Chapter 1: Introduction

1.1 Background:

Coronavirus disease 2019, also known as COVID-19, is an infectious disease caused by a newly discovered virus strain called SARS-CoV-2. It was first identified in Wuhan, China, in December 2019 and since then, it has spread globally, which has led to a pandemic that has affected millions of people worldwide with a high transmission rate (WHO, 2020a).

The World Health Organization (WHO) first informed about the outbreak of the virus, although the etiology was not identified then. On January 30, 2020, WHO declared that the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) epidemic is a public health emergency of international concern (PHEIC). On February 11, 2020, WHO termed this disease as coronavirus disease 2019 (COVID-19) (Bi et al., 2020). Later, The International Committee on Taxonomy of Viruses (ICTV) named the virus SARS-CoV-2 (Lai et al., 2020). The virus has spread to nearly every country globally, with thousands of new cases being reported daily, and some countries have been hit harder than others. As of February 28, 2023, 758,390,564 confirmed COVID-19 cases have been reported globally, while the cumulative death is 6,859,093 (WHO, 2023). The impact of the pandemic has been felt most severely in countries with feeble healthcare systems, limited resources, and high population densities. However, countries with advanced healthcare systems have even struggled to combat the pandemic due to its sheer impact.

COVID-19 has harshly affected the Health Care System of Bangladesh, including public health, where the healthcare system is under-resourced and overburdened. It affects almost every aspect of life including the economy, education, and social interactions. The government reported the first case of COVID-19 on March 8, 2020, and since then, the country has been grappling with the pandemic. According to the World Health Organization, over 20 million confirmed cases of COVID-19 and nearly thirty thousand deaths were reported in Bangladesh till February 28, 2023 (Worldometer, 2023). However, the country's actual number of cases and deaths may be higher than the reported figures due to factors such as limited testing capacity, underreporting and the absence of obligatory autopsy to find the actual cause of death. Moreover, asymptomatic cases could not be identified. An investigation discovered that

up to 23% of the patients diagnosed with COVID-19 from December 2020 to February 2021 in Bangladesh were asymptomatic (Hossain et al., 2021). Chittagong, the second largest city of Bangladesh, is at high risk of rapid transmission of COVID-19 due to its high population density and weak healthcare infrastructure. Moreover, being the port city and one of the most crowded economic and trading centers of Bangladesh, it is classified as a high-risk zone for SARS-CoV-2 transmission (Rana et al., 2020). On April 3, 2020, Chattogram city witnessed its first Coronavirus Disease 2019 (COVID-19) positive case, followed by the first death on April 9. During an infectious disease outbreak, seroprevalence studies are essential for detecting undiagnosed infections in the community and preventing post-pandemic recurrence (Bryant et al., 2020). The real burden of infection must also be estimated for epidemic forecasting and response planning. Seroprevalence studies are successful at recognizing the number of undetected missing cases with mild or no symptoms or who are unable to undergo testing, which may play a substantial role in the transmission (Shakiba et al., 2021). While nucleic acid amplification, such as polymerase chain reaction (PCR), is the gold standard for diagnosing acute SARS-CoV-2 infection and is widely recommended, the antibody-based approach improves diagnosis accuracy by capturing asymptomatic testing and recovered infections (Vogl et al., 2021; Thomas et al., 2021). According to numerous research, seropositivity fluctuates considerably depending on parameters such as location and time (Shakiba et al., 2021). However, vaccination coverage and seroprevalence among the public must be investigated nationwide to know the herd immunity. Antibody prevalence monitoring at a large scale in population studies can reveal parameters linked to vaccine immunogenicity and response durability.

Nevertheless, numerous interventions were undergone by the government to respond to the COVID-19 pandemic. The lockdown was an effective approach to slow down the COVID-19 transmission rate by deterring people from all kinds of public gatherings. Moreover, the government-imposed laws for travel restrictions and social distancing and made wearing masks compulsory during the pandemic. Vaccination campaigns were launched to immunize the population against the virus to impede disease transmission. The development of potent SARS-CoV-2 vaccines has proven to be a highly effective strategy for minimizing COVID-19 illness burden as well as death (Higdon et al., 2022; Bar-On et al., 2021). Vaccines consistently protect more than 90% of the population against hospitalizations and (Chuenkitmongkol et al., 2022). Bangladesh began the administration of COVID-19 vaccines on January 27, 2021, with

first dose, the second dose on April 7, 2021, and the third dose on December 28, 2021 (DGHS dashboard). As of February 28, 2023, 88.43 (%) 1st dose, 80.52% 2nd dose, and 49.14% 3rd dose had been administered (WHO, 2023).

Centers for Disease Control and Prevention (CDC) and World Health Organization (WHO) recommended that healthcare personnel should be the first ones to be offered the 1st dose of vaccine, and, therefore, HCWs have been at the forefront of SARS-CoV-2 vaccination program internationally (CDC, 2021; WHO, 2021). It is also worth mentioning that the government of Bangladesh has prioritized the HCWs for vaccination since the beginning of the vaccination campaign.

Vaccination against COVID-19 is crucial for protecting HCWs from getting infected, reduces the risk of transmission from patients, and prevents the collapse of the healthcare system. It also helps to build herd immunity, as they have the most potential for direct and indirect infections. Those directly involved in diagnosing, treating, and caring for COVID-19 patients are at risk of infection (Bi et al., 2020). Dr Li Wenliang (China), an ophthalmologist in Wuhan General Hospital, died on February 7, 2020, after treating patients of COVID-19 admitted in Intensive Care Unit (ICU) (Misra, 2020). Moreover, occupational exposures among HCWs have been documented in numerous nations as worrying (Mahmud et al., 2021). According to the latest data from the Bangladesh Medical Association, between March 8, 2020 to November 11, 2021, 9455 HCWs, including physicians, nurses, and other staff, were infected with COVID-19, and 188 doctors died as a result.

Antibodies serve as biomarkers of immunity; that's why the detection of specific antibodies can provide immunity against SARS-CoV-2. Quantitative assays detecting the anti-SARS-CoV-2 antibodies may help to determine the specific antibodies. A greater understanding of antibody responses to SARS-CoV-2 after natural infection might aid in the development of more successful vaccination strategies in the future. Despite the greater importance of this issue, it is still unclear whether the antibodies against SARS-CoV-2 correlate with protective immunity and for how long.

Numerous studies worldwide reported that seroprevalence to SARS-CoV-2 among HCWs was higher in comparison to the general (Shields et al., 2020; Eyre et al., 2020; Ristić et al., 2022). Nevertheless, minimal studies exist regarding seroprevalence in targeted populations like HCWs in Bangladesh. A recent study exploring seroprevalence showed a high prevalence among HCWs. Moreover, they also explored

antibody titers with a small duration (6 months) in different occupations in Bangladesh (Ara et al., 2022).

Due to the course of their work and potential exposure to workplace hazards to a SARS-CoV-2 infection, HCWs and those employed in healthcare system (Administrative staff) have a higher risk of infection. Finally, it is vital to know whether the vaccination improves immunity and for what duration among them. Therefore, the immunity to antibodies of extended duration (after six months) in Bangladesh may help to develop a proper vaccination strategy for their occupations. Considering the facts, the present study was conducted to report population-based anti-SARS-CoV-2 seropositivity among vaccinated HCWs of various government and private hospitals of CMP area, as determined by Immunochromatographic Test (ICT) and Qualitative measures; enzyme-linked immunosorbent assay (ELISA). Moreover, we measured the antibody titer by quantitative enzyme-linked immunosorbent assay (ELISA) to assess the durability of antibody titer over a long duration. Furthermore, the results of both tests (seropositivity and antibody titer) were evaluated to see if there was an agreement between these two. The research will also aid in determining the duration of antibodies following vaccination and the appropriate time interval between booster doses in HCWs.

1.2. Rationale:

1.2.1 Justification of the study

Since the first cases of COVID-19 were described in December 2019, a health system along with major social and economic world scientific communities was focused on the development of an effective vaccine. Moreover, a large proportion of people being asymptomatic though they can spread infection, was observed, and asymptomatic individuals do not seek tests. So, a wide range of populations remain undiagnosed (Vogl et al., 2021). Therefore, the Seroprevalence study might provide the true prevalence of the SARS-CoV-2 infection in the community and the detection of under-detecting COVID-19 infection, including asymptomatic and past infection.

To evaluate the immune response to the vaccines, the quantification of serum-neutralizing type G immunoglobulins (IgG) produced after vaccination should be determined (Widge et al., 2021). These antibodies are specific to the receptor binding domain (RBD) of the subunit S1 of the spike protein of SARS-CoV-2, and therefore they are called anti-RBD antibodies (Widge et al., 2021). Besides, various factors can

influence the immunological response. For example, an altered response can be expected in cases of immunosuppressive patients or having comorbidities (Colucci et al., 2021), while increased antibody production can be related to a previous exposure to the infectious agent that the vaccine intends to prevent (Chia et al., 2021). Individual factors, such as smoking, obesity, and hypertension, can also possibly influence the response. There are very few studies that have explored the seroprevalence of anti-SARS-CoV-2 antibodies in Bangladesh so far.

It is worth mentioning that due to the potential exposure hazards to a SARS-CoV-2 infection, health Care Workers, as well as those employed in the healthcare system (Administrative Staff), are more critical in the ongoing response than the general population (Shields et al., 2020). However, there was no study on the occupational variance in HCWs in Bangladesh as well as in Chattogram.

1.2.2 Significance of the study:

To gain insights into the real magnitude of anti-SARS-CoV-2 antibody among vaccinated HCWs and for decision-making purposes, especially to formulate vaccination strategies (how many doses of vaccine on what duration) to prevent the infection, it is very important to determine the level of antibody developed after vaccination along with its longevity in the human body. Moreover, we can learn about the duration of dose interval needed to be maintained among the risk population. Our study provides crucial information on the persistence of circulating antibodies against SARS CoV-2 in more than 8 months intervals after vaccination in case of 1st and 2nd doses and a few months after the 3rd dose as well as associated factors that can alter the immune response.

1.3 Objectives:

1.3.1 General Objective:

To evaluate the seroprevalence and titer of anti-SARS-CoV-2 antibodies among the vaccinated COVID-19 Health Care Providers.

1.3.2 Specific Objectives: `

1. To evaluate the immune response to different doses of the vaccine among health care workers.
2. To identify the potential variables that might be associated with different titers and seroprevalence of anti-SARS-CoV-2 antibodies.
3. To evaluate the diagnostic performance of ICT compared to ELISA.

Chapter 2: Literature Review

2.1 COVID-19 Pandemic and causative agent

The COVID-19 pandemic outbreak is the most astounding scene ever experienced in the XXI century. COVID-19 was first reported in Wuhan, Hubei province, China, in late 2019; it had spread rapidly to more than 180 countries by early 2020. As of February 9, 2021, more than 100 million COVID-19 cases, including 2 316 389 deaths, had been reported in 223 countries or regions (WHO, 2021). Consequently, on February 11, 2020, the WHO officially named the current outbreak of coronavirus disease as Coronavirus Disease-2019 (COVID-19) (Chuenkitmongkol et al., 2022).

The emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) rapidly evolved into a global pandemic. Following exponential growth, COVID-19 was declared a world pandemic by the World Health Organization—WHO (WHO, 2020a) on March 11. Globally, As of December 31, 2022, 640395651 confirmed cases of COVID-19, including 6618579 deaths, were reported to WHO (WHO, 2022b). However, these case counts might inevitably underestimate the true cumulative incidence of infection because of limited diagnostic test availability, barriers to testing accessibility, and asymptomatic infections.

2.2 SARS-CoV-2 Structure

Coronaviruses belong to the subfamily *Coronavirinae* in the family of Coronaviridae, and the subfamily contains four genera: *Alphacoronavirus*, *Betacoronavirus*, *Gammacoronavirus*, and *Deltacoronavirus*. The genome of CoVs (27–32 kb) is a single-stranded positive-sense RNA (+ssRNA) more extensive than any other RNA viruses. In addition, the SARS-CoV-2 genome encodes 23 putative nonstructural proteins. The nucleocapsid protein (N) forms the capsid outside the genome, and the genome is further packed by an envelope that is associated with three structural proteins: membrane protein (M), spike protein (S), and envelope protein (E) (Lai et al., 2020). Furthermore, the SARS-CoV-2 genome comprises 10Open Reading Frame (ORF). ORF1ab encodes a large polyprotein that is proteolytically cleaved into 16 nonstructural proteins (NSP1–16). In the first ORF (ORF1a/b), about two-thirds of viral is present that encodes for polyprotein1a and polyprotein 1b. ORF1ab encodes for a large polyprotein that is

proteolytically cleaved into 1–16 nonstructural proteins (NSP1–16). (Satarker and Namphoothiri, 2020). Other ORF3a, ORF3b, ORF6, ORF7a, ORF7b, ORF8 and ORF10 may encode for proteins, but their functions are yet to be determined. However, it is unclear whether neutralizing antibodies to S protein are the major contributor to a protective immune response.

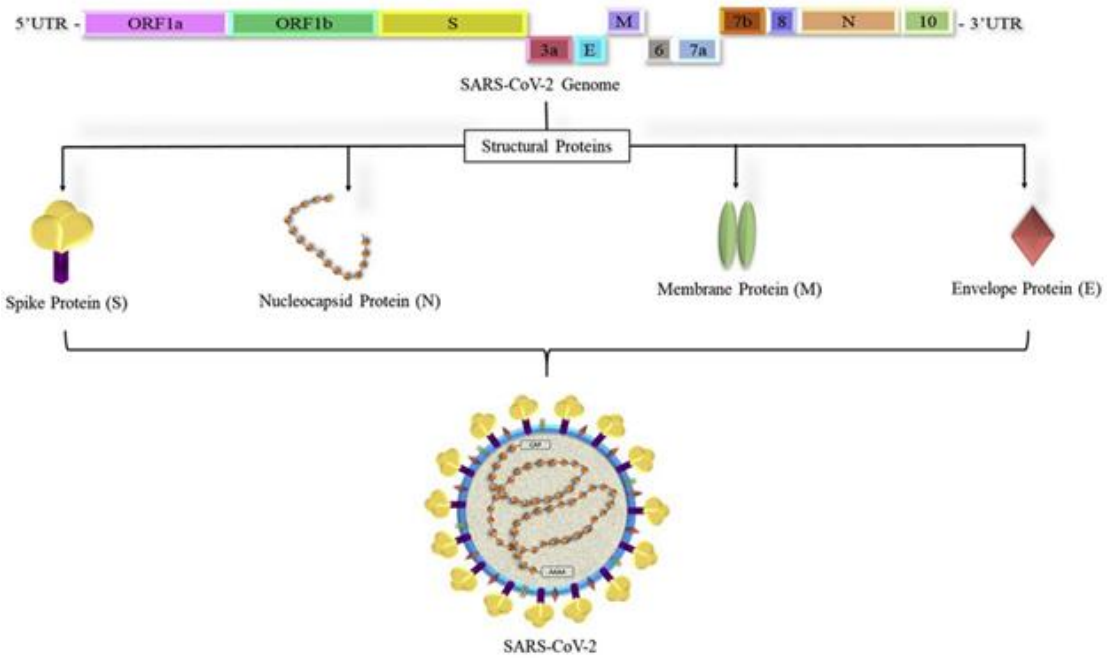


Figure 2.1: Genomic sequence of SARS-CoV-2. ORF–Open Reading Frame, UTR-Untranslated region, S-Spike protein, M-Membrane protein, E-Envelope protein, N-Nucleocapsid protein

Source: (Satarker & Nampoothiri, 2020).

2.3 Pathogenesis and host response

The SARS-CoV-2 virus can infect a wide range of cells and systems of the body. COVID-19 is most known for affecting the upper respiratory tract (sinuses, nose, and throat) and the lower respiratory tract (windpipe and lungs). The lungs are the organs most affected by COVID-19 because the virus accesses host cells via the receptor for the enzyme angiotensin-converting enzyme 2 (ACE2), which is most abundant on the surface of type II alveolar cells of the lungs. The virus uses a particular surface glycoprotein called a "spike" to connect to the ACE2 receptor and enter the host cell (Harrison et al., 2020).

Multiple viral and host factors affect the pathogenesis of the virus. The spike protein is the viral component that attaches to the host receptor via the ACE2 receptors. It includes two subunits: S1 and S2. S1 determines the virus-host range and cellular tropism via the receptor-binding domain. S2 mediates the membrane fusion of the virus to its potential cell host via the H1 and HR2, which are heptad repeat regions. Studies have shown that the S1 domain induced IgG and IgA antibody levels at a much higher capacity. The focus spike protein expression is involved in many effective COVID-19 vaccines (Dai & Gao, 2021).

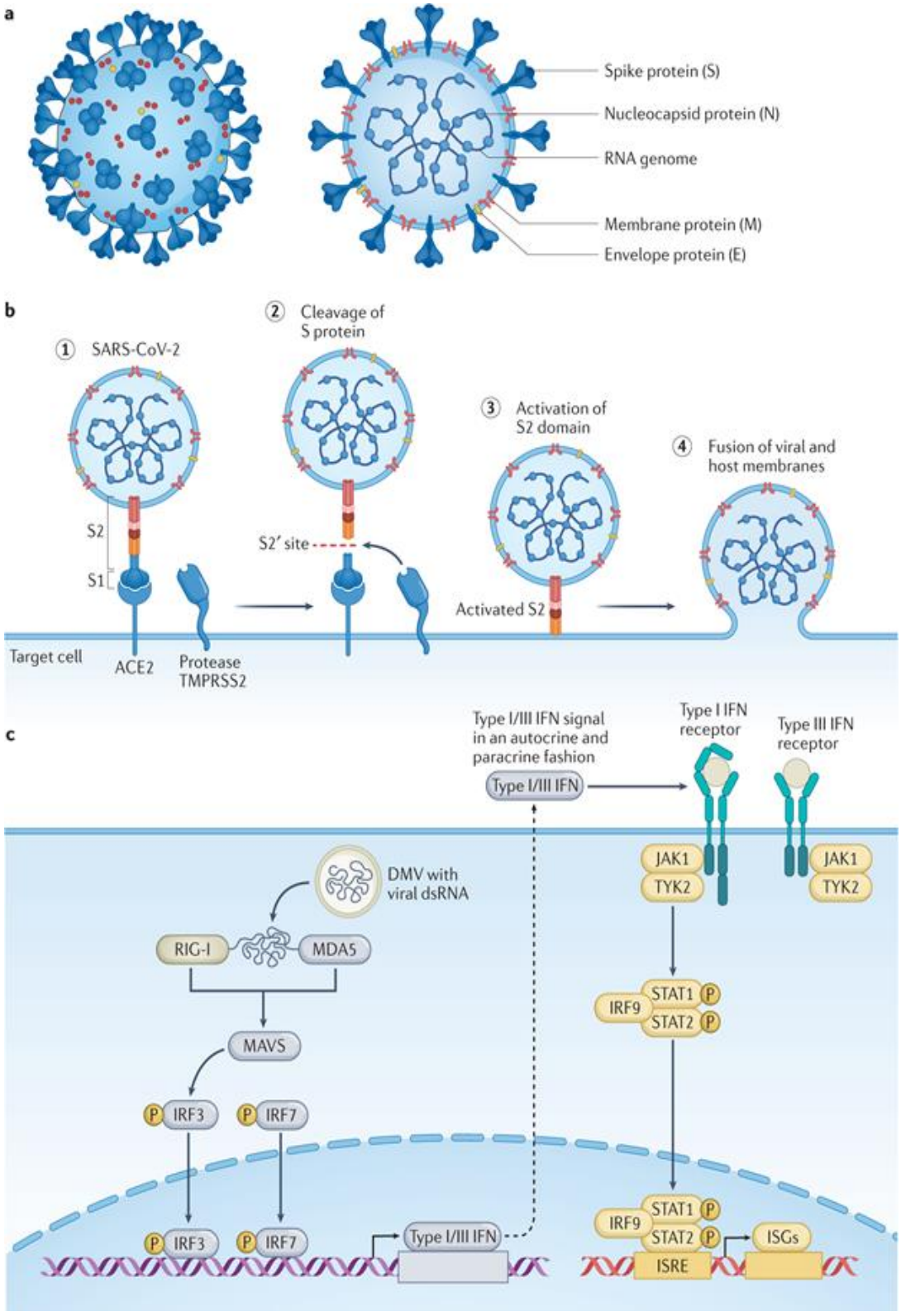


Figure 2.2: Pathogenesis of COVID-19

Source: (Harrison et al., 2020).

2.4 Immunopathogenesis of SARS CoV-2

Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) directly interacts with the host's epithelial and immune cells, leading to inflammatory response induction, which is considered the hallmark of infection. The host immune system is programmed to facilitate the clearance of viral infection by establishing a modulated response. However, SARS-CoV-2 takes the initiative, and its various structural and non-structural proteins directly or indirectly stimulate the uncontrolled activation of injurious inflammatory pathways through interaction with innate immune system mediators.

Indeed, it is pretty apparent now that the extensive tissue and organ damage and high mortality following SARS-CoV-2 infection cannot be attributed to the limited pathogenic effect of viral propagation alone. Instead, the outcome of the disease caused by this virus is forcefully dependent on the dilemma of protective or pathogenic host immune response. This emphasizes the central role of the improperly severe inflammatory aspects of immune response in the pathogenesis of the COVID-19 disease (Shahgolzari et al., 2021).

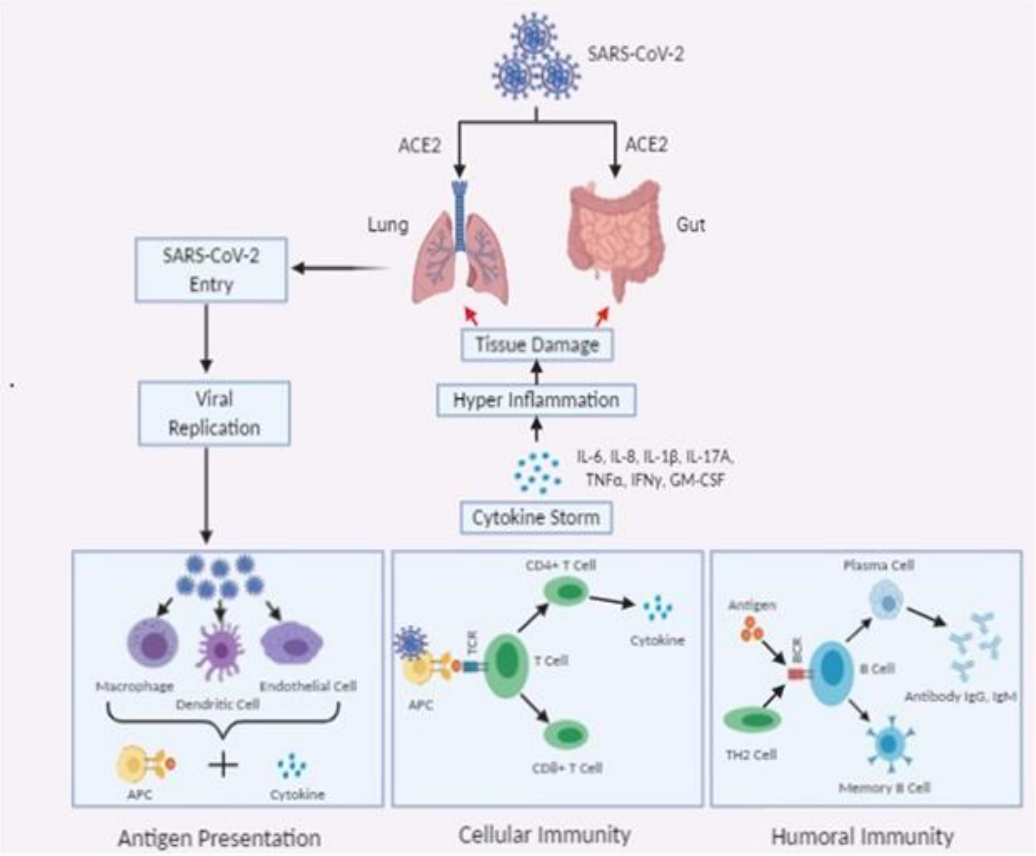


Figure 2.3: Immunopathogenesis Against COVID-19

Source: (Chatterjee et al., 2020).

2.5 Variants of SARS-CoV-2:

Viruses naturally accumulate mutations over time; since SARS-CoV-2 was first identified in China, thousands of mutations have been recorded (Alam et al., 2021). Variants are grouped into either clades or lineages in collaboration with partners, expert networks, national authorities, institutions and researchers; the WHO has been monitoring and assessing the evolution of SARS-CoV-2 since January 2020 WHO gathered scientists from the Technical Advisory Group on Virus Evolution (now known as the Technical Advisory Group on Virus Evolution), the WHO COVID-19 reference laboratory network, representatives from GISAID, Nextstrain, Pango, and additional experts in virological, microbial nomenclature, and communication from several countries and agencies to discuss simple-to-pronounce and non-stigmatizing labels for variants of interest (VOA) and variants of concern (VOC) (Happi et al., 2021).

Since the emergence of the SARS-CoV-2 virus, many variants have been reported; some are particularly important due to their increased virulence and transmissibility, as well as their abilities to evade the immune response. The more essential variants of SARS-CoV-2 include cluster 5, lineages B.1.1.7 (Alpha variant), B.1.351 (Beta), P.1 (B.1.1.28/Gamma), B.1.427/B.1.429 (Epsilon), B.1.526 (Iota) and B.1.617.2 (Delta) confer mutations in their respective spike proteins, which enhance viral fitness by improving binding affinity to the ACE2 receptor and lead to an increase in infectivity and transmission. These spike protein mutations provide resistance against immune responses, either acquired naturally or induced by vaccination (Salleh et al., 2021).

The Alpha variant (formerly called the UK variant) was first found in London and Kent, the Beta variant (formerly called the South Africa variant), the Gamma variant (formerly called the Brazil variant), and the Delta variant (formerly called the India variant).

Another variant, the Omicron variant (B.1.1.529), a fifth variant of concern, contains a large number of mutations that were previously reported in other VOCs, including at least 32 mutations in the spike protein alone compared to the 16 mutations in the already highly infectious delta variant. Omicron was quickly identified as being significantly more transmissible than Delta, the preceding variant of concern. Within four weeks, as the Omicron wave travelled worldwide, it replaced Delta as the dominant variant (WHO, 2020). This variant mutated 50 times and holds more than 30 changes in its spike protein. Another vital variant, Lineage B.1.427/B.1.429, also known as CAL.20C or Epsilon variant (WHO), was initially detected in California. Another

discovered variant is B.1.526 (lota variant, WHO) has a set of common mutations in the S glycoprotein: L5F, T95I, D253G, E484K, D614G and A701V (Salleh et al., 2021).

2.6 Transmission:

The remarkable spread of SARS-CoV-2 infection plays an essential role in seropositive ty, which might have a higher capability to develop immunity against SARS-CoV-2 infection. Seropositivity may be proportionally related to the spread of infection with time. Therefore, it is a matter of research to see if the immunity develops after infection with duration. Furthermore, asymptomatic COVID-19 infection is possible, and person-to-person transmission can occur from asymptomatic COVID-19 carriers to the community (Yu & Yang, 2020; Verma et al., 2023) measured the seropositivity and found 38% of participants were symptomatic, while 61.1% were asymptomatic. Moreover, symptomatic ones have more antibody titers than asymptomatic ones. They also had a high seroprevalence rate (Verma et al., 2023). Coronavirus transmission may be through fomite, droplets and aerosols that CDC describes.

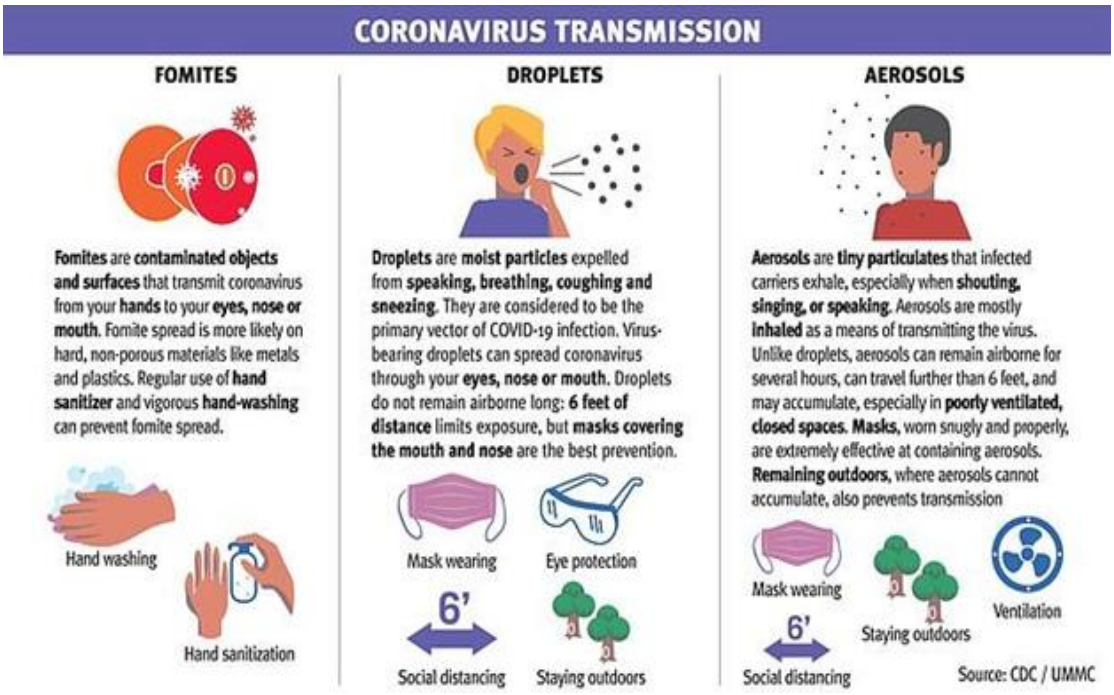


Figure 2.4: Mode of spread of COVID-19

Source: Centers for Disease Control and Prevention (CDC)

For SARS-CoV-2, various modes of transmission had been proposed. They are; aerosol-generating, respiratory droplets, Droplets on surfaces, and contact with contaminated surfaces (Harrison et al., 2020; Mehraeen et al., 2020). Moreover, oral and faecal secretions can be the mode of transmission. Consequently, the human environment will become a potential medium of virus transmission (Mehraeen et al., 2020).

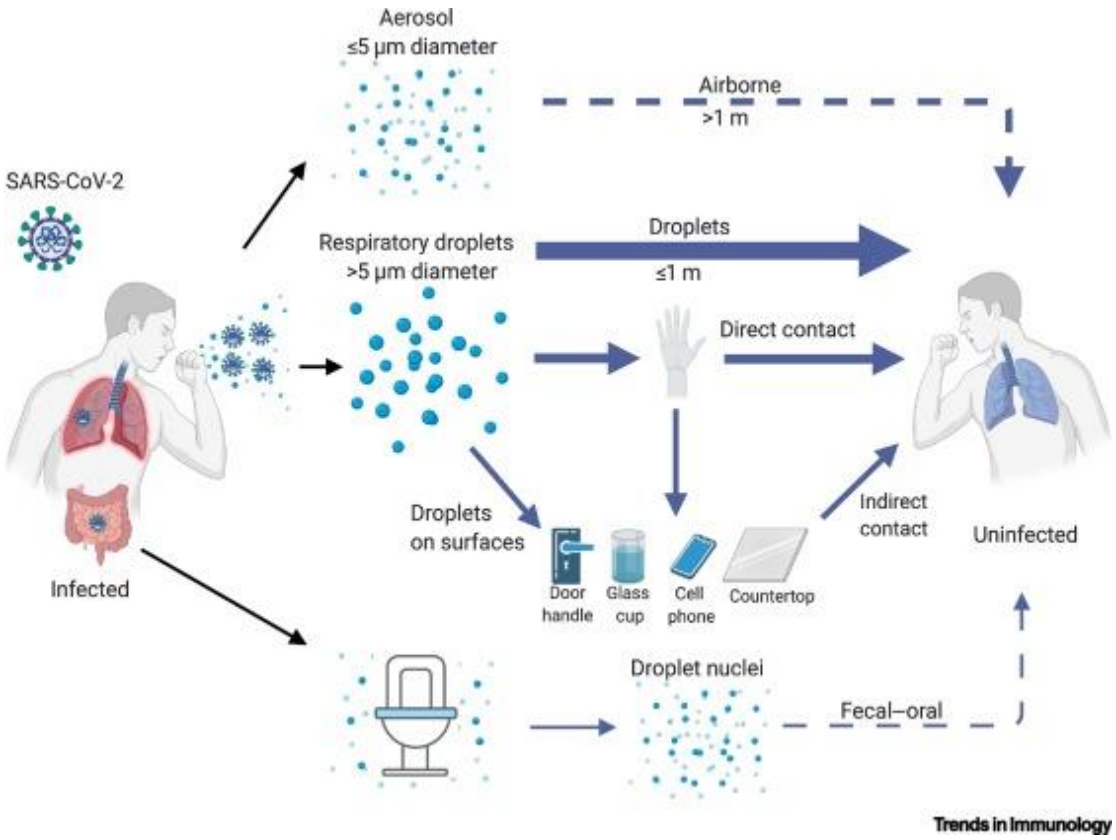


Figure 2.5: Proposed Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Transmission Routes

Source: (Harrison et al., 2020).

2.7 Symptoms and Signs of COVID-19

COVID-19 showed both symptomatic and asymptomatic infections. In a narrative review of 16 cohort studies on the prevalence of asymptomatic SARS-CoV-2 infection, it is estimated that approximately 40–45% of people with a positive swab remain asymptomatic (Oran & Topol, 2020).

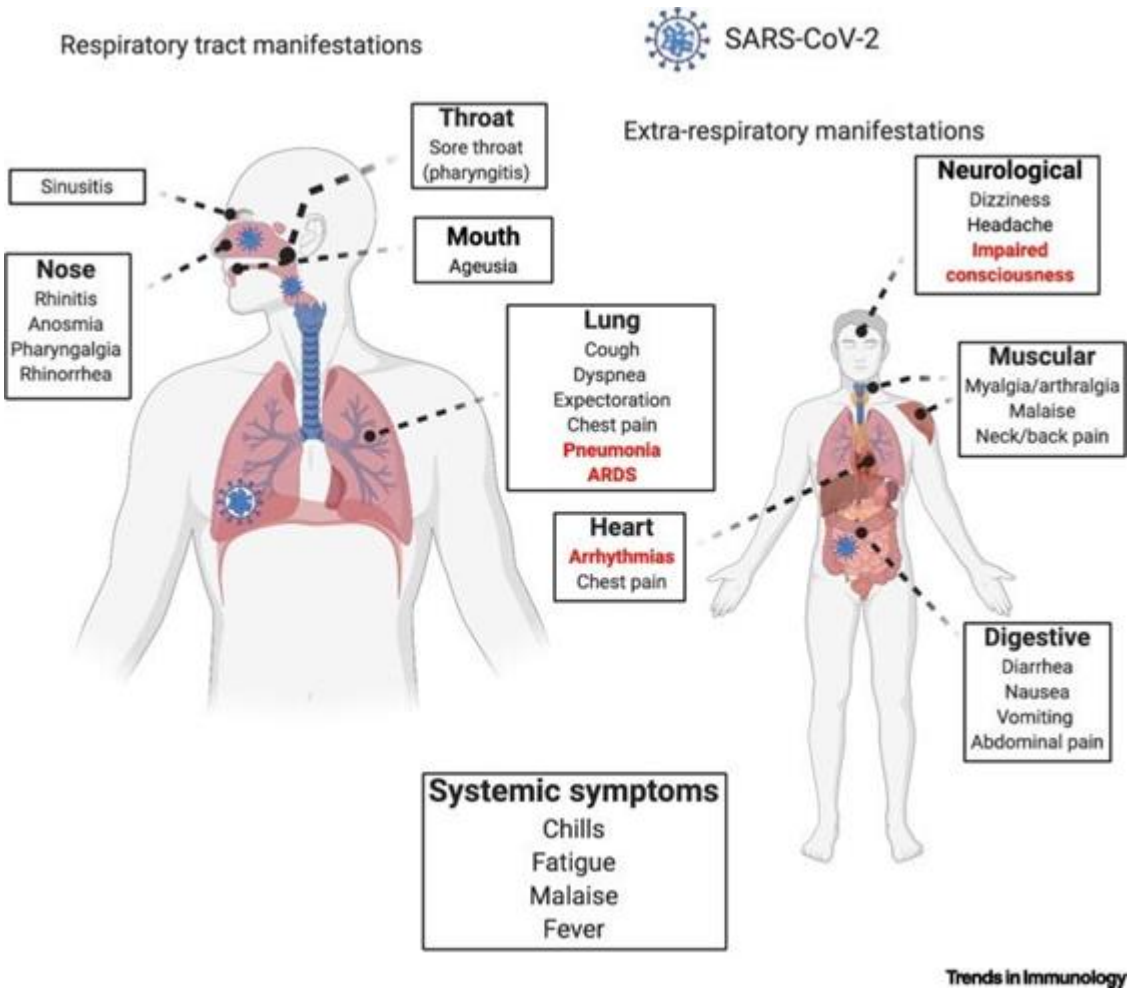


Fig 2.6: Coronavirus disease 2019 Clinical Symptoms (COVID-19)
Source: (Harrison et al., 2020).

On the contrary, human COVID-19 symptoms are reported to affect various bodily systems, with varied rates of onset and severity. The upper and lower respiratory tract manifestations and systemic symptoms that are most reported independent of illness severity are frequently the most obvious.

Considering the most common early symptoms of COVID-19 patients include fever, cough, fatigue, and dyspnea. A systematic review and meta-analysis of all articles published from January 1, 2020, to April 2, 2020, showed multiple Clinical Symptoms of COVID-19. They found; fever 81.2% (95% CI: 77.9-84.4); cough: 58.5% (95% CI: 54.2-62.8); fatigue 38.5% (95% CI: 30.6-45.3); dyspnea: 26.1% (95% CI: 20.4-31.8); and the sputum: 25.8% (95% CI: 21.1-30.4). However, chest tightness, diarrhoea, sore throat, hemoptysis, headache, my lgia, etc., were less common (Alimohamadi et al.,

2020). In one retrospective study from January to February 2020, the clinical features of the patients as well as the pattern, morphology, and concomitant symptoms of lung lesions, were studied in all patients with SARS-CoV-2 infection, identified by real-time polymerase chain reaction (PCR). Most infected individuals reported fever and cough and had a history of exposure. 59% of patients had more than two lobes affected. Apart from this, more than half of the patients had bilateral, multifocal lung lesions with peripheral dissemination. Ground glass opacities were seen in 72%, consolidation in 12%, crazy paving pattern, interlobular thickening in 37%, pleural thickening in 56%, and combined linear opacities in 61%, consolidation in 13%, crazy paving pattern in 12% of the patients. Lymphadenopathy, pericardial effusion, and pleural effusion were unusual observations (Cao et al., 2020).

Although COVID-19 is most well-known for causing substantial respiratory pathology, it can also result in several extrapulmonary manifestations. These conditions include thrombotic complications, myocardial dysfunction and arrhythmia, acute coronary syndromes, acute kidney injury, gastrointestinal symptoms, hepatocellular injury, hyperglycemia and ketosis, neurologic illnesses, ocular symptoms, and dermatologic complications (Karia et al., 2020).Typical symptoms include fever, fatigue, cough, shortness of breath, and anosmia (loss of smell), present in the majority of individuals, whereas atypical symptoms range from gastrointestinal discomfort (diarrhoea, nausea) to dizziness/confusion. (Kimball et al., 2019; Kim et al., 2020).

People with COVID-19 have reported a wide range of symptoms – ranging from mild to severe illness. Symptoms may appear 2-14 days after exposure to the virus. Anyone can have mild to severe symptoms. Possible symptoms include fever or chills, cough, shortness of breath or difficulty breathing, fatigue, muscle or body aches, headache, loss of taste or smell, sore throat, congestion or runny nose, nausea or vomiting, and diarrhea. However, this list does not include all possible symptoms. Symptoms may change with new COVID-19 variants and can vary depending on vaccination status. Older adults and people who have underlying medical conditions like heart or lung disease or diabetes are at higher risk of getting very sick from COVID-19 (WHO, 2022c).

2.8 Diagnosis of COVID-19

COVID-19 showed both symptomatic and asymptomatic infections. Due to the unavailability of detecting asymptomatic COVID-19 patients, the global prevalence of SARS-CoV-2 infections remains unknown. Moreover, COVID-19 diagnosis is challenging due to the variance in symptoms.

An estimate suggests that about 40-45% of asymptomatic COVID-19 patients remain asymptomatic worldwide and have the tremendous potential to spread silently and deeply (Oran & Topol, 2020). On 3 February 2020, 19.2% of passengers tested positive for COVID-19 in Japan. 46.5% of individuals having tested positive were asymptomatic at the time of testing. While later, several of them showed subclinical lung abnormalities. When 76 of these people's computed tomography images were evaluated, 54% had lung opacities (Oran & Topol, 2020).

Another study revealed the proportion of asymptomatic persons as 17.9%. The proportion of asymptomatic individuals appears to be 16.1% (35/218) before 13 February, 25.6% (73/285) on 15 February, 31.2% (111/355) on 16 February, 39.9% (181/454) on 17 February, 45.4% (246/542) on 18 February, 50.6% (314/621) on 19 February and 50.5% (320/634) on 20 February (Mizumoto *et al.,* 2020). Widespread outbreaks of COVID-19 in the correctional facilities of several states of the USA (Arkansas, North Carolina, Ohio, and Virginia) have led to large-scale screening programs. In this study, 3277 (69.8%) tested positive, of whom 3146 (96%) had no symptoms at the time of testing (Kimball et al., 2019). Currently, the diagnosis of COVID-19 in sputum samples, RT-PCR, continues to be the gold standard. The diagnosis is confirmed by the detection of SARS-CoV-2 via real-time reverse transcription polymerase chain reaction (qRT-PCR) assays that target open reading frame-1 antibodies, envelope proteins, nucleocapsid proteins, RNA-dependent RNA polymerase genes, and the N1, N2, and N3 target genes, among suspected cases with an exposure history and signs/symptoms of SARS-CoV-2 infection (Lai et al., 2020).

Rapid antigen detection (RAD) immunoassays have emerged as a valuable alternative to RT-PCR for diagnosing SARS-CoV-2 infection in patients presenting with clinically compatible COVID-19. RAD tests are simple to carry out and return results quickly, thus being well-suited for point-of-care testing (POCT). RAD assays may be used for SARS-CoV-2 culture. However, the study shows; the overall sensitivity and specificity of RAD were 48.1% and 100% (Torres et al., 2021).

A systematic review with a meta-analysis of sixteen studies was evaluated regarding diagnosing COVID-19; it showed that computed tomography has high sensitivity 91.9% but low specificity 25.1%. The combination of IgM and IgG antibodies demonstrated promising results for both parameters. For RT-PCR tests, rectal stools/swabs, urine, and plasma were less sensitive, while sputum presented higher sensitivity 97.2% for detecting the virus. To achieve acceptable sensitivity and specificity, it is strongly advised to combine different diagnostic tests (Böger et al., 2021).

These difficulties may restrict our understanding of the scope of SARS-CoV-2 infection and further affect the implementation of infection control and prevention policies. Adopting a serological test to identify anti SARS-CoV-2 antibodies might be a more accurate approach to estimating the actual burden of SARS-CoV-2 infection and help improve understanding of the associated epidemiology (Lai et al., 2020). Therefore, seroprevalence studies have vast importance for compounding the scenario of COVID-19 prevalence with asymptomatic.

Currently, more than 300 tests are available for SARS-CoV-2 antibody detection. These tests have been produced in several formats, and they detect different types of antibodies, including IgG, IgM or IgA subtypes or total immunoglobulin. In addition, the target proteins used to detect antibodies may vary between the tests. Commercial tests are usually designed to detect antibodies against SARS-CoV-2 nucleocapsid (N), spike1 (S1), spike2 (S2), or receptor-binding domain of the spike (S-RBD) protein, or their combinations, though not all commercial providers specify the viral proteins used. Given the large variability in antibody tests, discrepancies between test results are expected. Concordantly, at the moment, no agreement exists upon which viral protein should be used as a gold standard in the serodiagnosis of COVID-19 patients. Naaber et al. (2020) highlighted the importance of considering clinical symptoms, time of testing and using more than one viral antigen in SARS-CoV-2 antibody testing. Most clinical studies and validations of commercial tests have been performed in patient groups with severe disease, and thus reported sensitivity data may not be the same for COVID-19 patients with mild symptoms (Naaber et al., 2020).

2.9 Seroprevalence of SARS-CoV-2

COVID-19 has been one of the major concerns for the last three years. One of the challenges of this disease is to determine its prevalence. Serological surveys help to

determine the extent of infection by a viral agent in a population and identify associated risk factors. SARS-CoV-2 infections are symptomatic and asymptomatic; asymptomatic individuals have similar viral loads to those who are symptomatic and have a considerable role in transmitting the disease. Except for seroprevalence studies, most asymptomatic infections cannot be detected. Nevertheless, seroprevalence estimates vary widely depending on the country, region and risk groups, infection, and vaccination coverage.

Since January 2020, serological surveys of SARS-CoV-2 have been carried out in the general population on all continents. The seroprevalence was estimated to be 3.2% (interquartile range 1-6.4%) in 184 studies conducted in the general population. An association between a positive serological test and demographic characteristics have been reported (Bobrovitz et al., 2021).

An updated systematic review and meta-analysis of a few cohort and cross-sectional studies worldwide based on the year from December 2019 to December 2021 studies have been conducted in 88 countries: (Eastern Mediterranean, Africa, America, Europe, Western Pacific). Results showed that SARS-CoV-2 seroprevalence is between 3 and 15%. There is a variation in the pooled estimate of seroprevalence SARS-CoV-2. According to this study, in the Eastern Mediterranean; it is 15% (CI 95% 5-29%), and in Africa; it is 6% (CI 95% 1-13%), In America; 8% (CI 95% 6-11%), and in Europe; 5% (CI 95% 4-6%) (Azami et al., 2022).

After the first epidemic peak, Iquitos had one of the highest rates of seroprevalence of anti-SARS-CoV-2 antibodies worldwide, where also, variation perceived in the region. A study conducted in the four districts of Iquitos by an immunochromatographic assay, COVID-19 IgG/IgM Rapid Test Cassette (Zhejiang Orient Gene, Biotech, China) on capillary blood samples among a specific age group of sex from household members were reported about seroprevalence in July 2020, reported a seroprevalence of 29·7% in the region of Lambayeque; and another done in December 2020, found a seroprevalence of 39·3% in the regions of Lima and Callao, using population-based sampling techniques. Moreover, the seroprevalence rate increases with time (Álvarez-Antonio et al., 2021).

There are few published serosurveys in low-income and middle-income countries of Asia. However, the studies which have been done show a similar pattern. A Nationwide COVID-19 serosurvey conducted in India, the nearest country to Bangladesh, indicated an increase in seroprevalence. Two national serosurveys were

done in India among the general population and third and fourth serosurveys among the general population and HCWs. The first national serosurvey on severe acute respiratory syndrome (SARS-CoV-2) in India, done in May–June 2020, among adults aged 18 years or older, found a SARS-CoV-2 IgG antibody seroprevalence of 0·73% (95% CI 0·34–1·13). In the second serosurvey, August–September 2020, it is 0 7.1%. According to their study, adjusted seroprevalence was found to be higher in slums than in non-slums. The higher prevalence in slums could be driven by population density, lower adherence to distancing measures, and poorer hygiene. Following 1st And 2nd study, a third serosurvey was conducted between December 2020 and January 2021. Weighted and assay-characteristic-adjusted seroprevalence against either of the antibodies was 24.1% [95% confidence interval (CI) 23.0–25.3%]. It's worth mentioning that, Among HCWs, the seroprevalence of anti-S1-RBD IgG antibodies was 25.6% (95% CI 23.5–27.8%) (Murhekar *et al.,* 2021) Again, the fourth nationwide serosurvey had done to estimate the prevalence of SARS-CoV-2 antibodies between 14 June and 6 July 2021 in the same 20 states. Nearly two-thirds of individuals aged ≥ 6 years from the general population and 85% of HCWs had antibodies against SARS-CoV-2 by June–July 2021 in India. Vaccination started in January 2021, which is also a factor related to increased antibody response and seroprevalence was significantly higher among individuals who had received the vaccine than those unvaccinated (Murhekar et al., 2021).

During these studies, Various factors were observed associated with seroprevalence. A significant association was observed between positive anti-SARS-CoV-2 IgG and a history of contact with a confirmed case; the transmission rate within households was approximately 30. The total antibody seropositivity was higher in males than females (OR 1.22, 95% CI 1.110–1.340) (Javed et al., 2022). In symptomatic individuals, antibodies are developed more, while it is less in infected asymptomatic individuals (Shirin et al., 2020; Shields et al., 2020). The symptomatic subjects had 2.18 times higher odds of IgG seropositivity while 1.2 times for IgM seropositivity than the asymptomatic subjects (Javed et al., 2022). In asymptomatic patients, IgG levels were lower compared to symptomatic patients, tending to decline after four months since the onset of the symptoms. Asymptomatic patients showed lower levels of antibodies during the 5-month follow-up. IgG antibodies tended to decrease over this period regardless of symptoms (Rodeles et al., 2021).

2.10 Seroprevalence of SARS-CoV-2 among HCWs

Healthcare workers (HCWs) are a vulnerable and critical population in responding to the SARS-CoV-2 pandemic. As HCWs continue to be the front line of the fight against coronavirus disease-19, they have a high potential for direct or indirect exposure to patients or infectious materials with SARS-CoV-2.

A cross-sectional study evaluating 6038 HCWs of 17 hospitals in Spain showed that the Seroprevalence of IgG-SARS-CoV-2 antibodies in HCWs is higher than in the general population and varies depending on regional COVID-19 incidence (Varona et al., 2021). There was a wide variation in the positivity rate between regions and cities in Saudi Arabia, ranging from 0% to 6.31% (Alserehi et al., 2021). (Chen et al., 2021) reported in their study that close contacts (18·0%, 95% CI 15·7–20·3) and high-risk healthcare workers (17·1%, CI 9·9–24·4) had higher seroprevalence compare to low-risk healthcare workers (4·2%, CI 1·5–6·9) and the general population (8·0%, 6·8–9·2) (Chen et al., 2021).

Figueiredo-Campos et al. (2020) quantified IgM, IgG, and IgA antibodies recognizing the SARS-CoV-2 receptor-binding domain (RBD) or the Spike (S) protein over six months following COVID-19 onset. They reported the detailed setup to monitor the humoral immune response from over 300 COVID-19 hospital patients, healthcare workers, 2500 university staff, and 198 post-COVID-19 volunteers. Anti-SARS-CoV-2 antibody responses follow a classic pattern with a rapid increase within the first three weeks after symptoms. Although titers were reduced subsequently, the ability to detect anti-SARS-CoV-2 IgG antibodies remained robust, with confirmed neutralization activity for up to 6 months in a large proportion of previously virus-positive screened subjects. Their work provides detailed information for the assays used, facilitating further and longitudinal analysis of protective immunity to SARS-CoV-2. Importantly, it highlighted continued circulating neutralizing antibodies in most people with confirmed SARS-CoV-2 (Figueiredo-Campos et al., 2020).

In a study, Shields et al. (2020) found that the overall seroprevalence of SARS-CoV-2 antibodies was 24.4%. Participants who reported prior symptomatic illness had higher seroprevalence (37.5% vs 17.1%, $\chi 2$ =21.1034, $p<0.0001$). They identified differences in the occupational risk of exposure to SARSCoV-2 between hospital departments and confirmed that asymptomatic seroconversion occurs in healthcare workers. Seroprevalence was most significant among those working in housekeeping

(34.5%), acute medicine (33.3%) and general internal medicine (30.3%), with lower rates observed in participants working in intensive care (14.8%) (Shields et al., 2020). COVID-19 vaccination in January 2021, initially targeting HCWs and frontline workers. As a result, antibodies were higher among HCWs (Goenka et al., 2020). Eighty-five per cent of HCWs had antibodies against SARS-CoV-2 by June–July 2021 in India after the initiation of vaccination. Significantly higher seroprevalence among individuals who had received the vaccine compared to unvaccinated, which may be related to vaccination as well as infection (Murhekar et al., 2021). Again, Seropositivity to SARS-CoV-2 significantly ($p<0.0001$) increased with the number of SARS-CoV-2 infections combined with the number of doses of the SARS-CoV-2 vaccines received (Ristić et al., 2022).

After immunization, nearly all subjects (99.8%) exhibited measurable levels of spike IgG antibodies. Importantly, spike IgG GMTs were significantly larger in SARS-CoV-2-infected vaccines than in SARS-CoV-2-nave vaccines at all sampling time points (P-<0.001). A prospectively selected 1072 HCWs from a university hospital in Turkey were tested for antibodies to the spike protein of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and the presence of an adaptive immune response among them after vaccination using the chemiluminescent microparticle immunoassay. As found, Seropositivity was higher among females (84.6%) than males (70.6%) ($P < 0.001$). Before immunization, individuals with coronavirus disease-2019 had considerably greater antibody titers than those without (P<0.001). Moreover, HCWs with chronic conditions had substantially lower median antibody titers and antibody positivity than those without (p=0.05 and p=0.001, respectively). Also, after the completion of two vaccines, a comparatively high frequency (99.6%) of humoral immunity was established in HCWs between the ages of 18 and 59 (Bayram et al., 2021). Total anti-SARS-CoV-2 antibodies response in healthcare workers had been evaluated among HCWs having two vaccination doses. Overall, anti-SARS-CoV-2 antibody levels tend to decline three months after the first vaccination. Moreover, such was announced in the seropositive group than in the seronegative cohort compared to the value evaluated at 30 days following the second vaccination dose (Salvagno et al., 2021).

Evolution of SARS-CoV-2 nucleocapsid antibodies in seronegative and seropositive individuals according to the time since the first vaccine dose administration had been done. The percentage of seroconversion in seronegative people was 95.7% 14 days following the initial dosage. All individuals showed detectable anti-S antibodies

from day 28 to day 90, and between days 28 and 42, the maximum antibody response was attained. Considering each time point separately, anti-S titer of seropositive individuals were always statistically higher compared to seronegative individuals ($P <$ 0.0001). The greatest mean antibody response at three months was associated with a mean antibody drop of 37.9% and 44.7% in seronegative and seropositive persons, respectively. Nonetheless, it's crucial to note that all subjects had a strong antibody response at three months (Favresse et al., 2021).

Bonnet et al. (2022) monitored the Humoral and T-cell immune responses elicited by mRNA vaccine 110 HCWs having both doses of vaccines with 21days apart; till six months after the second dose injection. Among all participants (99.1%) had evidence of previous infections. Anti-RBD antibodies were detected above the seropositivity threshold in all volunteers at three months (M3) and six months (M6). Specifically, At M6, a positive SARS-CoV-2-specific memory T-cell response was observed in 104/110 (94.5%) HCWs. Anti-RBD IgG titer decreased in all individuals between 3 and 6 months, regardless of age or baseline comorbidities, and were lower in HCWs over 60 years old three months after the second dosage. Antibody titers were found 3-4 times higher in those who had COVID-19 than those who didn't. Additionally, One HCW, aged 60, was symptomatic and tested positive for SARS-CoV-2 166 days after her last vaccination dose, the day before the M6 time point. At M3 and M6, antibody titer was 118 and 53 BAU/mL, respectively, and they rose to 4209 BAU/mL 17 days following positive RT- PCR results (Bonnet et al., 2022). Another study evaluated immunogenicity among 274 health care workers receiving two vaccine doses 21 days apart. All have measurable Anti-SARS-CoV-2 antibodies for six months. Here, antibodies were measured one day prior to vaccination (T0) and followed up at different time points (T1=21; T2= 28, T3= 49; T4= 84, T5= 168). It is essential to be noted that measurable anti-SARS-CoV-2 antibodies existed until day 168. In T1, after only receiving one dose of the vaccine, they had already identified a humoral response with Ab GMC of 56.69 AU/mL, which was higher than the cut-off value. During T2, the Ab GMC reached its highest level (299.89 AU/mL; 95% CI: 263.53-339.52) and increased significantly compared to the baseline (p=0.0001). At T3 (271.09 AU/mL; 95% CI: 254.71-289.26), T4 (175.37 AU/mL; 95% CI: 165.51-186.06), and T5 (134.64 AU/mL; 95% CI: 123.25-146.54), a steady decline was then seen. Noteworthy at T5, the most enormous antibody response was observed as a median drop of 59.6% in COVID-19-negative persons and a median decrease of 67.8% in COVID-19-positive individuals (Campo et al., 2021).

Verma et al. (2023) inferred detectable antibodies up to 9 months, participants without detectable ASAb before vaccination, and 94 and 99 developed ASAb after the first dose. While follow-up specimens were collected at days 60, 150, and 270 following the second doses, all subjects who had developed detectable ASAb after the second doses (Verma et al., 2023).

Following full immunization, they compared the ASAb and NAb between those who developed COVID-19 infection (n = 29) and those who did not (n = 106). Their ASAb titer was comparable after the first dosage ($p = 0.362$), but in those who developed an infection, they were considerably greater at days 60 ($p<0.001$) and 150 ($p<0.001$). Following full immunization, they compared the ASAb and NAb between those who developed COVID-19 infection (n = 29) and those who did not (n = 106). Similarly, their NAb percentages were comparable at day 28 ($p = 0.071$) but significantly higher at day 60 and 150 in those who developed an infection.

Following group stratification, the titer of previously infected subjects was approximately 30 times higher (2210 AU/ml, IQR 1040-3310) than those who had not been exposed to SARS-CoV-2 before receiving the vaccine (75 AU/ml, IQR 52- 107) after the first vaccine dose and six times higher at the same time points after the second vaccine dose (1935 AU/ml vs. 328 AU/ml. Most individuals (n = 59) showed a significant increase in antibody titer after the second dose of vaccine ($p < 0.0001$) (Figueroa-Hurtado et al., 2022).

From January 2021 to April 2022, Lau et al. (2022) assessed total S-Ab, IgG, and N-Ab levels at specified intervals. Up to 90 days after receiving the booster dosage following taking the BNT162b2/ CoronaVac vaccines, overall spike antibody (S-Ab), IgG S-Ab, and neutralizing antibody (N-Ab) responses have been identified. 20–30 days after vaccination, the levels of all antibodies peaked. All antibodies began to decrease 90 days after the booster. The mRNA vaccine generated more robust total S-Ab, IgG and N-Ab responses after the second and third vaccinations (Lau et al., 2022).

2.11 The Bangladesh context of the disease:

The Government of Bangladesh reported the first case of COVID-19 in Bangladesh on 8 March 2020 (GARDAWORLD, 2020).

In Bangladesh, the Institute of Epidemiology, Disease Control and Research (IEDCR) directed a national-level investigation to evaluate the prevalence of COVID-19, in collaboration with the International Centre for Diarrhoeal Disease Research,

Bangladesh (ICDDR,B), with support from the US Agency for International Development (USAID) and the Bill and Melinda Gates Foundation. Much epidemiological information about this newly emerging disease remains to be discovered, including estimates of the proportion of COVID-19 cases in the community, particularly for lower-income regions and countries such as Bangladesh, making it difficult for government policymakers to design optimal containment and mitigation strategies. Prior research has indicated that there may be a considerable number of asymptomatic cases of COVID-19. Other areas requiring further exploration include the incidence rate, prevalence rate, secondary infection rate, incubation period, serial interval and reproductive number of COVID-19 in various settings. Although there have been attempts to gather some of this data in previous studies worldwide, most estimates have been based on small-scale data or information collected from relatively narrow geographic regions (Widge et al., 2021). It is also essential to determine and characterize the immune responses to SARS-CoV-2 infection to understand how well the response protects people against future SARS-CoV-2 infection and how long this protection lasts. In this context, a serological investigation has the potential to provide information about the actual number of SARS-CoV-2 infections, allowing for robust estimates of the infection fatality rates and guiding public health decision-making (Bhuiyan et al., 2022).

2.12. Seroprevalence in Bangladesh

Since February 2020, there have been reports of persons infected with SARS-CoV-2 but did not develop symptoms of COVID-19. In some cases, the viral load of such asymptomatic persons has been equal to that of symptomatic persons, suggesting a similar potential for viral transmission (Bi et al., 2020).

There has been an extensive spread of (SARS-CoV-2) infection in Bangladesh by October 2020, evidenced by the First national-level cross-sectional study in Bangladesh incorporated data from April 2020 to October 2020. The study was conducted over 120 households from 32 districts randomly out of 64 districts, including Dhaka, in both slum and non-slum areas. In Bangladesh, 51.81% of individuals tested seropositive against severe acute respiratory syndrome coronavirus (SARS-CoV-2). Detection of IgG/IgM antibodies evidenced by ELISA against RBD of the spike protein. The national seroprevalence rates of immunoglobulin IgG and IgM were estimated to be 30.4% and 39.7%, respectively. The highest seroprevalence rate (57% for IgG and 64% for IgM) was observed in August 2020. IgM was more prevalent in younger age participants.

Moreover, Follow-up specimens from patients with COVID-19 and their household members suggested that both IgG and IgM seropositivity increased significantly with day passed, increasing at days 14 and 28 compared to day 1 of enrollment. In addition, seroprevalence was more significant in slum areas than non-slum areas within Dhaka city and outside Dhaka; it was more in the urban than the rural areas. In Dhaka, the seroprevalence of IgG was 35.4% in non-slum regions while 63.5% in slum areas. In areas outside Dhaka, IgG seroprevalence was 37.5% in urban areas and 28.7% in rural areas (Bhuiyan et al., 2022).

Another cross-sectional serosurvey was done involving 3200 participants from slum and non-slum dwellers of Dhaka and Chattogram cities during the time period of October 2020 to February 2021, where an overall weighted SARS-CoV-2 seroprevalence of 67.3% was seen with the seropositivity rate being higher in slums (71.0%) than in non-slum localities (62.2%). The significant factors associated with the seroprevalence of SARS-CoV-2 among the study population included education, income, certain preventive behavior, pre-existing chronic conditions etc. In this study, A higher weighted seroprevalence was observed in Dhaka city (72.9%) than in Chattogram (54.2%). The odds of seropositivity were 1.55 folds higher for residents who exhibited symptoms in the preceding six months than those without. Moreover, the individuals having pre-existing infections had higher seropositivity compared to those who didn't have them (Raqib et al., 2022).

Bhuiyan et al. (2022) did another serosurvey in Chattogram (Shitakunda) over two periods, March to April and May to June 2021, to understand the prevalence of total antibodies (IgA, IgM, and IgG) against the receptor binding domain of SARS-CoV-2 Ab ELISA following manufacturer instructions. In this survey, among 2307 participants, 1443 were vaccinated with one or two doses. 62% of participants in each household found seropositive—31% of the total variability in seropositivity in the community. In addition, seroprevalence is associated with population density. Participants living in higher population density areas are more likely to be seropositive. 69% of participants living in the most population-dense areas were seropositive, which is higher than the 52% of participants living in the least population-dense areas. After adjusting for age, sex, household clustering and test performance, they estimated that the seroprevalence of SARS-CoV-2 in Sitakunda was 64.1% (95% credible interval [CrI] 60.0%–68.1%) among all participants and 63.4% (95% CrI 59.2%–67.6%) when only unvaccinated

participants were considered. However, among the vaccinated population, seroprevalence was 98% (Bhuiyan et al., 2021).

Shirin et al. (2020) reported that, in Bangladesh, mildly symptomatic individuals developed IgM and IgA responses by day 14 in 72% and 83%, respectively, while 95% developed IgG responses and rose to 100% by day 30. In contrast, individuals infected with SARS-CoV-2 who remained asymptomatic developed antibody responses significantly less frequently, with only 20% positive for IgA and 22% positive for IgM by day 14, and 45% positive for IgG by day 30 after infection (Shirin et al., 2020).

From February, 2021 to September, 2021, a cross-sectional study was conducted among the vulnerable groups (e.g., HCWs, indoor and outdoor patients, and garments workers); due to their workplace hazards in the Chattogram metropolitan area to estimate the seroprevalence to assess the degree of transmission in the community. Qualitative and quantitative ELISA was used to identify and quantify antibodies (IgG) in the serum sample. SARS-CoV-2 S1-RBD IgG determined the concentration of IgG antibodies. In this study, SARS-CoV-2 IgG antibodies were detected in 498 (66.99%) of 748 individuals. Ara et al. (2022) reported in this study the overall adjusted seroprevalence estimate of SARS-CoV-2 antibodies was 66.99% (95% CI: 63.40-70.4%) in the CMA. Indoor/outdoor patients amongst the different donor groups had a positivity rate of 81.37% (144 of 179) compared to 68.99% (248 of 362) in the HCWs and 50.56% in the garments workers (104 of 205); the difference was statistically significant ($p< 0.001$). Both vaccine receivers showed significantly ($p<0.001$) higher Seropositivity than one dose or no vaccine receivers. Furthermore, in/outpatients had the highest mean titer of 197.18 DU/mL, followed by HCWs (163.30 DU/mL) and garment workers (77.05 DU/ mL), which is statistically highly significant. The level of IgG-spike antibodies in recipients of both doses of vaccine was higher (255.46 DU/mL) than in those who received one dose (159.08 DU/mL) or no quantities (53.71 DU/ mL). Additionally, the mean titer was higher (170.89) in those participants who had contact with confirmed cases compared to noncontact (116.45), and the difference is statistically significant ($p<0.001$) (Ara et al., 2022).

Anti-S-protein immunoglobulin-G anti-nucleocapsid (N)-protein immunoglobulin-G and immunoglobulin-A levels were measured by ELISA. The Delta variant was found in 40 out of 40 (100%) SARS-CoV-2 RT-PCR positive COVID-19 patients between 13 September and 23 October 2021 and Omicron variants in 90 out of

90 (100%) RT-PCR positive COVID-19 patients between 9 January and 10 February 2022 (Ghosh et al., 2022).

Only a few studies have investigated the antibody responses in asymptomatic persons. Several studies have shown more robust antibody response in patients with severe disease than mildly symptomatic ones. Also, a higher rate of absence of seroconversion in asymptomatic patients has been described. However, other studies have failed to find any correlation between clinical course and immune response. Since most COVID-19 cases are asymptomatic, the performance of the tests in this group is vital to evaluate the reliability of antibody tests in seroepidemiological studies and clinical diagnostics.

2.13 Vaccine Status and Strategy:

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) 's rapid global spread, and alarming clinical severity have accelerated demand for vaccines that safely and effectively prevent disease or reduce its severity.

The WHO Director-General declared a Public Health Emergency of International Concern; COVID-19 continues to be a global threat to health and safety. To minimize deaths, severe disease, and overall disease burden; curtail the health system impact; and fully resume socio-economic activity, three strategies to achieve Global COVID-19 Vaccination issued by WHO (WHO, 2022a). There is still a long road ahead, and the future direction of the pandemic remains uncertain. According to Watson et al. (2022) conducted a mathematical study to see the Global impact of the 1st year of COVID vaccination shows: most at-risk populations who remained unvaccinated in many countries led to unnecessary death. Estimates showed that approximately 600,000 deaths could have been averted globally if all countries had reached 40% primary series vaccination coverage by the end of 2021. It was, therefore, crucial to sustaining momentum for vaccination (Watson et al., 2022). This mathematical modelling study concluded that vaccination prevented 14.4 million deaths from COVID-19 across 195 countries from 2020 to 2021 (Eyre et al., 2020).

Complete vaccine schedules, including booster doses, are recommended by WHO and are an essential part of building immunity against virus strains circulating in communities worldwide. In the future, additional doses with currently updated vaccines may be recommended if these are meaningfully enhanced protection (WHO, 2022b).

Moreover, every country has been affected by COVID-19 with excess mortality. As a result, significant progress has been made on the vaccination front. As of January 16 2023, 13,131,550,798 vaccine doses have administered; among them, 5,479,851,178 persons were vaccinated with at least one dose while, 5,035,835,135 persons fully vaccinated (WHO, 2022d). In Bangladesh, 88.5% completed at least one dose, and 77.7% completed all amounts. As of January 15, 2023, in Bangladesh, a total of 346,605,580 vaccine doses have been administered, and 150,584,398 persons have been vaccinated with at least one dose. And 130,405,812Persons fully vaccinated (Data source: Bangladesh: MIS unit; DGHS).

This massive and unprecedented COVID-19 vaccine development has led to significant reductions in severe disease, hospitalization and deaths. Ghosh et al. (2022) conducted a study regarding clinical signs and symptoms of COVID-19 during the Delta wave; from September to October 23, 2021, and reported that, the Delta variant was associated with hospitalization (80%, 74%, and 40%) and oxygen support (60%, 57%, and 40%) in the no vaccine, dose-1, and dose-2 vaccinated cases (Ghosh et al., 2022).

Evidence from clinical trials and observational studies overwhelmingly supports the safety, efficacy, and effectiveness of numerous COVID-19 vaccines, especially against severe disease and death in fully vaccinated individuals.

A systematic review of the COVID-19 vaccine through February 2022 showed vaccine metrics collected include protection against asymptomatic infection, any infection, symptomatic COVID-19, and severe outcome including hospitalization and death, for a partial outcome or complete vaccination, and against variants of concern Alpha, Beta, gamma, Delta, and omicron. Efficacy and effectiveness estimates after two doses in the general population, pre-Omicron, ranged from 90% to 99% for death, 80% to 100% for severe infection, 70% to 100% for symptomatic disease, 65% to 98% for any infection, and 65% to 90% for asymptomatic infection. These values were lower after only a single dose: 70%–90% for death, 55%–95% for severe illness, 35%–93% for symptomatic disease, and 30%–80% for any infection (Higdon et al., 2022).

Notwithstanding achievements to date, COVID-19 vaccination and immunization progress need to be sustained and momentum enhanced. The updated goals and tactics of the vaccination strategy developed to achieve two goals; (1) sustain and enhance momentum to reduce mortality and morbidity, protect the health system, and resume socio-economic activities with the existing vaccines; (2) accelerate development and

access to improve vaccines to achieve durable, broadly protective immunity, and reduce transmission (WHO, 2022d).

Even if one has already had COVID-19, one should be vaccinated. The protection someone gains from COVID-19 will vary greatly from person to person. The immunity people get from being vaccinated after having a natural infection is consistently very strong. Even if one has had COVID-19, getting vaccinated means they are more likely to be protected for longer. Furthermore, there is currently no evidence to determine the optimal time to wait to be vaccinated after COVID-19 (WHO, 2022d).

Reported side effects of COVID-19 vaccines have mostly been mild to moderate side effects, like a low-grade fever or muscle aches, which are normal and not a cause for alarm: they are signs that the body's immune system is responding to the vaccine, specifically the antigen (a substance that triggers an immune response), and is gearing up to fight the virus and go away within a few days on their own. As shown in the results of clinical trials, more severe or long-lasting side effects are possible. Vaccines are continually monitored to detect adverse events. Typical side effects include pain at the injection site, fever, fatigue, headache, muscle pain, chills and diarrhea (WHO, 2020).

Chapter 3: Materials and Methods

3.1 Study design:

An observational cross-sectional study design was followed to conduct the research.

3.2 Place of study:

This study was conducted in six government and private hospitals in the CMP Area. All hospitals belonging to the study area were stratified according to their affiliation status, government and private.

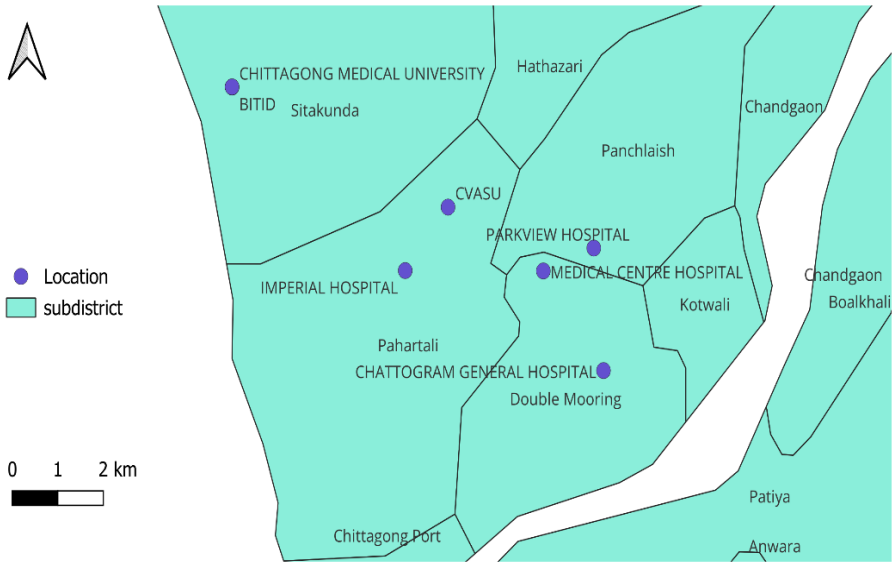


Figure 3.1: Geographical Locations of collected samples for the study

3.3 Period of study:

The study was carried out from January 2022 to April 2022.

3.4 Study population:

HCWs (e.g., doctors, nurses, other administrative staff, ward boy, and cleaner) of 4 government and 3 private hospitals in CMA.

3.5 Sampling technique:

Hospitals under the study area were selected by stratifying it as government and private and simple random sampling was applied to select study hospitals. Purposive sampling technique was used to select sampling units (individual HCWs) according to the availability of the study subjects who fulfilled the inclusion and exclusion criteria and willingly participated in the study.

3.6 Sample size determination:

The sample size was determined by using the following formula to estimate proportion:

$Sample\ size\ n = \frac{Z^2pq}{d^2}$ (Cochran,1977)

where,

z = z value of standard normal distribution at given level of significance or 99%

Confidence level, Z= 2.576

p= Expected prevalence of anti-SARS-CoV-2 antibody is 70% (expected prevalence; 66.99 % = 0.67, (Ara *et al.*, 2022)

q = 1-p

= (1- 0.70) = 0.30

d = Absolute error or precision (0.5)

$$Sample\ size\ n = \frac{Z^2pq}{d^2}$$

$$Therefore, \quad \frac{(2.5762)^2\ x\ 0.70\ x\ 0.30}{(0.05)^2}$$

= 557.40

Considering time and available resources, we enrolled 530 HCWs in our study.

3.7 Selection criteria:

3.7.1 Inclusion criteria:

Asymptomatic: Only asymptomatic subjects were included to ensure the presence of antibodies, i.e., participants had no COVID-19 related clinical signs, e.g., fever, coughing, runny nose, sore throat, dyspnea, shortness of breath, aches, and pain at the time of sample collection were considered to enroll into the study.

In case of having past confirmed COVID-19 status (by RT PCR):

Participants who had already passed at least 28 days after a negative Rt-PCR test.

Participants who did not take a repeated test to ensure negativity had passed at least 42 days after the first COVID-19 test.

3.7.2 Exclusion criteria:

People under 18 were excluded, as were those with an incomplete questionnaire.

Respondents who did not give informed written consent were not willing to participate.

3.8 Variables:

- Independent variables:
- Donor type
- Age
- Gender
- Education
- Occupation
- Vaccination
- Confirmed COVID-19 case
- Contact with COVID-19
- Side effects
- Day passed after 1^{st} dose/ 2^{nd} dose/ 3^{rd} dose
- Seroprevalence of anti-SARS-CoV-2 antibody
- Taking immunosuppressive drug
- Comorbidities
- Dependent variables:
- Anti-SARS-CoV-2 antibody titer
- Presence of IgG/IgM

3.9 Data collection instruments:

To collect data a structured questionnaire was followed during the study period. A detailed literature review was done before constructing questionnaire to identify the potential variables of interest after obtaining the study subjects. After correcting, with the final questionnaire including written consent, we conducted face to face interviews with individuals to collect information. The study process included answering a questionnaire and drawing blood to check for anti-SARS-CoV-2 antibodies.

3.10 Baseline blood collection and processing:

Blood specimens (6mL) were collected and transported to the clinical pathology laboratory (CPL) of Chattogram Veterinary and Animal Sciences University (CVASU) within three hours of collection. The serum was separated by centrifugation and stored at -20 ˚C till serological analysis.

3.11 Serological test examination :

Antibody was determined by a commercial qualitative assay using COVID-19 IgG ELISA test (Beijing Kewei Clinical Diagnostic Reagent Inc., China; Ref: 601340) as per the manufacturer's instructions. The assay is an enzyme-linked immunoassay (ELISA) that detects IgG against the SARS-CoV-2. An index (Absorbance/Cut-off) of <1 was interpreted as negative, 0.9 to 1.1 as borderline (retesting of these specimens in duplicates was done to confirm results), and ≥1 index as positive. Per the manufacturer, the sensitivity and specificity of the assay for IgG are 93.8% and 97.3%, respectively. Positive and negative controls were included in all assay batches. Repeated testing using the same specimen yielded the same interpretation.

3.11.1 Material amd component :

This kit contains reagents strips fixed on white strip holder. The plate is sealed in alumium pouch with desiccant.

- Microplate
- Desiccant
- Negetive control
- Positive control
- Conjugate

- Sample diluent
- Wash buffer
- Substrate solution A

Figure 3.2: kit contains reagents

3.11.2 Specimen preparation :

Stored sample (-20 ˚C)

↓

Stable for 3 days at 2-3 ˚C

↓

Ensure the patients samples, calibrators, and controls are ambient temperature (18-25 ˚C) before measurement.

↓

Adjust the incubator to 37 ˚C

↓

Prepare wash solution

3.11.3 Test procedure:

Adding sample diluent and sample: 100 µL diluent + 10ml sample to each serum diluent

↓

Incubating: Cover the plate with the plate lid then incubate 30 min at 37 ˚C

↓

Washing: after incubating remove and discard the plate cover

↓

Wash each well 5 times with dilute wash buffer

↓

Each time allow the micro well to soak for 30-60 second

↓

After the final washing cycle, turn down the plate

↓

Clean towel and tap it to remove any remainders

↓

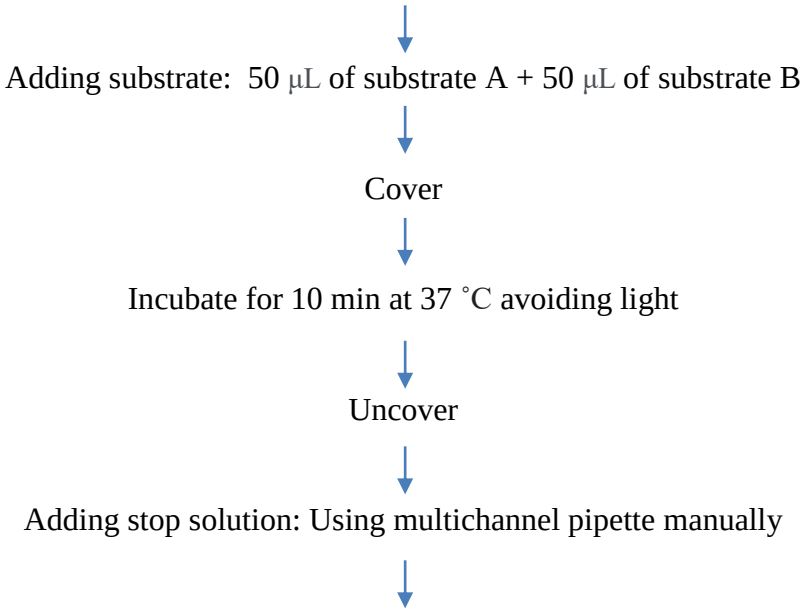
Adding conjugate: substrate: Add 110 µL of conjugate in each well Except blank

↓

Mix by tapping the plate gently

↓

Then wash 5 times



3.11.4 Measuring the Absorbance:

Read the absorbance of each well at 450nm (using 620 to 630 as the reference wavelength to minimize well imperfections) in a micro plate reader. The results should be read within 30 minutes of adding the stop solution.

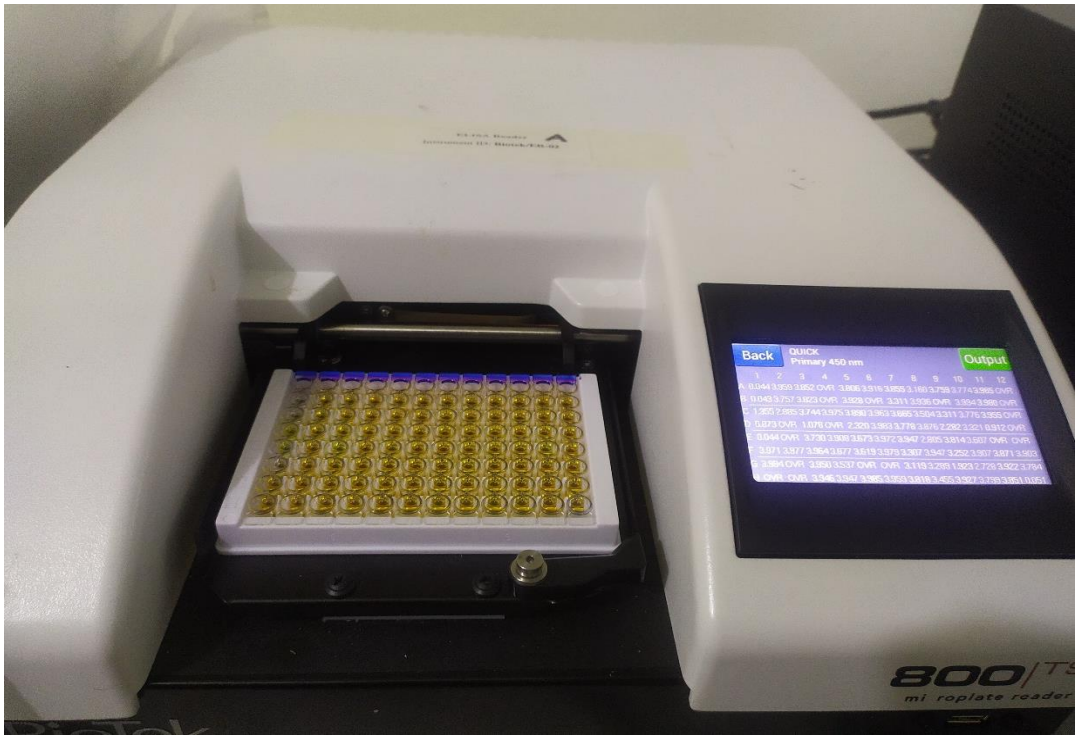


Figure 3.3: Placing of microplate for absorbance of each well.

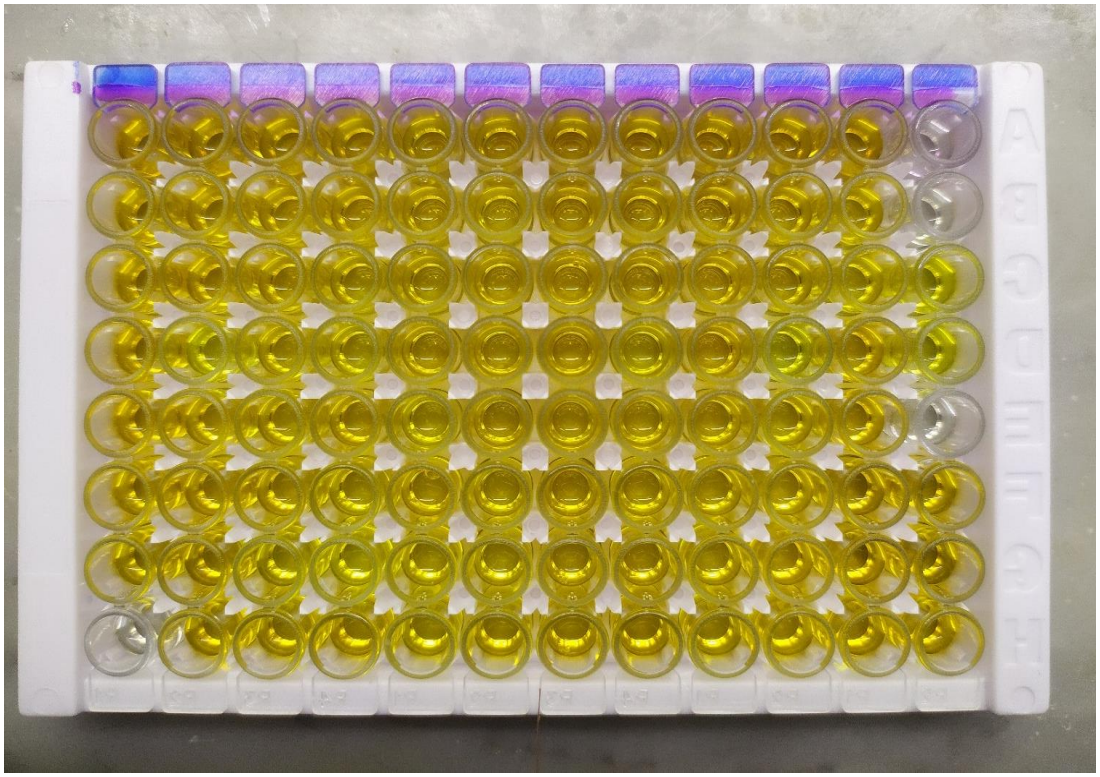


Figure 3.3(a): Reading the absorbance of each well in microplate

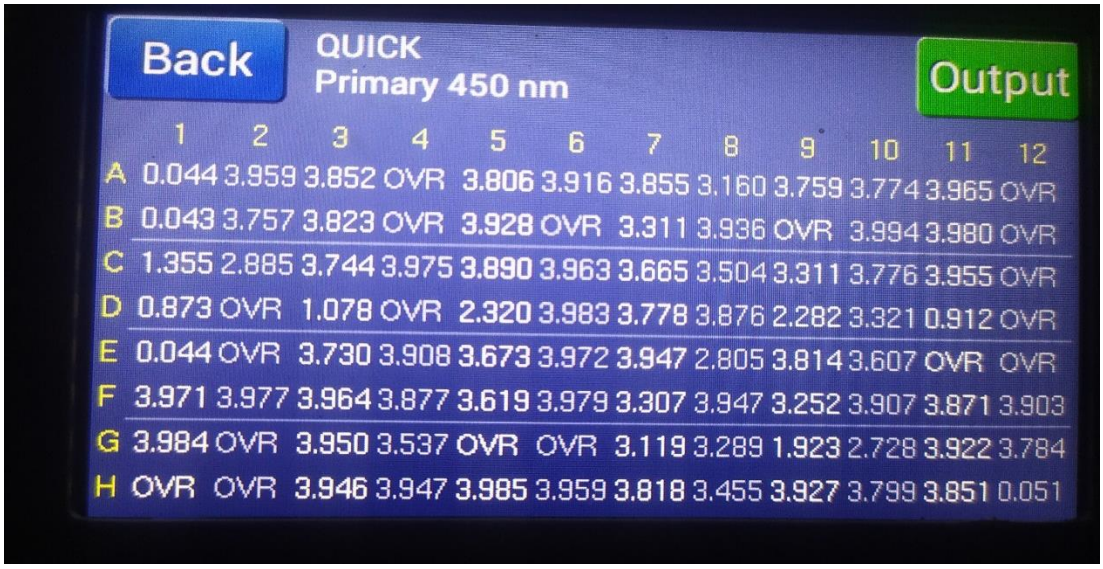


Figure 3.3(b): Reading the absorbance of each well in microplate.

3.11.5 Calculation: record the absorbance from the microplate reader.

Quantitative results were calculated as a ratio of the extinction of the control or tested specimen over the extinction of the calibrator. Results were reported in standardized units for the quantitative kits that included six calibrators to quantify the antibody concentration (i.e., DiaSino units/mL)

Expected values: A value of <10 DU/mL was considered negative, and values >10 DU/mL are considered positive for the presence of SARS-CoV-2 S1- RBD IgG.

3.11.6 Immunochromatography (ICT):

Immunochromatographic test (ICT) was also used in conjunction with ELISA (qualitative and quantitative) to evaluate the diagnostic performance of various assays. The test sensitivity and specificity of ICT [Rapid SARS-CoV-2 Antibody (IgM/IgG) (In Tec Products, INC., China, Ref: ITP16001-TC25 and ITP16002-TC25)] for IgM/IgG detection, according to the manufacturer, were 94.41% (95% CI: 84.89- 98.10) and 98% (95% CI: 96.10-98.98), respectively.

3.11.7 Materials:

- Droppers
- Sample diluents
- Alcohol Swab
- Safety Lancets

Figure 3.4: Materials for ICT

3.11.8 Procedure:

Specimen collection (whole blood collection with finger stick)

↓

Add sample diluent

↓

Wait for 15- 20 minutes

↓

Observe the coloured line

3.11.9 Interpretation:

Positive in presence of coloured line and test is negetive in absence of coloured line. Either of any or both IgM or IgA counted as positive.

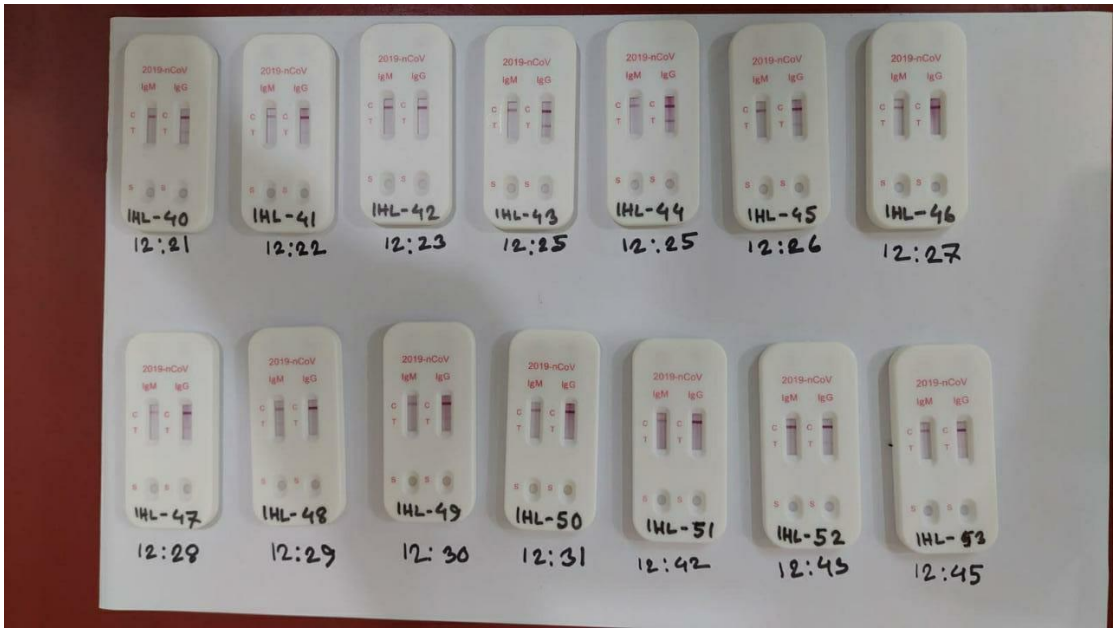


Figure 3.5: Reading of presence or absence of (IgG/M) in lancet

3.12 Data management:

The linearity of the quantitative variables was evaluated by categorizing them into four categories using quartiles as cut-off values. Logistic regression analysis was conducted on the categorized variables, and parameter estimates were observed for an increasing or decreasing trend. In case of linear increase or decrease in the parameter estimates, linearity in the quantitative variable was assumed and used without modification. In the case of nonlinearity, a quartile was used to categorize it. However, some quantitative variables were categorized considering research interest. For instance, the number of days between the first, second and third dose of vaccine and quantification of antibody titer was categorized as 'up to 8 months ' and 'after 8 months' and between the third dose of vaccine and quantification of antibody titer was categorized as 'up to two months' and 'after two months'.

3.13 Statistical analysis:

In the study period, a total of 530 qualitative and quantitative test results were included in the analysis.

Data from questionnaire survey and laboratory analysis was collated and entered into a spreadsheet (Microsoft Excel). T-test or ANOVA, $\chi 2$ test, where appropriate, was used to analyze differences in antibody detection and levels according to age, sex, gender etc. and the day since received 1st, 2nd and 3rd dose of vaccine. The effects of different

potential explanatory variables on the binary outcome - presence/absence of anti-SARS-CoV-2 antibody was evaluated using univariable logistic regression models. Effect of variables on the mean titer of the antibody was assessed by t-test and one way ANOVA. P-values <0.05 were considered as significant throughout the analysis. All statistical analyses were performed in STATA 11. Data were presented by tables, graphs and charts as needed.

3.14 Ethical aspect:

- Institutional ethical approval was taken from the authorized committee of Chattogram Veterinary and Animal Sciences University (CVASU), Bangladesh.
- Permission for the study was taken from the concerned departments from where we collected our study subjects.
- Written consent of all the study subjects was taken free of pressure and without exploiting any weakness of the subjects.
- The entire study subject was thoroughly appraised about the nature, purpose, and implications of the study, as well as the entire spectrum of benefits and risks of the study.
- The interest of the study subjects was not compromised to safeguard their rights and health.
- As this study needs only 8 ml of blood of study subjects, the chances of complications were very unlikely. But in case any complications like slight discomfort, mild pain, weakness, or vertigo occur, they were treated with assurance, analgesics.
- Subjects were assured about their confidentiality and freedom to withdraw them from the study anytime.
- For safeguarding confidentiality and protecting anonymity, each of the population were given an unique ID number which was followed in same collection, transport to lab and reporting, in each step of the procedure.

Chapter 4: Results

Total of 530 patients were selected in CMA, fulfilling the inclusion and exclusion criteria to evaluate the seroprevalence of anti-SARS-CoV-2 antibodies among the vaccinated HCWs against COVID-19. Data were analyzed using the appropriate statistical procedures and P-values <0.05 was considered significant throughout the analysis. Outcomes of the analysis are presented in this chapter through tables and graphs, and figures.

4.1 Characteristics of study participants:

A total of 530 respondents from different hospitals of CMA gave written informed consent and completed the questionnaire. Table 1 states that among 530 respondents from hospitals of CMA, 89 (16.79%) were doctors, 132 (24.91%) were nurses, 28 (5.28%) were lab techs, 24 (4.53%) were cleaner and ward boy and the remaining 257 (48.49%) were other administrative staffs of hospitals. The majority (n=334; 63.02%) were males. Most of the participants were from 18-27 years (27.45%), followed by 43-71 years (24.95%) and 34-42 years (24.57%) of age group. Among the study population, 67.57% were COVID-19 patient sometimes in the past confirmed with RT-PCR Test. 61.32% of the subjects had known contact with COVID-19, while 10% of the subjects were not sure about contact history, and 32.43% of the subjects reported that they don't have any known contact history (Table:1).

In the total population, the majority received both the 1st and 2nd doses of the vaccine. 14 (2.64%) respondents received only the first dose of the vaccine, 379 (71.51%) received both doses of the vaccine, and 137 (25.85%) received three doses of the vaccine. The greater number of the respondents (418; 78.87%) had no side effects after vaccination, while a good number (112; 21.13%) experienced side effects. The complications were mostly like fever (26.79%) and 32.14% had multiple complications. The pie chart illustrates 27% developed fever, 19% developed body ache. Moreover, 22.32% mentioned nonspecific symptoms side effects (Table:1)

The proportion of respondents taking immunosuppressives were 2.2%. One hundred and fourteen (22.44%) participants had pre-existing medical conditions such as diabetes mellitus, asthma, hypertension, carcinoma etc. (Table:1)

Table 1: Demographic Data of study participants

Variable	Level	Frequency	Percentage
Donor type	Doctor	89	16.79
	Nurse	132	24.91
	Lab Tech	28	5.28
	Cleaner/ Ward boy	24	4.53
	Hospital administrative Staffs	257	48.49
Gender	Female	196	36.98
	Male	334	63.02
Age	18-27 years	143	27.45
	28-33 years	120	23.03
	34-42 years	128	24.57
	43-71 years	130	24.95
Confirmed COVID-19 Case	Yes	75	67.57
	No	36	32.43
Contact with COVID-19	Yes	325	61.32
	No	152	28.68
	Don't Know	53	10.00
Vaccine taken	1st Dose	14	2.64

	2nd Dose	379	71.51
	3rd Dose	137	25.85
Side effect	Yes	112	21.13
	No	418	78.87
Day passed after 1st Dose of	Up to 8 months	10	71.43
	> 8 months	4	28.57
Day passed after 2nd Dose	Up to 8 months	254	67.20
	> 8 months	124	32.80
Day passed after 3rd Dose	Up to 2 months	87	65.91
	> 2 months	45	34.09
Side effect type	Fever	30	26.79
	Body ache	21	18.75
	More than one complication	36	32.14
	Non-Specific	25	22.32
Taking Immunosuppressive Drug	Yes	12	2.38
	No	492	97.62
Comorbidities	Yes	114	22.44
	No	394	77.56

Table 1: Demographic Data of study participants

4.2 Prevalence of antibody (IgG/IgM):

The overall prevalence of the presence of antibodies in serum samples of the study population was 99.62% in quantitative ELISA and 60.98% in ICT (Figure 4.1).

As nearly all samples were positive in ELISA, therefore, the data was not used in further statistical analysis (significance test). In ICT, 47.22% of samples from the doctors were positive. On the other hand, 15 out of 20 samples (75%) from hospital ward boys and cleaners were positive in ICT. It was observed that samples from those who received only the first dose of the vaccine were 55.56% positive in ICT. It is observed that the percentage increased to 57.87% and 77.50% when the donor received 1st and 2nd doses and all three doses, respectively, and the association was statistically significant (0.05) (Table 2). Prevalence was higher (70.135) in symptomatic individual than asymptomatic (57.58%) and association is significant (P= 0.05). ICT positivity was higher in person who had contact with COVID-19 (63.87), compared to who did not (59.68%), and who did not know about any contact history. ICT positivity did not vary significantly among different age groups. Moreover, 57.58% of subjects who were asymptomatic, and 70.13% of the subjects who were symptomatic, were positive in ICT and the difference was found statistically significant. Antibody was present in 50% up to 8 months and 66.67% after 8 months when they received 1st dose. It was found in 63.04% up to 8 months and 45.75% after 8 months when they received 2nd dose and the association was significant (P< 0.05) (Table:2). Moreover, Prevalence of IgG/IgM increases proportionally with the number of vaccination dose taken by different donor types (Figure 4.2)

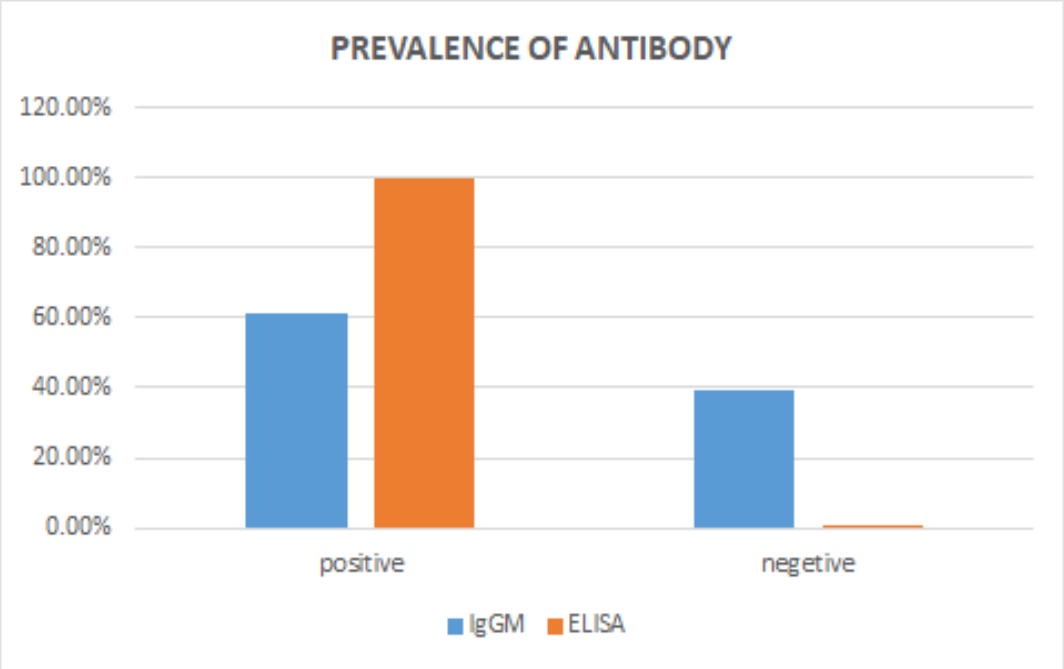


Figure 4.1: Prevalence of presence of antibody

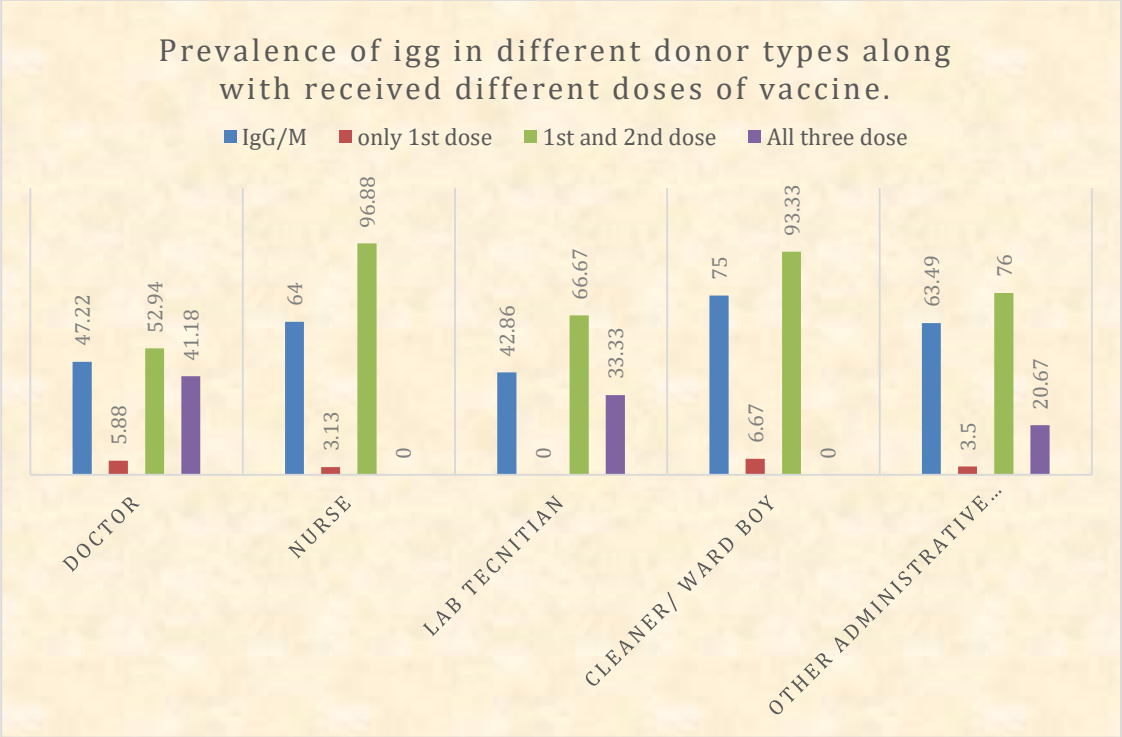


Figure 4.2: Prevalence of IgG in different donor types along with received different doses of vaccine.

Table 2: Univariable analysis (X2 test, logistic regression) to evaluate the association of different variables with seroprevalence of anti-SARS-CoV-2 antibodies tested with ICT.

Variable	Level	Observation	ICT(Frequency)	ICT*(percentage)	Odds ratio	P-value
Donor type	Doctor	36	17	(47.22)	Ref.	0.137
	Nurse	50	32	(64)	1.98	
	Lab tech	14	6	(42.86)	0.83	
	Cleaner/ Ward boy	20	15	(75)	3.35	
	Other administrative staff	126	80	(63.49)	1.94	
Gender	Male	167	101	60.48	Ref	0.7
	Female	78	49	62.03	0.13	
Confirmed RT-PCR	Yes	31	20	64.52	ref	0.6
	No	14	10	71.43	1.37	
Contact with COVID-19	Yes	155	99	63.87	Ref	0.28
	No	62	37	59.68	0.83	
	Don't Know	29	14	48.28	0.52	
Symptoms	Asymptomatic	165	95	57.58	ref	0.05

	Symptomatic	77	54	70.13	1.7	
Age	18-27 years	69	45	65.22	Ref	0.74
	28-33 years	63	40	63.49	0.93	
	34-42 years	55	31	56.36	0.69	
	43-71 years	54	32	59.26	0.78	
Vaccination	1st Dose	9	5	55.56	ref.	0.05
	2nd Dose	197	114	57.87	1.09	
	3rd Dose	40	31	77.50	2.75	
Day Passed after 1st Vaccination	Up to 8 months	6	3	50		0.63
	> 8 months	3	2	66.67	2	
Days Passed after 2nd Vaccination	Up to 8 months	138	87	63.04	ref.	0.02
	> 8 months	59	27	45.76	0.49	
Days Passed after 3rd Vaccination	Up to 2 months	25	18	72.00		0.3
	> 2 months	14	12	85.71	2.33	
Comorbidities	Yes	58	30	51.72	ref	0.10
	No	182	116	63.74	0.60	
	yes	6	6	100	ref	-

Immunosuppressive drug user	No	234	139	59.40	-	

Table 2*:* Univariable analysis (X2 test, logistic regression) to evaluate the association of different variables with seroprevalence of anti-SARS-CoV-2 antibodies tested with ICT.

4.3 Concentration of antibody (DU/ml:

All HCWs have detectable GMT up to 8 months after vaccination. After 8 months, all vaccinated HCWs still had considerable GMT up to a certain period. A considerable GMT is 200.441 DU/ml up to 8 months and 263.942 DU/ml at > 8 months had been observed among the vaccinated population who completed 1st dose of vaccine. It is also observed that, a considerable amount of GMT 264.72 DU/ml is found in those samples who had received a second dose of vaccine up to 8 months and it is 285.09 DU/ml at > 8 months. Apart from this, among the population who had received 3rd dose of vaccination, 51.59% developed GMT 332.08 DU/ml up to 2 months and 326.86 DU/ml after 8 months (Table: 3).

GMT was higher among doctors 307.68 DU/ml followed by Nurses 284.88 and the association was significant (P<0.05). It was revealed that, asymptomatic individuals developed lower GMT 278.50 DU/ml in comparison to symptomatic individuals 298.50 DU/ml which also showed significant association (P=0.006).

People with the age between 28 to 42 years GMT were stable at 284.64 DU/ml though it was moderately increased in those above 42 years (Figure: 4.3) and the association was significant (P<0.05) (Table:3)

It is clearly found that mean concentration of antibodies increases among population groups with the doses of vaccine received. Those who completed 1st dose developed GMT 218.54 DU/ml which increases to GMT 271.584 DU/ml, and it rises to GMT 331.411 DU/ml among those who received 3rd dose of vaccine (Figure: 4.4) and the association is highly significant (P=0.000) (Table:3).

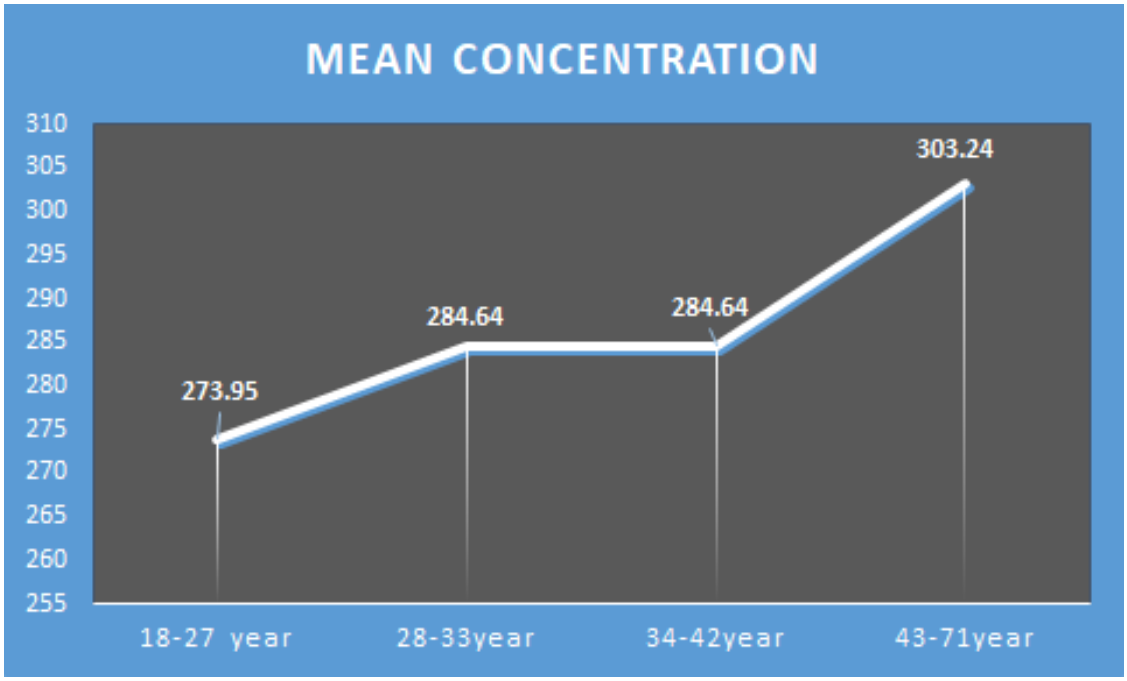


Figure 4.3: Mean concentration of antibody (DU/ml) among different age groups that received vaccine

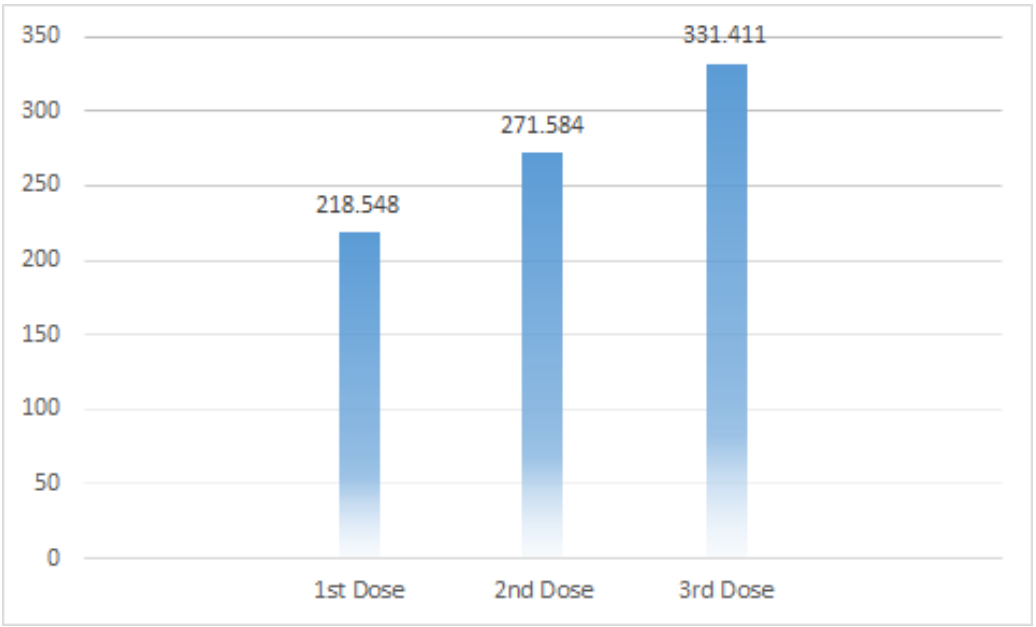


Figure 4.4: Mean concentration of antibody (DU/ml) among population groups received different doses of vaccine.

Table 3: Univariable analysis (t-test, One way Anova) to evaluate the mean difference of quality of anti SARS-Cov2 antibodies in serum sample

Variable	Level	Geometric Mean titer	SD	P-Value
Donor type	Doctor	307.68	85.35	0.04
	Nurse	284.88	74.04	
	Lab tech	274.27	109.65	
	Cleaner/ Ward boy	277.47	67.4006	
	Other stuff	280.47	81.97	
Gender	Male	285.97	83.47	0.76
	Female	285.74	79.29	
Contact with COVID-19	Yes	289.56	79.53	0.42
	No	277.93	85.18	
	Don't Know	283.90	88.27	
Symptomatic	Yes	298.56	72.20	0.006
	No	278.50	85.47	
Confirmed RT-PCR	Yes	299.115	84.45	0.26
	No	315.98	43.05	
Age	18- 27 years	273.95	69.02	0.01
	28- 33 years	284.64	86.40	
	34- 42 years	284.64	90.27	

	43- 71 years	303.24	78.03	
Vaccination	1st Dose	218.584	75.26	0.000
	2nd Dose	271.60	84.43	
	3rd Dose	331.411	51.59	
Day Passed after 1st Vaccination	Up to 8 months	200.441	80.82	0.16
	> 8 months	263.942	33.30	
Days Passed after 2nd Vaccination	Up to 8 months	264.72	84.32	0.02
	> 8 months	285.09	83.41	
Days Passed after 3rd Vaccination	Up to 2 months	332.08	50.05	0.58
	> 2 months	326.86	54.59	
Side effect Developed	Yes	311.444	76.20	0.0002
	No	278.752	82.17	
Side effects	Body ache	313.88	71.67	0.99
	Fever	311.07	87.53	
	Multiple symptom	313.004	61.38	
	Non-specific	307.44	88.31	
Comorbidities	Yes	296.08	80.90	0.11
	No	283.08	81.01	
Immunosuppressive drug user	Yes	309.867	79.84	0.27
	No	283.85	82.27	

Table 3: Univariable analysis (t-test, one way Anova) to evaluate the mean difference of quality of anti SARS-Cov2 antibodies in serum sample

4.4 Agreement between ELISA and ICT:

In the present study, it was observed that nearly all samples were positive in quantitative ELISA except 1 which was positive in ICT. On the other hand, 95 samples out of 244 samples were negative in ICT. The agreement between the test was 61.1% (kappa: 0.0046) (Table 4).

Table 4: Agreement between ELISA and ICT

			IgG/M		Total	Kappa
			Negative	Positive		
ELISA	Negative	Count	1	1	2	
		% within ELISA	50.0%	50.0%	100.0%	
	Positive	Count	95	149	244	
		% within ELISA	38.9%	61.1%	100.0%	0.0046
Total		Count	96	150	246	
		% within ELISA	39.0%	61.0%	100.0%	

Chapter 5: Discussion

This cross-sectional population-based study was carried out to dictate the seroprevalence of anti-SARS-CoV-2 antibodies among the asymptomatic and confirmed COVID-19 population. A total of 530 Health Care Workers (e.g., doctors, nurses, administrative staff, ward boys, and cleaners) of six government and private hospitals in the CMP area were included in this study.

There has been an extensive spread of SARS-CoV-2 infection in Bangladesh. Antibodies serve as biomarkers of immunity; detection of specific antibodies may give a testimony about adaptive immunity against SARS-CoV-2. Based on the current investigation, the study revealed a considerably high overall prevalence of anti-SARS-CoV-2 antibodies among the healthcare workers (HCWs) in the studied region, registering at 99.62% positivity when using ELISA among the vaccinated population. However, when assessed through the Immunochromatographic test, 60.98% displayed a positive outcome. The rise in seropositivity was evident as the number of doses increased.

Previously, cross sectional study conducted over 32 districts out of 64 of Bangladesh, showed an overall 51.81% seroprevalence till October 2020, where participants were selected randomly (Bhuiyan et al., 2022). Nevertheless, from October 2020 to February 2021, overall seroprevalence increased (67.3%) with time, and it was higher in slum areas (71.0%) in comparison to non-slum areas (62.2%). It was suggested that seropositivity is strongly associated with population density (Raqib et al., 2022) and that the prevalence might have increased due to either high infection levels or a positive response to the national immunization campaign in its early phases (Ward et al., 2021). Most importantly, in a recent study in CMP area of Chattogram, seroprevalence was reported as 66.99% (95% CI: 63.40%-70.4%) by using ELISA, in which there was inclusion of both vaccinated and non-vaccinated populations of garments worker and HCWs (Ara et al., 2021). Also, highest seroreactivity to SARS-CoV-2 was noticed among the unvaccinated participants regardless of the clinical forms of COVID-19 they had (asymptomatic, mild, severe) (Ristić et al., 2022).

In this cross-sectional study, by qualitative study(ICT) and quantitative study (ELISA), HCWs from private and government hospitals represented a high seropositivity in ELISA test (nearly all were positive) while study was conducted among

all vaccinated HCWs, dealing with patients of COVID-19. Previously reported study showed higher seroprevalence among HCWs compared to the general population. In India, there was a greater seroprevalence among close contacts and high-risk healthcare workers compared to low-risk healthcare workers and the general population (Chen et al., 2021). With vaccination rollout commencing in January 2021, initially focusing on healthcare workers and frontline personnel, higher antibody levels were observed among healthcare workers (Goenka et al., 2020).Ara et al. (2022) showed higher seropositivity in in/outpatients following HCWs assessed by quantitative ELISA (Ara et al., 2022). Overall prevalence among HCWs were 92.96% when tested with Anti-SARS-CoV-2 QuantiVac ELISA in the near time period of this study (March to June, 2022) in Serbia (Ristić et al., 2022). That also revealed high seroprevalence among HCWs. Therefore, it can be affirmed that there is a discernible difference in seroprevalence based on occupational diversity.

In this study, sensitivity of ICT (IgG/IgM) is 61.07% and specificity 50%. Therefore, it may state that, ELISA is preferable to see antibodies in comparison to ICT. Kontou et al. (2020) reported IgG tests were shown to be more accurate than IgM testing when samples were collected later after the onset of symptoms (Kontou et al., 2020). Furthermore, SARS-CoV-2 IgM-detecting tests, which are used to find COVID-19 in the acute phase, had a significant percentage of false-negative results, ranging from 10 to 44% (Mohit et al., 2021). Additionally, a systematic and meta-analysis review conducted by Vengesai et al. (2021), revealed that the best overall diagnostic test accuracy is obtained by IgG-IgM-based ELISA (Vengesai et al., 2021) which also supports our study.

In the present study, 47.22% of doctors and 64% of nurses were seropositive in ICT. On the other hand, 15 out of 20 samples (75%) from hospital ward boys and cleaners were positive in ICT. The observed seropositivity rate was greater among cleaning staff compared to physicians, nurses, lab technicians, and administrative staff. This correlation echoes the findings of Bayram et al., 2021, indicating a higher seroprevalence specifically among cleaners and ward boys, reaching 75%. In this study, Nurses displayed nearly twice the seropositivity rate of doctors, while cleaners and ward boys showed over three times higher odds, potentially due to increased exposure to contaminated surfaces and lower awareness. Doctors exhibiting higher antibody levels in quantitative ELISA (with a GMT of 303.3 DU/ml) compared to other HCWs might stem from their potentially heightened exposure to higher viral loads or strict adherence

to infection control protocols. Additionally, variances in immune responses, differences in vaccination timelines and number of vaccine doses received by different groups could influence antibody levels., roles involving closer patient contact, and other underlying factors such as genetic predisposition or prior exposure to related viruses might contribute to this difference. All these may collectively contribute to the observed disparity in antibody levels among healthcare professionals in this context.

Regardless of confirmed RT-PCR for COVID-19 and contact with COVID-19 cases, no statistically significant differences in seropositivity and GMT was observed when vaccinated HCWs were the study population. But previous study showed, individuals who had COVID-19 before vaccination had considerably greater antibody titers than those who did not ($p< 0.001$) (Bayram, et al., 2021). Havervall et al. (2022) also found that spike IgG GMTs were markedly higher in previously SARS-CoV-2-infected vaccinees than in native vaccinees with all P-Values < 0.001 (Havervall et al., 2022).

Again, it can be noted that symptomatic infected individuals were more seropositive (70.13%) than asymptomatic (57.58%) with statistically significant P value ($P<0.05$). This finding corroborates with previous study where development of IgG and IgA was found more in symptomatic individuals than asymptomatic (Shirin et al., 2020). Another study revealed symptomatic subjects had 2.18 times higher odds of IgG seropositivity and 1.2 times for IgM seropositivity than asymptomatic (Javed et al., 2022).

Since the first COVID-19 cases were reported in December 2019, a global health crisis with significant social and economic repercussions has developed. As a result, the development of a potent vaccine was the focus of the scientific community's research. Durable protective immunity following illness or immunization is based on immunological memory. Despite extensive research, the kinetics, duration, and evolution of the immune system's response to vaccination cannot be predicted based on the early effector phase. Hence, monitoring responses over several months is crucial to figuring out how long the immune response will last. In this study, seropositivity for COVID-19 IgG/IgM was risen dramatically and clearly showed trends in vaccine-induced antibodies among the health care workers. We found, after the 1st dose, 55.56% seropositivity in ICT, which increased to 57.87% when donor received 1st and 2nd dose and reached up to 77.50% after having all three doses. Similarly, GMT developed to 218.584 DU/ml after receiving 1st dose and 271.584 after 2nd dose and 331.441 DU/ml after the 3rd dose. Comparative study in India revealed that the seroprevalence (COVID-19 IgG by ELISA) among the vaccinated and unvaccinated HCW were 91.7% and

38.2%, respectively (Elangovan et al., 2022). Another study reported that seropositivity increased with the number of vaccines received (Ristić et al., 2022). Seropositivity rates after the first and second doses of CoronaVac vaccination were found to be 77.8% and 99.6%, respectively in study by (Bayram et al., 2021). (Ara et al., 2022) found that the IgG antibody was produced in 61.66% of the participants who received the first dose of COVID-19 vaccination. This number increased to 100% among individuals who received a second dose. In an early study, it was observed that previous infection with SARS CoV-2 boosted the immune response after receiving the first vaccination and it was roughly 30 times higher (2210 AU/ml, IQR 1040-3310) than those without previous infection (75 AU/ml, IQR 52-107) (Figueroa-Hurtado et al., 2021). Another study showed, after the first vaccine, antibody titers were found to be 3-4 times higher in those who had COVID-19 than those who didn't ($P<0.001$) (Bonnet et al., 2022). Moreover, when combined the data about previous SARS-CoV-2 infections and vaccination against COVID-19, the seropositivity to the SARS-CoV-2 virus significantly ($p<0.0001$) increased with the number of infection and the number of vaccines received (Ristić et al., 2022). Seropositivity for COVID-19 IgG by ELISA was higher (70.4%) among vaccinated HCW than the vaccine-native HCWs (29.6%) (Elangovan et al., 2022). Therefore, it can be stated that vaccination has an important role to enhance immunity among individuals.

Consequently, regarding the persistence of antibody, we found that either IgG/IgM antibody was found in 50% of participants who passed up to 8 months after vaccination and 66.67% of the participants who passed more than 8 months who received the first dose of vaccine. IgG/IgM persisted in 63.04% participants up to 8 months and in 45.76% participants who passed more than 8 months among individuals who received a second dose. Moreover, 72% had antibody up to 2 months and 85.71% had antibody who passed more than 2 months when they received 3rd dose of vaccine. However, the relationship between presence of antibodies and their persistence with time who received both 1st and 2nd dose showed statistically significant ($P=0.02$). In quantitative ELISA, we found a slightly increased titer after 8 months of vaccination in participants received both 1st and 2nd doses. It is important to highlight that considerable antibody titers were found up to 8 months and after 8 months of receiving 2nd dose of vaccine. On the other hand, Ara et al. (2022) reported, the overall seroprevalence of anti-SARS-CoV-2 antibody among the HCWs of the study area were noticeably high when tested with ELISA. However, the seroprevalence and antibody titers were dropping over time. By the 2nd

month following the initial dose, the mean IgG titer in the body dropped by nearly 25% and 21% by four months (Ara et al., 2022). To determine the long-term impact of prior SARS-CoV-2 infection on immune responses after COVID-19 vaccination, Havervall et al. (2022) measured the immune response over several months and found that enhanced immune responses sustained over 3-7 months following vaccination with predominantly previously infected SARS-CoV-2 individuals. They also observed that effect of a previous infection followed by vaccination on immune responses is not a temporary phenomenon. Moreover, the memory compartment continues to evolve after both natural infection and vaccination (Havervall et al., 2022). Verma et al. (2023) found that second dose boosted the immune response by more than 6-fold (from 77 U/ml to 512 U/ml). Besides, a considerable amount of titer found (2079U/ml) up to 270 days following 2nd dose of vaccination which supports our study. They also observed, the titer was significantly higher at day 60 (14019U/ml) with significant P value ($P<0.001$) and day 150 (2062 U/ml) with significant P value ($P< 0.002$) in (21.5%) participants, who have acquired infection after 2nd dose of vaccination (Verma et al., 2022). (Bonnet et al. (2022) monitored immune response observed up to 6 months after 2nd dose of vaccination. They found Anti-RBD IgG titers decreased in all individuals between 3 and 6 months where 99.1% had no evidence of prior SARS-CoV-2 infection. Meanwhile, one HCW 0.99% tested positive for SARS-CoV-2 166 days after the last dose of vaccination, and he developed antibody titers 4209 BAU/ml. However, 17days following positive RT-PCR, he had antibody titers 118AU/ml and 53AU/ml at month 3 and month 6 respectively. This supports that repeated infection can increase antibody titer (Bonnet et al., 2022).

Above all findings implies that, after repeated infection, antibody titers may increase drastically. Furthermore, the booster effects of the vaccine are likely to be more intense in presence of natural infection induced immunity as compared to vaccine induced immunity after the 2nd dose. This suggests that the effect of a previous infection followed by vaccination, immune responses is not a temporary phenomenon. The memory compartment continues to evolve after both natural infection and vaccination.
In our study seropositivity was not varied significantly between male (78/49; 62.03%) and females (101/167; 60.48%). According to this study, when concentration of antibody is measured through quantitative ELISA, there was no gender variation in titer too. Ara et al. (2022) also reported similar findings.

Seropositivity did not vary significantly according to presence and absence of comorbidities in the population; 51.72% participants having comorbidity found seropositive and 63.74% without comorbidities found seropositive. Moreover, in our study 100% immunosuppressive drug users had IgG/IgM. However, in a previous study, seropositivity was significantly higher among the subjects with comorbidities (Ristić et al., 2022). Ara et al. (2022) also reported 80.91% participants were seropositive who were taking immunosuppressive drugs while 65.32% were seropositive who didn't take immunosuppressive drugs which also supports our study. In the present study, GMT was slightly higher 296.08 DU/ml among individuals having comorbidities compared to those who didn't have (GMT; 283.08 DU/ml). Moreover, higher concentration (GMT; 309 DU/ml, with SD of 88.31) was found among subjects who were immunosuppressive drug users. However, none of the above associations were statistically significant might be because of low number of observations in the group of comorbid and immunosuppressive drug users. Reported side effects of COVID-19 vaccines have mostly been mild to moderate, like a low-grade fever or muscle aches, are normal and not a cause for alarm (WHO, 2020). In this study, the most noticeable adverse effects were: fever, myalgia/arthralgia. Additionally, other adverse effects were runny nose, sore throat, malaise, bone pain, GIT upset etc. Regardless of whether it was 1st or 2nd or booster dose, in our study, 112/530 (21%) of HCWs reported adverse effects with the administration of the vaccine, while 78.87% did not develop any side effects. 32.14 % respondents had developed more than one adverse effect while 30% developed fever and 26.79% developed body ache/arthralgia/myalgia. Furthermore, 22.32% had non-specific adverse effects. We found no adverse effects led to serious ill health. Nevertheless, symptoms remove automatically after 4-5 days. The frequent combination of symptoms encountered in other studies were body pain with low grade fever 12% and in combination with pain at the injection site 12% (Elangovan et al., 2022). (Figueroa-Hurtado et al. (2022) observed 80% of HCWs reported an adverse effect with the administration of the vaccine. Most noticeable adverse effects were headache, pain, myalgia. Less common adverse effects include diarrhea, abdominal pain etc. After the 2nd dose of vaccine there was a significant increase of headaches in comparison to after the 1st dose of vaccination. Fatigue was more prevalent in females after the 2nd dose while pain arthralgia/myalgia was more frequently seen adverse effects after both doses of vaccine. However, none classified as a severe side effect (Figueroa-Hurtado et al., 2022).

In those respondents who developed side effects, they had a high antibody titer (mean titer > 310.44 DU/ml). Those who had body ache had a summary mean of 308.32 DU/ml, fever only 311.07 DU/ml, multiple symptoms had 313. 004 DU/ml, and even those who had multiple nonspecific symptoms also had 307.600 DU/ml. Reported side effects of COVID-19 vaccination are signs that the body's immune system is responding to the vaccine, specifically the antigen (a substance that triggers an immune response), and is gearing up to fight the virus, and go away within a few days on their own (WHO, 2020). There was a highly significant ($p<0.001$) difference in antibody titer between two groups who developed side effects (mean titer: 311.444 DU/ml) compared to those who didn't (mean titer: 278.752 DU/ml). Higher antibody titer, evidenced here, may reflect the body's immune system is responding to the vaccine.

Chapter 6: Conclusion

In the present study, we observed that, since the COVID-19 vaccine commenced in our country as well as in Chattogram, high seropositivity for COVID-19 has risen dramatically and clearly showed trends in vaccine-induced antibodies among the moderate when tested with ICT might be due to sensitivity issues of the test. These results add the evidence for the impact of the COVID-19 vaccine on the seroprevalence of SARS-CoV-2 as well as development of immunity.

With increased number of vaccines doses the seroprevalence and the titer of antibody increased significantly. It was more prevalent in people who had a 2nd dose and passed more than 8 months than those received only the 1st dose. These findings imply that immunity as well as considerable GMT developed after the 2nd dose of vaccination and persisted after 8 months. Again, who had symptomatic COVID-19 prior infection, had significant GMT. Therefore, prior SARS-CoV-2 infection should be taken into consideration when planning booster doses for health professionals and design of current and future COVID-19 vaccine programs. Further study with more time period will add value to see the immunity for the booster.

Chapter 7: Limitations of the study

- We included only HCWs, so seroprevalence represents only a specific occupational group.
- We can’t measure mRNA vaccine effectiveness and inactivated vaccines separately.
- During the study period people were affected by Omicron variants, few developed neutral immunity besides vaccine induced immunity. We could not assess the immunity developed by nature and vaccine induced specifically.
- ICT had been done on about half of population due to limited resource facilities.

Chapter 8: Recommendations

Based on the finding of this study, following recommendations are made:

- New cases of COVID-19 continuously appear even under the strict interventions adopted in Bangladesh. The present findings suggest that asymptomatic seropositive individuals contribute to virus transmission. The strategy of applying PCR tests to suspected patients and quarantining should be continued. In addition, screening for SARS-CoV-2 using anti-SARS-CoV-2 antibody tests in the asymptomatic populations could be considered in the risk region with high incidence rate.
- Past SARS-CoV-2 infection should be addressed when designing booster doses for healthcare workers and existing and future COVID-19 vaccination programs.
- ELISA is more preferable to see antibody in comparison to ICT. So, it can be taken into account regarding antibody detection.

Chapter: 9 Appendix Questionnaire survey

Anti-SARS-CoV-2 (COVID-19) Sero-survey Questionnaire

One Health Institute, Chattogram Veterinary and Animal Sciences University (CVASU)
&
Bangladesh Institute of Tropical and Infectious Diseases (BITID)

Specimen ID		**Date of specimen collection**	____/____/ 2021

Site*	**Hospital***					**Garments name & Location**	**Slum name & Location**
	Medical Centre	CMC	USTC	Memon	Imperial		
Patient*		Health Worker*					
Indoor	Outdoor	Doctor	Nurse	Lab tech	Other		

Name of Interviewee:						Temp:	°F
Mobile no.:				Relationship with mobile owner*		Own/Spouse/Child/Other	
Age:	__ __ year	Sex*:	Male	Female*	Other	No. of family members:	
				Pregnant / Non- Pregnant			
Profession:				Education:		Blood group:	
Present address:	House no./ Road/Village:				Union/ Ward no:		
Upazila/ Thana:				District:			
Travelling history- last six months (if any)*	Yes/ No			Location/ Area		Duration of stay	

Did you take vaccine against COVID-19?*	Yes	Date.....	Dose:* 1st/ 2nd
		Hospital registered.....	
		Any side effect*	Yes / No
	No		

Anti-SARS-CoV-2 (COVID-19) Sero-survey Questionnaire

One Health Institute, Chattogram Veterinary and Animal Sciences University (CVASU)
&
Bangladesh Institute of Tropical and Infectious Diseases (BITID)

If COVID-19 Diagnostic test (Rt PCR) done

Test date:			**Test Lab name:**		
Test result*:	**(+ve)**	**(-ve)**	If (+ve), negative confirmation test done?*	Yes, Date of test.....	No
Symptoms during test*	Yes *	Fever (.....ºF)/Cough/Shortness of breath/Sore throat/ Aches & pain/ Anosmia/ Ageusia/ Dysgeusia/ Headache/ Rash/ Diarrhoea/ Other.....			Onset of symptom (.....) days
	No				
Similar Symptoms any time after Test		Yes, Time & duration.....		No	
Maintained isolation?*	Yes	Home Isolation*: Separate bed/ separate room			
		Hospital*: General bed/ ICU/ HDU/Other.....			
	No				
Contact with any confirmed case?*		Yes	No	Don't know	
Any family member infected?*		Yes, No. of family member infected:		No	
Comorbidity*	DM/HTN/Heart disease/Liver disease/Renal disease/Asthma/COPD/Bronchitis/Other.....				
Do you take any immunosuppressive drug?*		Yes*, Steroidal/Other.....		No	

If COVID-19 Diagnostic test (Rt PCR) not done

Any COVID like symptoms within last six months? *	Yes*	Fever (.....ºF)/Cough/Shortness of breath/Sore throat/Aches & pain/Anosmia/Ageusia/Dysgeusia/ Headache/Rash/Diarrhoea/ Other.....			
	No				
If yes, Time & onset of symptoms	.....months before, ...days				
If symptoms present, Maintained isolation?*	Yes	Home Isolation*: Separate bed/ separate room			
		Hospitalized			
	No				
Contact with any confirmed case? *	Yes	No	Don't know		
Any family member had COVID like symptoms within last six months?*	Yes, No. of family member infected:			No	
COVID-19 (Rt-PCR) done for any family member?*	Yes*, (+ve)/ (-ve)	Date of test.....		No	
Comorbidity*	DM/HTN/Heart disease/Liver dis/Renal disease/Asthma/COPD/Bronchitis/Cancer/Other				
Do you take any immunosuppressive drug?*	Yes, Steroidal/Other.....			No	

(*) Put tick mark at appropriate option

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Interviewer

Chapter 10: References

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Chapter 11: Biography

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