximum value with mean and standard deviations.

4.4 Outcome according to Age

Study shows that the minimum age of our respondents were 25 years and maximum age was 73 years. The mean age was 50.23 years. The ages were split in to two groups. One group was 50 years and below, while the other age group was above 50 years. On conducting a t- test to find an association between age of study subjects and death, a highly significant association (p=0.001) was found between the two with older age groups having a significantly higher proportion of death. The chart below demonstrates the findings.

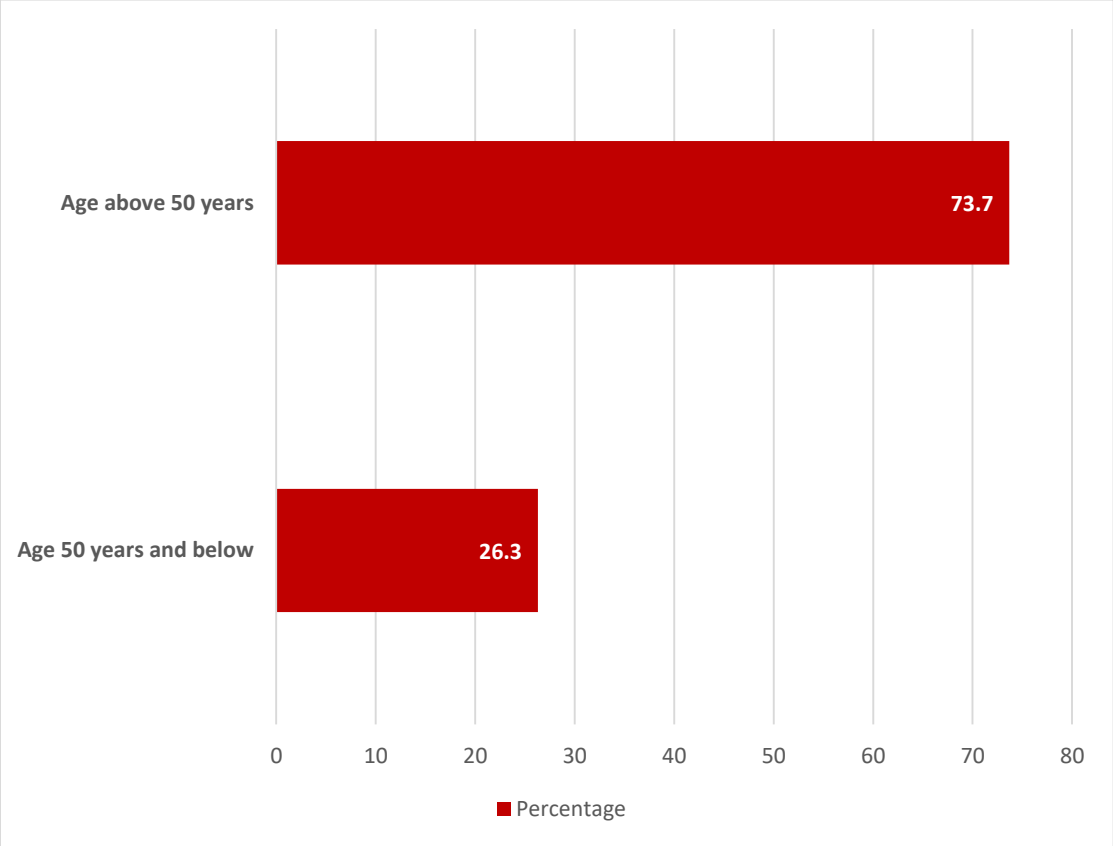

Figure 5: Outcome according to Age

4.5 Clinical symptoms of COVID 19 Patients according to disease outcome

The most common symptom observed among the study subjects was fever (100%) followed by cough (94.17%), headache (n=62.14%), dyspnea (55.34%), anosmia (47.57%) and diarrhea (11.65%) respectively. On conducting a t- test, significant association was found between the presence of dyspnea and patient outcome (P Value 0.005). Table 3 below displays the findings.

Table 3: Clinical symptoms of COVID 19 patients according to disease outcome (N=103)

Symptoms	Frequency	Percentage (%)	Alive (n=84)	Died (n=19)	P value
Fever	103	100	83	18	0.245
Cough	97	94.17	78	19	0.230
Headache	64	62.14	55	9	0.142
Dyspnea	**57**	**55.34**	**41**	**19**	**0.005**
Anosmia	49	47.57	42	7	0.300
Diarrhea	12	11.65	9	3	0.533

4.6 Hematological profile of COVID 19 Patients according to disease outcome

Table 4: Comparison of Hematological Profile of COVID 19 Patients with disease outcome

Parameters	Mean ± SD		P value
	Alive	Dead	
Hemoglobin (gm/dl)	10.99±1.40	10.91±1.17	0.795
ESR (mm in 1st hour)	46.31±10.06	49.94±6.73	0.799
Total WBC Count ($\times 10^9$/L)	6.38±1.19	6.93±1.85	0.226
Neutrophil (%)	76.07±6.32	75.74±11.81	0.865
Lymphocyte (%)	17.87±5.37	17.763±9.44	0.945
Platelet Count ($\times 10^9$/L)	180.93±41.13	168.42±54.89	0.265

The table 4 shows the comparison of hematological profile among alive and dead patients. We found mean±SD value for hemoglobin for alive and dead patients was 10.99±1.40 and 10.91±1.17 respectively (p=0.795). The mean±SD value for ESR for alive and dead patients was 46.31±10.06 and 49.94±6.73 respectively (p=0.799). For the parameters like Total WBC Count, Platelet Count, the mean±SD value for alive and dead patients were 6.38±1.19, 6.93±1.85, 180.93±41.13 and 168.42±54.89 respectively with p value 0.226 and 0.265. The mean±SD value for neutrophil and lymphocyte for alive and dead patients was 76.07±6.32, 75.74±11.81, 17.87±5.37 and 17.763±9.44 respectively with p value 0.865 and 0.945 respectively. No significant difference has been observed among the alive and dead patients for these parameters.

4.7 Biochemical Profile of COVID 19 patients according to disease outcome

Finally, the biochemical parameters were compared among patients who were alive during discharge and patients who had died during hospital stay. In the following table 5, significant differences are observed between a few variables.

Table 5: Comparison of Biochemical Profile of COVID 19 patients with disease outcome

Parameters	Mean ± SD		P Value
	Alive	Dead	
CRP	121.87±124.99	207.58±157.87	**0.012**
Ferritin	400.69±229.06	646.56±336.62	**0.000**
D Dimer	0.84±0.67	1.63±1.27	**0.000**
Procalcitonin	0.06±0.06	0.05±0.01	0.563
RBS	9.48±3.52	11.24±3.76	0.061
SGPT	44.00±13.11	45.53±12.67	0.641
SGOT	50.58±11.26	58.13±11.47	**0.021**
Creatinine	1.13±0.77	1.39±0.69	0.180
NT Pro BNP	236.90±114.96	542.68±415.05	**0.000**
hs Troponin I	4.29±4.28	99.09±176.07	**0.004**
PT	14.65±0.89	15.35±0.93	**0.010**
Sodium	134.76±3.76	131.26±5.52	**0.001**
Potassium	3.67±0.46	3.34±0.28	**0.004**
Chloride	98.45±3.15	97.11±3.41	0.101
Bicarbonate	23.46±1.18	23.63±2.52	0.664

As observed in the table 5, CRP (p=0.012), D-dimer (p=0.000), Serum Ferritin (p=0.000), SGOT (p=0.021), NT Pro BNP (p=0.000) and High Sensitive Troponin I (p=0.004), PT

(p=0.010) were significantly raised (p<0.05) among the patients who expired. Additionally, Serum Sodium (P 0.001) and Serum Potassium (P 0.004) were significantly reduced (p<0.05) among the patients who expired. Rest of the parameters have shown no significant difference in relation to outcome.

4.8 Multiple Regression between various significant factors effecting the disease outcome.

Among many factors significantly effecting the outcome of COVID 19 patients, multiple regression was done to identify the single most significant factor effecting the disease outcome. The following tables below demonstrates the findings.

Table 6: Multiple Regression between Various Significant Factors Effecting Disease Outcome: (Model 1)

Factors	Standardized Coefficients Beta	P Value	95% Confidence Interval	
			Lower Bound	Upper Bound
CRP	0.046	0.824	-0.001	0.001
PT	-0.263	0.209	-0.298	0.069
D-Dimer	-0.133	0.573	-0.237	0.135
SGOT	-0.041	0.817	-0.016	0.013
NT-pro BNP	-0.398	**0.046**	-0.001	0.000
hs Trop I	-0.105	0.570	-0.002	0.001
Na+	-0.097	0.661	-0.061	0.039
K+	0.414	0.044	0.013	0.879

In model 1, 8 significant variables such as CRP, PT, D Dimer, SGOT, NT Pro BNP, High Sensitive Troponin I, Na+ and K+ were included for multiple regression and it is seen that NT Pro BNP is most significantly associated with disease outcome (-0.398); (-0.001~0.000); (p value=0.046) among all.

Table 7: Multiple Regression among Various Significant Factors Effecting Disease Outcome: (Model 2)

Factors	Standardized Coefficients Beta	P Value	95% Confidence Interval	
			Lower Bound	Upper Bound
CRP	-0.010	0.958	-0.001	0.001
D-Dimer	-0.237	0.231	-0.262	0.066
SGOT	-0.021	0.884	-0.013	0.012
NT-pro BNP	-0.386	**0.026**	-0.001	0.000
hs Trop I	-0.163	0.313	-0.002	0.001
Na+	-0.009	0.955	-0.036	0.034
K+	0.273	0.074	-0.031	0.647

In model 2, Prothrombin Time was excluded and rest of the 7 significant factors such as CRP, D Dimer, SGOT, NT Pro BNP, High Sensitive Troponin I, Na+ and K+ were included and still NT Pro BNP remains to be the most significant among all (-0.386); (-0.001~0.000); (p value=0.026).

Table 8: Multiple Regression among Various Significant Factors Effecting Disease Outcome: (Model 3)

Factors	Standardized Coefficients Beta	P Value	95% Confidence Interval	
			Lower Bound	Upper Bound
CRP	-0.040	0.828	-0.001	0.001
D-Dimer	-0.211	0.283	-0.249	0.075
SGOT	0.001	0.992	-0.012	0.012
NT-pro BNP	-0.486	**0.001**	-0.001	0.000
Na+	0.000	0.999	-0.035	0.035
K+	0.289	0.058	-0.011	0.663

In model 3, we can see that if we eliminate High Sensitive Troponin I and consider the rest 6 factors such as CRP, D Dimer, SGOT, NT Pro BNP, Na+ and K+, NT Pro BNP is still remains to be the most significant factor effecting the disease outcome (-0.486); (-0.001~0.000); (p value=0.001).

Table 9: Multiple Regression among Various Significant Factors Effecting Disease Outcome: (Model 4)

Factors	Standardized Coefficients Beta	P Value	95% Confidence Interval	
			Lower Bound	Upper Bound
CRP	0.078	0.690	-0.001	0.001
D-Dimer	-0.307	0.142	-0.299	0.045
SGOT	-0.118	0.420	-0.018	0.008
Na+	-0.027	0.867	-0.040	0.034
K+	0.259	0.108	-0.067	0.652
hs Trop I	-0.382	**0.009**	-0.003	0.000

In model 4, If we eliminate the factor NT Pro BNP and retain High Sensitive Troponin I and rest 5 factors such as CRP, D Dimer, SGOT, Na+ and K+, it is seen that High Sensitive Troponin I becomes the most significant factor effecting the disease outcome (-0.382); (-0.003~0.000); (p value=0.009).

Therefore, after comparing all the above models we can assume that among all the significant variables effecting the disease outcome, NT Pro BNP is the most significant. If we eliminate NT Pro BNP then High Sensitive Troponin I becomes the most significant factor effecting disease outcome.

A possible explanation strongly indicates the cause of death to be associated with raised NT Pro BNP owing to heart failure or fluid overload. More over the second most significant factor is High sensitive Troponin I which may rise due to Myocardial Infarction or Viral myocarditis and thus aggravating the heart failure which is evident by the rise in NT Pro BNP levels.

Chapter 5

Discussion

Since the emergence of this pandemic, a plethora of studies have been conducted on COVID-19 with each focusing on various aspects of the disease. The novelty of this coronavirus coupled with the rapid spread of the COVID-19 pandemic has led to a tremendous burden on our already overloaded healthcare system. With the overwhelming number of cases, access to adequate care has become a challenge. In order to provide optimal care for patients, early diagnosis of vulnerable patients who are at risk of severe disease is needed. One option could be using biochemical and hematological testing to identify these patients. Hence this study was conducted in an attempt at identifying what parameters are significantly different among survivors and the deceased patients of COVID-19.

A total of 103 cases were enrolled in this study. Among them, 84 patients recovered and were discharged successfully, while 19 patients progressed in disease severity until they died. The most common age group was 55 to 64 years and the male to female ratio among study subjects was 7.4:1. On comparing age with patient outcome, a significant proportion of deaths (n=14) (p=0.001) occurred in the older age group (above 50 years group). This study showed a greater male: female ratio for hospital admissions due to COVID-19 as compared to other studies of a similar nature. One study in Wuhan China showed an almost equal ratio among the two genders (Wang et al., 2020). A similar observation was also found in a Danish study (Hodges et al., 2020) as well as a Turkish study (Gemcioglu et al., 2021). However, studies conducted in Italy higher risks have been reported in men than in women partly due to higher smoking rates and associated comorbidities (Jordan et al., 2020). One explanation for such a high male female ratio is the existence of gender disparity in access to care among men and women in Bangladesh. Such discrepancies are present in many developing nations of the world. Moreover small sample size might be responsible behind this findings. A study conducted by (Akter and Kim, 2020) reports that women living in such nations are less likely to get tested for Covid-19 and more likely to die without being diagnosed for the disease (Akter and Kim, 2020). Furthermore, male

patients are more likely to have better access to care as compared to women. This is also a plausible reason as to why a significant number of male are reported to have been infected by COVID-19 as compared to females in such countries. As for age group, many studies have reported that elderly population are more likely to be admitted to the ICU or die from the disease (Jordan et al., 2020; Saghazadeh and Rezaei, 2020; Niu et al., 2020). Hence this information is similar to what was found in our study.

For clinical features on admission, the most common symptoms observed among the study subjects was fever followed by cough, headache, dyspnea, anosmia and diarrhea. According to the Centers for Disease Control and Prevention (CDC), the main symptoms of COVID-19 be very mild to severe and include a fever, cough, and shortness of breath (Sheikhi et al., 2020). All of these symptoms were reported among our study subjects. Fever, cough, dyspnea and myalgia were the most common symptoms among admitted patients in several studies (Gemcioglu et al., 2021; Chen et al., 2020; Badedi et al., 2021). Other symptoms such as headache, nausea, diarrhea, anosmia, ageusia, arthralgia, etc. were also reported, but were not as common as the earlier mentioned symptoms (Gemcioglu et al., 2021). Our study showed dyspnea to be the only symptom that had a significant association with death of the patient ($p=0.005$). Although a similar finding was not commonly observed, Gemcioglu et al reported a significant association between case severity and dyspnea. Presence of other symptoms such as cough, fever, myalgia, anosmia, ageusia and arthralgia also had a significant association with disease severity in that study (Gemcioglu et al., 2021).

When hematological parameters were looked at, there were no significant difference found among the patients who expired and lived. Dissimilar observations were found in a study by Badedi et al where deceased patients were reported to have significantly low hemoglobin levels, lymphopenia and thrombocytopenia (Badedi et al., 2021). However, unlike our study, significantly raised WBC count as well as neutrophilia were also reported among the expired patients in that study. Another study by Bairwa et al also reported results that were somewhat not comparable to our study (Bairwa et al., 2021). Here, significantly raised WBC and neutrophil counts; and a significantly low lymphocyte and hemoglobin levels were observed. Like our study or the Badedi study, platelet counts were not

significantly reduced for expired patients (Badedi et al., 2021). On the contrary, other findings such as prothrombin time (PT) and partial thromboplastin time (aPTT) were significantly raised among non-survivors.

In case of electrolyte imbalances, our study showed serum sodium levels (p=0.001) and serum potassium levels (p=0.004) to be significantly low among non-survivors as compared to survivors. Similarly to our study, Bairwa et al found significantly low potassium levels among non-survivors when compared to survivors (Bairwa et al., 2021). Another study by Sjostrom et al found hyponatremia and hypokalemia on admission to be associated with increased risk of mechanical ventilation, however, hyponatremia was not associated with increased risk of death among the patients. This is because, the condition was corrected as soon as it was identified (Sjöström et al., 2021). Another study by Asgar et al found hypernatremia to be significantly associated with COVID-19 deaths which is similar with our study in terms of findings (Asghar et al., 2021). This hypernatremia occurred as a result of over treatment of the patient. Nevertheless, this study agreed that significantly low bicarbonate levels were also associated with death of COVID-19 patients, which is not the case in our study.

Finally, on comparing the biochemical profile of the two patient groups, certain parameters such as CRP (p=0.01), D-dimer (p=0.000), Ferritin (p=0.000), SGOT (p=0.021), NT Pro BNP (p=0.000) and High Sensitive Troponin I (p=0.004), PT (p=0.010) were significantly higher among dead patients. Among the mentioned parameters, D-dimer (Wang et al., 2020; Hodges et al., 2020; Gemcioglu et al., 2021), serum ferritin (Moradi et al., 2021; Tural et al., 2021; Ahmed et al., 2021) serum creatinine (Gemcioglu et al., 2021; Tian et al., 2020) and serum procalcitonin (Bairwa et al., 2021; Hodges et al., 2020) were significantly raised in many other studies. Procalcitonin and IL 6 was not done in all patients of our study sample probably owing to unavailability of the tests or its high cost. Unlike our study, one study by Liu et al. showed HbA1C to be significantly raised among patients who have expired (Liu et al., 2021). Additionally, markers such as LDH, CRP, IL6, and troponin I, were also significantly raised among COVID 19 patients who expired in those studies.

Surprisingly, in our study raised NT Pro BNP was found to be the most significant (p=0.001) factor effecting disease outcome. After comparing all the above models of multiple regression we can assume that among all the significant variables effecting the disease outcome, NT Pro BNP is the most significant. If we eliminate NT Pro BNP then High Sensitive Troponin I becomes the most significant factor effecting disease outcome.

A possible explanation strongly indicates the cause of death to be associated with raised NT Pro BNP owing to heart failure or fluid overload. More over the second most significant factor is High sensitive Troponin I which may rise due to Myocardial Infarction or Viral myocarditis and thus aggravating the heart failure which is evident by the rise in NT Pro BNP levels. This finding is also relatable with the most significant symptoms effecting the disease outcome and justifies Dyspnea to be the most significant symptom associated with adverse disease outcome.

Chapter 6

Conclusion

Aging of the patients were associated with a significantly higher number of deaths in this study (p=0.001). Among all clinical signs and symptoms, dyspnea was the only symptom that had a significant association with death of the patient (p=0.005).When hematological parameters were looked at, there were no significant difference found among the patients who expired and lived. On comparing biochemical parameters, significant differences were observed for certain parameters between patients who survived versus patients who died. Among the parameters were CRP (p=0.01), D-dimer (p=0.000), Ferritin (p=0.000), SGOT (p=0.021), NT Pro BNP (p=0.000) and High Sensitive Troponin I (p=0.004), IL 6 (p=0.015), PT (p=0.010) were significantly higher among the patients who expired. In case of electrolyte imbalances, our study showed serum sodium levels (p=0.001) and serum potassium levels (p=0.004) to be significantly low among non-survivors as compared to survivors. Raised NT Pro BNP was found to be the most significant (p=0.001) factor effecting disease outcome. After comparing all the above models of multiple regression we can assume that among all the significant variables effecting the disease outcome, NT Pro BNP is the most significant. If we eliminate NT Pro BNP then High Sensitive Troponin I becomes the most significant factor effecting disease outcome. A possible explanation strongly indicates the cause of death to be associated with raised NT Pro BNP owing to heart failure or fluid overload. More over the second most significant factor is High sensitive Troponin I which may rise due to Myocardial Infarction or Viral myocarditis and thus aggravating the heart failure which is evident by the rise in NT Pro BNP levels. .

Chapter 7

Limitations

The study had several limitations.

- First of all, owing to time restrictions only 103 cases could be enrolled in our study. A study with a larger sample size would have given more accurate results.
- Secondly, this study was limited to only four hospitals.
- Thirdly, only a limited number of biochemical markers could be studied, thus limiting the findings' implications. In fact, some biochemical markers (particularly IL-6 and LDH) were more likely to be measured among patients with more severe disease, suggesting confounding by indication.
- Finally, the study only included patients admitted to the hospital with COVID-19. Symptomatic patients who are not RT PCR positive were excluded from the studies. As RT PCR is not 100% sensitive, there is a high chance of missing a large number of patients.
- Further study is needed on larger samples to distinguish the etiology behind raised NT Pro BNP.

Chapter 8

Recommendations

- Further studies regarding the biochemical markers using a larger sample size is necessary.
- Additional biochemical parameters such as serum albumin also needs to be included in future studies along with IL-6 and LDH. One study suggested high-density lipoprotein cholesterol could also be a prognostic indicator and hence should be considered for evaluation.
- Complications that develop during the course of the disease is another important factor that needs to be identified in future studies.
- There is a term commonly stated as the long COVID syndrome. Patients suffering from various long term complications of COVID 19 and its treatment needs to be evaluated in separate studies.
- Attempts to early identification of biochemical abnormalities and their prompt management might help us in reducing mortality to further extent.

Chapter 9

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Appendix-I

Informed written consent form for subjects

Title: Association between biochemical markers and adverse outcome of COVID-19 patients without comorbidity

Principal Investigator: Dr. Aditi Goswami

Name of participant: ----------
Name of Investigator: Dr. Aditi Goswami

1. I consent to participate in the project named above, the particulars of which including details of interviews and questionaries' have been explained to me. A written copy of the information's has been given to me to keep with.
2. I authorize the researcher to use with me the interviews and questionaries' referred to under (1) above.
3. I acknowledge that:
   a. The possible effects of the interviews and questionaries' have been explained to me to my satisfaction.
   b. I have been informed that I am free to withdraw myself from the project at any time without explanation or prejudice and to withdraw any unprocessed data previously supplied.
   c. The project is for purpose of research.
   d. I have been informed that the confidentiality of the information's will be safeguarded.
e. I have been informed regarding the interviews. I have also been informed that because of the number of people to be interviewed are small ; it is possible that someone may still be able to identify me on the basis of any references to personal information that might allow someone to guess my identity. However, I will be referred by pseudo name or identified by a different name in any publications arising from the research.

Signature date

(Participant)

Signature date (Witness to consent)

Appendix - II

Questionnaire (English)

ASSOCIATION BETWEEN BIOCHEMICAL MARKERS AND OUTCOME OF COVID-19 PATIENTS: A COMPARATIVE STUDY

Thesis for MPH One Health done by: Dr. Aditi Goswami

SUPERVISER: PROF. DR. S K M AZIZUL ISLAM
Professor, Department of Physiology and Pharmacology, CVASU, Chattogram

1. Demographic profile:

1.1 Name/ID (due to confidentiality)	
1.2 Age (In years Completed)	
1.3 Sex	
1.4 Occupation	
1.5 Area	Rural ☐ Urban ☐

2. Clinical Features:

2.1 Fever	
2.2 Cough	
2.3 Anosmia	
2.4 Headache	
2.5 Dyspnoea	
2.6 Diarrhoea	

3. Heamatological Profile of study subjects

3.1 Hb% (gm/dl)	
3.2 WBC Total Count (x10^9/L)	
3.3 Neutrophil Count %	
3.4 Lymphocyte Count %	
3.5 ESR (in mm in 1stHr)	
3.6 Neutrophil Lymphocyte Ratio	
3.7 Platelet Count (x 10^9/L)	

4. Electrolyte Profile of study subjects

4.1 Serum Sodium (mmol/L)	
4.2 Serum Potassium (mmol/L)	
4.3 Serum Chloride (mmol/L)	
4.4 Serum Bicarbonate (mmol/L)	

5. Biochemical Profile of study subjects

5.1 Prothrombin Time	
5.2 CRP	
5.3 D-Dimer	
5.4 LDH	
5.5 Interleukin 6	
5.6 Serum Ferritin	
5.7 High sensitive Troponin I	
5.8 Serum Creatinine	
5.9 Serum Procalcitonin	
5.10 Random Blood Sugar	
5.11 SGPT	
5.12 SGOT	
5.13 NT Pro BNP	

6. Outcome of disease

6.1 Cured	
6.2 Death	