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# Evaluation of the humoral immunity to CoVID-19 among immunized Healthcare Workers in Omdurman hospitals, in Khartoum state 2024.

A dissertation submitted in partial fulfillment for the requirements of M.Sc. Degree in Medical Laboratory Sciences (Medical Microbiology).

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# الآية

قال تعالى:

"قَالُواْ سُبۡحَٰنَكَ لَا عِلۡمَ لَنَآ إِلَّا مَا عَلَّمۡتَنَآۖ إِنَّكَ أَنتَ ٱلۡعَلِيمُ

ٱلۡحَكِيمُ"

سورة البقرة"32"

## Dedication

*All praise to **ALLAH**, today we fold the days' tiredness and the errand summing up between the cover of this humble work.*

*To the utmost knowledge lighthouse, to our greatest and most honored prophet Mohamed - May peace and grace from Allah be upon him.*

*To my motherto whom he strives to bless comfort and welfare and never stints what he owns to push me in the success way who taught me to promote life stairs wisely and patiently, To my father who taught me to trust in myself and believe in hard work and keeping fight for knowledge may his sole rest in peace.*

*To my brother and sisters. To my wife who to push me in the success way who encouraged me and gave me positive energy to continue this work. To my honored teachers.*

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# Acknowledgment

**First of all, thanks to Allah and his messenger our prophet Mohammed all pace from Allah upon him.**

**I would like to express my deeply thanks to my supervisor Dr. Hadia Abbas for repeated direction and dissolving my problems.**

**Also our thanks to Clinical Microbiology Department for continuous helping.**

# Abbreviations

| | |
|---|---|
| **ACE2** | **Angiotensin-Converting Enzyme 2** |
| **ADRS** | **Acute respiratory distress syndrome** |
| **CK** | **Creatine kinase** |
| **CLpro** | **Cysteine like protease** |
| **DNA** | **Deoxyribo nucleic acid** |
| **ELIZA** | **Enzymes linked immuno sorbentassy** |
| **IFN-γ** | **Interferon Gamma** |
| **IL-10** | **Interleukin 10** |
| **MERS _Cov** | **Middle East Respiratory Syndrome Corona Virus** |
| **Nabs** | **Neutralizing antibodies** |
| **NPS** | **Nonstructural protein** |
| **PLpro** | **Papain like protease** |
| **RBD** | **Receptor-binding domain** |
| **RNA** | **Ribo nucleic acid** |
| **ROC** | **Receiver operating curve** |
| **SARS_Cov2** | **Sever acute respiratory syndrome coronavirus2** |
| **SGPT** | **Glutamic-pyruvic transaminase** |
| **TNF-α** | **Tumer necrosis factor alpha** |
| **WHO** | **World health organization** |

# Abstract

**Background:** Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a non-segmented positive-sense, single-stranded ribonucleic acid (RNA) beta coronavirus .That was first reported in Wuhan, China. The SARS-CoV-2 infection causes the coronavirus disease 2019 (COVID-19) that became a global pandemic and public health crisis.

**Aim:** To evaluate the antibodies titer against COVID19 in immunized Healthcare Workers in Omdurman hospitals in Khartoum state.

**Methods:** This study was a cross-sectional study conducted in Khartoum state. During the period from January to April 2023. A list of the healthcare workers were chosen randomly by simple random sampling technique, 20of healthcare workers (HCWS) was un-vaccinated and 70 HCWS was vaccinated group. ELISA technique was used to detect the immunoglobulin G for vaccinated and not vaccinated group collected data were competent and analyzed by using the application SPSS version 25.

**Results:** The positive rate of anti-S antibodies after vaccination and post infection was 100%. Prevalence of antibody was higher in males than in females in post infected group only *P.value* (0.305) and (0.873) in post infected and vaccinated groups respectively. The highest titer level was seen in age group (43 – 54) years among vaccinated and un-vaccinated HCWS. The mean titer of anti-RBD IgG levels was insignificantly diverse among different types of vaccines. (*P.value*= 0.247) The highest titers of antibody was in vaccinated groups (mean 56.5) at duration (>=3 Month) more than post infected groups (mean 34.6), but there was no significant differences. The mean of antibodies titer was insignificantly different among workers occupied at the departments serving COVID- 19 patients than among those working in other departments. (*P.value*=.940)& (*P value= 0.226*) on post infected and vaccinated groups respectively.

**Conclusion:** The study's findings contribute to the growing body of evidence that both natural infection and vaccination induce strong and durable immune responses in healthcare workers. The lack of significant differences across various subgroups suggests that current vaccination strategies are broadly effective across different demographics. However, the study also highlights the need for ongoing monitoring of immune responses, particularly as new variants of SARS-CoV-2 emerge and as booster programs continue. Further research could explore the long-term durability of

immune protection, particularly beyond the 10-month mark, and how this may be influenced by factors such as prior infection, booster doses, and variant exposure.

## المستخلص

**الخلفية:** فيروس كورونا 2 المتلازمة التنفسية الحادة الوخيمة (SARS-CoV-2) هو عبارة عن فيروس كورونا بيتا لحمض الريبونوكلييك (RNA) غير المجزأ ذو الإحساس الإيجابي غير المجزأ (RNA) الذي تم الإبلاغ عنه لأول مرة في ووهان ، الصين. تسبب عدوى SARS-CoV-2 مرض فيروس كورونا 2019 (COVID-19) الذي أصبح وباءً عالميًا وأزمة صحية عامة.

**الهدف:** هدفي الدراسة هو تقييم عيار الجسم المضاد ضد فيروس كورونا 2019 لدي العاملين في مجال الرعاية الصحية المحصنين بمستشفيات أم درمان بولاية الخرطوم.

**الطرق:** هذه الدراسة عبارة عن دراسة مقطعية أجريت في ولاية الخرطوم. خلال الفترة من يناير إلى آبريل 2023. تم اختيار قائمة بالعاملين في مجال الرعاية الصحية بشكل عشوائي من خلال تقنية أخذ العينات العشوائية البسيطة ، 20 من العاملين في مجال الرعاية الصحية كانوا غير ملقحين وتم تلقيح 70 من العاملين في مجال الرعاية الصحية (HCWS). تم استخدام تقنية ELISA للكشف عن الغلوبولين المناعي G للمجموعة المحصنة وغير المحصنة التي تم جمع البيانات المختصة وتحليلها باستخدام تطبيق SPSS النسخة 25.

**النتائج:** كان المعدل الإيجابي للأجسام المضادة للبروتينات الشوكية بعد التطعيم وبعد الإصابة 100٪. كان انتشار الأجسام المضادة أعلى في الذكور منه في الإناث ولكن لم يكن الاختلاف بينهما فقيمة ص كانت (0.305) & (0.873) في المجموعتين اللاحقتين المصابة والمحصنة على التوالي . شوهد أعلى مستوى عيار في (54-43) سنة في عاملين الرعاية الصحية المحصنة وغير الملقحة. كان العيار المتوسط لمستويات IgG المضادة لـ RBD متنوعًا بشكل ضئيل بين أنواع اللقاحات المختلفة. كان أعلى عيار من الأجسام المضادة في المجموعات المحصنة (متوسط 56.5) لمدة (> = 3 أشهر) أكثر من المجموعات المصابة بعدوى (متوسط 34.6) ، لكن لم يكن هنالك فرق كبير. لم يكن عيار الجسم المضاد المتوسط مختلفًا بشكل كبير بين العاملين في الأقسام التي تخدم مرضى COVID  19 عن أولئك الذين يعملون في الأقسام الأخرى.

**الخلاصة:**تساهم نتائج الدراسة في مجموعة متزايدة من الأدلة على أن العدوى الطبيعية والتطعيم يحفزان استجابات مناعية قوية ودائمة لدى العاملين في مجال الرعاية الصحية. يشير عدم وجود اختلافات كبيرة بين المجموعات الفرعية المختلفة إلى أن استراتيجيات التطعيم الحالية فعالة على نطاق واسع عبر التركيبة السكانية المختلفة. ومع ذلك، تسلط الدراسة الضوء أيضًا على الحاجة إلى المراقبة المستمرة للاستجابات المناعية، خاصة مع ظهور سلالات جديدة من فيروس SARS-CoV-2 ومع استمرار برامج التعزيز. يمكن إجراء المزيد من الأبحاث لاستكشاف متانة الحماية المناعية على المدى الطويل، خاصة بعد مرور 10 أشهر، وكيف يمكن أن يتأثر ذلك بعوامل مثل العدوى السابقة، والجرعات المعززة، والتعرض المتنوع.

# Chapter One

## *Introduction*

## *Rationale*

## *Objectives*

# 1.1Introduction

A newly discovered coronavirus is the causative agent of the current coronavirus disease and the virus is called Severe AcuteRespiratory Syndrome Coronavirus 2 (SARS-CoV-2) (Wu*et al.*, 2020).Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a non-segmented positive-sense, single-stranded ribonucleic acid (RNA) beta coronavirus. (Kovski *et al.*, 2020).It has been reported in Sudan since March 13, 2020. To date, more than thirty seven thousand people have been infected with over 2,800 deaths (World meter, 2020). Nearly a year after the first case, Sudan received over 800,000 doses of AstraZeneca vaccine on March 3, 2021, through COVID-19 Vaccines Global Access (COVAX) Facility and vaccinations started on March 9 2021 prioritizing healthcare workers, the aged population, and those with chronic medical conditions. (UNICEF, 2021). Two shots of the vaccine have been delivered thereby allowing some people to get fully vaccinated.(UNICEF, 2021).Understanding the factors affecting humoral immune response to COVID-19 vaccines among healthcareworkers(HCWs) is essentialtopredicttheirlevelofprotection. Vaccination elicitsantibodiesagainstSARS-CoV-2spikeprotein(anti-S).(WHO, 2021).In the initial stage of coronavirus disease 2019 (COVID-19) pandemic, the World Health Organization (WHO) reported that 14%ofCOVID-19caseswerehealthcareworkers (HCWs) (WHO .,2021). Globally, between 80,000 and 180,000 deaths occurred among HCWs between January 2020 and May 2021 (WHO, 2021).Therefore, HCWs have had the priority for receipt of the COVID-19 vaccine. Developinganeffectiveimmunityisthegoalofallvaccineswithdifferenttechnologies. The humoral immune response is much easier to detect and far standardized than all other aspects of the immune response (Gundlapalli, 2021;McDonald,2021). COVID-19 vaccines induce detectable humoral antibodies directed against different antigens of SARS-CoV-2(Krammer, 2020). One of the main immunogenic antigens in the post-vaccine immune response is the Transmembrane spike (S) which is a receptor-binding domain (RBD) that protrudes from the viral surface andmediatesviralentryintohost cells (Bosch ,2003; Rottier, 2003). However, the post-vaccination immune responseandantibodytiterremainmarkedlyunpredictable, andmanyfactorsmayaffectit (Formica, 2021; Albert, 2021;Robinson and Plested, 2021).Ithad been reported to be age and gender-dependent, with higher titer in females and younger age groups (Coppeta *etal*., 2021).Associated comorbidities that may weaken the immune response; such as cardiovascular

disease, hypertension, diabetes, and obesity had been associated with lower levels of protective antibodies (Calendar, 2020); (Bates andMairesse, 2020).Additionally, vaccine type, number of doses and duration since the last vaccination markedly affect the immune response. Antibody level peaks twoweeks after the second dose of vaccine (independently of age or gender) (Trougakos*etal.*, 2021).Accumulating evidence suggests that the presence of antibodies following infection offers some level of protection from reinfection. Evidence includes the following: reduced incidence of infection among persons with SARS-CoV-2 antibodies followed for 3 months or longer, viral neutralization demonstrated with serum from persons following infection and data demonstrating that vaccination, which also results in antibody production, can reduce the incidence of illness and decreased disease severity, and even prevention, of infection associated with administration of monoclonal antibodies external icon. IgG antibodies, including IgG against the S and N proteins, persist for at least several months in most HCWS, but the precise duration of time that antibodies persist after infection is unknown. Despite the present of antibodies in the blood, reinfection occurs this may be due to neutralizing antibodies might not be detected among patients with mild or asymptomatic disease. The humoral immune response appears to remain intact even with loss of specific antibodies over time because of the persistence of memory B-cells. (Callendar, 2020).

## 1.2 Rationale

The SARS-CoV-2 virus, responsible for the COVID-19 pandemic, emerged from an outbreak in Wuhan, China, in December 2019. Despite efforts to contain it, the virus spread globally, resulting in over 520 million cases and 6.26 million deaths worldwide as of 2020. Healthcare workers (HCWs) are at particularly high risk of infection and were prioritized for COVID-19 vaccination. Inactivated SARS-CoV-2 vaccines have been used to induce an antibody response among HCWs. However, there has been some hesitancy among HCWs regarding vaccination. Understanding the factors that influence the humoral immune response to the COVID-19 vaccine in HCWs is crucial for predicting their level of protection.

HCWs are frequently exposed to the virus, especially during patient care, and could potentially contribute to hospital-acquired infections if infected. Therefore, assessing the durability of immune protection in this group is important. The transmembrane spike (S) protein, specifically the receptor-binding domain (RBD), is a key target of the immune response after vaccination and infection. However, it is still uncertain how long antibody levels remain protective against reinfection in HCWs and what concentrations are necessary for effective protection. Few studies, particularly in Sudan, have evaluated the humoral immunity to COVID-19 among vaccinated HCWs in Khartoum hospitals. HCWs, being role models in their communities, can significantly influence public health attitudes, making it important to assess whether their antibody levels result from natural infection or vaccination.

# 1.3 Objectives

## 1.3.1 General objective

To detect and evaluate the IgG antibodies titer against CoVID19 in Immunized Healthcare Workers in Omdurman hospitals.

## 1.3.2 Specific objectives

1. To quantify S (spike) antibody titer against CoVID19 in post infected Healthcare Workers by using ELISA.

2. To quantify S (spike) antibody titer against CoVID19 in vaccinated Healthcare Workers by using ELISA.

3. To associate between antibodies titer and if there was effect in age, gender and occupational in study groups.

# Chapter Two

## *Literature Review*

# *Literature Review*

## 2.1Coronavirusvirus

The name "coronavirus" is derived from Latin corona, meaning "crown" or "wreath", itself a borrowing from Greek κορώνηkorṓnē, "garland, wreath", the name was coined by June Almeida and David Tyrrell who first observed and studied human coronaviruses.(Tyrrell and Fielder,2002).The word was first used in print in 1968 by an informal group of virologists in the journal Nature to designate the new family of viruses.(Sturman *etal.,* 1983).The scientific name Coronavirus was accepted as a genus name by the International Committee for the Nomenclature of Viruses (later renamed International Committee on Taxonomy of Viruses) in 1971. (Lalchhandama, 2020).As the number of new species increased, the genus was split into four genera Alphacoronavirus, Betacoronavirus, Deltacoronavirus, and Gammacoronavirus in 2009, the common name coronavirus is used to refer to any member of the subfamily Orthocoronavirinae. As of 2020, 45 species are offi cially recognised.(Carstens, 2010).

### 2.1.1 History

Human coronaviruses were discovered in the 1960s using two different methods in the United Kingdom and the United States.(Kahn and McIntosh, 2005).Kendall, Bynoe, and David Tyrrell working at the Common Cold Unit of the British Medical Research Council collected a unique common cold virus designated B814 in 1961(Monto, 1984).The virus could not be cultivated using standard techniques which had successfully cultivated rhinoviruses, adenoviruses and other known common cold viruses. In 1965, Tyrrell and Bynoe successfully cultivated the novel virus by serially passing it through organ culture of human embryonic trachea, the new cultivating method was introduced to the lab by Bertil Hoorn. The isolated virus when intranasal inoculated into volunteers caused a cold and was inactivated by ether which indicated it had a lipid envelope.Hamre and Procknow at the University of Chicago isolated a novel cold from medical students in 1962. They isolated and grew the virus in kidney tissue culture, designating it 229E. The novel virus caused a cold in volunteers and, like B814, was inactivated by ether. (Tyrrelland Bynoe, 1965). A research group at the National Institute of Health the same year was able to isolate another member of this new group of viruses using organ culture and named one of the samples OC43 (OC for organ culture). (McIntosh*et al.*, 1967). Like B814, 229E, and IBV, the novel cold

virus OC43 had distinctive club-like spikes when observed with the electron microscope(McIntosh*et al.*, 1967).The IBV-like novel cold viruses were soon shown to be also morphologically related to the mouse hepatitis virus.(Decaro, 2011). This new group of viruses were named coronaviruses after their distinctive morphological appearance.(Almeida *et al.*,1968).Human coronavirus 229E and human coronavirus OC43 continued to be studied in subsequent decades.(Myint, 1995)(Geller*et al.*, 2012).The coronavirus strain B814 was lost. It is not known which present human coronavirus it was. Other human coronaviruses have since been identified, including SARS-CoV in 2003, HCoV NL63 in 2003, HCoV HKU1 in 2004, MERS-CoV in 2013, and SARS-CoV-2 in 2019. There have also been a large number of animal coronaviruses identified since the 1960s. (Groot *et al.*, 2011).

### 2.1.2 Classification

Coronaviruses form the subfamily Orthocoronavirinae, (Fan *et al.*, 2019).Which is one of two sub-families in the family Coronaviridae, order Nidovirales, and realm Ribavirin. (Su *et al.*,2016) They are divided into the four genera: Alphacoronavirus, Betacoronavirus, Gammacoronavirus and Deltacoronavirus. Alphacoronaviruses and betacoronaviruses infect mammals, while gammacoronaviruses and deltacoronaviruses primarily infect birds.

Genus: Alphacoronavirus; Species: Alphacoronavirus 1 (TGEV, Feline coronavirus, Canine coronavirus), Human coronavirus 229E, Human coronavirus NL63, Miniopterus bat coronavirus 1, Miniopterus bat coronavirus HKU8, Porcine epidemic diarrhea virus, Rhinolophus bat coronavirus HKU2, Scotophilus bat coronavirus 512.(Su, *et al*, 2016).

Genus Betacoronavirus; Species: Betacoronavirus 1 (Bovine Coronavirus, Human coronavirus OC43), Hedgehog coronavirus 1, Human coronavirus HKU1, Middle East respiratory syndrome-related coronavirus, Murine coronavirus, Pipistrellus bat coronavirus HKU5, Rousettus bat coronavirus HKU9, Severe acute respiratory syndrome–related coronavirus (SARS-CoV, SARS-CoV-2), Tylonycteris bat coronavirus HKU4(Su, *et al*, 2016). Genus Gammacoronavirus; (Neuman et al, 2006) Species: Avian coronavirus, Beluga whale coronavirus SW1. Genus Deltacoronavirus Species: Bulbul coronavirus HKU11, Porcine coronavirus HKU15 Phylogenetic tree of coronaviruses**.** (Su, *et al*, 2016).

### 2.1.3 Genome

Coronaviruses contain a positive-sense, single-stranded RNA genome. The genome size for coronaviruses ranges from 26.4 to 31.7 kilobases. (Woo *et al.*, 2010). The genome size is one of the largest among RNA viruses. The genome has a 5′ methylated cap and a 3′ polyadenylated tail. (Fehr and Perlman, 2015). The genome organization for a coronavirus is 5′-leader-UTR-replicase (ORF1ab)-spike (S) envelope (E)-membrane (M)-nucleocapsid (N)-3′UTR-poly (A) tail. The open reading frames 1a and 1b, which occupy the first two-thirds of the genome, encode the replicase polyprotein (pp1ab). The replicase polyprotein self cleaves to form 16 nonstructural proteins (nsp1–nsp16). (Fehr and Perlman, 2015).The later reading frames encode the four major structural proteins: spike, envelope, membrane, and nucleocapsid. (Snijder *et al.*, 2003) Interspersed between these reading frames are the reading frames for the accessory proteins. The number of accessory proteins and their function is unique depending on the specific coronavirus. (Fehr and Perlman, 2015).

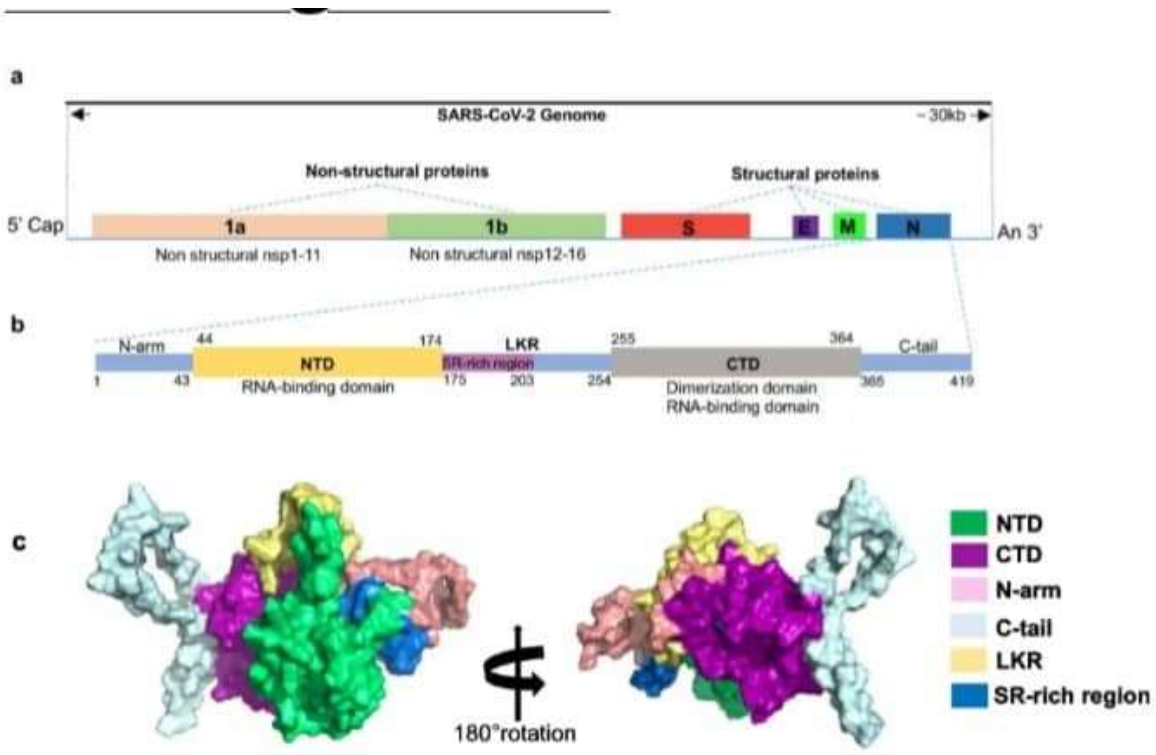



**Fig. 2.1 Genome and nonstructural proteins of SARS-CoV-2** (Dandekar and Perlman, 2005)

### 2.1.4 Structure

Coronavirus have a large (30+ bk ) single –stranded positive sense RNA genome encased by a helical nucleocapsid (N) and an outer envelope comprised of matrix protein (M) envelope protein (E) and spike protein (S) size 60-220nm .(Oke and Heneghan, 2020).The spike or S glycoprotein is a Trans membrane protein with amolecularweightofabout150kDafoundintheouterportionofthevirus. S protein forms homotrimers protruding in the viral surfaceandfacilitatesbindingofenvelopevirusestohostcellsbyattractionwith angiotensin-converting enzyme 2 (ACE2) expressed in lowerrespiratory tract cells. This glycoprotein is cleaved by the host cellfurin-like protease into 2 sub units namely S1 and S2. Part S1 isresponsibleforthedeterminationofthehostvirusrangeandcellular tropism with the receptor binding domain make-up whileS2functionstomediatevirusfusionintransmittinghostcells.(Guo*etal.*, 2019);(Fehr Perlmanand, 2015).Anotherimportantpart ofthisvirusisthemembraneorMprotein,whichisthemoststructurallystructuredprotein andplays a role in determining the shape of the virus envelope. Thisprotein can bind to all other structural proteins. Binding with Mprotein helpsto stabilize nucleocapsidsorN proteins andpromotes completion of viral assembly by stabilizing N protein-RNAcomplex, insidetheinternalvirion.Thelastcomponentistheenvelope or E protein which is the smallest protein in the SARS-CoVstructurethatplaysaroleintheproductionandmaturationofthisvirus. (Schoeman andFielding 2019). In supporting the process of entry of the virus into the host cell,SARS-CoV2 binds to the ACE2 receiver that is highly expressed inthelowerrespiratorytractsuchastypeIIalveolarcells(AT2)ofthelungs, upper esophagus and stratified epithelial cells, and othercellssuchasabsorptiveenterocytesfromtheileumandcolon,cholangiocytes, myocardial cells, kidney proximal tubule cells, andbladder urothelial cells. Therefore, patients who are infectedwith this virus not only experience respiratory problems such aspneumonialeadingtoAcuteRespiratoryDistressSyndrome (ARDS),butalsoexperiencedisordersofheart,kidneys,and digestivetract.(Walls*et al.*, 2020).

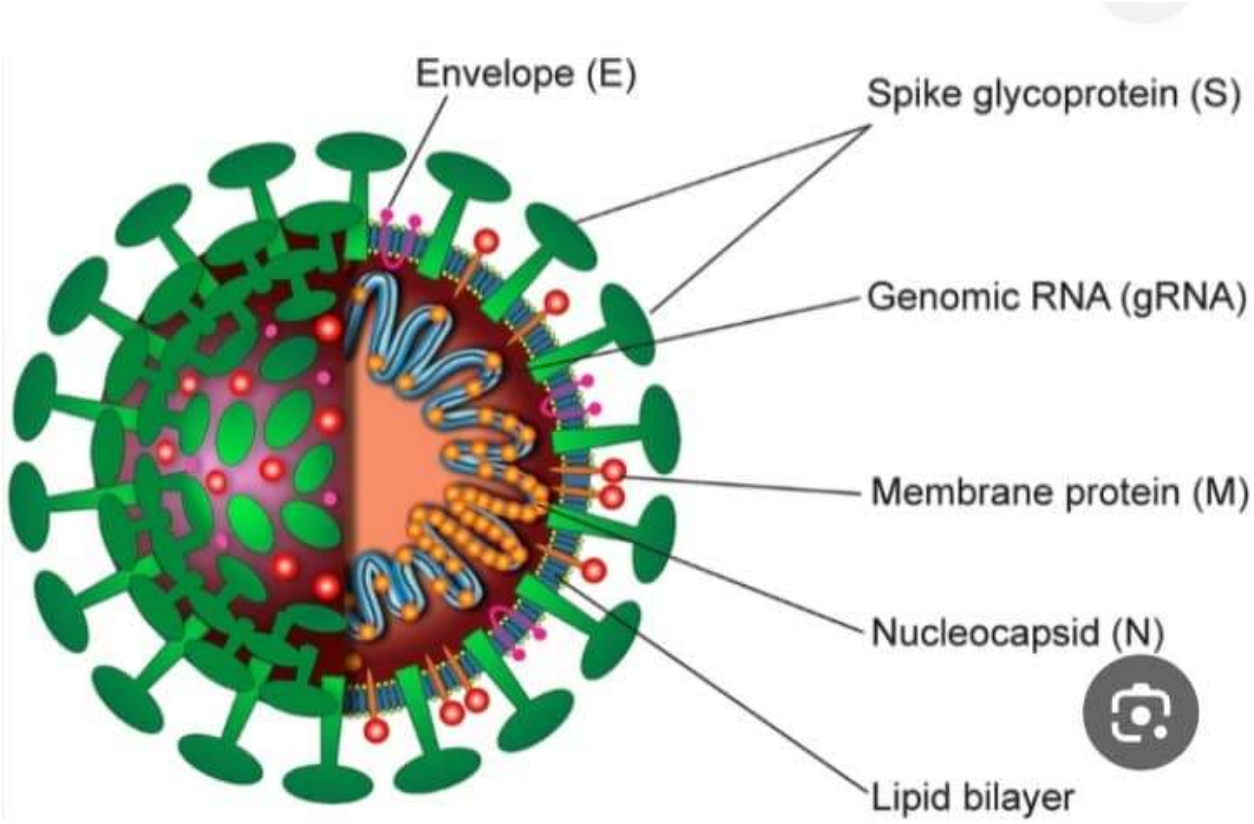



**Fig 2.2: Structure of SARS-CoV-2** (Tai*et al.*, 2020).

### 2.1.5 Life cycle of coronavirus

As a member of the Nidovirus family, coronavirus infection (SARS-CoV2) can be contracted from animals such as bats, and fellow humans. This virus can enter the human body through its receptors, ACE2 which are found in various organs such as heart, lungs, kidneys, and gastrointestinal tract, thus facilitating viral entry into target cells. The process of CoV entering into the host cell begins through the attachment of the S glycoprotein to the receptor, the ACE2 in the host cells (such as in type II pneumocytes in the lungs).(Rabi*et al.*, 2020).This attachment occurs in the binding domain of S protein of SARS-CoV-2 receptors which are present at 331 to 524 residues, and can bind strongly to human ACE2 and bat ACE2 (Tai*et al.*, 2020).The entry and binding processes are then followed by fusion of the viral membrane and host cell.(Walls*etal.*, 2020).After fusion occurs, the type II Transmembrane serine protease (TMPRSS2) that is present on the surface of the host cell will clear the ACE2 and activate the receptor-attached spike-like, S proteins.(Rabi*et al.*, 2020).Activation of the S proteins leads to conformational changes and allows

the virus to enter the cells (Simmons*et al.,* 2013). Both of these proteins (TMPRSS2 and ACE2) are the main determinants of the entry of this virus. Based on the research of Sungnak et al. nasal epithelial cells, specifically goblet/secretory cells and ciliated cells, display the highest ACE2 expression throughout the respiratory tract.(Sungnak*et al.*,2020).Furthermore, entered-SARS-CoV-2 will subsequently release its genomic material in the cytoplasm and become translated in the nuclei.(Sungnak*et al.,* 2020).The genomic material released by this virus is mRNA that is ready to be translated into protein. In its genome range, this virus is complemented by about 14 open reading frames (ORF), each of which encodes a variety of proteins, both structural and non-structural that play a role in its survival as well as virulence power. In its phase of transformation, the gene segments that encode nonstructural polyproteins are the ones this process first translates into ORF1a and ORF1b to produce two large overlapping poly- proteins, pp1a and pp1ab by contributing a ribosomal frame shifting event. (Masters, 2006). The polyproteins are supplemented by protease enzymes namely papain-like proteases (PLpro) and a serine type Mpro (chymotrypsin-like protease (3CLpro)) protease that are encoded in nsp3 and nsp 5. Subsequently, cleavage occurs between pp1a and pp1ab into nonstructural proteins (nsps) 1e11 and 1e16, respectively. The nsps play an important role in many processes in viruses and host cells.(Chen*et al.,* 2020).Many of the nsps subsequently form replicase-transcriptase complex (RTC) in double-membrane vesicles (DMVs), which are mainly an assembly by RNA-dependent RNA polymerase (RdRp)- and helicase-containing subunits, the canonical RdRp domain residing of CoV nsp 12 and AV nsp9. Furthermore, the complex transcribes an endogenous genome template of viral entry to negative-sense genes of both the progeny genome and sub genomic RNA as intermediate products and followed by transcription to positive-sense mRNAs that are mainly mediated by RdRp. (Posthuma*et al.*,2017).Next, the sub genomic proteins become translated into structural and accessories proteins such as M, S, and E proteins that subsequently are insulated in the endoplasmic reticulum and then moved to the endoplasmic reticulum-Golgi intermediate compartment (ERGIC). Meanwhile, the previously replicated genome program can directly join the N protein to the nucleocapsid form and move into the ERGIC. In this compartment, nucleocapsids will meet with several other structural proteins and form small wallet vesicles to be exported out of the cell through exocytosis.(Sun *et al.,* 2012).

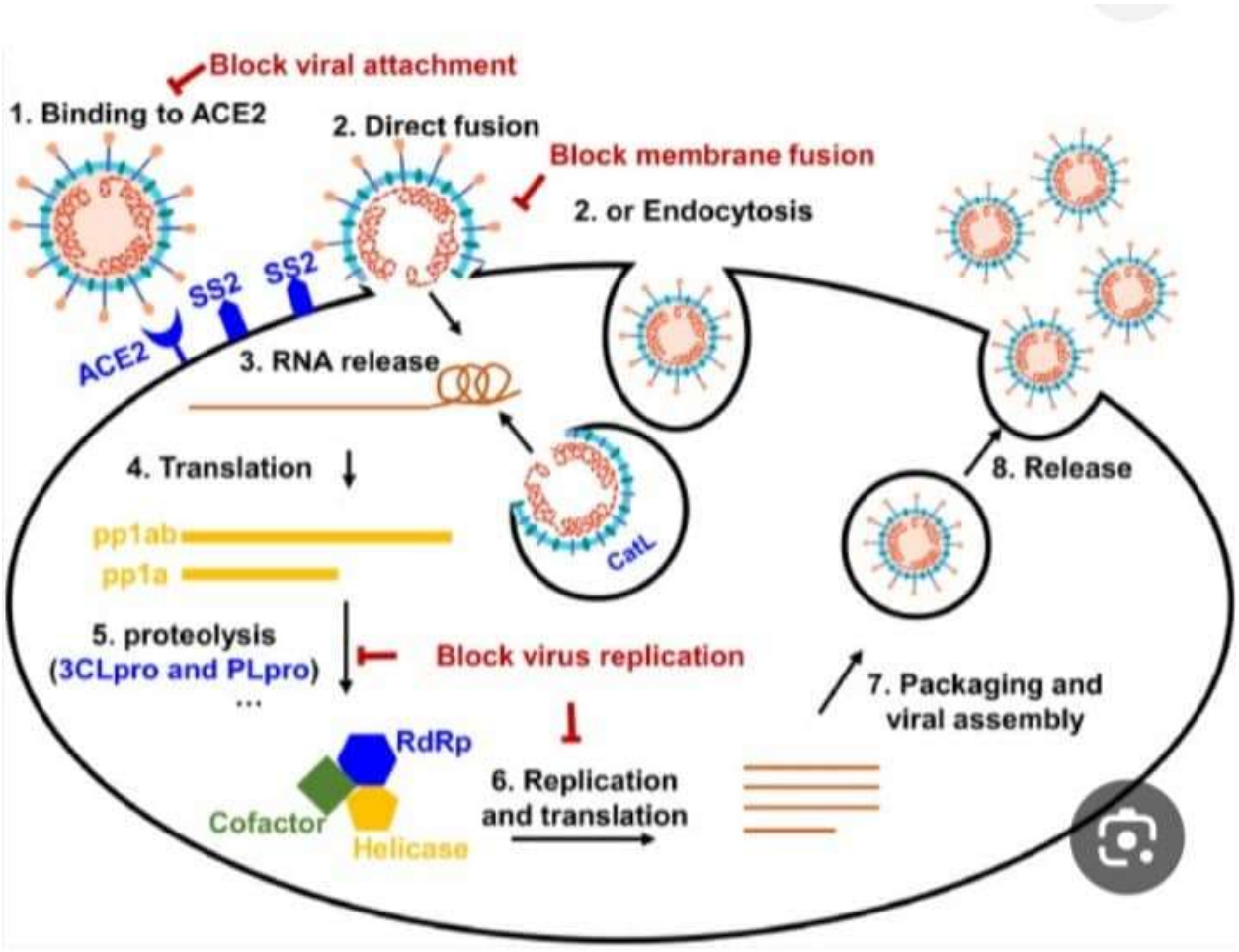



**Fig. 2.3 Life cycle of SARS CoV-2** (Rabi*etal.*, 2020).

### 2.1.6 Epidemiology

In 2003, following the outbreak of severe acute respiratory syndrome (SARS) which had begun the prior year in Asia, and secondary cases elsewhere in the world, the World Health Organization (WHO) issued a press release stating that a novel coronavirus identified by several laboratories was the causative agent for SARS. The virus was offi cially named the SARS coronavirus (SARSCoV). More than 8,000 people from 29 countries and territories were infected, and at least 774 died**.** (Pasley and James, 2020).In September 2012, a new type of coronavirus was identified, initially called Novel Coronavirus 2012, and now offi cially named Middle East respiratory syndrome coronavirus (MERS CoV). (Doucleef, 2012).The World Health Organization issued a global alert soon after. The WHO update on 28 September 2012 said the virus did not seem to pass easily from person to person. (Falco, 2012). However, on 12 May 2013, a case of human-to-human

transmission in France was confirmed by the French Ministry of Social Affairs and Health. (Kelland, 2012). In addition, cases of human-to-human transmission were reported by the Ministry of Health in Tunisia. Two confirmed cases involved people who seemed to have caught the disease from their late father, who became ill after a visit to Qatar and Saudi Arabia. Despite this, it appears the virus had trouble spreading from human to human, as most individuals who are infected do not transmit the virus. (CDC, 2019) By 30 October 2013, there were 124 cases and 52 deaths in Saudi Arabia**.** (WHO, 2013).In December 2019, a pneumonia outbreak was reported in Wuhan, China. (CDC, 2019). On 31 December 2019, the outbreak was traced to a novel strain of coronavirus, which was given the interim name 2019-nCoV by the World Health Organization, later renamed SARS-CoV-2 by the International Committee on Taxonomy of Viruses. As of 11 October 2022, there have been at least 6,559,055 confirmed deaths and more than 622,057,900 confirmed cases in the COVID-19 pandemic. The Wuhan strain has been identified as a new strain of Betacoronavirus from group 2B with approximately 70% genetic similarity to the SARS-CoV. (WHO, 2019). The virus has a 96% similarity to a bat coronavirus, so it is widely suspected to originate from bats as well. (Hui *et al.*, 2020).As of 15 April 2020, 210 Countries and Territories around the world have reported over 1.998.111 confirmed cases and 126.604 deaths of COVID-19 and show the presence in six continents. According to the medical journal hosted by Johns Hopkins University. Though the proportion of confirmed cases outside China is steadily increasing. (Murphy *et al.*, 2019).

### 2.1.7Transmission

Coronavirus is spread through two modes of transmission of COVID-19 exist—direct and indirect(WHO, 2019).The direct mode includes (1) transmission via aerosols formed via surgical and dental procedures and/or in the form of respiratory droplet nuclei; (2) other body fluids and secretions, for example, feces, saliva, urine, semen, and tears; and (3) mother-to-child. SARS-CoV-2 is thought to commonly spread via respiratory droplets formed while talking, coughing, and sneezing of an infected person. The exposure and, hence, risk of transmission are increased if the infected person is present within1-m length of susceptible host .Less number of infected patients has shown to shed virus from sources other than the respiratory tract(WHO, 2019). Though not high, the risk of transmission through modes other than respiratory tract can still be possible. Indirect transmission may

occur via (1) fomites or surfaces (e.g., furniture and fixtures) present within the immediate environment of an infected patient and (2) objects used on the infected person (e.g., stethoscope or thermometer)(Sun*et al.*,2020). Several of these modes may be underestimated and cause increased spread of virus. The goal of this paper is to briefly review how SARS-CoV-2 is shown to transmit via various modes and propose measures to reduce the risk of spread within the population and operating personnel (Sun*et al.*, 2020).

### 2.1.8 Pathogenesis

Infection begins when the viral spike protein attaches to its complementary host cell receptor. After attachment, a protease of the host cell cleaves and activates the receptor-attached spike protein. Depending on the host cell protease available, cleavage and activation allows the virus to enter the host cell by endocytosis or direct fusion of the viral envelope with the host membrane. (Seow *et al.*, 2020).And fall genome was sequenced and published by (Qiu*et al.*,2020). .The infection fatality rate death amongst all people infected a symptomatic and not tested is a better estimate of population mortality and is modelled to be between 0.1% and 0.41%. (Tian*et al.*,2020).While SARS-CoV-2 is a new virus and, therefore, the exact correlates of protection are not completely defined, there are precedents from other respiratory infections in general and corona viruses in particular.(Seydoux*et al.*,2020).There has been discussion that natural immunity to SARS-CoV-2 declines quickly; whether this is the case is still unclear. It is our speculation that because vaccines aim to evoke an immune response they could be more immunogenic than the virus itself, which might have mechanisms to dampen immune response: whether this speculation is correct or not is yet to be determined. (Chen*et al.*, 2020).The T cell response is important in the control of other respiratory infections, and therefore likely to be important in COVID. (Chen*et al.*, 2020).

### 2.1.9 Clinical manifestation

Clinical manifestations of covid 19 infection have similarities with SARS-CoV where the most common symptoms include fever, dry cough, dyspnea, chest pain, fatigue and myalgia(Huang*etal.*,2020; Zhu*et al.*, 2020).Less common symptoms include headache, dizziness, abdominal pain, diarrhea, nausea, and vomiting(Li*et al.*, 2020). Based on the report of the first 425 confirmed cases in Wuhan, the common symptoms include fever, dry cough, myalgia and fatigue

with less common are sputum production, headache, hemoptysis, abdominal pain, and diarrhea. Approximately 75% patients had bilateral pneumonia(Chen*et al.*, 2020).Different from SARS-CoV and MERS-CoV infections, however, is that very few COVID-19 patients show prominent upper respiratory tract signs and symptoms such as rhinorrhea, sneezing, or sore throat, suggesting that the virus might have greater preference for infecting the lower respiratory tract. Pregnant and non-pregnant women have similar characteristics(Chen*et al.*, 2020).Severe complications such as hypoxemia, acute ARDS, arrhythmia, shock, acute cardiac injury, and acute kidney injury have been reported among COVID-19 patients (Huang*et al.*, 2020).A study among 99 patients found that approximately 17% patients developed ARDS and, among them, 11% died of multiple organ failure. The median duration from first symptoms to ARDS was 8 days(Wang*et al.*, 2020).

### 2.1.10 Laboratory diagnosis of COVID-19

The gold standard for diagnosis is the identification of viral genome targets by real-time polymerase chain reaction (RT-PCR) in respiratory tract materials during the first week of symptoms. Serological tests should be indicated from the second week of symptoms onwards. A wide range of different tests is available, with variable sensitivity and specificity, most of which require validation. Laboratory tests such as complete blood count, C-reactive protein (CRP), D-dimer, clotting tests, lactic dehydrogenase (LDH), ferritin, and procalcitonin identify risk of disease with greater severity, thromboembolic complications, myocardial dam- age, and/or worse prognosis. Imaging tests may be useful for diagnosis, especially when there is a compatible clinical picture, and other tests presented negative results or were unavailable (Sethuraman*etal.*,2020).Tests for Covid 19 may be direct, identifying genetic material of SARS-CoV-2, or indirect, determining the humoral immune response to SARS-CoV-2, the most commonly used method for identifying genetic material from SARS-CoV-2 is real-time polymerase chain reaction (RT-PCR). This method involves reverse tran- scription of the genetic material of the virus (RNA) to complementary DNA (cDNA), followed by amplification of some regions of the cDNA. Probes (DNA/RNA marked sequences to identify the genetic target in the material) and primers (DNA/RNA sequences that promote replication of the genetic material found in the sample) were created after the SARS-CoV-2 genome was sequenced. Several serial amplification cycles are performed to identify these targets: the more cycles are needed, the lower the viral load of the material under study(Sethuraman*et al.*, 2020).Serological tests identify the presence of humoral response to SARS-CoV-2. Antibodies of IgA, IgM, and IgG isotypes specific

to different virus proteins are detected by enzyme-linked immunosorbent assay (ELISA) or chemiluminescence immunoassays (CLIA), and the latter has been shown to be more sensitive(Deeks*et al.*, 2020).The gold standard for the diagnosis of SARS-CoV-2 infection is the identification of viral genetic material by RT-PCR, in different samples, with greater sensitivity in broncho alveolar lavage and nasopharyngeal swab. Many factors related to the individual, the collection procedure, and the test technique interfere with the sensitivity of these tests. Therefore, a negative test in a patient with a characteristic clinical picture should not discard the possibility of COVID-19 (Deeks*et al.*, 2020).The available serological tests are different from each other and many factors influence their sensitivity and specificity. Not all patients who have SARS-CoV-2 infection will have detectable levels of antibodies, particularly if they have milder symptoms. The absence of antibodies does not imply the absence of contact or protection against the virus, since there may be an efficient specific cellular immune response. In turn, the presence of antibodies does not rule out the possibility that the individual is still infectious, as no immediate reduction in the elimination of the virus has been identified. The support laboratory and imaging tests show alterations that are characteristic of COVID-19, but they lack specificity, the diagnosis of COVID-19 should be based on clinical and epidemiological history, tests for etiological diagnosis, and tests to support the diagnosis of infection and/or its complications. (Azkur*et al.*, 2020).

**2.1.10.1Support tests**

These are laboratory or imaging tests that demonstrate characteristic manifestations of COVID-19, its complications, and/or risk factors for complications (Azkur *et al.*, 2020).

1. **Laboratory tests**

Complete blood count - lymphopenia, eosinopenia, and neutrophil/lymphocyte ratio 3.13 are related to greater severity and worse prognosis. Thrombocytopenia is related to a higher risk of myocardial damage and a worse prognosis. Lymphopenia results from a multifactorial mechanism that includes the cytopathic effect of the virus, induction of apoptosis, IL1-mediated pyro ptosis, and bone marrow suppression by inflammatory cytokines(Azkur*et al.*, 2020).High values of C-reactive protein (CRP), ferritin, D-dimer, procalcitonin, lactic dehydrogenesis (DHL), prothrombin time, activated partial thromboplastin time, amyloid serum protein A, creatine kinase (CK), glutamic-pyruvic transaminase (SGPT), urea, and creatinine are risk factors for more severe

disease, thromboembolic complications, myocardial damage, and/or worse prognosis(Chen*et al.*, 2020).Immunological markers that may also represent risk factors for greater severity and/or worse prognosis are: decreased values of CD4 + T and CD8+ lymphocytes, and NK cells and increased values of IL6, IL-8, IL-10, IFN-γ, TNF-IL-2R, TNF-α, GM-CSF, and IL-1 β. (Vabret*et al.*, 2020).

**2. Imaging tests**

Imaging tests for the diagnosis of COVID-19 have gained relevance, given the unavailability of tests for etiological diagnosis (Udugama*et al.*, 2020).The alterations described in these tests can also be found in influenza or mycoplasma infections, in inflammatory processes of different origins, or in eosinophilia lung diseases (Foust*et al.*, 2020).Although the findings in these tests are not specific to COVID-19, given a compatible clinical picture and/or the presence of confirmed or possible history of contact, they may help in the diagnosis. Plain chest X-rays are less sensitive than computed tomography, but may evidence sparse bilateral consolidations accompanied by ground glass opacities, peripheral/sub pleural images, predominantly in the lower lobes(Foust*et al.*, 2020).Computed tomography of the chest presents greater sensitivity and reveals multifocal, bilateral, periph- eral/subpleural ground glass opacities, generally affecting the posterior portions of the lower lobes, with or with- out associated consolidations(Carusom *et al.*, 2020). Children have a similar presentation to that found in adults, albeit with a milder involvement (Foust *et al.*, 2020).The halo sign, described as a consolidation area involved by ground glass opacities, was identified in 50% of the children(Foust*et al.*, 2020). An inverted halo sign, in which areas of ground glass opacities are surrounded by condensation halo, has also been described(Faria*setal.*, 2020).Pulmonary ultrasonography has good sensitivity; the typical findings are B-lines, consolidations and pleural thickening(Boccatonda*et al.*, 2020). The advantages of this method are its lower cost, absence of radiation exposure, and the fact that it does not require sedation or transportation of unstable patients(Zhang*et al.*, 2020).Most studies on diagnostic methods presented here refer to adults; however, studies specific to the pediatric age group show very similar data(Hoang *et al.*, 2020).The data presented suggest that the diagnosis of COVID-19 should be based on clinical manifestations, contact history, imaging tests, laboratory tests, and not only on serological tests and the search for the genetic material of the virus. In addition, strategies to increase sensitivity, specificity, and speed of diagnosis are fundamental (Watson *et al.*, 2020).

**2.1.11Immune response to coronavirus infection**

### 2.1.11.1Humoral and cellular immunity

Antigen presentation subsequently stimulates the body's humoral and cellular immunity, which are mediated by virus-specific B and T cells. Similar to common acute viral infections, the antibody profile against SARS-CoV virus has a typical pattern of IgM and IgG production. The SARS-specific IgM antibodies disappear at the end of week 12, while the IgG antibody can last for a long time, which indicates IgG antibody may mainly play a protective role (Xu *et al.*,2020).The SARS-specific IgG antibodies primarily are S-specific and N specific antibodies. Comparing to humoral responses, there are more researches on the cellular immunity of coronavirus. The latest report shows the number of CD4þ and CD8þ T cells in the peripheral blood of SARS-CoV-2-infected patients significantly is reduced, whereas its status is excessive activation, as evidenced by high proportions of HLA-DR (CD4 3.47%) and CD38 (CD8 39.4%) double positive fractions(Fan*et al.,* 2019). Similarly, the acute phase response in patients with SARS-CoV is associated with severe decrease of CD4þ T and CD8þ T cells. Even if there is no antigen, CD4þ and CD8þ memory T cells can persist for four years in a part of SARS-CoV recovered individuals and can perform T cell proliferation, DTH response and production of IFN-g (Fan*et al.,* 2019). Six years after SARSCoV infection, specific T-cell memory responses to the SARS-CoV S peptide library could still be identified in 14 of 23 recovered SARS patients (Tang*et al.,* 2011). The specific CD8þ T cells also show a similar effect on valuable information for the rational design of vaccines againstSARS-CoV-2.Antibodies are likely to be an important part of vaccine induced protection. In SARS-CoV-1 the antibody response is short lived immunoglobulin IgM and IgA responses last less than 6 months and IgG lasts approximately 1 year, this is possibly the same for SARS-CoV-2. Although this is in the context of more than 25 million recorded cases globally suggesting that it is a rare event. Because of the overlap between SARS-CoV-1 and SARS-CoV-2 spike proteins antibodies could be cross neutralizing (Oke and Heneghan, 2020) .

### 2.1.11.2 Coronavirus immune evasion

To better survive in host cells, SARS-CoV and MERS-CoV use multiple strategies to avoid immune responses. The evolutionarily conserved microbial structures called pathogen-associated molecular patterns (PAMPs) can be recognized by pattern recognition receptors (PRRs). However, SARS-CoV and MERS-CoV can induce the production of double-membrane vesicles that lack PRRs and then replicate in these vesicles, thereby avoiding the host detection of their

dsRNA.(Snijder *et al.*,2003).The For SARS-CoV-2, T cell responses have been observed to a range of antigens, including S, M, N and other ORFs.(Channappanaar *et al.*, 2014).T cell memory can be long lived; SARS-CoV-1 T cells were detected 4 years after infection(Grifoni*et al.*,2020). S ARSCoV-2-specific T cells have been detected in individuals who had asymptomatic or mild COVID-19 (Channappanavar *et al.*, 2014).And SARS-CoV-2-specific T cells have been observed in contacts of infected individuals(Channappanavar*et al.*, 2014).Patients suffering from COVID-19 had fewer T cells than healthy controls (Gallais*et al.*, 2020). T cells, especially CD4+ T cells, can influence the immune response through the production of cytokines, and elevated cytokines have been associated with exacerbated disease(Tan *et al.*,2020).The skewing of the CD4 + T cell response is likely to be important T helper type 1 (Th1) responses are central to the successful control of SARS-CoV-1 and MERS-CoV (Braun *et al.*,2020). Th17 responses have been speculated to be deleterious and increased Th2 cytokines were seen in severe disease regulatory T cells are important in the resolution of infection, and were observed to be elevated in COVID-19 patients (Hotez *et al.*, 2020). Circulating follicular T helper cells, important in defining recall antibody response to infection, have been observed in a small number of individuals with COVID-19 (Lucas, et al 2020). It is not clear whether the 'cytokine storm' is a cause or effect of disease understanding this relationship is critical in monitoring vaccine safety (Hotez *et al.*, 2020).Coronavirus S protein has been reported as a significant determinant of virus entry into host cells.(Yang*et al.*, 2020).Observed that antibody was rarely seen in the first 7 days of infection, but rises in the second and third weeks post-infection. It is unclear whether antibodies correlate with COVID-19 severity(Tian *et al.*, 2020).

### 2.1.12 Ttreatment

A confirmed patient of COVID 19 needs complete bed rest and supportive treatment, ensuring adequate calorie and water intake to reduce the risk of dehydration. Water electrolyte balance and homeostasis need to maintain along with the of monitoring vital signs and oxygen saturation; keeping respiratory tract unobstructed and inhaling oxygen in more severe cases; measuring blood count, C reactive protein, urine test, and other blood biochemical indexes including liver and

kidney function, myocardial enzyme spectrum, and coagulation function according to patient's conditions. Chest imaging should be continuously re-examined and blood gas analysis should be performed when required (Arabi *et al.*, 2019).Control measures are needed for patients with a high fever. Antipyretic drug treatment should be performed in case the temperature exceeds 38.5 °C. Warm water bath and antipyretic patches are preferred as a preventive measure to lower the temperature. Common drugs include ibuprofen orally, 5–10 mg/kg every time; acetaminophen orally, 10– 15 mg/kg every time. Need to administer sedative arises in case the child suffers from convulsions or seizure (Siegel *et al.*, 2017).The chances of hypoxia are increased as the virus targets the lungs. Nasal catheter, mask oxygen should be immediately provided to the patient. In emergency conditions, Non-invasive or invasive mechanical ventilation should be provided to the patient (Shen and Yang, 2020).Group of antiviral drugs including interferon α (IFN-α), lopinavir/ritonavir, chloroquine phosphate, ribavirin, and arbidol are therapeutically useful for the Prevention, Diagnosis, and Treatment of Novel Coronavirus-induced Pneumonia by the National Health Commission (NHC) of the People's Republic of China for tentative treatment of COVID-19(Liying *et al.*, 2020).

### 2.1.13 Prevention

On top of basic illness prevention and real defense against disease is a strong immune system. People body is better able to fight off disease when the immune system is humming and people should put to get their perfect body shape. This is a time to focus on all the health habits people may have been putting off, Dr. Tom Moorcroft, an osteopathic doctor who specializes in infectious disease says, start daily activities and food choices that support people's health and turn them into habits that will lead to life-long improvements in health. During this critical situation, get adequate sleep and some fresh air and sunlight daily. People also, stay hydrated, minimize overly processed foods and make sure to eat enough micronutrients when they can try their best with what they can find at grocery stores right now(Capritto, 2020).People should stay aware of the latest information on the COVID-19 outbreak provided by WHO and Follow the directions of your local health authority and prevent secondary infections, interrupt human-to-human transmission to your close contacts, health care workers and prevent further international spread. Most of the people who infected, experience mild illness and recover it, but its infection can be more severe for other individuals, to take care of your health and protect others take the subsequent steps:(WHO, 2019).

- Wash your hands regularly and thoroughly with soap and water for at least 20 seconds or with an alcohol based hand rub (hand sanitizer that contains at least 60% alcohol) completely cover your hands and rub them together until they do not dry especially after you have been visited a public place, or after blowing your nose, sneezing or coughing.(WHO, 2019).
- Hands touch many surfaces and pick up viruses and these contaminated hands, can transfer the virus to your nose, eyes or mouth So, avoid touching these organs with unwashed hands. Because from there, the virus can enter the body and may cause persons to sick.
- Maintain social distancing (maintain at least 1 metre or 3 feet distance between yourself and anyone) and avoid close contact with people who are sick (who is coughing or sneezing). When infected individuals cough or sneezes, they spray small droplets from their nose or mouth which may contain COVID-19 virus. The person can breathe in these droplets (CDC, 2019).
- Avoid large events and mass gatherings

A number of vaccines using different methods have been developed against human coronavirus SARS-CoV-2. Vaccines are available for animal coronaviruses IBV, TGEV, and Canine CoV, although their effectiveness is limited. In the case of outbreaks of highly contagious animal coronaviruses, such as PEDV, measures such as destruction of entire herds of pigs may be used to prevent transmission to other herds (Fehr *et al.*, 2015).

### 2.1.14Vaccination

As of 1 May 2021, there have been 152 661 445 Covid- 19 cases with 3 202 256 deaths globally. This pandemic led to the race to discover a vaccine to achieve herd immunity and curtail the damaging effects of Covid- 19.(WHO, 2021). On 31 December 2020, the Pfizer COVID- 19 vaccine (BNT162b2) was issued for emergency use listing by WHO. This was followed by the AstraZeneca/Oxford COVID- 19 vaccine, manufactured by the Serum Institute of India and SKBio on 15 February 2021, and most recently, on 12 March 2021, the Ad26.COV2.S, developed by Janssen (Johnson & Johnson) and Moderna on 30 April.(WHO, 2021). COVAX, coordinated by

WHO, Gavi: The Vaccine Alliance, the Coalition for Epidemic Preparedness Innovations (CEPI), acts as a programmed that supports the development of COVID- 19 vaccine candidates and negotiates their pricing to ensure low- and- middle- income countries have a fair shot at receiving vaccines.(Herzog *et al.*, 2021).

**2.1.14.1Vaccine Subtypes**

**2.1.14.1.1mRNA**

**A.1BNT162b2/ Pfizer**

This is a lipid nano particle–formulated, nucleoside- modified RNA vaccine that works against the S protein of the SARS- CoV- 2 virus.9 this vaccine allows for the body to create an antibodies response to neutralize the virus which is dependent on the S protein for entry via the ACE2 receptor on type 2 alveolar cells(WHO, 2021).

**B. mRNA-1273/Moderna**

This is a lipid nanoparticle–encapsulated nucleoside- modified messenger RNA (mRNA) – based vaccine. It encodes the prefusion stabilized full- length spike protein of SARS- CoV- 2. This spike glycoprotein moderates host cell attachments. Hence, it is essential for viral entry and thus the primary vaccine target. The vaccine gives rise to a vigorous binding and neutralizing antibody response(Jackson*et al.*, 2020).This also includes CD4+ T- cell and CD8+ cytotoxic T- cell response to eliminate the virus(Umakanthan *et al.*, 2021).

**2.1.14.1.2Adenoviruses**

Viral vectors provide an avenue for vaccines. The vectors may generally be classified as replicating or non- replicating vectors. Adenoviruses (Ads) are an example of vectors with both traits(Sumirtanurdin *et al.*, 2019).This platform was explored by the Oxford/AstraZeneca vaccine and the Janssen Pharmaceuticals vaccine by Johnson & Johnson. Both these vaccines encode the S protein of the SARS- CoV- 2 virus (Garcia*et al.*, 2021).After vaccination, it is expected that the surface spike protein is produced, encouraging the immune system to attack when it encounters the SARS- CoV- 2 virus. ChAdOx1- S- (AZD1222) uses a chimpanzee adenovirus vector while

Ad26. COV2.S relies on a recombinant human- based adenovirus vector. However, the Janssen vaccine is advantageous over the other candidate, as it is administered in only one dose, which reduces manufacturing costs.(Garcia *et al.*, 2021).

### A. Oxford/AstraZeneca/AZD1222

The University of Oxford and the British- Swedish pharmaceutical company AstraZeneca partnered to develop a non- replicating chimpanzee viral vector vaccine, formerly known as ChAdOx1nCoV- 19 and now called AZD1222.14 It is branded and popularly known as the 'AstraZeneca Vaccine' or 'Covishield Vaccine' if manufactured by the Serum Institute of India. 'Covishield' is produced based on the same technology by the Serum Institute of India to supply low- to- middle- income countries through COVAX.(Garcia*et al.*, 2021)

### B. Janssen vaccine/Ad26.COV2.S

This is a non- replicating, recombinant human adenovirus type 26 which contains a full- length SARS- CoV- 2 S protein that induces an antibody response against the SARS- CoV- 2 infection. Antibody directed against the S protein prevents invasion of the SARS- CoV- 2 virus in type 2 alveolar cells of the lungs, thus reducing the severity and morbidity of the infection. (Sadoff *etal.*, 2021). Advantages of adenoviral vectors are adjuvant qualities, scalability and their broad tissue tropism. On the downside, there is likely to be a slower pace of vaccine manufacturing in an outbreak setting, such as the current pandemic, as these laboratories need to have biosafety level 2. In addition, there is the possibility of pre- existing immunity to viral vectors, decreasing the effectiveness of the vaccine. The Oxford/AstraZeneca was able to overcome this disadvantage by using the Chimpanzee adenovirus (ChAdOx1) which represents an alternative to the human Ad vector and lacks preexisting immunity in humans (Koirala *et al.*, 2020 ; Belete, 2021).

## 2.2 COVID 19 and Health care workers

Healthcare personnel are all paid and unpaid persons working in healthcare settings who have the potential for exposure to infectious materials, these personnel include those involved in direct patient care, students and trainees, contractual staff, and personnel not directly involved in patient care but potentially exposed to infectious agents (Lorenc *et al.*, 2017).Vaccination of all HCWs will play a key role in avoiding sickness from this preventable disease, which can protect not only

HCWs themselves but also patients against infection (Lorenc, *et al.*, 2017). Based on the Strategic Advisory Group of Experts on Immunisation values framework, WHO identified HCWs as a high-priority group for COVID-19 vaccination(WHO, 2019).To date, many countries have initiated COVID-19 vaccination campaigns for priority populations including HCWs. Vaccination is an important measure to control the global pandemic of COVID-19. The most hopeful way of controlling COVID-19 could be universal vaccination to achieve herd immunity.1 Adequate COVID-19 vaccination coverage is the guarantee of herd immunity. Studies indicate that when basic reproduction number (R0) is estimated to be three, the threshold of herd immunity for COVID-19 is approximately 67% (Randolph and Barreiro, 2020**).**This means that when acquired immunity of population is over 67%, the incidence of COVID-19 infection will begin to decline (Fontanet and Cauchemez, 2020).The public's attitudes towards vaccine were unclear and affected by many factors, making the achievement of herd immunity a challenge(Fisher *et al.*, 2020;Malik*et al.*, 2020).Among the public, healthcare workers (HCWs) will be key to the success of COVID-19 vaccination.(Wang A *et al.*, 2020). Serving as a trusted source of vaccination information, HCWs' recommendations play a major role in patients' vaccination decisions. During the COVID-19 pandemic, HCWs are at high risk of infection. (Chersich *et al.*, 2020).Vaccination of all HCWs will play a key role in avoiding sickness from this preventable disease, which can protect not only HCWs themselves but also patients against infection. Based on the Strategic Advisory Group of Experts on Immunisation values framework, WHO identified HCWs as a high- priority group for COVID-19 vaccination(Lorenc *et al*, 2017).To date, many countries have initiated COVID-19 vaccination campaigns for priority populations including HCWs. Vaccine hesitancy is a behaviour that includes refusal of vaccines or delay of vaccination despite available services. (MacDonald, 2015).The WHO regarded vaccine hesitancy as a global health threat in 2019, Vaccine hesitancy in public has also been linked to the level of vaccine hesitancy among HCWs (WHO, 2019).

## 2.3 Previous study

In turkey 2022 this study examined the relationship between post vaccine seropositive rate, age, gender, smoking, body mass index (BMI) and antibody titer total of 314 HCWS who were not previously infected with covid 19 and who had received two dose of corona inactivated vaccine, 99.6% of the volunteer developed seropostively 4 weeks after the second dose of

vaccine.it was observed that seropostivity developed in almost all participant after CoronaVac vaccine. (Uysal E *et al.*, 2022).

In European 2020 this study examined Seroprevalence of anti-SARS-CoV-2 antibodies in COVID-19 patients and healthy volunteers up to 6 months post disease onset found no significant difference in IgG titers between males and females, however which showed that gender differences in antibody responses are often minimal or non-significant. (Figueiredo-Campos *et al.* 2020)

In London 2020 the study examined pandemic peak SARS-CoV-2 infection and serocoversion rates in London frontline health care workers found that the health care workers in direct contact with covid 19 patients may develop higher antibody levels than indirect contact due to repeated exposure to patients. . (Houlihan *et al.* 2020)

# Chapter Three

## *Materials and Methods*

# Materials and Methods

### 3.1 Study design

This is analytical cross-sectional study.

### 3.2 Study area

This study was conducted at different hospitals in Khartoum State (Omdurman Teaching Hospitals, Libya Specialist Hospital, Specialist Hospital Qtaralanda, Ombada Teaching Hospital and Rajhi Hospital).

### 3.3 Study duration

The study was conducted during the period between Januarys to April 2023.

### 3.4 Study population

Both vaccinated and post infected health care workers (HCWs) were enrolled in the study.

#### 3.4.1 Inclusion criteria

1. HCWs include physicians, nurses, technicians and pharmacists working at frontline departments (Intensive care units, emergency room, internal medicine wards, radiology, or laboratory) and low-risk departments (others).
2. Vaccinated HCWs without a history of infection prior to vaccination.

#### 3.4.2 Exclusion criteria

1. HCWs with chronic Illness (Diabetics, Hypertension) will excluded from the study.

2. HCWs with immunodeficiency diseases will excluded from the study.

### 3.5 Ethical considerations

Ethical considerations was taken from Provincial Ethical Committee, department of microbiology, Faculty of Medical Laboratory Sciences, informed consent was taken from all participants.

### 3.6 Sample Size

Sample size was calculated according to the following formula

$$N = Z2*(p)*Q/D2$$

N= is the sample size.

Z= is the confidence interval= (1.96).

P= Prevalence of disease = (50%)

Q= (1-p).

D= percentage of error which equal 0.05%.

Sample size= 384 samples.

Limitations of sample size due to the high cost of materials total of 90 blood samples were collected in this study.

### 3.7 Sampling technique

A simple sampling technique was employed to recruit participants into the study.

### 3.8 Data collection

Data was collected using structured questionnaire **(Appendix 1)** including all socio-demographic and clinical data.

### 3.9 laboratory examination

#### 3.9.1 Specimen collection

The blood specimens was collected using the venipuncture for collection. (5ml) ml was collected using sterile syringe to collect the blood after cleaning the skin area with alcohol pads and the blood was dispensed in a sterile plain container. Blood specimens after clotting , was centrifuged at 3000 round/minute for 5 minutes to obtain serum, and then the obtained sera were collected in clean sterile containers properly labeled and kept at -20 ºC till used.

#### 3.9.2 Enzyme Link Immunosorbent Assay test

For detection of SARS-CoV-2 IgG Spike antibodies on human sera using commercial AESKULISA ELISA kits (AESKU Germany) Qualitative and Quantitative kits. The test is based on the ELISA principle (Enzyme Linked Immunosorbent Assay).The Antigen is bound to solid phase. The specific immunoglobulins are bound to the antigen through incubation with diluted human serum. After washing to eliminate the proteins which have not reacted, incubation is performed with the conjugate, composed of anti-human IgG monoclonal antibodies conjugated to horseradish peroxidase. The unbound conjugate is eliminated, and the peroxidase substrate added. The blue color which develops is proportional to the concentration of specific antibodies present in the serum sample.

### 3.9.3 Assay procedure

All reagents were allowed to reach room temperature. The needed strips had been set in strip-holder and sufficient numbers of wells including four calibrators (A, B, C, and D), one positive controls and one negative control and remain wells for samples. The samples were diluted 1:101(e.g. 10 µl+1000µl) with 1x sample buffer and mixed thoroughly, then added 100 µl of calibrators, controls and diluted samples into the appropriate wells. Incubated the plate for 30+/-3 minutes at 20-32°c/68-89 F. After that aspirated the solution, fill each well with 300 µl 1x wash buffer, aspirated the washing procedure another two times; after the final washing cycle, the plate was dried. Then 100 µl of HRP-conjugate reagent was added into each well and mixed gently. Then the plate was covered with the sealer and incubated for 30 minutes at 20-32°c/68-89 F. After that the sealer had been removed and each well was washed 3 times with diluted wash buffer. After the final washing cycle, the strep plate was dried then 100 µl f substrate solution were dispensed into each wells Then the plate was covered with the sealer and incubated for 30 minutes at 20-32°c/68-89 F with light avoidance. The enzymatic reaction between TMB substrate solution and the HRP-conjugate produced blue color in CAL wells, positive controls wells and in positive specimens. Then the plate sealer was removed and 100µl of stop solution was added into each well and mixed gently. The blue color turned yellow after stopping the reaction in CAL wells, positive controls and positives specimens. Then incubated for 5 minutes and the optical density (O.D) was read at 450 nm against a recommended reference wavelength of 620 nm.

### 3.9.4 Calculation of the result

By use the qualitative AESKULISA immunoassay, the variation of the OD values of the cut off calibrator, tested in duplicated, must not be higher than 20%.

The result were expressed in index (OD sample\OD cut-off).

And the results were calculated quantitatively by using certain formula and the result was expressed by U/ml.

Y= 57.539*-24.432

Y= concentration

*= OD

### 3.9.5 Interpretation of results

The mean optical measurements signal of the calibrator B (cut off calibrator CAL B) tested in duplicated. If the patient's samples reached OD values within the borderline range of +/-20 % around the mean OD of the cut off calibrator CAL B, the sample is considered as equivocal. Samples with higher OD values are evaluated as positive, samples with lower OD values are evaluated as negative.

## 3.10 Data analysis

Raw data was entered into Microsoft Excel to be cleaned and order it, and then it was analyzed using Statistical Package for Social Sciences (SPSS) v25. Numerical variables were presented in term of Mean, Minimum, Maximum and standard Deviation, categorical data were presented in term of frequency and percent tables, bar, pie chart. a p-value of <0.05 will considered significant and Odd ratio with confidence interval 95% and paired sample t-test, ROC test were used.

# Chapter Four

## *Results*

## 4.1 Results

During the period from January to April 2023, this study involved 90 blood samples from a volunteered HCWs from different hospitals in Omdurman hospital in Khartoum state; the number of vaccinated HCWs were70 (77.8%). They represented 41(46%) vaccinated by AstraZeneca and 29(32%) by Janssen while 20 (22.2%) Non-vaccinated group **(Figure4.1).**In consider to study population 47.8% (n=43) were males and 52.2% (n=47) were females **(Figure4.2).** And the age ranged from 19 to >=55 years with mean of post infected and vaccinated HCWS were 51.65 and 51.87 years, respectively. Moreover, this study showed the distribution of age groups with highest proportion among <=30 years old. **(Figure 4.3).**This study showed the distribution of occupation among HCWs was divided into two groups according to contact with Covid pts. As follow direct contact (52%) and indirect contact (48%) **(Figure 4.4)** with highest proportion among Medical Laboratories Technologist 33.3%. **(Figure4.5).** In consider the duration after infection and vaccination divided into four groups as follow:**(**>=3), **(**4 – 6), (7 – 9) and >=10 month**.** With mean 51.65 and 51.87 month in post infection and vaccination respectively. **(Figure 4.6)& (Figure 4.7).**The positive rate of anti-S antibodies after vaccination and post infection was 100% after vaccination and infection. According to the gender prevalence of antibody was higher in males than in females only on post infected group showed as follow: there was insignificant differences between IgG titers and gender among post infected groups.(*P value 0*.305)**(Table 4.1)**. Also study showed that insignificant differences between IgG titers and gender among vaccinated groups. (*P value* 0.873)**(Table 4.1).**The frequency of positive antibodies titers on post infection HCWS was divided into four groups according to age as follow: (<=30) years (55.42), (31-42) & (43 – 54) the highest percent in post infected groups (mean 71.50) & and (>=55 years) groups. No significant differences between age groups >=10 Month and antibodies titers in post infection groups was observed. (*P value*.324)**(Table 4.2).**While the frequency on vaccinated groups was as follow: (<=30 years), (31-42), (43 – 54) as highest percent in vaccinated groups (mean 64.00) and (>=55 years) groups. No significant differences between age groups and antibodies titers in vaccinated groups was observed. (*P value=0*.584)**(Table 4.2).**In consider the mean of antibodies titer was insignificantly different among workers occupied at the departments serving COVID- 19 patients than among those working in other departments. (*P.value=*.940*)*& (*P value= 0.226*) on post infected and vaccinated groups respectively. **(Table 4.3).** The vaccinated and post infected HCWS

was divided into four groups according to duration of vaccination and post infection, within groups the highest proportion of IgG titers was presented in duration (4 - 6 Month) on post infected groups, the differences was not reach statistical significance in duration and antibody titers in post infected groups was observed. (*P value*.139) , and the highest proportion of IgG titers was presented in duration (7 - 9 Month) on vaccinated groups, there was no significant differences in duration and antibody titers in this groups was observed. (*P value*.58)**(Table 4.4).**Moreover another parameters that was compared in this study the titer of IgG antibody and duration between two groups The highest titers of antibody was in vaccinated groups (mean 56.5) at duration (>=3 Month) more than post infected groups (mean 34.6), the result showed no significant difference was found between duration and IgG titers in the study groups at different duration**. (Table 4.5).**This study showed Association between types of vaccine and IgG titers in the vaccinated groups there was no significant difference found. (*P value* 0.247)**(Table 4.6).**Furthermore, study was showed the antibody titers based on booster dose of AstraZeneca vaccine there was no significant difference was found between types of vaccine and IgG titers in the vaccinated groups.(*P value* 0.405)**(Table 4.7).**Moreover another parameters that was compared in this study the titer of IgG antibody among vaccinated groups regard of time of taken Johnson Vaccine, The highest titers of antibody in this groups was (mean59.67) at duration (4-6 month) result showed no significant difference was found between duration and IgG titers in this groups**. (***P value*0.55)and the highest titers of antibody in this groups was (mean56.17) at duration (<=3 month)since there was no significant difference between the mean duration in month to sample acquisition following the AstraZeneca vaccine in vaccinated groups (*P value*0.416), it is less likely that there is any effect of vaccination time and sample collection on seroconversion rate or titer measured in the serum of population groups. **(Table 4.8)& (Table 4.9)**. The probability of being seropositive for the IgG antibodies after vaccination remained constant until the more than ten month in the vector vaccine.

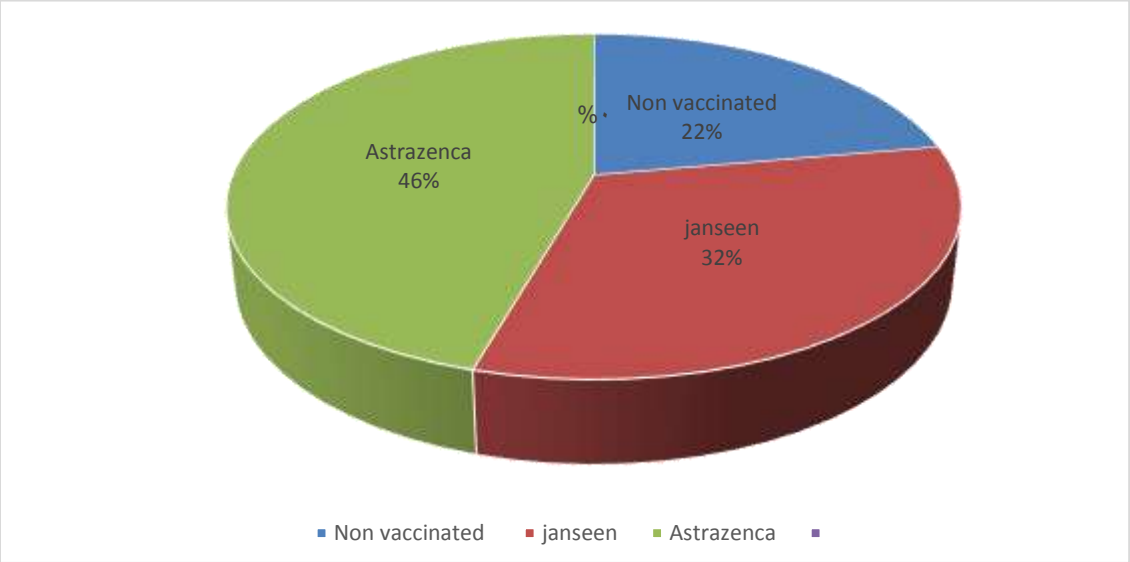



**Figure (4.1): Distribution of study groups among the study population**

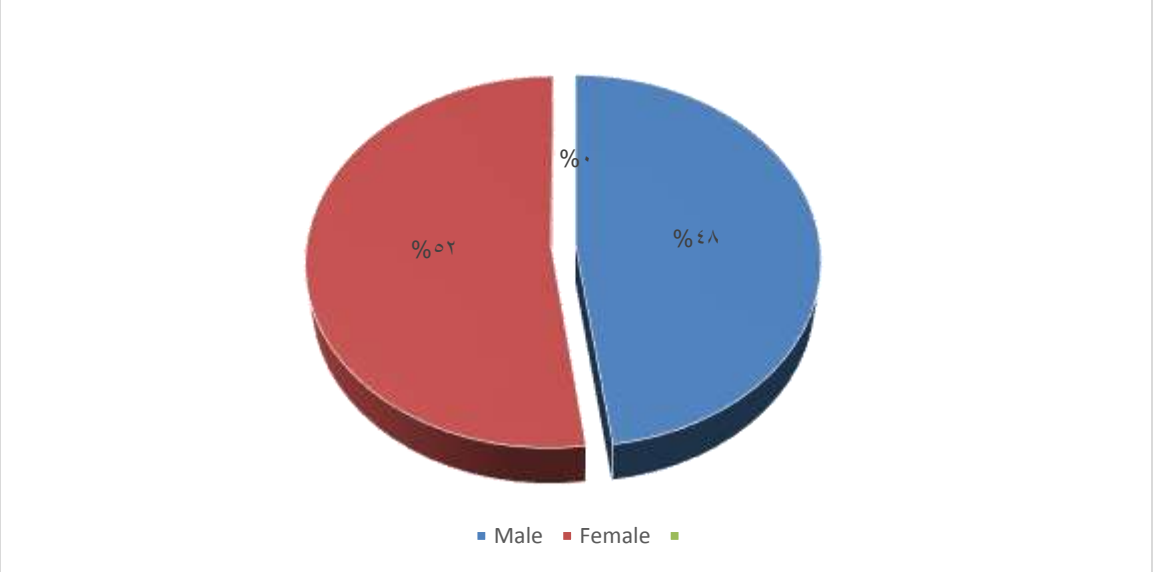



**Figure (4.2): Distribution of gender among the study population.**

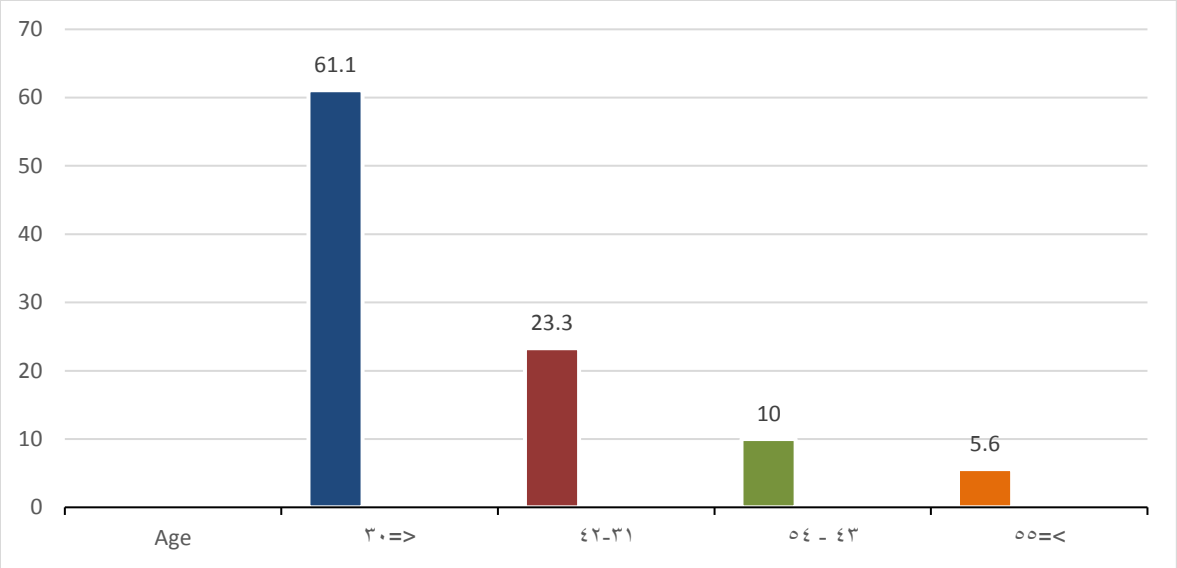
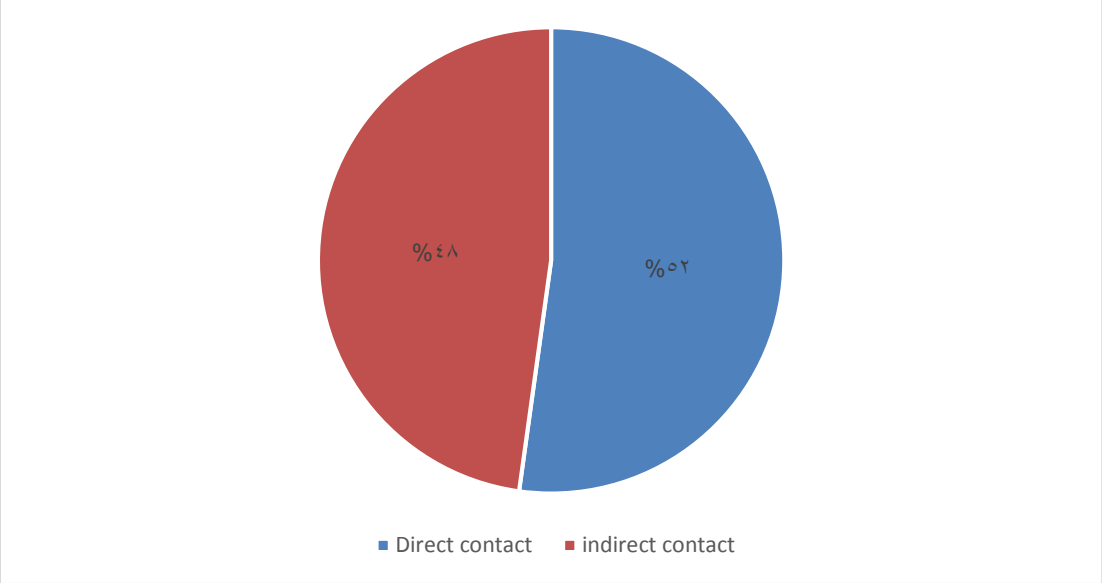



**Figure (4.3) Frequency of age groups among the study population.**



**(Figure 4.4) Distribution of population according to direct contact with patients.**

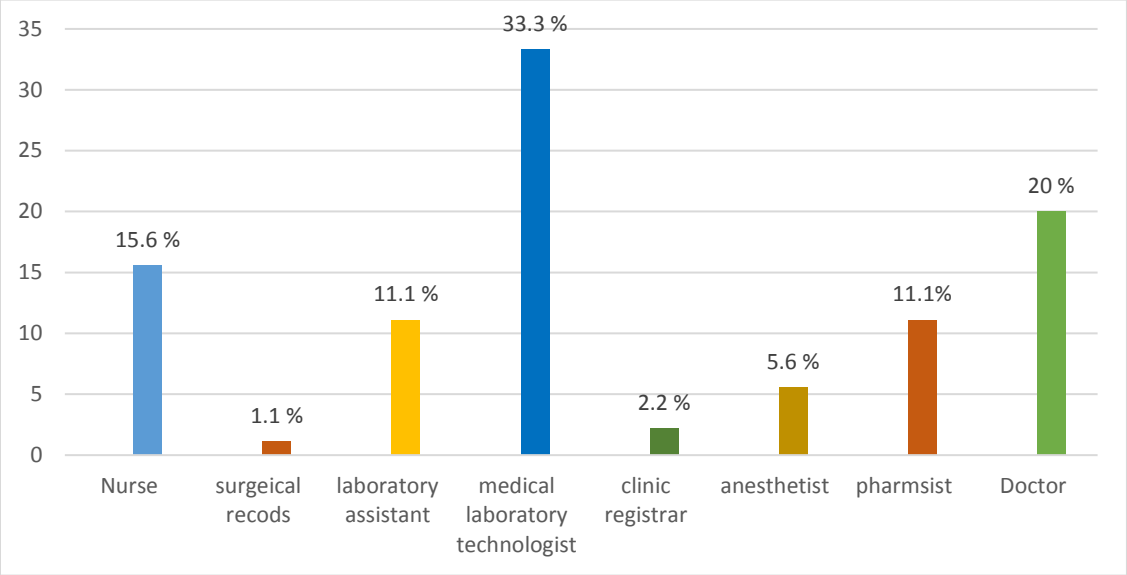



**Figure 4.5 Frequency of study population according occupation.**

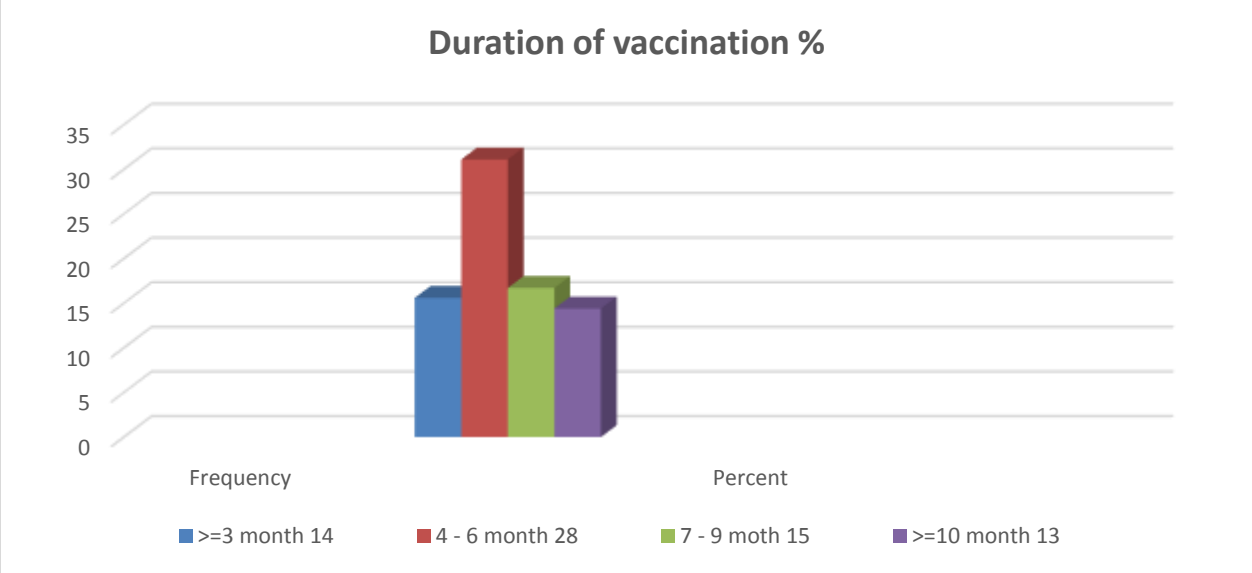



**Figure 4.6 Duration of vaccination among vaccinated HCWS.**

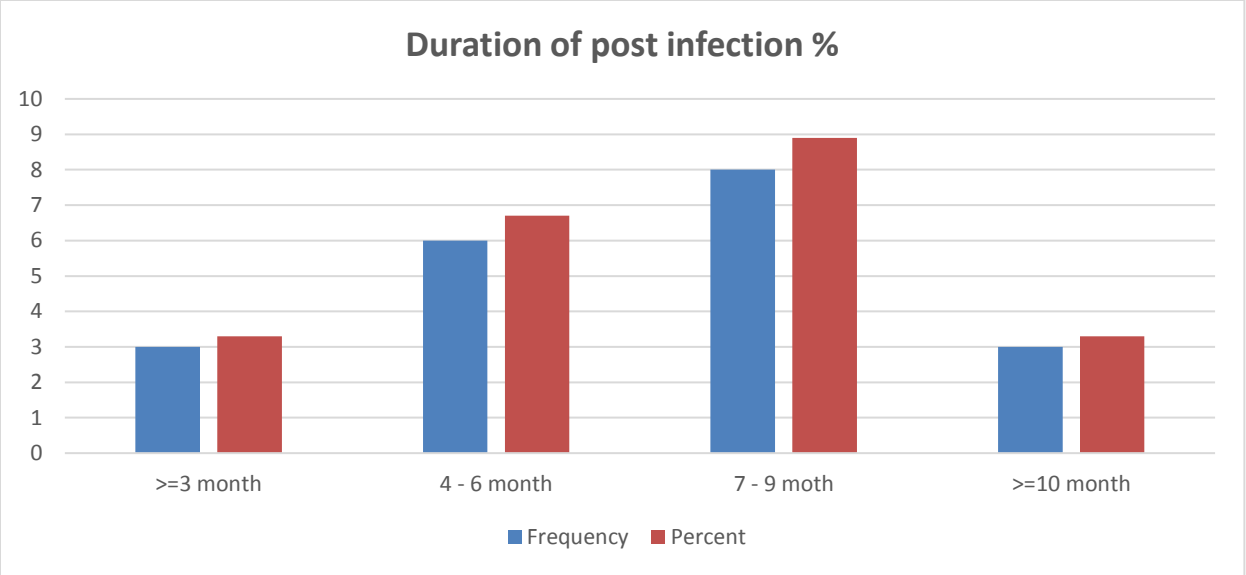



**Figure (4.7) Duration of Post infection among non-vaccinated HCWS.**

**Table (4.1): Association between antibodies titers and Gender in post infected and vaccinated groups.**

| Antibody titer | Gender group of Post infection | | Total | **P=value** | Gender group of vaccinated peoples | | Total | **P=value** |
|---|---|---|---|---|---|---|---|---|
| | Male | Female | | | Male | Female | | |
| Frequency | 8 | 12 | 20 | **0.305** | 35 | 35 | 70 | **0.873** |
| Mean | 60.375 | 45.83 | 51.65 | | 51.40 | 52.34 | 51.87 | |

**Table (4.2): Association between antibodies titers and age in post infected and vaccinated groups.**

| Antibody titer | Age group of Post infection | | | Total | P=value | Age group of vaccinated peoples | | | | Total | P=value |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | <= 30 | 31-42 | 43-54 | | | <= 30 | 31-42 | 43-54 | <=55 | | |
| Frequency | 12 | 6 | 2 | 20 | 0.324 | 43 | 15 | 7 | 5 | 70 | 0.584 |
| Mean | 55.42 | 37.50 | 71.50 | 51.65 | | 50.42 | 50 | 64 | 53 | 51.87 | |

**Table (4.3): Association between antibodies titers and occupation in post infection and vaccinated groups. (*Direct include nurse, doctors, laboratory assistant)(*indirect include medical laboratory technologist, clinic registrar, anesthetist, pharmsist)**

| Contact groups | Post infection group | | | Vaccinated group | | |
|---|---|---|---|---|---|---|
| | N | Mean | *P value* | N | Mean | *P value* |
| *Direct contact | 14 | 52.00 | 0.940 | 33 | 48.12 | 0.226 |
| *Indirect contact | 6 | 50.83 | | 37 | 55.22 | |

**Table (4.4): Association between antibodies titers and duration of infection and vaccination in post infected and vaccinated groups.**

| **Duration Within group** | **Post infection group** | | | **Vaccinated group** | | |
|---|---|---|---|---|---|---|
| | **N** | **Mean** | ***P value*** | **N** | **Mean** | ***P value*** |
| **>=3 Month** | **3** | **34.67** | | **43** | **50.42** | |
| **4 - 6 Month** | **6** | **63.33** | **.139** | **15** | **50.00** | **0.583542** |
| **7 - 9 Month** | **8** | **46.63** | | **7** | **22.494** | |
| **>=10 Month** | **3** | **58.67** | | **5** | **35.616** | |

**Table (4.5): Comparison between antibody titers and duration of infection and vaccination between two groups.**

| Duration | Group | No | Mean | *P value* |
|---|---|---|---|---|
| >=3 Month | Post infected | 3 | 34.6 | 0.2 |
| | Vaccinated | 14 | 56.5 | |
| | Total | 17 | | |
| 4-6 Month | Post infected | 6 | 63.3 | 0.5 |
| | Vaccinated | 28 | 56.4 | |
| | Total | 34 | | |
| 7-9 Month | Post infected | 8 | 46.6 | 0.3 |
| | Vaccinated | 15 | 39.3 | |
| | Total | 23 | | |
| >=10 Month | Post infected | 3 | 58.6 | 0.6 |
| | Vaccinated | 13 | 51.5 | |
| | Total | 16 | | |

**Table (4.6): Association of antibodies titers and time of taken Johnson Vaccine among vaccinated groups**.

| Duration | Mean | N | Std. Deviation | *P value* |
|---|---|---|---|---|
| <=3 month | 56.75 | 8 | 31.653 | |
| 4-6 month | 59.67 | 15 | 22.959 | |
| 7-9 month | 44.33 | 3 | 5.508 | |
| | | | | 0.55 |
| <=10 month | 46.33 | 3 | 43.132 | |
| Total | 55.90 | 29 | 26.096 | |

**Table (4.7): Association of antibody titers among vaccinated groups regard of time of taken AstraZeneca Vaccine.**

| Duration | Mean | N | Std. Deviation | *P value* |
|---|---|---|---|---|
| <=3 MONTH | 56.17 | 6 | 19.188 | |
| 4-6 MONTH | 52.38 | 13 | 29.528 | |
| 7-9 MONTH | 38.08 | 12 | 16.003 | 0.416 |
| > 10 MONTH | 53.10 | 10 | 21.497 | |
| Total | 48.93 | 41 | 23.095 | |

# Chapter Five

***Discussion***

***Conclusion***

***Recommendation***

## 5.1 Discussion

HCWS are particularly exposed to SARS Cov2 infection during management of patients with COVID19, and several recommendation have been made to protect them. Moreover infected HCWS could serve as a source of hospital acquired COVID19. It therefore important to assess the durability of immune protection after SARS Cov2 infection in this population. (WHO, 2021). Measurement of the seroprevalence of antibodies, especially neutralizing antibodies, against SARS-CoV-2 from population-based epidemiological surveys is informative for the assessment of the proportion of the population who have at some point been infected with the virus and provides insight into the design of vaccination programs. During a period from January to April blood samples from 90 HCWS (43 males) and (47 females) were included in this study. (70) Of them vaccinated by AstraZeneca and Johnson vaccine and (20) post infected by Cov-19 were examined for IgG antibody using enzyme-linked immunosorbent assays (ELISA). The duration and effectiveness of humoral immunity directed against SARS-CoV-2 after primary infection and vaccination are key questions in understanding the corona virus disease 2019 pandemic.

According to the gender this study found no significant difference in IgG titers between males and females, both in post-infection and post-vaccination groups. This is consistent with some studies, such as those by Figueiredo-Campos *et al.* (2020), which showed that gender differences in antibody responses are often minimal or non-significant. However, other studies, by Klein *et al*. (2020), suggest that females may generally produce higher antibody levels post-vaccination. The discrepancy could be due to variations in study populations, vaccine types, or the methods used to measure antibody levels.

According to the age this study observed that the highest proportion of positive antibody titers was among HCWs aged 43-54 years in both vaccinated and post-infection groups, but the differences were not statistically significant. This aligns with findings from the COV002 trial, which showed that while older adults (aged ≥56 years) had slightly lower antibody titers post-vaccination, the differences were not significant enough to affect overall vaccine efficacy (Ramasamy *et al*., 2020). The lack of significant differences across age groups in the current study may suggest that both natural infection and vaccination induce a robust immune response across a wide age range.

Duration after Vaccination/Infection and Antibody Titers the study found that antibody titers were highest at 4-6 months post-infection and 7-9 months post-vaccination, with no significant difference across different time intervals. This is consistent with studies like those by Dan *et al.* (2021), which showed that antibody levels peak several months post-infection or vaccination before gradually declining. However, despite this decline, memory B cells and T cell responses may still provide long-term immunity, as indicated by the persistence of antibodies in HCWs even up to 10 months post-vaccination. and differs may be due to limitation of sample size or due to study population HCWS are particularly exposed to SARS Cov2 infection during management of patients with COVID19so they have the possibility of getting infection several times and The humoral immune response appears to remain intact even with loss of specific antibodies over time because of the persistence of memory B-cells. (Calendar, 2020).The durability of humoral response against SARS-CoV-2 on vaccination needs to be further clarified with a longer follow-up time.

In this study, it was observed that the IgG antibody titer is highest in vaccinated groups (mean 56.5) at duration (>=3 Month) more than post infected groups (mean 34.6). It may be due to the viral neutralization demonstrated with serum from persons following infection. (Callendar, 2020).According to Occupational Exposure This study found no significant difference in antibody titers between HCWs with direct contact with COVID-19 patients and those with indirect contact. This finding contrasts with some studies that have suggested HCWs in direct contact with COVID-19 patients may develop higher antibody levels due to repeated exposure (Houlihan *et al*., 2020). However, the lack of difference in this study could be due to effective use of personal protective equipment (PPE) or other infection control measures that minimize exposure risk.

Moreover, This study found no significant difference in antibody titers between HCWs vaccinated with AstraZeneca and those vaccinated with Janssen (Johnson & Johnson). This result is in line with data from clinical trials showing comparable efficacy between these vaccines in preventing severe disease, although the antibody titers induced by adenovirus-vectored vaccines like AstraZeneca and Janssen might be lower than those induced by mRNA vaccines (Falsey *et al*., 2021). The lack of significant differences in this study could suggest that both vaccines provide sufficient protection, especially in the context of severe outcomes.

Finally No significant difference in antibody titers was observed based on the timing of booster doses of the AstraZeneca vaccine. This finding is supported by research from Amirthalingam *et al*. (2021), which demonstrated that booster doses of the AstraZeneca vaccine significantly enhance immune responses, but the timing of the booster's administration (within a certain window) might not drastically alter antibody titers .Our study differs from previous studies which they suggested the booster dose from a different type of vaccine induced a significantly higher titer of IgG antibodies (15,832 AU/ml) compared to all types of vaccines including the mRNA type of vaccines. (Sughayer, *et al,* 2022*)*. This may be due to limitation of sample size and it was no compared to all types of vaccines.

**5.2 Conclusion:**

The study's findings contribute to the growing body of evidence that both natural infection and vaccination induce strong and durable immune responses in healthcare workers. The lack of significant differences across various subgroups suggests that current vaccination strategies are broadly effective across different demographics. However, the study also highlights the need for ongoing monitoring of immune responses, particularly as new variants of SARS-CoV-2 emerge and as booster programs continue. Further research could explore the long-term durability of immune protection, particularly beyond the 10-month mark, and how this may be influenced by factors such as prior infection, booster doses, and variant exposure.

**5.3 Recommendation:**

- Continued Monitoring of Antibody Levels.
- Targeted Booster Dose Administration.
- Personal Protective Equipment (PPE) and Infection Control Measures.
- Vaccine Type Considerations.
- Further studies with large sample size is recommend to detect the covid IgG in vaccinated and un-vaccinated HCWS and compare between all types of vaccines.
- Detection of anti-spike antibody before vaccination.
- Preventive measure should be delivered to community through the different media.
- Further studies must be include HCWS who have chronic disease or impaired immunity.

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## Appendix 1

## Questionnaire

# Evaluation of the humoral immunity to CoVID-19 among immunized Healthcare Workers in Omdurman hospitals, in Khartoum state 2023.

1. Serial number: ☐

2. Gender. Female ☐ Male ☐

3. Age ☐

4. Occupational ----------

Medical history:

5. What types of vaccine?

Janssen and Janssen ☐ AstraZeneca ☐

6. DO you have previous infection of COVID -19?

Yes ☐ No ☐

7. What the time you take the vaccine?

Three Months ☐ Six months ☐ or more ☐

8. Did you take booster doses?

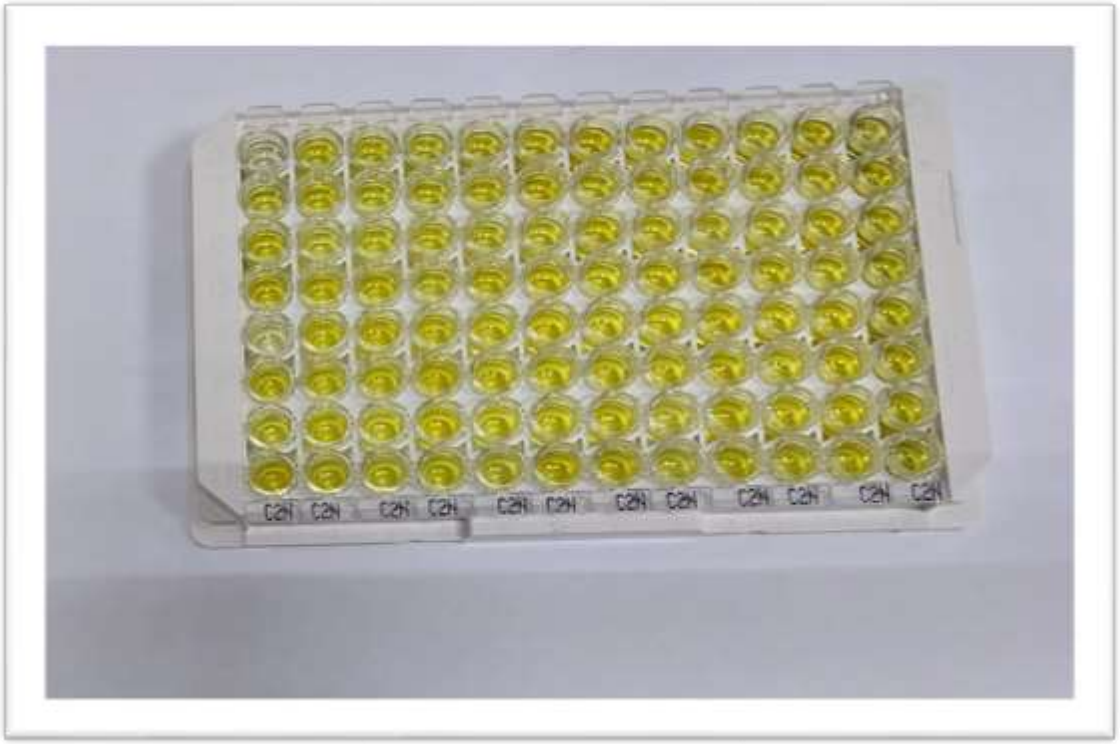
One dose ☐ two doses ☐

# Appendix-2

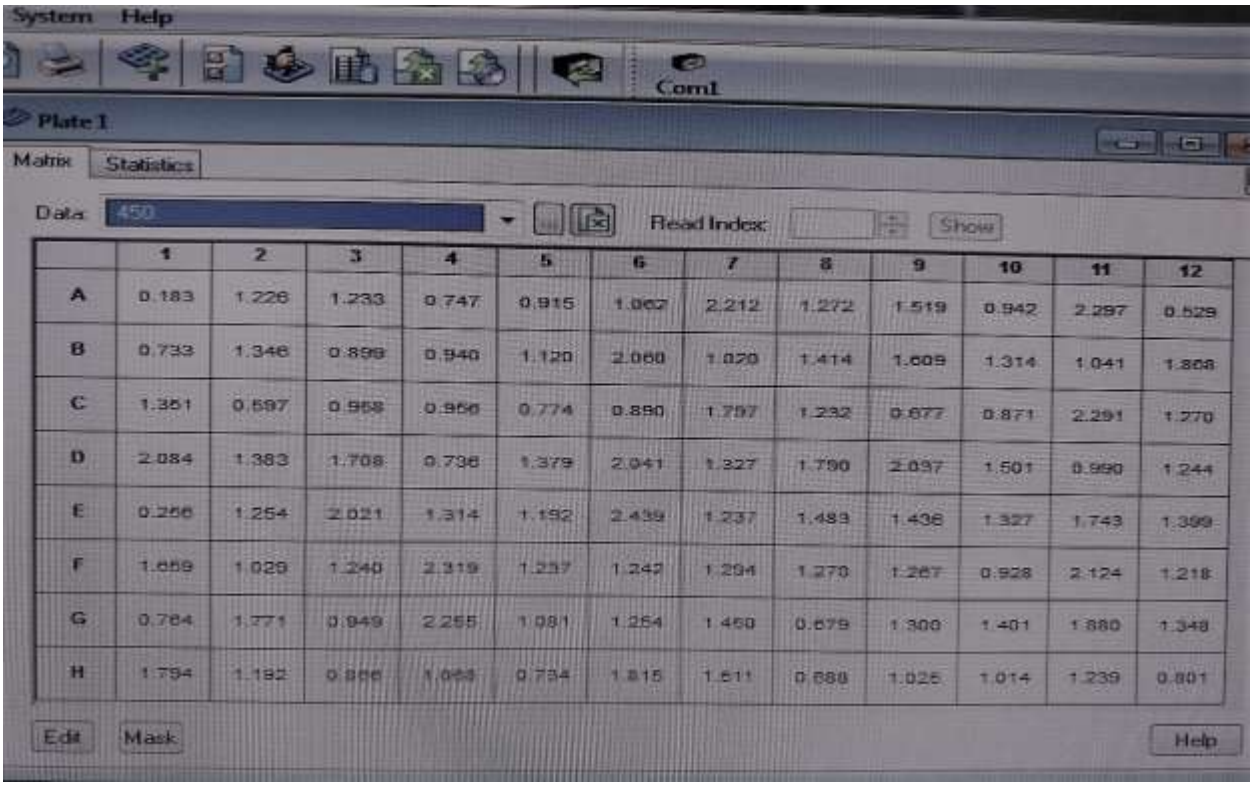
## Color plates

**Color plate (1)AESKULISA ELISAKits.**


**Color plate (2) Blue color after added substrate solution (positive reaction)**



**Color plate (3) Yellow color after added stop solution (positive reaction)**




| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 0.183 | 1.226 | 1.233 | 0.747 | 0.915 | 1.067 | 2.212 | 1.272 | 1.519 | 0.942 | 2.297 | 0.529 |
| B | 0.733 | 1.346 | 0.899 | 0.940 | 1.120 | 2.068 | 1.020 | 1.414 | 1.609 | 1.314 | 1.041 | 1.868 |
| C | 1.351 | 0.597 | 0.958 | 0.956 | 0.774 | 0.890 | 1.797 | 1.292 | 0.677 | 0.871 | 2.291 | 1.270 |
| D | 2.084 | 1.383 | 1.708 | 0.736 | 1.379 | 2.041 | 1.327 | 1.790 | 2.097 | 1.501 | 0.990 | 1.244 |
| E | 0.266 | 1.254 | 2.021 | 1.314 | 1.192 | 2.439 | 1.237 | 1.489 | 1.436 | 1.327 | 1.743 | 1.399 |
| F | 1.659 | 1.029 | 1.240 | 2.319 | 1.257 | 1.242 | 1.294 | 1.270 | 1.267 | 0.928 | 2.124 | 1.218 |
| G | 0.764 | 1.771 | 0.949 | 2.255 | 1.081 | 1.264 | 1.460 | 0.679 | 1.300 | 1.401 | 1.880 | 1.348 |
| H | 1.794 | 1.182 | 0.866 | 1.068 | 0.734 | 1.815 | 1.611 | 0.888 | 1.025 | 1.014 | 1.239 | 0.801 |

**Color plate (4) ELISA optical density**


**Color plate (4) ELISA Readder**

# Appendix-3

## Instruction manual