University of Kerbala

Evaluation the IgG and IFN-γ levels among different types of COVID-19 vaccine

A thesis

Submitted to the council of the
College of Applied Medical Sciences – University of Kerbala
In Partial of Fulfillment of the Requirements for the Degree of Master in Clinical Laboratories

by

(Rawaq Taleb Hassan Hadi)

B.Sc. in Applied Medical Sciences/University of Kerbala, 2017

Supervised by

Prof. Suhad Hadi Mohammed (Ph. D)

Medical Microbiology

College of Applied Medical Science

July (2022) **Dhul Hijjah (1444)**

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيم

{وَلَنَبْلُوَنَّكُمْ بِشَيْءٍ مِنَ الْخَوْفِ وَالْجُوعِ وَنَقْصٍ مِنَ الْأَمْوَالِ وَالْأَنْفُسِ وَالثَّمَرَاتِ ۗ وَبَشِّرِ الصَّابِرِينَ ﴿١٥٥﴾}

صدق الله العلي العظيم

سورة البقرة الآيه ١٥٥

Supervisor's Declaration

I certify that this entitle thesis (**Evaluation the IgG and IFN-γ levels among different types of COVID-19 vaccine)** was prepared under my supervision at the department of Clinical Laboratories, College of Applied Medical Sciences, University of Kerbala, as a partial fulfillment of the requirements for the degree of Master in science of Clinical Laboratories.

Signature

Suhad

Prof. Dr. Suhad Hadi Mohammed

Medical Microbiology

Collage of Applied Medical Science

University of Kerbala

Date: / / 2022

In view of the available recommendation, I forward this thesis for debate by the examining committee.

Signature

Dr. Linda Hamid Turki

Head of the Department of Clinical Laboratories

College of Applied Medical Sciences

University of Kerbala

Date: 29/ 12/2022

Dedication

For those who helped me start this life, my mother and father, who are present in my heart and my reality, so they were my support from the softness of the roots to what I am now

To my loved ones, my extended family and friends, and to everyone who supported me of all kinds

To my life partner, my husband, you are part of all my successes

Rawaq

Discussion Committee Certification

We are member of the discussion committee certify that we read this thesis entitled **" Evaluation the IgG and IFN-γ levels among different types of COVID-19 vaccine"** and we discussed Mr. student **Rawaq Taleb Hassan** in its contents. It is adequate for the award of the certification of Master of Clinical Laboratories.

Signature

Prof. Dr. Wafaa Sadiq Mohsin Al-Wazni

(Chairman)

/ 12 / 2022

Signature A.M.Ghareeb

Assist. Prof. Dr. Abdulameer Mohammed Ghareeb

(Member)

/ 12 / 2022

Signature

Assist. Prof. Dr. Israa Saeed Abbas

(Member)

/ 12 / 2022

Signature

Prof. Dr. Suhad Hadi Mohammed

(Member & Supervisor)

/ 12 / 2022

I have certified upon the discussion of the examining committee.

Signature

Assist. Prof. Dr. Jwadat Noori Ghaaib

Dean of the College of Applied Medical Sciences / University of Kerbala

29 / 12 / 2022

Acknowledgments

Thank for God, who has increased my knowledge and eased all difficulties

After God, a special thanks to the virtuous professor, Dr. Suhad Hadi, the owner of professional and scientific supervision, who was the best role model and teacher during every minute of the research period.

The honorable Dean and Head of the Department of Pathological Analysis have our sincere gratitude.

All thank to Al-kafeel specialist hospital for their help and forgiving me to conduct the experiment.

All gratitude and thanks to my sisters and friends for their efforts to help me complete my scientific career.

All the gratitude and love to my first family, especially my mother and uncle, for their constant love and prayers. And to my second family, I want to express my love and gratitude for helping me to realize my dream.

to the first supporter and my life partner all my love and gratitude

Supervisor's Declaration

I certify that this entitle thesis (**Evaluation the IgG and IFN-γ levels among different types of COVID-19 vaccine)** was prepared under my supervision at the department of Clinical Laboratories, College of Applied Medical Sciences, University of Kerbala, as a partial fulfillment of the requirements for the degree of Master in science of Clinical Laboratories.

Signature

Prof. Dr. Suhad Hadi Mohammed

Medical Microbiology

Collage of Applied Medical Science

University of Kerbala

Date: / / 2022

In view of the available recommendation, I forward this thesis for debate by the examining committee.

Signature

Dr.Linda Hamid Turki

Head of the Department of Clinical Laboratories

College of Applied Medical Sciences

University of Kerbala

Date: / /2022

Linguistic Certification

I Certify that the master's thesis tagged: (Evaluation the IgG and IFN-γ levels among different types of COVID-19 vaccine) by the master's student (rawaq taleb hassan), in the Department of Clinical Laboratories - College of Applied Medical Sciences - University of Karbala, has been reviewed from the linguistic point of view, and all the grammatical and stylistic errors in it were corrected, and the thesis is eligible for discussion.

Signature:

Linguistic component:

Academic title:

College of:

Date: / / 2022

List of contents

Item No	Subject	Page
	List of Tables	**VII**
	List of Figures	**VIII**
	List of Abbreviations	**IX**
Chapter One Introduction		
1.1	Introduction	**1**
Chapter Two Literatures Review		
2	Literatures Review	**6**
2.1	Coronavirus disease (COVID-19)	**6**
2.2	Epidemiology	**7**
2.3	Coronavirus structure	**8**
2.4	Transmission	**11**
2.5	Risk factor	**12**
2.6	Clinical signs	**13**
2.7	Diagnosis	**14**
2.8	Treatment	**16**
2.9	Immune response to coronavirus infection	**17**
2.9.1	Cellular immune response	**18**
2.9.2	Humoral immune response	**19**
2.10	Antigenic escape from immune cell	**20**
2.11	Covid vaccines	**21**
2.12	Vaccination platforms	**21**
2.13	Current and most common COVID-19 vaccines	**22**
2.13.1	Pfizer	**22**
2.13.2	AstraZeneca	**24**
2.13.3	Sinopharm	**26**
Chapter Three Materials and Methods		
3.1	Subjects	**28**
3.2	Sample collection	**28**
3.3	Materials	**30**
3.3.1	Devices and equipment	**30**
3.3.2	Kits and Chemicals Materials	**30**
3.4	Methods	**30**
3.4.1	Determination Human IFN-γ (Interferon Gamma) using ELISA Kit	**30**
3.4.1.1	Principle	**31**
3.4.1.2	Kit components	**31**
3.4.1.3	Procedure	**32**
3.4.2	Determination Human anti-SARS-CoV-2 S1(RBD) IgG using	**33**

3.4.2.1	Principle	**34**
3.4.2.2	Procedure	**34**
3.5	Statistical analysis	**34**
3.6	Ethical consideration	**35**
Chapter Four Results and Discussions		
4.1	Demographic data	**36**
4.2	Comparison of IgG and IFN-γ concentration among the three types of vaccines	**38**
4.3	Differences in IgG and IFN γ according to age groups	**40**
4.4	Differences in IgG and IFN γ levels with Sex	**43**
4.5	Differences of IgG and IFN γ concentrations according to dose	**46**
4.6	Differences in immune response for vaccine within weeks	**49**
4.7	Differences in IgG and IFN γ level between previously Infected and uninfected subjects	**53**
4.8	Differences in IgG and IFN γ according to BMI	**56**
4.9	Correlation of IgG with IFN γ	**59**
Conclusions		**61**
Recommendations		**63**
References		**64**
Appendices		**87**

List of Tables

Table NO	Tables	Pages
3.1	Device and equipment	**30**
3.2	Kits and chemicals	**30**
3.3	ELISA kit details	**32**
4.1	Demographic data	**37**
4.2	Comparison of IgG and IFN-γ concentration among the three types of vaccines	**39**
4.3	Differences in IgG and IFN γ according to age groups	**40**
4.4	Correlation between ages /male/female and IgG / IFN γ	**42**
4.5	Differences in IgG and IFN γ levels with Sex	**44**
4.6	Differences of IgG and IFN γ concentrations according to dose	**47**
4.7	Compare between doses for IgG and IFN-γ	**48**
4.8	Doses in weeks for types of vaccines	**48**
4.9	Differences in IgG for vaccine within weeks	**49**
4.10	Differences in IFN-γ for vaccine within weeks	**51**
4.11	Differences in IgG and IFN γ level between previously Infected and uninfected subjects	**54**
4.12	Differences in IgG according to BMI	**56**
4.13	Differences in IFN γ according to BMI	**67**
4.14	Correlation of IgG with IFN γ	**59**

List of Figures

Figure NO	Figures	Pages
2.1	Structure of SARS-COV 2	**10**
3.1	Study design	**29**
4.1	Different in IgG according to age group	**43**
4.2	Compare between vaccines and sex for IgG concentration	**46**
4.3	Difference in IgG for vaccine within weeks	**52**
4.4	Difference in IFN γ for vaccine within weeks	**52**

List of Abbreviations

Abbreviations	Items
AB	Anti-body
ACE2	Angiotensin-Converting Enzyme 2
AdV	Adenovirus
BMI	Body Mass Index
CD8	cluster of differentiation 8
CMIA	Chemiluminescent Microparticle Immunoassay
COVID-19	Coronavirus Disease
CVST	Cerebral Venous Sinus Thrombosis
DCs	Dendritic Cells
dsRNA	Double-Stranded Ribonucleic Acid
ELIZA	Enzyme Linked Immunoassay
EMA	European Medicines Agency
HCoV	Human Corona Virus
HE	Hemagglutinin-Esterase
HRP	Horseradish Peroxidase
IFN-γ	Interferon gamma
IL-2	Interleukin two
IM	Intramuscular
LSD	Less Significant difference
MERS-CoV	Middle East Respiratory Syndrome Coronavirus
MHC class I	Major Histocompatibility Complex Class 1
MHRA	Medicines and Health-Care Regulatory Agency
mRNA	Messenger Ribonucleic acid
OD	Optical Density
ORF	Open reading farm
PRRs	Porcine Reproductive and Respiratory Syndrome
RBD	Receptor-Binding Domain
RLU	Relative Light Unit
SARS-CoV	Sever Acute Respiratory Syndrome Corona virus
TCR	T Cell Receptor
Tcx	T Cytotoxic
TFH	T Follicular Helper cells
Th1	T Helper
VIPIT	Vaccine-Induced Prothrombotic Immune Thrombocytopenia
WHO	Would Health Organization

Summary

coronavirus disease 2019 (COVID-19) is a pandemic acute respiratory disease caused by a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). It is a highly transmissible coronavirus that emerged in China in late 2019 and it is considered as a public health emergency worldwide by the World Health Organization.

This pandemic has also changed the people's life style; caused extensive job losses and threatened the sustenance of millions of people, as businesses have shut down to control the spread of virus. The most effective and promising method of combating this widespread viral pandemic was through vaccination.

A cross sectional study was conducted in College of Applied Medical Sciences, Kerbala university. Sample collection was carried out for 6 months, starting from November /2021 to April/2022. The total number of participants were 174 vaccinated subjects divided into three groups; 105(60.3%) subjects had received the Pfizer vaccine, 59(33.9%) subjects had received the Sinopharm vaccine, and 10(5.7%) subjects of them had received AstraZeneca. ELIZA tests were done for all subjects to measure IFN-γ, and SARS-CoV-2 IgG II Quant antibody test was used to measure IgG anti spike.

From 174 subjects, 90 subjects (51.7%) were men and 84 (48.2%) were women with ages ranged from 18 to 70 years. The mean age of participant was 25.97 ± 9.327. Compares anti-spike (IgG) levels among the three types of vaccines reveals significant difference. AstraZeneca and Sinopharm's vaccines had lower IgG concentrations as compared to Pfizer's vaccine.

The overall antibody concentration in participants under the age of 25 was higher than that in people above the age of 25. Also, there were significant

differences among the three types of vaccine within both age groups, and the highest concentration was seen in participants vaccinated with Pfizer.

Regarding IFN-γ, there was no significant difference within and between the two age groups, exception the AstraZeneca vaccine there was significant difference between less and more than 25 age.

Additionally, there was a significant difference in IgG concentration among the three types of vaccines within male and female subjects, and the antibody production was higher in participants vaccinated with Pfizer. The mean IgG concentration was higher in males than females in subjects vaccinated with Pfizer and AstraZeneca. However, no significant difference between males and females' subjects was observed for each type of vaccine. Regarding the IFN γ, there were no significant differences either among the three types of vaccines, nor between males and females for each vaccine.

The anti-spike IgG concentration for vaccination with Pfizer varied significantly among the weeks after vaccination and the maximum concentration occurring between the sixth and seventh weeks. The weeks after vaccination do not significantly differ for the Sinopharm and AstraZeneca vaccinations.

There was no statistically significant difference in IgG and IFN-γ mean levels between vaccinated subject with confirmed previous infection group versus vaccinated subject without apparent previous infection in subjects vaccinated with Pfizer and Sinopharm. However, there was a significant difference in the case of the AstraZeneca vaccine regarding IgG levels but not for IFN-γ levels.

The antibody concentration is significantly different among the three types of vaccines in overweight and obese subjects. whereas no significant difference was

observed in the case of underweight subjects. The mean was higher in subjects vaccinated with Pfizer vaccine. Moreover, there was marginally significant difference between the mean of antibody concentration of normal weight subject vaccinated with Pfizer vaccine and those subjects in other groups (underweight, overweight, and obese). Regarding the IFN-γ level, there were significant difference among the three types of vaccines in obese subjects. The highest mean was observed in subjects vaccinated with Sinopharm vaccine. Additionally, there were highly significant difference among the four groups vaccinated with Sinopharm vaccine.

Finally, we conclude from this study the Participants vaccinated with Pfizer vaccine produces the highest antibody and IFN-γ concentration, younger participants under the age of 25 had higher antibody and IFN-γ concentrations than older participants, no difference between male and female in immune response to vaccine, no difference between first and second dose after vaccination and no effect for previous infection in improve the response to vaccine.

Chapter One
Introduction

Chapter one

Introduction

1.1 General overview

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) disease has caused a worldwide challenging and threatening pandemic (COVID-19), with huge health and economic losses. It was A novel and unique strain of RNA member of coronavirus that have not been previously observed in humans causing respiratory and gastrointestinal infections (Pal *et al.*, 2020). This virus, which has recently emerged in Wuhan, China, is capable of inflicting serious diseases on humans and has a broad range of host adaptability. The average number of days that COVID takes to incubate were two to seven (range of one to two weeks) (Neumann-Prochaska *et al.*, 2020).

The disease was defined by certain medical symptoms and indicators, including as dry cough, exhaustion, shortness of breath, and fever, were more frequently mentioned, but myalgia, sore throat, rhinorrhea, and nasal congestion were very uncommon. Sometimes, non-respiratory symptoms like palpitations, diarrhea, or headaches came first (Pal *et al.*, 2020).

It can spread by either direct or indirect contact with infectious respiratory droplets or fomites on mucous membranes (such as the eyes, nose, or mouth). Risks of transmission rise with time and proximity to contacts/infected people (Schilling-Loeffler *et al.*, 2022). Morphologically Coronaviruses were enclosed, positive-sense single-stranded RNA viruses with a diameter of 60–140 nm (Dutta *et al.*, 2020).

The coronavirus gets its name because of the envelope’s 20-nm-long spikes, which resemble the sun's corona under an electron microscope, Among the RNA viruses that are currently known, it has the biggest genome (Park, 2020). Within the

coronavirus particle A nucleoprotein (N) wraps the RNA genome to create a coiled tubular structure, this helical nucleocapsid was encased in the viral envelop (E). With the viral envelop are two or three structural proteins. The envelope contains the matrix protein (M). The target of the neutralizing antibody was the spike structural protein (S) anchored in the envelope. Numerous beta coronaviruses include the hemagglutinin esterase (HE) (Malik, 2020).

By March 5, 2020, there had been 80,555 confirmed cases in China and 17,821 confirmed cases in 90 countries outside of China during the first three months following the initial notification of the outbreak in Wuhan. By July 2022, COVID-19 had spread to every country in the world, infected 561million people, and killed more than 6. 37million. In Iraq, the disease causes a total of 2.4 million cases and 25,263 fatalities (Dong *et al.*, 2020) . The most effective and promising method of combating this widespread viral pandemic was vaccination (Bhavana *et al.*, 2020).

According to the most popular classification scheme, vaccines can be characterized as either classical or new generation depending on the platforms on which they were produced (Simões & Rodríguez-Lázaro, 2022). Pfizer, AstraZeneca, and Sinopharm vaccines were the most significant and widely used vaccines in Iraq.

The first mRNA vaccine, Comirnaty (Pfizer/BioNTech), was created within a year of the WHO's pandemic statement. The BNT162b2 mRNA, which encodes the whole SARS-CoV-2 spike protein, was the active component. The mRNA vaccination serves as an adjuvant and antigen, which, once inside the cell, triggers an immune response (Turner *et al.*, 2021).

In contrast, the Oxford/AstraZeneca COVID-19 vaccine was a replication-deficient simian adenovirus vector vaccine, meaning that several crucial genes were

removed and replaced by a gene encoding the spike protein. The chimpanzee adenovirus ChAdOx1 was modified and utilized as a vector (Jamkhande *et al.,* 2021).

The two vaccine formulations — mRNA encoding the SARS-CoV-2 spike (S) protein encapsulated in lipid nanoparticles or adenovirus (AdV) vectors encoding the S protein — gain entry into dendritic cells (DCs) at the injection site or in lymph nodes, where high levels of S protein are produced (Teijaro & Farber, 2021). The intrinsic adjuvant activity of the vaccines also activates innate sensors, which leads to the generation of type I interferon and many proinflammatory cytokines and chemokines(Fan *et al.,* 2022).

As a result, activated DCs deliver antigen and co-stimulatory molecules to naive T cells that were specific for the S protein. These effector cells then differentiate into cytotoxic T lymphocytes or helper T cells after becoming activated and differentiating(Yao *et al.,* 2018).

High affinity anti-S protein antibodies were produced by T follicular helper (TFH) cells, which also assist S protein-specific B cells in differentiating into plasma cells that secrete antibodies. IgG molecules are created and released by plasma B cells. IgG is the main type of antibody found in blood and extracellular fluid, allowing it to control infection of body tissues, IgG protects the body from infection. IgG antibodies are generated following class switching and maturation of the antibody response, thus they participate predominantly in the secondary immune response. it’s a major component of humoral immunity. Following vaccination, high affinity SARS-CoV-2 antibodies circulate along with S protein-specific memory T cells and B cells, which together help prevent subsequent SARS-CoV-2 infection (Teijaro & Farber, 2021).

As a result of the potential of mRNA and adenovirus vaccines to stimulate intracellular S protein synthesis and innate immune responses, both CD8+ and CD4+ T cells should be primed for effector and memory subset differentiation. IFN-γ, or type II interferon, is a cytokine that is critical for innate and adaptive immunity against viral. IFN-γ is an important activator of macrophages and inducer of major histocompatibility complex class II molecule expression, and its produced predominantly by natural killer cells (NK) as part of the innate immune response, and by CD4 Th1 and CD8 cytotoxic T lymphocyte (CTL) effector T cells once antigen-specific immunity develops. This cytokine represents cellular immunity (Pardi *et al.,* 2018).

Three vaccinations need two doses spaced 3–4 weeks apart to achieve optimum protection. Sinopharm, a state-owned Chinese business, is creating the Sinopharm/BIBP COVID-19 vaccine. is an inactivated vaccine that contains viral particles and is administered to the body as a dead copy of SARS-CoV-2 (Zahid *et al.,* 2021) . Thus, these vaccines are created using highly purified, non-contagious viruses. There was difference in the in immune response between individual according to age, where it was the majority of young individuals experience mild disease from SARS-CoV-2 (Brodin, 2020). Sex where men overrepresented among patients with severe disease, likely as a result of variations in the immunological responses that are induced (Takahashi *et al.,* 2020). and chronic disease including Obesity may be a risk factor, the immune system is suppressed in obese people especially in vulnerable people with multiple comorbidities (Jayanama *et al.,* 2021).

Despite the great published information globally, still there were little information about which type of vaccines are better for certain group of population (with specific age, sex, and other risk factors like BMI), especially among Iraqi

individuals residing in Karbala Province, and which type of vaccines produce more cellular and humeral immune response and how long these antibodies may persist.

Aim of this study

Evaluation and comparison of IgG and IFN γ concentrations generated against the available types of COVID-19 vaccine in Iraq.

Objectives of the study:

1. Measuring the humoral immune response by determining the titter of IgG antibodies against the S1 subunit of the virus's spike protein in subjects vaccinated with the available vaccines.
2. Measuring cellular immune response by determining the titter of IFN γ level in vaccinated subjects.
3. Comparison the mean levels of IgG and IFN γ produced in vaccinated subjects with different types of vaccines.
4. Comparing the level of IgG and IFN γ in concerning to the dose number, weeks after vaccination, and the presence of previous infection.
5. Analysis the association of different risk factors (like age, sex, and BMI) with the level of IgG and IFN γ
6. Study the presence of correlation between IgG and IFN γ production.

Chapter Two
Literatures Review

Chapter two

Literatures Review

2.1 Coronavirus disease (COVID-19)

COVID-19 was an infectious disease caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The majority of virus-infected individuals will experience mild to severe respiratory diseases and will recover without the need for special care. However, some people will get serious illnesses and need to see a doctor. Serious sickness is more likely to strike older people and those with underlying medical illnesses, including cancer, diabetes, cardiovascular disease, or chronic respiratory diseases. COVID-19 can cause significant illness or death in anyone, at any age (WHO, 2020) .

The average number of days that COVID takes to incubate was 2 to 7 (a range of 1 to 2 weeks). The clinical presentation is characterized by a high fever, cold, coughing, breathlessness or trouble breathing, diarrhea, muscle aches or tiredness, and hematuria. (Neumann-Prochaska *et al.,* 2020). Pneumonia can occur in patients with severe forms, and case fatality rates can vary greatly. Serious outcomes like heart failure, lung failure, and liver failure are more common in the elderly people (Pal *et al.,* 2020). Respiratory failure is the biggest problem with COVID-19; during the acute stage, at least half of the patients (mainly elderly individuals) need oxygen supply (Daher *et al.,* 2021) , acute respiratory distress syndrome, which requires invasive mechanical ventilator support, develops in roughly 20% of patients. However, the severity is often mild in infected young children (Precit *et al.,* 2020). Deaths have been reported as early as day 4 and as late as 108 days after the onset of symptoms (Baiocchi *et al.,* 2021) . It was shown that respiratory virus shedding

peaked around 10 day and then decreased. Additionally, the gastrointestinal tract's viral excretion was seen (Wölfel *et al.,* 2020).

2.2. Epidemiology

In December 2019, atypical unknown pneumonia was first recorded in Wuhan City. High temperatures (over 38 C), a dry cough, malaise, and breathing issues have all been present in the patients. The illness, known as COVID-19, has been connected to Wuhan, China's seafood industry (Sarhan *et al.,* 2020).

As of July 2022, there were 561 million global cases of COVID-19. while there had 6. 37 million deaths(Dong *et al.*, 2020). The seafood market was closed on January 1st in accordance with the World Health Organization. The virus was identified on January 7 as a coronavirus with > 95% homology to the bat coronavirus and > 70% resemblance to the SARS-CoV. Positive results from environmental samples from the Huanan Sea food market also indicated that the virus originated there (Singhal, 2020). There was an exponential rise in the number of cases, some of which did not involve the live animal market, pointing to the possibility of human-to-human transmission (Jagtap *et al.,* 2020). It quickly expanded to neighboring countries in Southeast Asia, followed by the Middle East and Europe. On May 18, 2022, 227 countries and territories around the world were affected, there has been a significant increase in COVID-19 cases.

In Iraq, the first confirmed case was reported on February 24. On February 25, four other cases from one family in the Kirkuk province were also recorded, this family had traveled to Iran previously (Jassim Abd-Alhussein *et al.*, 2020). On February 27, another case of a patient who had recently been travelled to Iran was reported in Baghdad. On July 2022 in iraq the total registered cases and death, where 2.4M and 25,263 respectively (Dong *et al.*, 2020).

2.3 Coronavirus structure

The Coronaviridae subfamily of viruses includes coronaviruses (CoVs), which are spherical enclosed, positive single-stranded RNA viruses with helical nucleocapsids, genus Coronavirus and the order Nidovirales (Mazzini *et al.*, 2021). They make up the majority of viruses that cause gastrointestinal and respiratory diseases. Alphacoronavirus, which includes the human coronavirus (HCoV)-229E and HCoV-NL63 (Liu *et al.*, 2021), Betacoronavirus, which includes HCoV-OC43, the Middle Eastern respiratory syndrome coronavirus (MERS-CoV), the human coronavirus for the severe acute respiratory syndrome (SARS-CoV-1), and the recently discovered Severe Acute Respiratory Syndrome Coronavirus 2 (Zhu *et al.*, 2020) . Infectious bronchitis virus-related viruses were only included in the terms Gammacoronavirus and Deltacoronavirus(Abdel-Moneim, 2017).

SARS-COV-2 is a rare strain of RNA virus that has never been seen in humans before (Pal et al., 2020) .The virus is capable of causing serious illnesses in a variety of hosts, including people, mice, camels, masked palm civets, cats, dogs, pigs, chickens, and bats (Mahdy *et al.*, 2020). In both people and animals, SARS-CoV-2 often causes gastrointestinal and illness respiratory (Luo *et al.*, 2020) .The capsid protein, the virus's outer covering, makes up the virus. The N protein participates in processes that affect the viral genome, the viral replication cycle, and the host cells' biological response to viral infection(Bakhshandeh *et al.*, 2021). The protein wraps the viral RNA genome and is needed for it to be copied and read.

The membrane protein, or M protein, is another significant protein that is widely distributed on the viral surface. This influences how the form of the virus envelope is determined (Cao *et al.*, 2022) . All other structural proteins can bind to this protein. It is thought that binding with M protein is the main way that

coronaviruses put themselves together. This is because binding with M protein helps to stabilize nucleocapsids or N proteins and makes it easier for viruses to put themselves together (Thomas, 2020).

The E-protein is a tiny membrane protein that is made up of 76 to 109 amino acids and minor viral particle components(Gupta *et al.,* 2021). It is important for virus assembly, the ability of the host cell membrane to let the virus in, and virus-host cell contact.

Hemagglutinin-esterase dimers (HE) have been identified on the surface of the virus. The HE protein may be involved in virus entry and appears to be essential for infecting the natural host cell, although it is not required for virus reproduction as shown in figure 2.1 (Kim, 2020).

It’s identified that the virus's surface has been enhanced with the S-protein. It facilitates the virus's attachment to the host cell's surface receptors and induces the membrane to fuse, making it simpler for the virus to enter the host cell (Alejandra Tortorici *et al.,* 2019). Two distinct protein domain segments, S1 and S2, which are related to cell identification and the joining of viral and cellular membranes, respectively, make up this glycoprotein. Each of its three identical chains contains 1273 amino acids (Mishra *et al.,* 2020). Many human cells, particularly those in the lungs, have angiotensin converting enzyme (ACE2) receptors on their surface, and when the coronavirus spike (S) protein attaches to these receptors, the virus can enter those cells(Ni *et al.,* 2020). The coronavirus S protein is cleaved at two locations known as the S1/S2 site, which is the boundary between the S1 and S2 subunits, by two host proteases called trypsin and furin (Örd *et al.,* 2020). Later cleavage occurs at the S2 domain (S20 site) to liberate the fusion peptide. This will cause the membrane fusion mechanism to begin working.

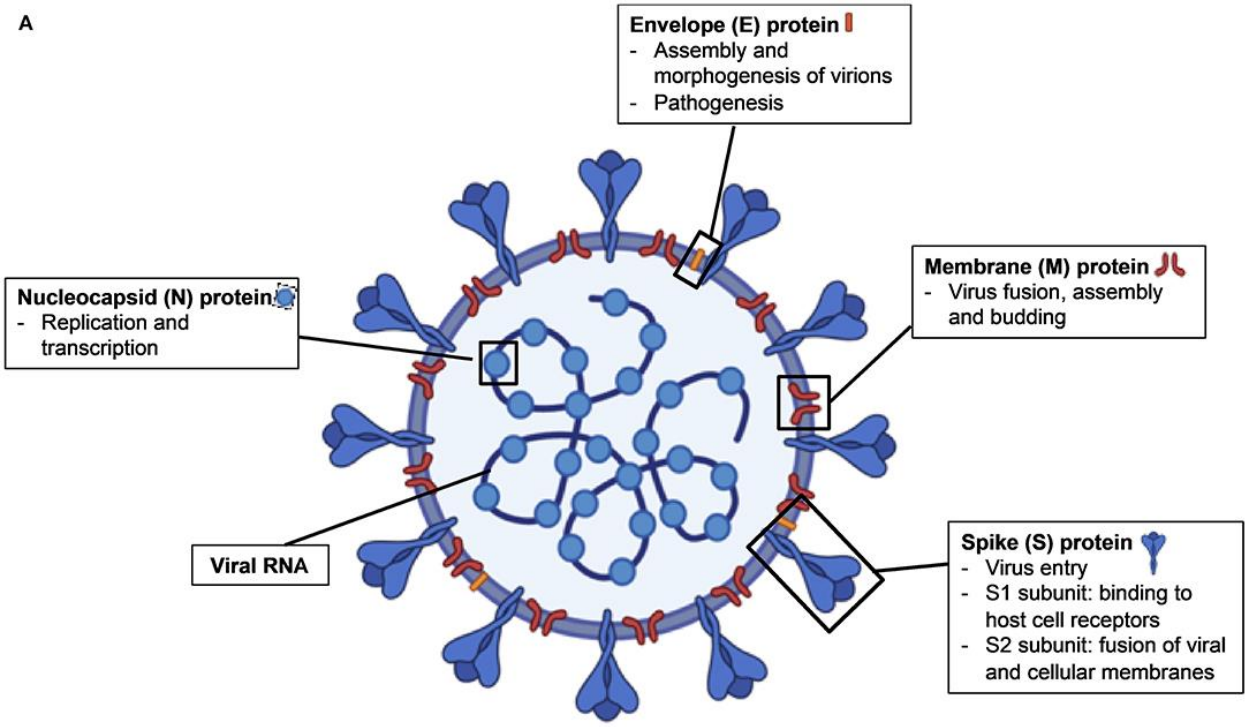

Figure 2.1 Structure of SARS-COV 2 *(Lee et al., 2020)*

2.4 Transmission

SARS-COV2 spread either direct or indirect contact with fomites or infectious respiratory droplets with mucous membranes (such as the eyes, nose, or mouth), or by aerosols, as well as during medical procedures and the handling of laboratory specimens. The pathogenesis and development of the disorders depend on specific structural proteins that may be found on the virus surface (Beig Parikhani *et al.,* 2021).

Additionally, it is possible for SARS-CoV to pass from bats to tree animals or mammal horses before infecting people(Frutos *et al.,* 2020). Human-to-human transmission or human infections and may result from reintroduction of an animal reservoir, persistent illness in previously ill individuals, or lab strains (Pal *et al.,* 2020). According to (Patel *et al.,* 2021) transmission risks rise related to time and proximity to contacts/infected people.

It is unknown how long SARS-CoV-2 will survive in the environment, according to recent studies, SARS-CoV-2 can survive for up to two weeks after being dried and for five days at40–50 % relative humidity and 22–25 °C before gradually losing viability (Chan *et al.,* 2011) . inversely, it has been documented that the virus declines after 24 hours at 38 °C and 80-90 % relative humidity (Riddell *et al.,* 2020). While bueckert documented that the virus can survive on various surfaces for 48 hours at 20°C and 40% relative humidity, but only for 8 hours at 30°C and 80% relative humidity (Bueckert *et al.,* 2020). This demonstrates that low humidity and temperature conditions are favorable for the virus's ability to survive in the environment. According to the evidence of a rapidly increasing prevalence of infections and the risk of transmission by asymptomatic carriers, SARS-CoV-2 can successfully spread between people and demonstrates great potential for a pandemic (Bandala *et al.,* 2021). The development and ease of international travel may also

contribute to the SARS-CoV2 virus's ability to spread throughout the world (Datta et al., 2020). Public health concerns arise from the potential for SARS-CoV-2 feco-oral transmission, particularly in unsanitary places (Odih *et al.,* 2020). In severe situations, the illness results in bleeding, pneumonia, septic shock, and metabolic acidosis (Helmy *et al.,* 2020) . According on age and prior infection history, the incubation time has been predicted to be between 5 and 14 days and may differ from patient to patient. Numerous investigations have shown that COVID-19 can spread between people by direct touch and nasal droplets in both symptomatic and asymptomatic patients (Jayaweera *et al.,* 2020).

2.5 Risk factor

It is obvious that there is a wide range in how (SARS-CoV-2) infection manifests itself. Public health professionals have identified a number of risk factors (Brodin, 2020) states that the vast majority of young people have moderate conditions. According to takahashi men are more likely to have severe illness than women, likely because of variations in the immunological responses that are induced between the sexes (Takahashi *et al.,* 2020). Despite the fact that there is a higher chance of developing severe disease as people age, a small subset of young and middle-aged people has severe COVID-19 disease, which is characterized by low oxygen saturation and significant lung inflammation (Felsenstein & Hedrich, 2020). Severe COVID-19 disease is linked to comorbidities such as hypertension, chronic obstructive pulmonary disease, and cardiovascular disease (Huang *et al.,* 2020) . Smoking is another risk factor since it increases angiotensin-converting enzyme 2 (ACE2) expression, which allows SARS-CoV-2 to penetrate cells and may even impact viral invasion in addition to its detrimental effects on lung function as a whole (Huang *et al.,* 2020). Numerous studies have sought to identify the mediating factors

of such hyperinflammatory disease presentations. Such cases require rapid management and intensive care.

Obesity, defined as an abnormal accumulation of body fat, is typically measured using body mass index (BMI), which is calculated by dividing body weight (kg) by height squared (m2) (Ridha Guedjati *et al.,* 2022) .In the entire world, there are more and more obese people. Adiposity influences harmful health consequences such as fatty liver disease, coronary artery disease, cerebrovascular disease, insulin resistance, and hypertension (Ghoorah *et al.,* 2016) . In addition to contributing to mechanical health issues, fat buildup also causes the release of a large number of adipokines, which are inflammatory mediators (Nimptsch *et al.,* 2019) . However, the immune system is weakened in obese people, especially in weak people with a lot of health problems (Jayanama *et al.,* 2021) (Misumi *et al.,* 2019) . Obese people may be more vulnerable to SARS-CoV-2 infection. A pathology of COVID-19 is a dysfunctional immune response that damages several organs, especially the lower airways (Gallo, 2021) Due to a similar cause, COVID-19's bad effects and severity may be linked to obesity (Jayanama *et al.,* 2021).

2.6 Clinical signs

In general, SARA-COVID-2 early clinical symptoms can resemble those of other seasonal viral respiratory diseases, making it difficult for doctors to recognize the condition in its earliest stages (Carpenter *et al.,* 2020). Between two and seven days following the start of the infection, respiratory symptoms frequently worsen and are typically accompanied by a nonproductive cough and dyspnea. Breathing issues include (Bertolino *et al.,* 2020). It is unusual to get more severe respiratory symptoms, such as rhinorrhea and sore throat (Y. Wang *et al.,* 2020). After 7–10

days of infection, patients with SARS-CoV positive lab results may exhibit advanced radiographic lung alterations indicative of pneumonia (Shan *et al.*, 2020) .

2.7 Diagnosis

It is best to collect samples from the upper respiratory tract (such as nasopharyngeal swabs, oropharyngeal swabs, nasopharyngeal aspirates, and nasal washes) or, if the patient is in a hospital or intensive care unit, also from the lower respiratory tract (such as bronchoalveolar lavages, endotracheal aspirates, and expectorated sputum) (Ahmadzadeh *et al.,* 2021). The diagnostic techniques comprise:

1-Molecular testing; When possible, SARS-CoV-2 nucleic acids are detected in nasopharyngeal fluids by molecular testing, such as real-time reverse-transcription polymerase chain reaction (RT-PCR). Serological tests are currently the best way to find viruses, but nucleic acid assays are more sensitive and specific(Kevadiya *et al.,* 2021).

2-Serological testing: Serological testing is a useful adjunct to viral identification since it can reveal previous infections that may be useful for therapeutic purposes. The qualitative detection of IgG or IgM antibodies is used in the enzyme-linked immunosorbent test, which detects antibodies. It cannot be utilized to diagnose acute SARS-CoV-2 infections on its own but it could be useful in a number of situations, such as when molecular tests come back negative, when patients show up late or have symptoms that last a long time, or in sero-surveillance studies.

3-The rapid diagnostic test

_Rapid antibody detection kits for the qualitative identification of SARS-CoV-2 IgG/IgM antibodies in serum, plasma, or whole blood have received approval.

_ Antigen testing is based on the direct identification of SARS-CoV-2 viral proteins in nasal swabs and other samples from the respiratory tract. This is done with a lateral flow immunoassay.

4-chest x-rays to stop the spread, all imaging procedures should be done in line with regional policies for preventing and controlling infections. In some facilities, lung ultrasonography is employed as a diagnostic technique in place of chest x-rays and chest computed tomography. Its accuracy as a diagnostic tool is only backed up by evidence with a very low level of certainty, but it might be useful as an extra or different imaging modality.

5-A new test for identifying SARS-CoV-2 viral RNA is called RT-LAMP tests, which uses reverse transcription loop-mediated isothermal amplification. Assays are quick and easy, but there is less support for their use.

6- virus isolation as a standard diagnostic method, viral isolation is not advised. For any process in cell culture that needs to isolate a virus, qualified people and biosafety level 3 (BSL-3) facilities are needed(Kampf *et al.*, 2020).

2.8 Treatment

To stop the virus from spreading to other people, patients, and healthcare workers, the first step is to make sure the person is properly isolated (Singhal, 2020).

-The management of mild illnesses at home should include education on warning indicators (Greenhalgh *et al.*, 2020) .

-The standard guidelines include keeping hydrated, eating well, and managing fever and cough, it has been documented those antibiotics and antiviral like oseltamivir shouldn't be used routinely in cases where they have been proven to work (Costa *et al.*, 2022).

It is recommended to provide oxygen to hypoxic patients using nasal prongs, a face mask, a high flow nasal cannula (HFNC), or non-invasive ventilation (Costa *et al.*, 2022) It may be necessary to use artificial breathing or even extracorporeal membrane oxygen support.

-Possible requirement for renal replacement treatment (Singhal, 2020).If co-infections are thought to exist or are confirmed, antibiotics and antifungals are necessary.

The antivirals being tested for SARS-CoV-2 can be split into two categories: those that target the virus's proteins or RNA and those that target the host proteins, such as host proteases. Researchers in the lab and in the real world will find that the following types of drugs are effective against SARS-CoV-2 (Zhang *et al.*, 2022):

1) Antiviral targets the viral genome replication process by acting as an RNA-dependent RNA polymerase inhibitor for example Remdesivir

2) Antiviral inhibitors of viral protease for example Lopinavir

3) Antiviral inhibitors virus proliferation in the cell for example Ribavirin

4) Antiviral inhibitors both viral entry and the inflammatory response for example Baricitinib

5) Antiviral inhibit the fusion process during viral entry into the host cells for example umifenovir

6) Antiviral inhibitors of viral entry for example Hydroxychloroquine

The most abundant and efficient therapies use in Iraq and world were blood plasma transfusion, the combination of hydroxyl-chloroquine with azithromycin, and remdesivir(Faraj *et al.*, 2022).

2.9 Immune response to coronavirus infection

The COVID-19 pandemic can be controlled, people can be protected from life-threatening illness, and viral dissemination can be restricted by developing immunity against the SARS-CoV-2(Chang & Radbruch, 2021). Immunity to viral infection is caused by a variety of specific and nonspecific mechanisms. The activation of different immune functions and the duration and magnitude of the immune response depend on how the virus interacts with host cells (on whether it is a cytolytic, steady-state, latent, and/or integrated infection) and on how the virus spreads (by local, primary hematogenous, secondary hematogenous, and/or nervous system spread). Therefore, viral antigens may be present in different parts of the body depending on the route of spread and phase of infection(Shah *et al.*, 2020) . The host has multiple immune defense functions that can eliminate virus and/or viral disease. In this sense, the immune response can be divided into:

2.9.1 Cellular immune response

The term cell-mediated immunity refers to the recognition and/or killing of virus and virus-infected cells by leukocytes and the production of different soluble factors (cytokines) by these cells when stimulated by virus or virus-infected cells. the T lymphocytes prevent virus multiplication by destroying infected cells before mature, infectious virus particles can be assembled. This hypothesis assumes that viral antigens appear on the plasma membrane before the release of virus progeny, a view that is substantiated by studies of many, but not all, infections (G. Li *et al.*, 2020). Several types of T cells are involved in this response.

Exposure to a virus-infected cell can cause the antigen-specific T lymphocytes to differentiate into cytotoxic effector T cells, which can lyse virus infected or virally transformed cells. The CD8+ T cytotoxic cells directly recognize viral peptides presented by major histocompatibility complex (MHC) class I at the surfaces of infected cells, which destroy the infected cell by release cytoplasmic granular and granzymes, causing apoptosis (a form of programmed cell death) and preventing the virus from spreading further (Fialkowski *et al.,* 2020).

Helper T cells may be as important as cytotoxic T cells in the immune response to a virus infection. Helper T cells are required for the generation of cytotoxic T cells and for optimal antibody production (Duckworth & Groom, 2021). These neutralizing antibodies can recognize whole viruses and act by blocking the virus from infecting cells (Asarnow *et al.,* 2021). In addition, helper T cells, and cytotoxic T cells produce a number of important soluble factors (lymphokines) that can recruit and influence other cellular components of the immune and inflammatory responses (Tay *et al.*, 2020).

Alveolar macrophages identify the viruses that have been neutralized and the apoptotic cells that have been eliminated by CD8+ T cells and phagocytose them or

will use MHC class II to present the antigen to T helper 1 cell (Singh *et al.*, 2021). Th1 release IFN-γ antiviral properties activator of macrophages (Increases lysosome activity of macrophages, activates inducible nitric oxide synthase (iNOS) release free radical and O_2) and induce of MHC class II molecule expression, it has the ability to inhibit viral replication directly, which is considered the most important function (Kak *et al.*, 2018). Activation of Th1 cells and release IFN-γ could stimulate CD8 T cytotoxic cells (Toor *et al.*, 2021).

2.9.2 Humoral immune response

The bone marrow produces an immature B lymphocyte at the beginning, which is one of the Antigen presenting cell (APC), and on its surface it has express MHC Class II and specific type of antibody, usually IgM and express CD40 that needed it to attachment with T cell (Upasani *et al.*, 2021). B cell settles in the lymph node, waiting for it to recognize a specific antigen. When virus enters the body as a product or outside the cell, it recognized by antibody (AB) on B cell (Shah *et al.*, 2020).

B cell will be activated by linking the antigen with AB, and this is the primary signal, so it needs a secondary signal to complete the activation process, by presenting the antigen to the T cells, which in turn releases certain cytokines that complete the activation and transformation of the B cell to plasma cell that can secreted specific antibodies to virus antigen (they produce antibodies that can recognize viral proteins).

Antibody response can be directed against all viral proteins, although Spike and nucleocapsid are considered the main targets of humoral response. Antibodies against receptor-binding domain (RBD), appear earlier in the course of infection than those antibodies against nucleocapsid, and it became clear that plasma cells can differentiate into memory cells and release antibodies to provide long-lasting

defense. In the bone marrow, memory plasma cells can be preserved for decades, if not a lifetime (Brochot *et al.,* 2020).

2.10 Antigenic escape from immune cell

In order to better survive and infect host cells, viruses, particularly coronaviruses, have a variety of techniques to evade the immune system cells (X. Li *et al.,* 2020). this a process is introduced before it enters the cell or after it has already reached the host cell. It can employ avoidance tactics by creating duplicate vesicles on the outside of the cell during the recognition process. As an intermediate viral replication product, the creation of these vesicles causes shield recognition of cytosolic Pattern Recognition Receptors (PRRs) to dsRNA (Astuti & Ysrafil, 2020). In addition to forming a double vesicle, this virus possesses 8 proteins that can suppress INF to evade the immune system (Kasuga *et al.,* 2021). For example, SARS-Covid-2 has a 5 cap smaller than the RNA of the host cell, which makes it simple for immune system cells to detect its existence and trigger an immunological response (Gorkhali *et al.,* 2021). The virus devised a technique to imitate the host capping mechanism in order to get around this. This method uses two non-structural proteins: nsp 14, which triggers cap formation, and nsp 16 which modifies the viral RNA caps such that they resemble host cell RNA and prevents PRRs from recognizing them (Etido *et al.,* 2021).

Additionally, other coronavirus nonstructural proteins that have the capacity to shield SARS-CoV from immune responses are used as agents in the virus' evasion of immune response-inducing pathogens (Gu *et al.,* 2022). SARS-CoV may also use its protein accessories to evade immune responses in addition to nonstructural proteins. For instance, the gene segment on open reading farm (ORF3b) of this virus can block the INF signaling pathway and prevent the activation of effector cells, which will stop the spread of the infection (Redondo *et al.,* 2021).

2.11 Covid Vaccines

Throughout history, vaccines have been shown to be the most efficient method of containing viral pandemics (Bhavana *et al.*, 2020). The outbreak started a race for vaccinations around the world. At the end of 2020, 259 COVID-19 vaccination studies were ongoing, with 11 in phase III clinical trials (Chakraborty *et al., 2021)*. According to the most popular classification scheme, vaccines can be characterized as either classical or new generation depending on the platforms on which they are produced (Simões & Rodríguez-Lázaro, 2022).

Three main processes are involved in vaccine action: antigen presentation and phagocytosis, T cell activation and cytokine generation, and B cell differentiation into plasma cells stimulated by cytokines. Therefore, an immunological response connected to vaccinations involves both B and T cells. As a result, choosing the right antigen and therapeutic target is essential for a vaccine's effectiveness. The S protein, in particular the RBD, will be the therapeutic target for COVID-19 because it blocks binding to the host receptors. This is the most common and effective method of targeting the virus. The S protein, which comprises two domains (S1 and S2), likewise functions as the neutralizing antibodies' principal target. The RBD is a component of the S1 domain that eventually interacts to the ACE2 receptor with a comparatively high affinity(Harvey *et al.*, 2021).

2.12 Vaccination Platforms

The following are the vaccination platforms used to prepare the various vaccines.

-mRNA vaccines have been developed by Moderna and Pfizer-BioNTech. A cutting-edge technique called RNA and DNA vaccines employs genetically

modified RNA or DNA produce a protein that safely elicits an immune response (Mascellino *et al.,* 2021).

- Several companies, including Astra-Zeneca, Johnson & Johnson, Reithera, and Sputnik, have created viral vector (adenovirus) vaccines. Viral vector vaccines use a virus that has been genetically modified to not only cause disease but also to create coronavirus proteins in order to safely elicit an immune response (Mendonça *et al.,* 2021).

- Sinopharm has developed inactivated viral vaccinations. Immunizations against weakened or inactivated viruses don't make disease, but they do boost the immune system.

-Protein subunit vaccines have been created by Novavax. In order to safely stimulate an immune response, protein-based vaccinations employ innocuous protein fragments or protein shells that resemble the COVID-19 (Gao *et al.,* 2020).

2.13 Current and Most Common COVID-19 Vaccines

2.13.1 Pfizer

According to the interim analysis ("Safety and Efficacy of the BNT162b2 MRNA Covid-19 Vaccine," 2021) Pfizer's mRNA vaccination was the first vaccine to be approved for emergency use against COVID-19. It has a prospective efficacy of more than 90%. This vaccine, which is given intramuscularly (IM), is made up of a lipid-enclosed, nucleoside-modified mRNA that specifies the shape of a COVID-19 spike protein that has undergone mutation (Walsh *et al.,* 2020) . Two 30 g doses make up the Pfizer vaccine, with the second dosage being given three weeks after the first (Cohen, 2020).

Lipid nanoparticles enable delivery of COVID-19 S gene mRNA into the host cell, maintaining the integrity of the mRNA and preventing it from being mistaken

with other RNA molecules, leading to expression of the COVID-19 spike protein antigen. Lipid nanoparticles are injected into the deltoid as part of the Pfizer mRNA vaccinations. These muscle cells have T cells, which are antigen-presenting cells that show CD4 and CD8 cells, natural killer cells, and blood vessels (Siddique & Ahmed, 2021).The lipid nanoparticles are ingested by the cells, then the mRNA reaches the ribosomes, which are then used to synthesize the viral spike proteins in processes referred to as translation (Pušnik *et al.,* 2021). The proteins are then recognized by antigen-presenting cells, such as B-cells, macrophages, and dendritic cells, which possess MHC-2 molecules on their surface or cells that possess MHC-1 molecules.

The binding of the protein acting as an antigen to the MHC molecules leads to immune recognition of the S-proteins by cytotoxic T-cells in the case of MHC-I cells and helper T-cells (Th) in the case of MHC-II cells. The CD4+ T-cell receptors on Th cells bind to the antigen-presenting MHC-II which triggers the production of cytokines such as IL-2, IL-4, and IL5 (Cox & Brokstad, 2020).

These interleukins cause our body's B-cells to mature into plasma cells, which create a significant quantity of Abs targeting viral spike proteins. In the meantime, interleukins (IL) result in memory T-cell proliferation. The TCRs of CD8+ T-cells are activated by the presentation of the spike protein antigens by MHC-1 proteins on cell membranes, resulting in the production of cytotoxic T-cells (Tcx) which directly cause the death of virus-infected cells using harmful molecules such as granzyme and perforin (Bettini & Locci, 2021).Tcx cells also secrete immune signals to further amplify the immune response (He *et al.,* 2021) .

The body develops immunity against the virus thanks to this process, which involves the vaccination eliciting an immune response to the spike protein. This immunity should last for six to nine months (Dan *et al.,* 2021).

The safety of vaccinations is concerned with the detection of adverse reactions either after the first dose or after the second. The first and second doses are given 21 days apart, and any potential symptoms are noted seven days after the previous dose. There are only moderate or negligible adverse effects recorded for both vaccinations. After the second dose, it's possible to notice a slight pinched pain at the injection site, a little bit of redness, fatigue, headaches, muscle and joint pain, and fever. Even though they are extremely rare, several severe side effects of the Pfizer vaccination have been documented in the literature, including the Guillain-Barre syndrome, anaphylactic reactions, and allergic reactions.

Twenty-one people out of the 1.893.360 who received the first dose of the Pfizer-BioNTech COVID-19 vaccine between December 10 and December 23, 2020, experienced life-threatening allergic reactions, including anaphylaxis. This rate of cases per million doses administered is estimated to be 11.1 cases. However, no anaphylaxis-related deaths have been reported. a rare case of Bell's palsy of facial paralysis, has been observed in Covid-19 patients. However, following vaccination, this incidence did not increase (Mascellino *et al.*, 2021).

2.13.2 AstraZeneca

The AstraZeneca adenovirus viral vector vaccine was developed in a collaboration between Oxford University and AstraZeneca in the United Kingdom and makes use of a genetically engineered vector carrying genetic information encoding a wild-type S protein. The immune response in the AstraZeneca vaccine is generated from the use of a modified replication-deficient chimpanzee DNA adenovirus, ChAdOx1, that human populations have not been exposed to. The DNA encodes a protein that elicits a similar immune response as the SARS-CoV-2 S-peptide along with a tissue plasminogen activator (Ramasamy *et al.*, 2020).

In human host cells, this vector leads to the production of new adenovirus viral proteins that generate this immunological response. The adenovirus latches on to the outside of the human host cell and DNA is then injected into the cytoplasm where it travels to the nucleus. The DNA is transcribed into mRNA by the host cells and this RNA is later translated into the appropriate proteins by ribosomes. MHC1 and MHC2 complexes are formed when the proteins are expressed on cell membranes. The processes of RNA and DNA vaccines are identical at this point, leading to the activation of T-cells, B-cells, and plasma cells along with the production of antibodies (Jones & Roy, 2021). Only 10% of vaccine recipients in the trials for the AstraZeneca vaccine reported adverse effects, with headache, nausea, and muscle soreness, pain at the injection site, and redness being the most frequent (Ricke, 2021). However, the MHRA (Medicines and Health-Care Regulatory Agency) recorded 6 cases of Bell's palsy and 13 cases of allergic reactions (all patients recovered well). These side effects were not definitively linked to the vaccine.

Out of more than 9 million vaccines, there have been 194 complaints of anaphylaxis, which is a side effect (*Mascellino et al.,* 2021) . The majority of the 143 people who were reported to have passed away soon after receiving a vaccination were elderly patients with underlying medical issues. Therefore, these fatalities did not necessarily point to the vaccine as the direct cause. The analysis of the negative consequences of the immunizations is still ongoing. There have been concerns expressed concerning the AstraZeneca vaccine's potential side effects, including as uncommon blood clots linked to bleeding-related or not-bleeding thrombocytopenia (including cerebral venous sinus thrombosis [CVST] or pulmonary embolism) or thromboembolic events (Pai *et al.,* 2021).

The European Medicines Agency (EMA) pulled the vaccine off the market because of these side effects on March 15, 2021, but it was approved again three days later on the grounds that the vaccine's advantages exceeded its drawbacks. The COVID-19 vaccination from AstraZeneca: EMA discovers a potential connection to extremely uncommon blood clots with low blood platelets. AstraZeneca has thus been outlawed in Germany and other European nations for those under the age of 60. It hasn't been shown that the vaccine is to blame for these problems, but it has been decided that more research is needed to figure out what's going on.

2.13.3 Sinopharm

Sinopharm, a state-owned Chinese company, it is creating the Sinopharm/BIBP COVID-19 vaccine. is a virus-containing inactivated vaccine that is administered to patients as a dead version of pathogen and are generally the quickest choice for antiviral immunization (Zahid et al., 2021) . Thus, these vaccines are created using highly purified, non-infectious viruses.

The Sinopharm vaccine is a complete viral vaccine that has been inactivated and created using Vero cells. The SARS-CoV-2 virus is produced by these cells in large quantities, and after being exposed to beta-propiolactone, the virus is rendered inactive by binding to its genes. Inactivated vaccines need adjuvants, which are introduced into the vitro cell once more to ensure that the virus don't multiply while maintaining their form (Siddique & Ahmed, 2021). These vaccines, which contain an adjuvant of aluminum hydroxide to modify the immune system, offer certain advantages because they can be delivered and kept at typical refrigerator temperatures. For the prevention of diseases brought on by viruses, these vaccinations have been found to be both secure and efficient (Joshi *et al.*, 2021).

The Sinopharm vaccines come in two doses and are injected intramuscularly (IM). Sinopharm's examination of their vaccinations, however, indicates that BBIBP-CorV has an efficacy of only about 79 percent, which is significantly lower than that of the vaccines produced by Pfizer and AstraZeneca. However, the information that is now available demonstrates that when both dosages are given, an effective humoral immune response is produced in all recipients. Studies for Sinopharm studies' adverse effects have been conducted in total. Headache, myalgia, fatigue, pain at the injection site, in the body, or in the muscles; shortness of breath; stomach pain; and diarrhea are all possible side effects(Attash *et al.*, 2022).

Chapter Three
Materials and Methods

Chapter three

Materials and Methods

3.1 Subjects

A cross-sectional study was conducted at College of Applied Medical Sciences/ University of Karbala, Figure (3.1). Out of 174 vaccinated healthy individuals were enrolled in this study age range 18-70 with one or two doses from different health centers in Karbala Province. Blood samples collection from the participant was done during the period from November 2021 to April 2022, serum samples were separated from blood to be used in the detection IgG and interferon game IFN levels. Every subject with certain disease like Diabetic patients, Hypertension patients, cardiovascular diseases, all kinds of Cancer, Smokers, and Pregnant woman were excluded from the study. while people who have vaccine and do not have chronic diseases, diseases that weaken immunity, and non-smokers are the ones who have been done included to this study**.**

3.2 Sample collection

Five milliliters of venous blood were drawn from all participants by using a disposable syringe. Drawn blood was put into gel tubes and centrifuged at 4000 xg for 20 mint to get serum used to analyze, IgG antibody, and interferon gamma automatically.

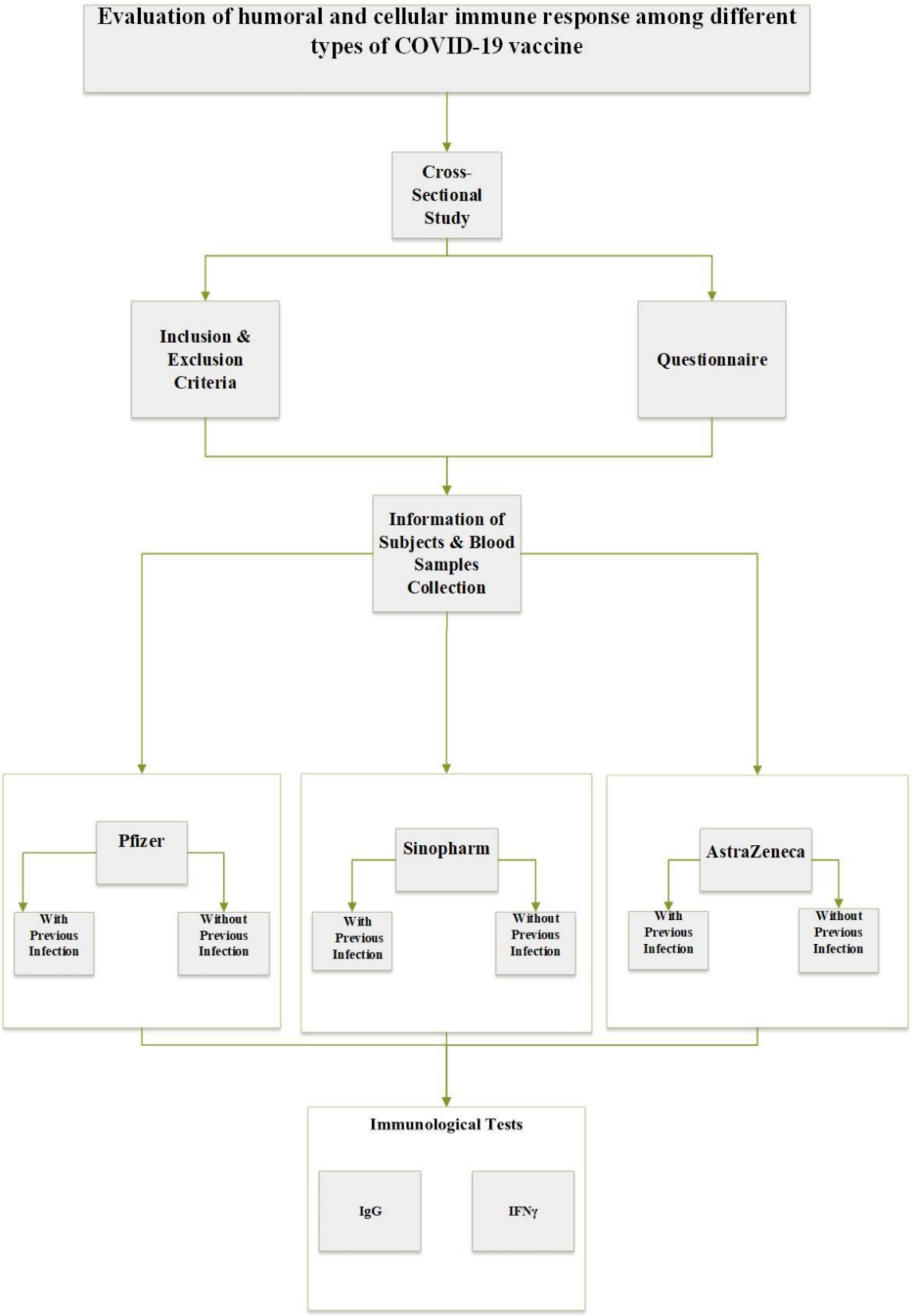

Figure 3.1study design

3.3 Materials

3.3.1 Devices and Equipment

Table (3.1) shows the instruments and apparatus that were used in this study.

Table 3.1 Devices and Equipment

No	Equipment	Manufacturer
1	Deep freeze	German
2	Micro centrifuge	German
3	Eppendorf tube	German
4	Micropipette	Iraq
5	Elisys Uno fully automated ELISA	Human (Germany)
6	ARCHITECT C4000 clinical chemistry analyzer	Abbott (Germany)

3.3.2 Kits and Chemicals Materials

Chemicals and kits used in current study are presented in table (3-2).

Table 3.2 Kits and Chemicals

NO	Kits	Manufacturer
1	SARS-CoV-2 IgG II Quant	Abbott (Ireland)
2	IFN gamma kit	Sun log (China)

3.4 Methods

3.4.1 Determination Human IFN-γ (Interferon Gamma) using ELISA Kit

Interferon Gamma was measured according to procedure mentioned by sun long (China).

3.4.1.1 principle

This ELISA kit uses the Sandwich-ELISA principle. The micro-ELISA plate provided in this kit has been pre-coated with an antibody specific to Human IFN-γ. Samples (or Standards) are added to the micro-ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for Human IFN-γ and Avidin-Horseradish Peroxidase (HRP) conjugate are added successively to each micro plate well and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain Human IFN-γ, biotinylated detection antibody and Avidin-HRP conjugate will appear blue in color. The enzyme-substrate reaction is terminated by the addition of stop solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The OD value is proportional to the concentration of Human IFN-γ. You can calculate the concentration of Human IFN-γ in the samples by comparing the OD of the samples to the standard curve.

3.4.1.2 Kit components

Kits can be kept unopened for one month at 2 to 8 °C. Once the kit is received, if it is not intended to be used within a month, the components must be stored individually and in accordance with the following guidelines. Table 3.3, shows the details of the kit.

Table 3.3 ELISA kit details

No	Items
1	Micro ELIZA strip plate
2	Standard :108pg/ml
3	Standard diluent
4	HRP-Conjugate reagent
5	Sample diluent
6	Chromogen Solution A
7	Chromogen Solution B
8	Stope solution
9	Wash solution

3.4.1.3 Procedure

1- Dilution of Standards: the standard was diluted by small tubes first, then the volume of 50ul was pipetted from each tube to microplate well, each tube uses two wells, total ten wells

2- In sample wells, 40µl Sample dilution buffer and 10µl sample were added. The samples were loaded onto the bottom without touching the well wall, and it was mixed well with gentle shaking

3- Incubation: the plat was incubated for 30 min at 37℃ after sealed with Closure plate membrane.

4- Dilution: the concentrated washing buffer was diluted with distilled water (30 times for 96T)

5- Washing: the wash solution was aspirated and refilled. the wash solution was discarded after resting for 30 seconds. the washing was repeated procedure for 5 times.

6- 50 µl HRP-Conjugate reagent was added to each well except the blank control well.

7. Incubation: the plat was incubated 30 min at 37℃

8. Washing: the washing procedure was repeated as step 5

9. Coloring: 50 µl Chromogen Solution A and 50 µl Chromogen Solution B were added to each well, mixed with gently shaking and incubated at 37℃ for 15 minutes.

10. Termination: 50 µl stop solution was add to each well to terminate the reaction. The color in the well should change from blue to yellow.

11. Read absorbance O.D. at 450nm using a Microtiter Plate Reader. The OD value of the blank control well is set as zero. Assay should be carried out within 15 minutes after adding stop solution.

3.4.2 Determination Human anti-SARS-CoV-2 S1(RBD) IgG using

On the ARCHITECT I System, the SARS-CoV-2 IgG II Quant test is a chemiluminescent microparticle immunoassay (CMIA) used to determine the quantity and quality of IgG antibodies to SARS-CoV-2 in human serum and plasma.

3.4.2.1 Principles

Using chemiluminescent microparticle immunoassay (CMIA) technology, this assay is an automated, two-step immunoassay for the qualitative and quantitative detection of IgG antibodies to SARS-CoV-2 in human serum and plasma.

The sample is mixed with the assay diluent, paramagnetic microparticles coated with the SARS-CoV-2 antigen, and then incubated. The SARS-CoV-2 antigen-coated microparticles attach to the IgG antibodies against SARS-CoV-2 that are present in the sample. The combination is cleaned. A reaction mixture is made by mixing in the conjugate of anti-human IgG that has been acridinium-labeled. Pre-Trigger and Trigger Solutions are added after a wash cycle. A relative light unit is used to measure the chemiluminescent reaction that results (RLU). The amount of SARS-CoV-2 IgG antibodies in the sample and the RLU picked up by the system optics are directly correlated.

3.4.2.2 Procedure

Serum sample in Eppendorf tube was entered to the equipment (Architect Abbott) and analyzed automatically to measure IgG.

3.5 Statistical Analysis

IBM SPSS VERSION 24 software was used for statistical analysis of data. Quantitative results are indicated as mean ± SD. Pearson test was used for analyzing correlations between parameters. The statistical significance level was set at $P<0.05$ ANOVA table to compare three vaccine and independent sample t test to compere groups and LSD to test less significant deference

3.6 Ethical considerations

This study was approved by Ethical Committee at College of Applied Medical Science/ University of Kerbala. All subjects involved in this work were informed and agreement was obtained verbally from each one before the collection of samples.

Chapter Four
Results and Discussion

Chapter four
Results and Discussions

4.1 Distribution of subjects

Serum sample was collected from One hundred and seventy-four patients, between 1 November 2021 to April 2022 in Karbala, Iraq. the most and main participant were medical student in Kerbala university. The information for each participant was documented according to the questionnaire form, which include age, sex, weight, high, type of vaccine taken, type and date of dose administration, date of previous infection if found, and other questions, as shown in questionnaire form in appendix. The participants' ages ranged from 18 to 70 years, the mean age was 25.97, they were divided into 2 groups: those under 25 years old 109 (62.6%), and those over 25 years old 65 (37.3%). 90 subjects (51.7%) were men and 84 (48.2%) were women. 105 out of 174 (60.3%) had received the Pfizer vaccine, 59 (33.9%) had received the Sinopharm vaccine, and 10 (5.7%) of them had received AstraZeneca. The sample was taken at various times and weeks. Some of them 69 had received one dose, with a percentage of (39.6%); while the other part 105 had received two doses, with a percentage of (60.3%). Additionally, some of them 59 (33.9%) had a confirmed infections prior to receiving the vaccine whereas others 115 did not (66%). Furthermore, according to BMI, 100 subjects (57.4%) had a normal weight and 50 (28.7%) had overweight, as shown in Table (4.1).

Table 4.1data distribution

Variables		**Frequencies (%)**
Gender	Female	84 (48.2%)
	Male	90 (51.7%)
Age (Mean ±SD 25.979±.327)	More than 25	65 (37.3%)
	Less than 25	109 (62.6%)
Dose	First	69 (39.65%)
	Second	105 (60.34%)
Type of vaccine	Pfizer	105 (60.34%)
	Sinopharm	59 (33.9%)
	AstraZeneca	10 (5.7%)
Previous infection	Vaccination without previous infection	115 (66%)
	vaccination with previous infection	59(33.9%)

Variables		**Frequencies**
Weeks	1 week	19 (10.9%)
	2-3 weeks	29 (16.6%)
	4-5 weeks	9 (5.1%)
	6-7 weeks	14 (8%)
	8-9 weeks	13 (7.4%)
	10& more weeks	90 (52.7%)
Body mass index	Underweight	9 (5.17%)
	Normal weight	100 (57.47%)
	Over weight	50 (28.73%)
	Obese	15 (8.62%)

4.2 Comparison of IgG and IFN-γ concentration among the three types of vaccines

As shown in table (4.2), Comparing anti-spike (IgG) levels among the three types of vaccines revealed significant difference. AstraZeneca and Sinopharm's vaccines had lower IgG concentrations as compared to Pfizer's vaccine.

This result is in agreement with previous study in which the author reported that the Pfizer BioNTech vaccination produce greater antibody readings after a first dose than the Oxford AstraZeneca vaccine (Eyre *et al.*, 2021).

Table 4.2 Comparison of IgG and IFN γ level among the three types of vaccines

Type of vaccine	IgG AU/ml	IFN-γ pg/ml
	Mean ± S. D	Mean ± S. D
Pfizer	16960.6 ±11092.0	64.6 ±14.7
Sinopharm	4118.3 ±1380.3	64.2 ±12.5
AstraZeneca	3195.6 ±658.6	60.9 ±12.1
P value	0.00**	0.719
ISD	2793	

LSD: Least Significant Difference, ** highly significant difference

Additionally, other study documented that comparison of ChAdOx1 (Oxford-AstraZeneca) and BNT162b2 (Pfizer-BioNTech) revealed that the mRNA vaccine BNT162b2 induces a stronger humoral response than the adenovirus-based ChAdOx1 vaccine, both after the first and second doses (Romero-Pinedo *et al.*, 2022). Moreover, and according to other investigation revealed that those who received two doses of BNT162b2 were much more likely to have anti-SARS-CoV-2 antibodies than those who received Sinopharm (99.4 % Vs 71.0 %, respectively) (Gómez de la Torre *et al.*, 2022).

Additionally, another study was conducted to evaluate the best effective vaccination. The results showed that there was no statistically significant difference between the antibody titers produced by the Sinopharm traditional inactivated virus vaccine and the Pfizer-BioNTech mRNA vaccine (Alqassieh *et al.*, 2021) .

Comparison of IFN γ levels among the three types of vaccines revealed no significant difference. Similarly, previous results showed that there were only marginally different variations in the cumulative number of IFN γ producing cells in participant vaccinated with mRNA (BNT162b2) and inactivated virus (Sinopharm) (Gómez de la Torre *et al.*, 2022).

4.3 Differences in IgG and IFN γ according to age groups

The overall antibody concentration in participants under the age of 25 was higher than that in people above the age of 25. Also, there were significant differences among the three types of vaccine within both age groups, and the highest concentration was seen in participants vaccinated with Pfizer, as shown in table (4.3).

Table 4.3 Differences in IgG and IFN-γ according to age group

Type of vaccine	IgG AU/ml			IFN-γ Pg/ml		
	Less than 25	More than 25	P value	Less than 25	More than	P value
	Mean ± S. D	Mean± S. D		Mean± S. D	Mean± S. D	
Pfizer	18329.5±11461.0	14714.2±10204.8	0.109	65.5±14.9	63.1±14.5	0.429
Sinopharm	4110.8±1274.8	4132.5±1597.1	0.955	64.5±13.1	63.8±11.7	0.852
AstraZeneca	3551.1±446.3	2662.4±580.9	0.025*	67.0±8.3	51.7±11.5	0.04*
Total	12505.6±11301.4	10589.8±9634.8		65.2±13.9	62.6±13.6	
P value	0.00**	0.00**		0.89	0.252	
LSD	3577	4405				

LSD: Least Significant Difference, ** highly significant difference, * significant difference

Despite this, the current study does not observe any significant difference in IgG concentration between persons younger and older than 25 whom vaccinated with Sinopharm and Pfizer vaccines, whereas, there is a significant difference between the two age groups in subjects vaccinated with the AstraZeneca vaccine.

The result of the current study is in agreement with previous research showed that S1 IgG levels caused by BNT162b2 immunization decreased with age, with the maximum amounts seen in people between the ages of 12 and 19(Wei *et al.*, 2022). Also, another study documented that the geometric mean titer of anti-spike IgG was

consistently lower in the older age group and declined following the second vaccination (Ikezaki *et al.,* 2022).

Inversely, Age-related differences in IgG antibody levels were evident in previous study, especially between participants in the younger (aged 21 to 30) and older age groups (Anastassopoulou *et al.,* 2022).

Elderly adults are also substantially more likely to have inadequate or no post-vaccination humoral response, and the values of anti-SARS-CoV-2 antibodies after vaccination are higher than in the elderly. (Collier *et al.,* 2021)

It has been found that mRNA vaccines (BNT162b2) induce a stronger humoral response, both after the first and the second dose, than the adenovirus-based ChAdOx1 vaccine on the contrary, IgG1 increased progressively reaching its maximum level around 2-4 weeks after the second, boosting, dose and decreasing slightly afterwards. Of note, ChAd vaccine induced delayed kinetics, highlighting a decreased potency compared to mRNA vaccines (Romero-Pinedo *et al.*, 2022).

Also, AstraZeneca Vaccine-Induced Prothrombotic Immune Thrombocytopenia (VIPIT)., adults under the age of 55 should not receive (Health Ontario, 2021). Additionally, it was the first vaccination to reach Iraq and its use was specified. so, the significant differences in IgG level between age groups may be due to the low antibody concentration and the small number of participants vaccinated with AstraZeneca.

This study approved that the exception of the AstraZeneca vaccine, there were no significant differences in IFN γ levels between the two age groups or within either group, as shown in table (4.3). However, the mean level of IFN γ for the subjects whom less than 25 years were higher than that in subjects with more than 25 years. This in agreement with previous study in which the author reported that older

participants produce less IFN γ from SARS-CoV-2 spike-specific T cells than younger participants did (Collier *et al.*, 2021).

Additionally, in table (4.4) there were no correlations was found between the overall age of participant and IgG, IFN γ levels and also no correlation was found between the male and female age in IgG and IFN γ levels. Similarly, in another study, Age and RBD-IgG have a poor correlation in males (r = 0.410) and no relationship was found in females (Fujigaki *et al.*, 2022). Inversely, the anti-spike IgG level was strongly inversely correlated with age in previous study (Ikezaki *et al.*, 2022).

Table 4.4 Relation between ages, sex and IgG /IFN-γ levels

Sample	variable p value	IgG AU/ml	IFN-γ Pg/ml
all sample	**Age**	0.023	0.013
	p value	0.762	0.867
Male	**Age**	0.033	0.011
	P value	0.758	0.918
Female	**Age**	0.067	0.019
	P value	0.552	0.864

Previous studies showed a link between the age and the potency of the humoral or cellular response (Ebersole *et al.*, 2018). In spite of the increase in age makes the immune system suffer from characteristic changes that lead to an increase in the severity and the extent of the spread of infectious diseases, as well as to a lack of complete protection after the vaccine (Weinberger *et al.*, 2008), But it was becoming clear that when considering the immune health, age is just a number, where age was not a measure to how well the immune system was. Some people actually have immune systems that are much older or younger than they are. Some 60-year-olds have the immune system of a 40-year-old, while others have an immune system more

similar to an 80-year-old. The environment and the right system free of harmful things can make an older person with immunity better than a person of a younger age but with a wrong health system (Simon *et al.,* 2015). Variations in the results of this study in comparison with other study the context with age and humoral and cellular immune response after vaccination and even after infection might possibly due to the nature of the place in which the study was conducted, the health system that individuals follow, and the differences in sample size and subgrouping of participants according to age.

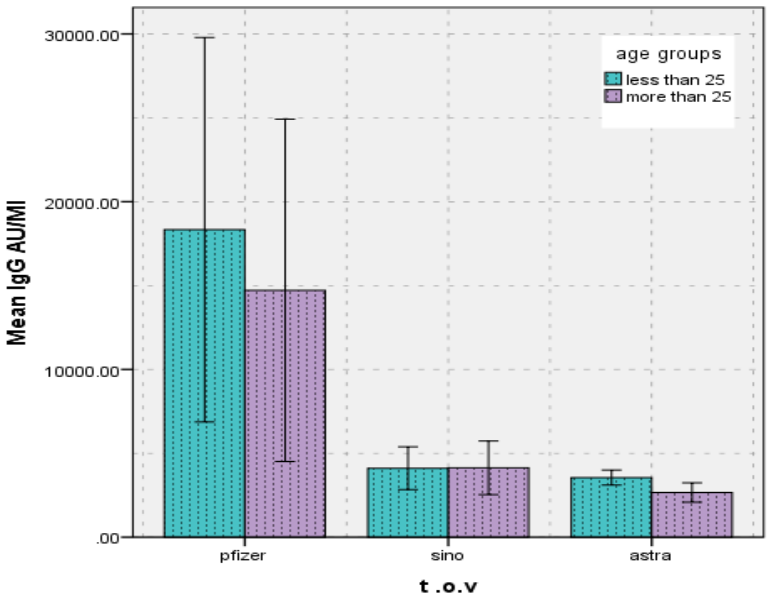

Figure 4.1 Different in IgG levels according to age group

4.4 Differences in IgG and IFN γ levels with Sex

As shown in table (4.5), the current study revealed that there was significant difference in IgG concentration among the three types of vaccines within male and female subjects and the antibody production was higher in participants vaccinated with Pfizer. The mean of the IgG concentration was higher in males than females in

subjects vaccinated with Pfizer and AstraZeneca. However, no significant difference between males and females' subjects was observed for each type of vaccines.

Table 4.5 Differences in IgG and IFN-γ level with sex

Type of vaccine	IgG AU/ml			IFN-γ Pg/ml		
	Male	Female	P value	Male	Female	P value
	Mean ± S. D	Mean± S. D		Mean± S. D	Mean± S. D	
Pfizer	17030.8±11011.8	16877.0±11305.4	0.945	62.7±14.5	66.9±14.7	0.155
Sinopharm	3927.1±1001.5	4273.7±1624.5	0.346	62.7±11.6	65.5±13.3	0.413
AstraZeneca	3251.4±546.2	2972.5±1300.4	0.622	63.3±12.3	51.1±3.3	0.216
Total	12020.4±10820.5	11554.6±10687.6	0.95	62.8±13.4	65.9±14.1	
P value	0.00**	0.00**		0.99	0.70	
LSD	4081	3928				

LSD: Least Significant Difference, ** highly significant difference

Similarly, in a previous study, where the Euroimmun anti-SARS-CoV-2 S1 IgG ELISA assay was used to monitor humeral response to COVID-19 mRNA BNT162b2 vaccine, did not show any statistically significant correlation between the sex of the individuals and the anti-spike protein antibody titers (Dörschug *et al.*, 2021). Additionally, the mean value for all types of vaccines (Sinopharm, AstraZeneca, Pfizer) showed no significant differences in IgG titer for vaccinated males and females(Abdul-Ghani, 2022). While inversely, significant difference in IgG concentration between males and females was observed previously. The anti-Spike-RBD IgG response were observed to be significantly more in females than in males after vaccination with BNT162b2(Gharpure *et al.*, 2021).

Regarding the IFN γ, there were no significant differences neither among the three types of vaccines, nor between males and females for each vaccine despite that the mean level of IFN γ in females were higher than that in males in subjects vaccinated with Pfizer and Sinopharm. The IFN γ level was higher in males in

comparison to females in subjects vaccinated AstraZeneca. Significant difference in the IFN γ levels between male and females in fully vaccinated subjects was observed by Kurteva (Kurteva *et al.*, 2022). CD4+ and CD8+ T cells in female generate more robust responses to viral infections (Raza *et al.*, 2021).This study reported lower T cell levels in males associated with worsening disease as compared to females. Moreover, number of activated CD8 T cells were significantly higher in females (Takahashi *et al.*, 2020). Higher activity of T cells may in turn contribute to potentially better antiviral adaptive immune response in females, which may lead to greater viral clearance.

It is well established that, compared to males, females develop stronger humeral and cellular immune response to foreign antigenic stimulation, vaccination and infections than male which is considered as benefit (Fink & Klein, 2015).Whereas, strong immune response generated by females to self-antigens make them susceptible to autoimmune diseases(Klein & Flanagan, 2016). Post-vaccination adverse events are more common in women, as are adverse events from pharmaceutical medications more broadly(Nyankerh *et al.*, 2022) .

There are also sex differences in antiviral immunity, "caused by sex steroid hormone signaling (i.e., testosterone, estrogens, and progesterone), genetics (e.g., immune function genes that escape X inactivation), and sex-specific composition of the microbiome". Women seem to recover better from infection as they induce a stronger immune response to the virus. Estrogens have also been shown to help heal acute lung injury. Sex hormones, for example, testosterone and estrogen, seems to play diverse roles in immune responses. While estrogen has immune-stimulatory roles, progesterone and androgens are immune-suppressive and counteract the pathways affected by estrogen(Ciarambino *et al.*, 2021).

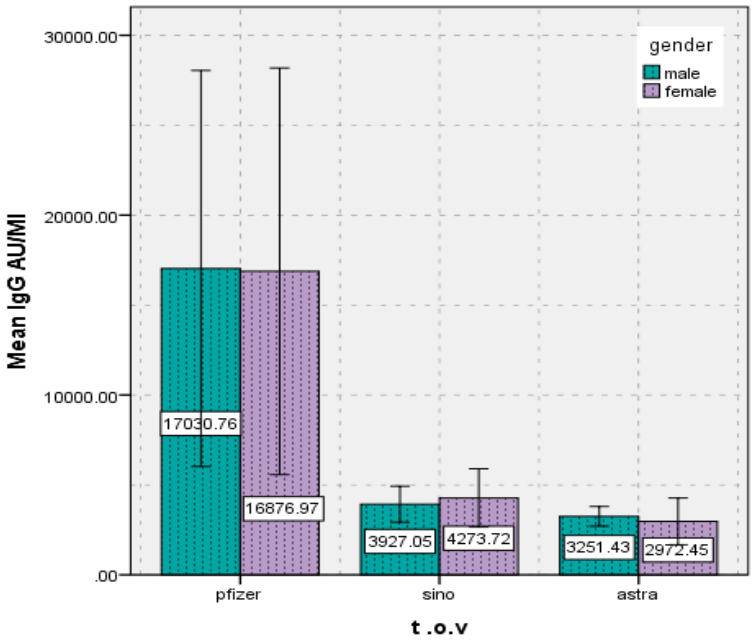

Figure 4.2 Differences in IgG concentration between sex among the three types of vaccines

4.5 Differences of IgG and IFN γ concentrations according to dose

As shown in table (4.6), there was no significant variation between the first and second dose for each type of the three vaccines However, there was a significant difference in IgG concentration between the Pfizer vaccine and Sinopharm for the first dose, and among the three types of vaccine in the second dose. The highest concentration was seen in Pfizer vaccine.

Regarding IFN γ, there were no significant difference between the first and second doses for each type of vaccines and among the first dose and second dose for all types of vaccines.

Additionally, comparison the levels between the first and second doses revealed the presence of significant difference in IgG and marginally significant in IFN γ levels, as shown in table (4.6). Furthermore, the mean level of both IgG and

IFN γ were decreased in the second dose. This might possibly due to that samples from more than 78%, 100%, and 75% of the subjects were collected after 10 weeks from the administration of the second dose with Pfizer, AstraZeneca, and Sinopharm, respectively, Table (4.6).

Table 4.6 Differences of IgG and IFN γ concentrations according to dose

Type of vaccine	IgG			IFN gamma		
	dose 1	dose 2	P value	dose 1	dose 2	P value
	Mean ± S. D	Mean± S. D		Mean± S. D	Mean± S. D	
Pfizer	18123±11062	15905±11116	0.313	66±13	64±16	0.247
Sinopharm	4498±1592	3934±1245	0.146	68±15	63±11	0.167
AstraZeneca		3196±659			61±12	
P value	0.00 **	0.00**		0.61	0.84	

LSD: Least Significant Difference, ** highly significant difference

The result of the current study is in agreement with other recent study which found that the second dose of the vaccination did not improve humoral or cellular immune responses since neither anti-spike IgG levels nor specific IFN γ producing T cells significantly increased (Busà *et al.,* 2022). In another study, stated that despite infected patients with COVID-19 showed robust humoral and antigen-specific responses to the first dose, these responses did not improve following the second dose of the vaccine at the time points examined (Samanovic *et al.,* 2022). Moreover, Fonseca, reported that following receiving the second dose of the vaccine, there was no increase in anti-S IgG in the group of healthcare professionals who had previously infected COVID-19)(Fonseca *et al.,* 2022). Tormo reported that IFN γ production by T cells improved over time following the second dose, reaching levels comparable to those seen following the first dose (Tormo *et al.,* 2022).

Inversely, other study which has been done in Bagdad clarified that the second dose of vaccine caused a significant higher increase in the mean levels of IgG (29.08 ± 2.37) as compared to the mean levels (23.42 ± 1.25) of those who were administered the first dose all types of vaccine (Abdul-Ghani, 2022). The differences in the result of the current study and this study might possibly due to of sample size.

Table 4.7 Differences in IgG and IFN γ according to dose

Dose		IgG AU/ML	IFN pg/ML
1	Mean	25200.597	67.109
	St. Deviation	62312.091	16.710
2	Mean	11563.508	62.374
	St. Deviation	15022.640	18.485
	P value	0.033*	0.08⁑

LSD: Least Significant Difference, * significant difference, ⁑ marginally significant

Table 4.8 Cross-tabulation between the type of dose and weeks after vaccination

Group			Dose		
			1	2	Total
Pfizer	Weeks after vaccination	2 Weeks	15(30%)	4(7.2%)	19 (18.1%)
		4 Weeks	20(40%)	3(5.4%)	23 (21.9%)
		6 Weeks	4(8%)	3(5.4%)	7(6.6%)
		8 Weeks	4(8%)	2(3.6%)	6(5.7%)
		10 Weeks	4(8%)	10(18.1%)	14 (13.3%)
		12 Weeks	0	4(7.25%)	4(3.8%)
		More than12 weeks	3(6%)	29(52%)	32 (30.4%)
	Total		50	55	105
AstraZeneca	Weeks after Vaccination	8 Weeks		1(10%)	1(10%)
		More than12 weeks		9(90%)	9(90%)
	Total			10	10
Sinopharm	Weeks after Vaccination	2 Weeks	4(21%)	1(2.5%)	5(8.4%)
		4 Weeks	4(21%)	4(10%)	8(13.5%)
		6 Weeks	2(10.5%)	1(2.5%)	3(5%)
		8 Weeks	0	4(10%)	4(6.7%)
		10 Weeks	0	1(2.5%)	1(1.6%)

		12 Weeks	0	8(20%)	8(13.5%)
		More than12 weeks	9(47%)	21(52.5%)	30(28.5%)
	Total		19	40	59

4.6 Differences in Immune Response to Vaccine According to Weeks

IgG antibody concentration for vaccination with Pfizer varied significantly among the weeks after vaccination, the concentration increases with weeks and the maximum concentration occurring between the sixth and seventh weeks and the lowest concentration being between the tenth and above weeks. The weeks after vaccination do not significantly differ for the Sinopharm and AstraZeneca vaccinations (AstraZeneca is the first type of vaccine introduced in Iraq and thus the number of participants were low and had the vaccine before long period of time from this study). Additionally, Comparison the antibody levels and their presence for weeks after vaccination among the three types of vaccines revealed that they were significantly different and that the Pfizer vaccine had the highest level of antibody, as shown in table (4.9).

Table 4.9 Differences in IgG against vaccine within weeks

IgG AU/ml								
Type of vaccine	**1 week**	**2-3 weeks**	**4-5 weeks**	**6-7 weeks**	**8-9 weeks**	**10 & more**		
	Mean ± S. D	**Mean ± S. D**	**Mean ± S. D**	**Mean ± S. D**	**Mean ± S. D**	**Mean ± S. D**	**P value**	**LSD**
Pfizer	16293.7 ±12400.9	19491.7 ±11842.4	21624.3 ±8893.2	25312.5 ±8736.1	20010.0 ±10850.7	12965.2 ±9786.9	.023	7054
Sinopharm	4388.4 ±1725.9	4318.7 ±1765.9	3814.9 ±111.9	3309.0 ±164.9	3637.7 ±10.4	4174.2 ±1411.8	.848	
AstraZeneca				3081.2		3208.3 ±697.2	.867	
Total	13787.3 ±12040.4	15306.1 ±12189.5	17666.6 ±10999.6	16125.4 ±13059.3	17491.2 ±11658.3	8225.1 ±8117.4		

P value	0.078⁑	0.001**	0.031*	0.002**	0.064⁑	0.00**		
LSD				4512		2995		

*Significant difference at 0.05 level, ** highly significant difference, ⁑ marginally significant difference

In the line of the current study, in previous studies, the mean anti-RBD IgG titer differs widely amongst various vaccination types. Participants who received a third booster shot of the vaccination had the highest titer levels, followed by the Pfizer/BioNTech, AstraZeneca, and Sinopharm vaccines. The Pfizer vaccination group had the highest mean titer levels of anti-RBD IgG antibodies after vaccination, but their levels began to decline after 60 days, in contrast to AstraZeneca and Sinopharm vaccine-induced antibodies, whose mean titers remained stable until 120 days but whose levels were significantly lower. The Sinopharm vaccination group suffered from the majority of breakthrough infections, which occurred at sporadic intervals for the three primary vaccine kinds.(Sughayer *et al.*, 2022)

The Pfizer/BioNTech vaccine is about 90% effective against illnesses with high viral loads, per the manufacturer's specifications, but only one month after the second dosage. However, after two months and three months, this effectiveness falls to 85% and 78%, respectively. These statistics show a loss of several percentage points in the vaccine's protective abilities. In contrast, the Oxford/AstraZeneca vaccine's effectiveness dropped by only six percentage points (the equivalent protection was 67 %, 65 % and 61 % over the same period) Consequently, this mRNA vaccine caused a rapid loss of antibodies in the first six months following the second dosage. (Pouwels *et al.*, 2021).

Concerning IFN γ, there were no significant differences in concentration either between three types of vaccines in each week nor between the weeks after

each vaccine, with higher concentration in the sixth to seven weeks as shown in table (4.10).

Table 4.10 Differences in IFN-γ for vaccine within weeks

IFN-γ Pg/ml							
Type of vaccine	**1 week**	**2-3 weeks**	**4-5 weeks**	**6-7 weeks**	**8-9 weeks**	**10 & more**	
	Mean ± S. D	**Mean ± S. D**	**Mean ± S. D**	**Mean ± S. D**	**Mean ± S. D**	**Mean ± S. D**	**P value**
Pfizer	67.0 ±16.1	64.0 ±15.0	72.7±14.5	72.8 ±15.1	57.6 ±14.0	63.1 ±13.7	0.174
Sinopharm	67.1 ±15.5	60.9 ±13.5	53.1 ±3.6	69.9 ±13.5	73.1 ±20.3	64.1 ±12.0	0.540
AstraZeneca				64.9		60.4 ±12.7	0.745
Total	67.0 ±15.5	63.2 ±14.4	68.4 ±15.3	71.2 ±13.4	60.0 ±15.2	63.3 ±12.8	
P value	0.99	0.617	.112 ±	0.863	0.195	0.736	

This result is in consistent with studies that show T cells produced much less IFN γ in the first two weeks following the second dose of the vaccine. This down regulation of the immune response may be due to regulatory T cells (Tormo *et al.*, 2022). Inversely, IFNγ production was found to be increased after 2 weeks from the second dose to levels similar to those achieved after the first dose (de Wolf *et al.*, 2017, Tormo *et al.*, 2022).

Figure (4.3), reflects that the antibody production appears to be higher in males than the females during the seven weeks from vaccination. However, females produce more antibodies than male after the seven weeks. Inversely, IFN γ levels were higher in females during the first 5 weeks and after the 7th week the cytokine level was higher in males as show in figure (4.4).

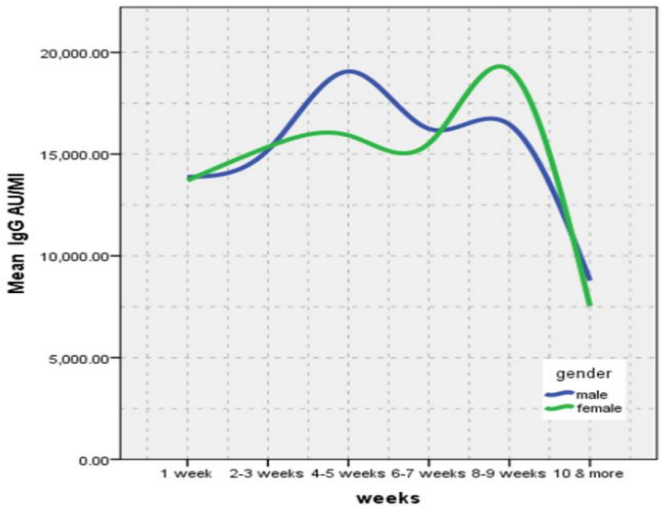

Figure 4.3 Differences IgG level, weeks, after vaccination

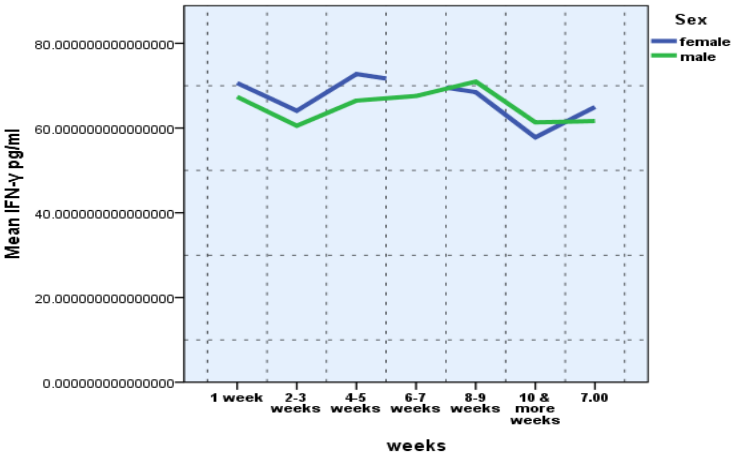

Figure 4.4 Sex difference in IFN γ level weeks after vaccination

Disagree with study published the evaluation of IgG titers between males and females showed levels increased in females in first (3 weeks) and second (4weeks and more) dose, and increase in igg concentration after second dose more than in female and decrease more than male after 6 months from second dose (Fonseca *et al.*, 2022).

inversely demonbreum documented that after 2 doses, IgG levels remained significantly higher for women compared to men while percent inhibition was similar (Demonbreun *et al.,* 2021). Importantly, these sex-based differences in humoral immunity contribute to variation in the responses to vaccines and may explain some disparities in vaccine efficacy between the sexes. Elevated humoral immunity in females compared with males is phylogenetically well conserved, suggesting an adaptive advantage of elevated antibody for reproductive success, including for the transfer of protective antibodies to offspring (Higher B cell activity, including antibody production and activity of memory B cells, in females might improve vaccine efficacy in females compared with males)(Fink & Klein, 2018).

4.7 Differences in IgG and IFN γ level between previously Infected and uninfected subjects

As shown in Table (4.11), there were no statistically significant difference in IgG and IFNγ mean levels between vaccinated subject with confirmed previous infection group versus vaccinated subject without apparent previous infection in subjects vaccinated with Pfizer (*p value*, 0.354 and 0. 53, respectively) and Sinopharm (p-value, 0.896 and 0.07, respectively). However, there were significant difference in case of AstraZeneca vaccine regarding IgG levels (*p-value*,0.04) but no significant difference concerning IFNγ level (*p-value*, 0.923). Additionally, there were significant differences in IgG antibody level (but not in IFNγ levels) among the vaccinated subjects with three types of vaccines within group of confirmed previous infection and among vaccinated subjects without confirmation of previous infection.

Table 4.11 Differences in IgG and IFN γ level between previously Infected and uninfected subjects

Type of vaccine	IgG AU/ml			IFN-γ Pg/ml		
	Vaccinated without confirmed previous infection	Vaccination with confirmed previous infection	P value	Vaccinated without confirmed previous infection	Vaccinated with confirmed previous infection	P value
	Mean ± S. D	Mean± S. D		Mean± S. D	Mean± S. D	
Pfizer	16159.3 ±11561.2	18331.3 ±10242.7	0.354	65.6 ±14.1	63.0 ±15.7	0.53
Sinopharm	4143.1 ±1228.6	4040.5 ±1831.0	0.896	65.9 ±12.7	58.9 ±10.6	0.07⁑
AstraZeneca	3663.2 ±539.2	2884.0 ±561.1	0.04*	61.0 ±16.9	60.8 ±9.5	0.923
P value	0.00**	0.00**		0.79	0.647	
LSD	3387	5178				

*****Significant difference at 0.05 level, ** highly significant difference, ⁑ marginally significant difference

These findings were inconsistent with other previous published data in which authors were reported that in people who were vaccinated after contracting COVID-19, antibody responses after the first dose of Pfizer/BioNTech vaccine were 6.8 times higher, and T-cell responses were 5.9 times higher than in people who had never had the disease (Tretyn *et al.*, 2021).

In another study, Memory CD4+ T-cell and total CD8 responses elicited by a single dose of vaccine were significantly higher in the previously infected group compared with the no prior exposure group (Sasikala *et al.*, 2021).

Tormo, found that participants who had previously been exposed to COVI-19 had fewer and slower increases in both cellular and humeral immunity markers than those who had not experienced the prior infection (Tormo *et al.*, 2022).

Moreover, Tretyn and Abdul-Ghani documented that in vaccinated individuals who had experienced COVID-19 infection, IgG levels were found to be at their greatest (Tretyn *et al.*, 2021, Abdul-Ghani, 2022).

There are only a handful of studies on the importance of previous infection in improve immune response to covid vaccine. However, researchers found that people who had hybrid immunity is more protective than either vaccination or infection alone. So, compared to individuals with only natural immunity, those who had been exposed to the virus and received a single dose of the COVID vaccination were 58% less likely to contract it again. Those with two-dose and hybrid immunity had a 66% lower chance of reinfection. The combination of a previous SARS-CoV-2 infection and a respective vaccination seems to confer the greatest protection against SARS-CoV-2 infections immunity acquired from a previous infection plus either one or two doses of a COVID-19 vaccine(Nordström *et al.*, 2022).

Vaccination after recovery from natural SARS-CoV-2 infection, or "hybrid immunity," has been reported to substantially increase both the potency and breadth of humoral response to COVID-19 (Wang *et al.*, 2021). This occurs as a result of the combined effect of acquired (vaccine) immunity and natural immunity, which produces stronger antibody responses than either kind of immunity alone. It provides 25 to 100 times more antibody responses than natural and vaccine-produced immunity alone.

The differences of the current study findings with other previously published data might be possibly due to the lack of confirmation for the absence of infection with COVID-19. It has been documented that there were high proportion of individuals who are infected with COVID-19 and had never develop symptoms or experience a very mild or almost unrecognizable symptoms. This proportion is difficult to quantify because it requires intensive prospective clinical sampling and symptom screening from a representative sample of individuals with and without infection(Oran & Topol, 2020). However, it has been reported that more than 30%

of population were infected without symptoms and 80% of population have a mild illness, much like normal flu or bad cold.

4.8 Differences in IgG and IFN γ according to BMI

The statistical analysis of the current study revealed that the antibodies concentration is significantly different among the three types of vaccines in overweight and obese subjects. Whereas no noticeable difference was found in case of underweight subjects. The mean was higher in subjects vaccinated with Pfizer than that in subjects vaccinated with other two types of vaccines. Moreover, the mean antibody concentration of patients with normal weight and those in other groups (underweight, overweight, and obese) who received the Pfizer vaccine showed a slightly significant difference (0.061), table (4.12).

Table 4.12 Differences in IgG according to BMI

IgG AU/ml					
Type of vaccine	**Underweight**	**normal weight**	**Overweight**	**Obese**	**P value**
	Mean± S. D	**Mean± S. D**	**Mean± S. D**	**Mean± S. D**	
Pfizer	10254.0±5650.7	19345.6±11409.7	13815.7±10416.2	13921.0±9247.9	.061⁑
Sinopharm	3398.1±	4315.52±16591.1	3847.5±834.0	3802.5±254.7	.604
AstraZeneca	3975.8±	3390±730.1	2827.2±710.1	2968.6±159.	.468
P value	0.493	0.00**	0.00**	0.05*	
LSD		3752	4878	7894	

*****Significant difference at 0.05 level, ** highly significant difference, ⁑ marginally significant difference

This result supports previous researches that found humoral response was seen in all study participants, with normal-weight groups showing higher values than pre-obesity and obese groups(Pellini *et al.*, 2021).

Kooistra, found that There was a statistically significant difference in IgG values between underweight and overweight BMI and between obese subjects and normal weight; and finally, between obese and overweight groups for IgG testing (Kooistra *et al.*, 2021).

Inversely, Other previous study showed that following vaccination, BMI had no real effect on RBD-specific IgG titers and simulated neutralizing titers(Bates et al., 2022a). Also Bates et al., documented that BMI had no influence on the size and persistence of the antibody response to mRNA-based vaccinations (Bates *et al.*, 2022).

Regarding the IFN γ level, there were significant difference among the three types of vaccines in obese subjects. The highest mean was observed in subjects vaccinated with Sinopharm vaccine. Additionally, there were highly significant difference among the four groups vaccinated with Sinopharm vaccine (the highest means was observed in underweight and obese subjects, Table (4.13).

Table 4.13 Differences in IFN γ according to BMI

IFN-γ Pg/ml					
Type of vaccine	**Underweight**	**normal weight**	**Overweight**	**Obese**	**P value**
	Mean± S. D	**Mean± S. D**	**Mean± S. D**	**Mean± S. D**	
Pfizer	59.9±14.7	65.3±15.9	62.7±13.2	68.6±11.1	.663
Sinopharm	88.6±	63.3±13.1	60.7±8.4	77.9±8.5	.007**
AstraZeneca	71.6±	62.1±13.3	60.9±11.0	53.0±16.9	.717
P value	0.337	0.774	0.836	0.05*	
LSD				12	

*****Significant difference at 0.05 level, ** highly significant difference,

Kooistra, reported no potential link between BMI and the cytokine response, (Kooistra *et al.*, 2021).

There is evidence that vaccination protects against severe COVID-19 to a degree comparable to that of persons who are of a healthy weight in those who are overweight or obese. People who were underweight had slightly reduced vaccination effectiveness, and they also had the lowest overall vaccination uptake. When compared to the vaccinated population who were of a healthy weight, there were higher chances of severe COVID-19 outcomes in the vaccinated cohort for those who were obese or underweight (Piernas *et al.*, 2022). On the other hand, other study reported that Current COVID-19 vaccine trials have shown no difference in the vaccine efficacy between normal and obese BMI groups (Kipshidze *et al.*, 2021)

BMI is an effect refers of someone's risk of developing weight-related health issues. It has also been used as an indicator of a potential weakened immunological response to vaccinations. (Louie *et al.*, 2009)

All age groups are affected by the global public health crisis of obesity and overweight. Both conditions are regarded as multifactorial illnesses that cause an abnormal buildup of fatty tissue, and obese or overweight patients exhibit immunological as well as metabolic type changes. Additionally, due to these immunological alterations, infections are more likely to occur and/or be more serious, and immunizations are less effective (Fariñas Guerrero & López Gigosos, 2021).

Obesity has been shown to impede the adaptive immunological response to infection. Growing evidence points to dysregulation of food, hormone, and adipokine levels in the obese as the cause of these T cell metabolic disturbances, which in turn suppress this immunological response. (Pugliese *et al.*, 2022).

Additionally, Obesity has been demonstrated to lower the number of T lymphocytes in the circulation as well as to affect their function by lowering IL-2 receptor expression and IFN-γ production (Green & Beck, 2017).

Additionally, adipocytes have been reported to have increased expression levels of the angiotensin converting enzyme 2 receptor, which SARS-CoV-2 uses to enter cells, in adipocytes from patients with obesity and type 2 diabetes. Therefore, it is possible that adipose tissue could act as a virus reservoir, causing more pronounced and long-lasting viral shedding that would result in an ongoing inflammatory response and poor outcome.(Gómez-zorita *et al.*, 2021).

4.9 Correlation of IgG with IFN γ

The current study revealed that there was positive significant correlation between IgG and IFN γ. This mean that their levels increase/or decreased simultaneously, table (4.14).

Table 4.14 Correlation between IgG and IFN γ

Correlations			
		IFN-γ pg/ml	IgG AU/Ml
IFN-γ pg/ml	Pearson Correlation	1	.178*
	Sig. (2-tailed)		.020
IgG AU/Ml	Pearson Correlation	.178*	1
	Sig. (2-tailed)	.020	
*. Correlation is significant at the 0.05 level (2-tailed).			

This is in agreement with previous study that showed direct association between IgG-S titers and the intensity of IFN- γ response against spike antigens(Jesús *et al.*, 2022).

Inversely, the results of the current study inconsistent with other previous study in which the author stated that the production of IgG and IFN-γ were closely associated, but on an individual basis, they observed that patients with high-antibody titers but low IFN-γ levels and vice versa (Schiffner *et al.,* 2021). Other study demonstrate that the strength of CD4+ and CD8+ T cell responses to almost all proteins is substantially linked. Follicular helper T cells correlate with humoral immunity in the memory phase, and spike-specific T cell responses, which are CD4+ dominated, are expected to enhance antibody production (Moss, 2022).

The main paracrine source of IFN-γ in adaptive immunity is T cells. Due to cytotoxic T cell activation, which enhances cell-mediated immunity, IFN-γ is essential for upregulating cell surface MHC class I, which is essential for the host response to intracellular pathogens like viral infection. IFN-γ is necessary for the generation of cytotoxic T cell precursor proliferation and functions directly as a cytotoxic CD8 T cell differentiation signal. IFN-γ also increases the expression of cell surface MHC class II on APCs, facilitating the activation of CD4 T cells in response to peptides. IFN-γ also releases IL2, which activates B lymphocytes to produce plasma cells and antibodies, resulting in a humoral immune response (Anaya JM, 2013).

Conclusions and Recommendations

Conclusions

Current study concludes the following:

1- Participants vaccinated with Pfizer vaccine produces the highest antibody and IFN-γ concentration as compared to AstraZeneca and Sinopharm vaccines.

2- Younger participants under the age of 25 had higher antibody and IFN γ concentrations than older participants vaccinated with Pfizer and Sinopharm but not for the significant level.

3- Regarding Sex, Pfizer vaccine produce higher antibody level and less IFN-γ in males than females. Sinopharm vaccine produce higher antibody and IFN γ levels in females whereas AstraZeneca produce lower antibody and IFN γ levels in females.

4- After the first dose with Pfizer and Sinopharm, antibody and IFN γ production were higher than that produced after the second dose but not for significant level.

5- The IgG level was significantly increased with weeks after vaccination with Pfizer and the maximum IgG concentration occurred between the sixth and seventh weeks. Regarding IFN γ, high level was seen during the first week after vaccination with Pfizer and Sinopharm and then raised after the 6-7 weeks.

6- Previous infection with covid 19 seems to have no effect on antibody level and IFN γ concentrations after vaccination with Pfizer and Sinopharm. Inversely, AstraZeneca produce significant difference in IgG level but not in IFN γ level.

7- Normal weight subjects might possibly respond better to vaccine and produce more antibody level while underweight subjects respond better in case of AstraZeneca.

8- The level of IFN γ and the concentration of IgG were closely related

Recommendations

Current study recommended the following:

1. Detection of IgG and IFN γ levels before and after vaccination to overcome the problem of asymptomatic or mild infections with COVID-19.
2. Design a cohort study with follow up of the subjects aimed to determine the IgG and IFN γ concentration after each dose in each subject.
3. Study the safety and efficacy of vaccines in immunocompromised individuals.
4. Detection of IgG and IFN- γ in subjects vaccinated with heterologous vaccine platform.

References

References

Abdel-Moneim, A. S. (2017). Coronaviridae: Infectious bronchitis virus. In *Emerging and Re-emerging Infectious Diseases of Livestock* (pp. 133–166). Springer International Publishing. https://doi.org/10.1007/978-3-319-47426-7_5.

Abdul-Ghani, M. N. (2022). Immune Response among Different Types of SARS-CoV-2 Vaccines in Iraq. *Journal of Communicable Diseases*, 103–108. https://doi.org/10.24321/0019.5138.202216.

Ahmadzadeh, M., Vahidi, H., Mahboubi, A., Hajifathaliha, F., Nematollahi, L., & Mohit, E. (2021). Different Respiratory Samples for COVID-19 Detection by Standard and Direct Quantitative RT-PCR: A Literature Review. In *Iranian Journal of Pharmaceutical Research* (Vol. 20, Issue 3, pp. 285–299). Iranian Journal of Pharmaceutical Research. https://doi.org/10.22037/IJPR.2021.115458.15383

Alejandra Tortorici, M., Walls, A. C., Lang, Y., Wang, C., Li, Z., Koerhuis, D., Boons, G. J., Bosch, B. J., Rey, F. A., de Groot, R. J., & Veesler, D. (2019). Structural basis for human coronavirus attachment to sialic acid receptors. *Nature Structural and Molecular Biology*, *26*(6), 481–489. https://doi.org/10.1038/s41594-019-0233-y

Alqassieh, R., Suleiman, A., Abu-Halaweh, S., Santarisi, A., Shatnawi, O., Shdaifat, L., Tarifi, A., Al-Tamimi, M., Al-Shudifat, A. E., Alsmadi, H., al Sharqawi, A., Alnawaiseh, H., Anasweh, Y., Domaidah, F. A., Jaber, H. A., Al-Zarir, M. R., & Bsisu, I. (2021). Pfizer-biontech and sinopharm: A comparative study on post-vaccination antibody titers. *Vaccines*, *9*(11). https://doi.org/10.3390/vaccines9111223

Anastassopoulou, C., Antoni, D., Manoussopoulos, Y., Stefanou, P., Argyropoulou, S., Vrioni, G., & Tsakris, A. (2022). Age and sex associations of SARS-CoV-2 antibody responses post BNT162b2 vaccination in healthcare workers: A mixed effects model across two vaccination periods. *PLoS ONE*, *17*(4 April). https://doi.org/10.1371/journal.pone.0266958

Anaya JM, S. Y. R.-V. A. et al. (2013). *Introduction to T and B lymphocytes*.

Asarnow, D., Wang, B., Lee, W. H., Hu, Y., Huang, C. W., Faust, B., Ng, P. M. L., Ngoh, E. Z. X., Bohn, M., Bulkley, D., Pizzorno, A., Ary, B., Tan, H. C.,

Lee, C. Y., Minhat, R. A., Terrier, O., Soh, M. K., Teo, F. J., Yeap, Y. Y. C., … Wang, C. I. (2021). Structural insight into SARS-CoV-2 neutralizing antibodies and modulation of syncytia. *Cell*, *184*(12), 3192-3204.e16. https://doi.org/10.1016/j.cell.2021.04.033

Astuti, I., & Ysrafil. (2020). Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2): An overview of viral structure and host response. *Diabetes and Metabolic Syndrome: Clinical Research and Reviews*, *14*(4), 407–412. https://doi.org/10.1016/j.dsx.2020.04.020

Baiocchi, G., Aguiar, S., Duprat, J. P., Coimbra, F. J. F., Makdissi, F. B., Vartanian, J. G., Zequi, S. de C., Gross, J. L., Nakagawa, S. A., Yazbek, G., Diniz, T. P., Gonçalves, B. T., Zurstrassen, C. E., Campos, H. G. do A., Joaquim, E. H. G., França e Silva, I. A., & Kowalski, L. P. (2021). Early postoperative outcomes among patients with delayed surgeries after preoperative positive test for SARS-CoV-2: A case-control study from a single institution. *Journal of Surgical Oncology*, *123*(4), 823–833. https://doi.org/10.1002/jso.26377

Bakhshandeh, B., Jahanafrooz, Z., Abbasi, A., Goli, M. B., Sadeghi, M., Mottaqi, M. S., & Zamani, M. (2021). Mutations in SARS-CoV-2; Consequences in structure, function, and pathogenicity of the virus. In *Microbial Pathogenesis* (Vol. 154). Academic Press. https://doi.org/10.1016/j.micpath.2021.104831

Bandala, E. R., Kruger, B. R., Cesarino, I., Leao, A. L., Wijesiri, B., & Goonetilleke, A. (2021). Impacts of COVID-19 pandemic on the wastewater pathway into surface water: A review. In *Science of the Total Environment* (Vol. 774). Elsevier B.V. https://doi.org/10.1016/j.scitotenv.2021.145586

Bates, J. T., Farmer, A. P., Bierdeman, M. A., Ederer, D. R., Carney, L. S., Montgomery, D. D., Lirette, S. T., & Marshall, G. D. (2022a). IgG Antibody Response to the Pfizer BNT162b2 SARS-CoV-2 Vaccine in Healthcare Workers with Healthy Weight, Overweight, and Obesity. *Vaccines*, *10*(4). https://doi.org/10.3390/vaccines10040512

Bates, J. T., Farmer, A. P., Bierdeman, M. A., Ederer, D. R., Carney, L. S., Montgomery, D. D., Lirette, S. T., & Marshall, G. D. (2022b). IgG Antibody Response to the Pfizer BNT162b2 SARS-CoV-2 Vaccine in Healthcare Workers with Healthy Weight, Overweight, and Obesity. *Vaccines*, *10*(4). https://doi.org/10.3390/vaccines10040512

Beig Parikhani, A., Bazaz, M., Bamehr, H., Fereshteh, S., Amiri, S., Salehi-Vaziri, M., Arashkia, A., & Azadmanesh, K. (2021). The Inclusive Review on SARS-CoV-2 Biology, Epidemiology, Diagnosis, and Potential Management Options. In *Current Microbiology* (Vol. 78, Issue 4, pp. 1099–1114). Springer. https://doi.org/10.1007/s00284-021-02396-x

Bertolino, L., Vitrone, M., & Durante-Mangoni, E. (2020). Does this patient have COVID-19? A practical guide for the internist. In *Internal and Emergency Medicine* (Vol. 15, Issue 5, pp. 791–800). Springer. https://doi.org/10.1007/s11739-020-02377-1

Bettini, E., & Locci, M. (2021). SARS-CoV-2 mRNA Vaccines: Immunological mechanism and beyond. *Vaccines*, *9*(2), 1–20. https://doi.org/10.3390/vaccines9020147

Bhavana, V., Thakor, P., Singh, S. B., & Mehra, N. K. (2020). COVID-19: Pathophysiology, treatment options, nanotechnology approaches, and research agenda to combating the SARS-CoV2 pandemic. In *Life Sciences* (Vol. 261). Elsevier Inc. https://doi.org/10.1016/j.lfs.2020.118336

Brochot, E., Demey, B., Touzé, A., Belouzard, S., Dubuisson, J., Schmit, J. L., Duverlie, G., Francois, C., Castelain, S., & Helle, F. (2020). Anti-spike, Anti-nucleocapsid and Neutralizing Antibodies in SARS-CoV-2 Inpatients and Asymptomatic Individuals. *Frontiers in Microbiology*, *11*. https://doi.org/10.3389/fmicb.2020.584251

Brodin, P. (2020). Why is COVID-19 so mild in children? In *Acta Paediatrica, International Journal of Paediatrics* (Vol. 109, Issue 6, pp. 1082–1083). Blackwell Publishing Ltd. https://doi.org/10.1111/apa.15271

Bueckert, M., Gupta, R., Gupta, A., Garg, M., & Mazumder, A. (2020). Infectivity of sars-cov-2 and other coronaviruses on dry surfaces: Potential for indirect transmission. In *Materials* (Vol. 13, Issue 22, pp. 1–16). MDPI AG. https://doi.org/10.3390/ma13225211

Busà, R., Sorrentino, M. C., Russelli, G., Amico, G., Miceli, V., Miele, M., di Bella, M., Timoneri, F., Gallo, A., Zito, G., di Carlo, D., Conaldi, P. G., & Bulati, M. (2022). Specific Anti-SARS-CoV-2 Humoral and Cellular Immune Responses After Booster Dose of BNT162b2 Pfizer-BioNTech mRNA-Based Vaccine: Integrated Study of Adaptive Immune System Components. *Frontiers in Immunology*, *13*. https://doi.org/10.3389/fimmu.2022.856657

Cao, Y., Yang, R., Wang, W., Jiang, S., Yang, C., Liu, N., Dai, H., Lee, I., Meng, X., & Yuan, Z. (2022). Probing the formation, structure and free energy relationships of M protein dimers of SARS-CoV-2. *Computational and Structural Biotechnology Journal*, *20*, 573–582. https://doi.org/10.1016/j.csbj.2022.01.007

Carpenter, C. R., Mudd, P. A., West, C. P., Wilber, E., & Wilber, S. T. (2020). Diagnosing COVID-19 in the Emergency Department: A Scoping Review of Clinical Examinations, Laboratory Tests, Imaging Accuracy, and Biases. *Academic Emergency Medicine*, *27*(8), 653–670. https://doi.org/10.1111/acem.14048

Chakraborty, S., Mallajosyula, V., Tato, C. M., Tan, G. S., & Wang, T. T. (2021). SARS-CoV-2 vaccines in advanced clinical trials: Where do we stand? In *Advanced Drug Delivery Reviews* (Vol. 172, pp. 314–338). Elsevier B.V. https://doi.org/10.1016/j.addr.2021.01.014

Chan, K. H., Peiris, J. S. M., Lam, S. Y., Poon, L. L. M., Yuen, K. Y., & Seto, W. H. (2011). The effects of temperature and relative humidity on the viability of the SARS coronavirus. *Advances in Virology*, *2011*. https://doi.org/10.1155/2011/734690

Chang, H. D., & Radbruch, A. (2021). Maintenance of quiescent immune memory in the bone marrow. In *European Journal of Immunology* (Vol. 51, Issue 7, pp. 1592–1601). John Wiley and Sons Inc. https://doi.org/10.1002/eji.202049012

Ciarambino, T., Para, O., & Giordano, M. (2021). Immune system and COVID-19 by sex differences and age. In *Women's Health* (Vol. 17). SAGE Publications Ltd. https://doi.org/10.1177/17455065211022262

Cohen, J. (2020). Vaccine wagers on coronavirus surface protein pay off. In *Science* (Vol. 370, Issue 6519, pp. 894–895). American Association for the Advancement of Science. https://doi.org/10.1126/science.370.6519.894

Collier, D. A., Ferreira, I. A. T. M., Kotagiri, P., Datir, R. P., Lim, E. Y., Touizer, E., Meng, B., Abdullahi, A., Baker, S., Dougan, G., Hess, C., Kingston, N., Lehner, P. J., Lyons, P. A., Matheson, N. J., Owehand, W. H., Saunders, C., Summers, C., Thaventhiran, J. E. D., … Gupta, R. K. (2021a). Age-related immune response heterogeneity to SARS-CoV-2 vaccine BNT162b2. *Nature*, *596*(7872), 417–422. https://doi.org/10.1038/s41586-021-03739-1

Collier, D. A., Ferreira, I. A. T. M., Kotagiri, P., Datir, R. P., Lim, E. Y., Touizer, E., Meng, B., Abdullahi, A., Baker, S., Dougan, G., Hess, C., Kingston, N., Lehner, P. J., Lyons, P. A., Matheson, N. J., Owehand, W. H., Saunders, C., Summers, C., Thaventhiran, J. E. D., … Gupta, R. K. (2021b). Age-related immune response heterogeneity to SARS-CoV-2 vaccine BNT162b2. *Nature*, *596*(7872), 417–422. https://doi.org/10.1038/s41586-021-03739-1

Costa, W. N. da S., Miguel, J. P., Prado, F. dos S., Lula, L. H. S. de M., Amarante, G. A. J., Righetti, R. F., & Yamaguti, W. P. (2022). Noninvasive ventilation and high-flow nasal cannula in patients with acute hypoxemic respiratory failure by covid-19: A retrospective study of the feasibility, safety and outcomes. *Respiratory Physiology and Neurobiology*, *298*. https://doi.org/10.1016/j.resp.2022.103842

Cox, R. J., & Brokstad, K. A. (2020). Not just antibodies: B cells and T cells mediate immunity to COVID-19. In *Nature Reviews Immunology* (Vol. 20, Issue 10, pp. 581–582). Nature Research. https://doi.org/10.1038/s41577-020-00436-4

Daher, A., Balfanz, P., Aetou, M., Hartmann, B., Müller-Wieland, D., Müller, T., Marx, N., Dreher, M., & Cornelissen, C. G. (2021). Clinical course of COVID-19 patients needing supplemental oxygen outside the intensive care unit. *Scientific Reports*, *11*(1). https://doi.org/10.1038/s41598-021-81444-9

Dan, J. M., Mateus, J., Kato, Y., Hastie, K. M., Yu, E. D., Faliti, C. E., Grifoni, A., Ramirez, S. I., Haupt, S., Frazier, A., Nakao, C., Rayaprolu, V., Rawlings, S. A., Peters, B., Krammer, F., Simon, V., Saphire, E. O., Smith, D. M., Weiskopf, D., … Crotty, S. (2021). Immunological memory to SARS-CoV-2 assessed for up to 8 months after infection. *Science*, *371*(6529). https://doi.org/10.1126/science.abf4063

Datta, P. K., Liu, F., Fischer, T., Rappaport, J., & Qin, X. (2020). SARS-CoV-2 pandemic and research gaps: Understanding SARS-CoV-2 interaction with the ACE2 receptor and implications for therapy. In *Theranostics* (Vol. 10, Issue 16, pp. 7448–7464). Ivyspring International Publisher. https://doi.org/10.7150/thno.48076

de Wolf, A. C. M. T., van Aalst, S., Ludwig, I. S., Bodinham, C. L., Lewis, D. J., van der Zee, R., van Eden, W., & Broere, F. (2017). Regulatory T cell frequencies and phenotypes following anti-viral vaccination. *PLoS ONE*, *12*(6). https://doi.org/10.1371/journal.pone.0179942

Demonbreun, A. R., Sancilio, A., Velez, M. E., Ryan, D. T., Pesce, L., Saber, R., Vaught, L. A., Reiser, N. L., Hsieh, R. R., D'Aquila, R. T., Mustanski, B., McDade, T. W., & McNally, E. M. (2021). COVID-19 mRNA Vaccination Generates Greater Immunoglobulin G Levels in Women Compared to Men. *Journal of Infectious Diseases*, *224*(5), 793–797. https://doi.org/10.1093/infdis/jiab314

Dong, E., Du, H., & Gardner, L. (2020). An interactive web-based dashboard to track COVID-19 in real time. In *The Lancet Infectious Diseases* (Vol. 20, Issue 5, pp. 533–534). Lancet Publishing Group. https://doi.org/10.1016/S1473-3099(20)30120-1

Dörschug, A., Frickmann, H., Schwanbeck, J., Yilmaz, E., Mese, K., Hahn, A., Groß, U., & Zautner, A. E. (2021). Comparative assessment of sera from individuals after s-gene rna-based sars-cov-2 vaccination with spike-protein-based and nucleocapsid-based serological assays. *Diagnostics*, *11*(3). https://doi.org/10.3390/diagnostics11030426

Duckworth, B. C., & Groom, J. R. (2021). Conversations that count: Cellular interactions that drive T cell fate. In *Immunological Reviews* (Vol. 300, Issue 1, pp. 203–219). Blackwell Publishing Ltd. https://doi.org/10.1111/imr.12945

Dutta, A., Roy, A., Roy, L., Chattopadhyay, S., & Chatterjee, S. (2020). Immune response and possible therapeutics in COVID-19. In *RSC Advances* (Vol. 11, Issue 2, pp. 960–977). Royal Society of Chemistry. https://doi.org/10.1039/d0ra08901j

Etido, A., Obeagu, E. I., Okafor, C. J., Chijioke, U. O., Vincent, C. C. N., & Mojo-Eyes, G. C. (2021). The Dynamics of Innate and Adaptive Immune Response to Sars Cov-2 Infection and Its Limitations in Human Beings. *Journal of Pharmaceutical Research International*, 10–25. https://doi.org/10.9734/jpri/2021/v33i45a32708

Eyre, D. W., Lumley, S. F., Wei, J., Cox, S., James, T., Justice, A., Jesuthasan, G., O'Donnell, D., Howarth, A., Hatch, S. B., Marsden, B. D., Jones, E. Y., Stuart, D. I., Ebner, D., Hoosdally, S., Crook, D. W., Peto, T. E. A., Walker, T. M., Stoesser, N. E., … Jeffery, K. (2021). Quantitative SARS-CoV-2 anti-spike responses to Pfizer–BioNTech and Oxford–AstraZeneca vaccines by previous infection status. *Clinical Microbiology and Infection*, *27*(10), 1516.e7-1516.e14. https://doi.org/10.1016/j.cmi.2021.05.041

Fan, J., Jin, S., Gilmartin, L., Toth, I., Hussein, W. M., & Stephenson, R. J. (2022). Advances in Infectious Disease Vaccine Adjuvants. *Vaccines*, *10*(7), 1120. https://doi.org/10.3390/vaccines10071120

Faraj, A. M., Qadir, S. A., Mohammed, O. A., Aziz, P. Y., Alkhafaji, M. H., Rahman, H. S., Abdul Aziz, J. M., Othman, H. H., & AL-Zubaidy, A. M. A. (2022). Current Potential Options for COVID-19 Treatment in Iraq- Kurdistan Region and the Rest of the World: A Mini-review. In *Iraqi Journal of Science* (Vol. 63, Issue 3, pp. 948–958). University of Baghdad-College of Science. https://doi.org/10.24996/ijs.2022.63.3.4

Felsenstein, S., & Hedrich, C. M. (2020). SARS-CoV-2 infections in children and young people. In *Clinical Immunology* (Vol. 220). Academic Press Inc. https://doi.org/10.1016/j.clim.2020.108588

Fialkowski, A., Gernez, Y., Arya, P., Weinacht, K. G., Kinane, T. B., & Yonker, L. M. (2020). Insight into the pediatric and adult dichotomy of COVID-19: Age-related differences in the immune response to SARS-CoV-2 infection. In *Pediatric Pulmonology* (Vol. 55, Issue 10, pp. 2556–2564). John Wiley and Sons Inc. https://doi.org/10.1002/ppul.24981

Fink, A. L., & Klein, S. L. (2015). Sex and gender impact immune responses to vaccines among the elderly. In *Physiology* (Vol. 30, Issue 6, pp. 408–416). American Physiological Society. https://doi.org/10.1152/physiol.00035.2015

Fink, A. L., & Klein, S. L. (2018). The evolution of greater humoral immunity in females than males: implications for vaccine efficacy. In *Current Opinion in Physiology* (Vol. 6, pp. 16–20). Elsevier Ltd. https://doi.org/10.1016/j.cophys.2018.03.010

Fonseca, M. H. G., de Souza, T. de F. G., de Carvalho Araújo, F. M., & de Andrade, L. O. M. (2022a). Dynamics of antibody response to CoronaVac vaccine. *Journal of Medical Virology*, *94*(5), 2139–2148. https://doi.org/10.1002/jmv.27604

Fonseca, M. H. G., de Souza, T. de F. G., de Carvalho Araújo, F. M., & de Andrade, L. O. M. (2022b). Dynamics of antibody response to CoronaVac vaccine. *Journal of Medical Virology*, *94*(5), 2139–2148. https://doi.org/10.1002/jmv.27604

Frutos, R., Serra-Cobo, J., Chen, T., & Devaux, C. A. (2020). COVID-19: Time to exonerate the pangolin from the transmission of SARS-CoV-2 to humans.

In *Infection, Genetics and Evolution* (Vol. 84). Elsevier B.V. https://doi.org/10.1016/j.meegid.2020.104493

Fujigaki, H., Yamamoto, Y., Koseki, T., Banno, S., Ando, T., Ito, H., Fujita, T., Naruse, H., Hata, T., Moriyama, S., Takahashi, Y., Suzuki, T., Murakami, T., Yoshida, Y., Yagura, Y., Oyamada, T., Takemura, M., Kondo, M., Iwata, M., & Saito, K. (2022). *Antibody Responses to BNT162b2 Vaccination in Japan: Monitoring Vaccine Efficacy by Measuring IgG Antibodies against the Receptor-Binding Domain of SARS-CoV-2*.

Gallo, O. (2021). Risk for COVID-19 infection in patients with tobacco smoke-associated cancers of the upper and lower airway. In *European Archives of Oto-Rhino-Laryngology* (Vol. 278, Issue 8, pp. 2695–2702). Springer Science and Business Media Deutschland GmbH. https://doi.org/10.1007/s00405-020-06456-z

Gao, Q., Bao, L., Mao, H., Wang, L., Xu, K., Yang, M., Li, Y., Zhu, L., Wang, N., Lv, Z., Gao, H., Ge, X., Kan, B., Hu, Y., Liu, J., Cai, F., Jiang, D., Yin, Y., Qin, C., … Qin, C. (2020). Development of an inactivated vaccine candidate for SARS-CoV-2. *Science*, *369*(6499), 77–81. https://doi.org/10.1126/science.abc1932

Gharpure, R., Patel, A., & Link-Gelles, R. (2021). First-Dose COVID-19 Vaccination Coverage among Skilled Nursing Facility Residents and Staff. In *JAMA - Journal of the American Medical Association* (Vol. 325, Issue 16, pp. 1670–1671). American Medical Association. https://doi.org/10.1001/jama.2021.2352

Ghoorah, K., Campbell, P., Kent, A., Maznyczka, A., & Kunadian, V. (2016). Obesity and cardiovascular outcomes: a review. In *European heart journal. Acute cardiovascular care* (Vol. 5, Issue 1, pp. 77–85). https://doi.org/10.1177/2048872614523349

Gómez de la Torre, J. C., Cáceres-Delaguila, J. A., Muro-Rojo, C., de La Cruz-Escurra, N., Copaja-Corzo, C., Hueda-Zavaleta, M., Siles, D. A., & Benites-Zapata, V. A. (2022). Humoral Immune Response Induced by the BBIBP-CorV Vaccine (Sinopharm) in Healthcare Workers: A Cohort Study. *Tropical Medicine and Infectious Disease*, *7*(5). https://doi.org/10.3390/tropicalmed7050066

Gómez-zorita, S., Milton-laskibar, I., García-arellano, L., González, M., & Portillo, M. P. (2021). An overview of adipose tissue ace2 modulation by diet and obesity. Potential implications in covid-19 infection and severity. In *International Journal of Molecular Sciences* (Vol. 22, Issue 15). MDPI. https://doi.org/10.3390/ijms22157975

Gorkhali, R., Koirala, P., Rijal, S., Mainali, A., Baral, A., & Bhattarai, H. K. (2021). Structure and Function of Major SARS-CoV-2 and SARS-CoV Proteins. In *Bioinformatics and Biology Insights* (Vol. 15). SAGE Publications Inc. https://doi.org/10.1177/11779322211025876

Green, W. D., & Beck, M. A. (2017). Obesity altered T cell metabolism and the response to infection. In *Current Opinion in Immunology* (Vol. 46, pp. 1–7). Elsevier Ltd. https://doi.org/10.1016/j.coi.2017.03.008

Greenhalgh, T., Koh, G. C. H., & Car, J. (2020). Covid-19: A remote assessment in primary care. *The BMJ*, *368*. https://doi.org/10.1136/bmj.m1182

Gu, W., Gan, H., Ma, Y., Xu, L., Cheng, Z. J., Li, B., Zhang, X., Jiang, W., Sun, J., Sun, B., & Hao, C. (2022). The molecular mechanism of SARS-CoV-2 evading host antiviral innate immunity. In *Virology Journal* (Vol. 19, Issue 1). BioMed Central Ltd. https://doi.org/10.1186/s12985-022-01783-5

Gupta, M. K., Vemula, S., Donde, R., Gouda, G., Behera, L., & Vadde, R. (2021). In-silico approaches to detect inhibitors of the human severe acute respiratory syndrome coronavirus envelope protein ion channel. *Journal of Biomolecular Structure and Dynamics*, *39*(7), 2617–2627. https://doi.org/10.1080/07391102.2020.1751300

Harvey, W. T., Carabelli, A. M., Jackson, B., Gupta, R. K., Thomson, E. C., Harrison, E. M., Ludden, C., Reeve, R., Rambaut, A., Peacock, S. J., & Robertson, D. L. (2021). SARS-CoV-2 variants, spike mutations and immune escape. In *Nature Reviews Microbiology* (Vol. 19, Issue 7, pp. 409–424). Nature Research. https://doi.org/10.1038/s41579-021-00573-0

He, Z., Ren, L., Yang, J., Guo, L., Feng, L., Ma, C., Wang, X., Leng, Z., Tong, X., Zhou, W., Wang, G., Zhang, T., Guo, Y., Wu, C., Wang, Q., Liu, M., Wang, C., Jia, M., Hu, X., … Wang, C. (2021). Seroprevalence and humoral immune durability of anti-SARS-CoV-2 antibodies in Wuhan, China: a longitudinal, population-level, cross-sectional study. *The Lancet*, *397*(10279), 1075–1084. https://doi.org/10.1016/S0140-6736(21)00238-5

Health Ontario, P. (2021). *COVID-19 Viral Vector Vaccines and Rare Blood Clots-Vaccine Safety COVID-19 Viral Vector Vaccines and Rare Blood Clots-Vaccine Safety Surveillance In Action*.

Helmy, Y. A., Fawzy, M., Elaswad, A., Sobieh, A., Kenney, S. P., & Shehata, A. A. (2020). The COVID-19 pandemic: A comprehensive review of taxonomy, genetics, epidemiology, diagnosis, treatment, and control. *Journal of Clinical Medicine*, *9*(4). https://doi.org/10.3390/jcm9041225

Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y., Zhang, L., Fan, G., Xu, J., Gu, X., Cheng, Z., Yu, T., Xia, J., Wei, Y., Wu, W., Xie, X., Yin, W., Li, H., Liu, M., … Cao, B. (2020). Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *The Lancet*, *395*(10223), 497–506. https://doi.org/10.1016/S0140-6736(20)30183-5

Ikezaki, H., Nomura, H., & Shimono, N. (2022). Dynamics of anti-Spike IgG antibody level after the second BNT162b2 COVID-19 vaccination in health care workers. *Journal of Infection and Chemotherapy*, *28*(6), 802–805. https://doi.org/10.1016/j.jiac.2022.02.024

Jagtap, V. S., Jha, U., More, P., & Urmilesh Jha, D. (2020). *The Pharma Innovation Journal 2020; 9(4): 07-10 A review of the 2019 novel coronavirus (COVID-19) based on current evidence*. http://www.thepharmajournal.com

Jamkhande, A., Khairnar, M. R., Gavali, N., Patil, Y., Kapare, S. S., & Bhosale, K. P. (2021). A review of approved COVID-19 vaccines. In *Roczniki Panstwowego Zakladu Higieny* (Vol. 72, Issue 3, pp. 245–252). NLM (Medline). https://doi.org/10.32394/rpzh.2021.0177

Jassim Abd-Alhussein, A., Arkan, D., Jawad, M., Alzhraa, F., & Mahdi, A. (2020). *Epidemiology of COVID-19 Infection in Iraq*.

Jayanama, K., Srichatrapimuk, S., Thammavaranucupt, K., Kirdlarp, S., Suppadungsuk, S., Wongsinin, T., Nanthatanti, N., Phusanti, S., Pitidhammabhorn, D., & Sungkanuparph, S. (2021). The association between body mass index and severity of Coronavirus Disease 2019 (COVID-19): A cohort study. *PLoS ONE*, *16*(2 Febuary). https://doi.org/10.1371/journal.pone.0247023

Jayaweera, M., Perera, H., Gunawardana, B., & Manatunge, J. (2020). Transmission of COVID-19 virus by droplets and aerosols: A critical review

on the unresolved dichotomy. In *Environmental Research* (Vol. 188). Academic Press Inc. https://doi.org/10.1016/j.envres.2020.109819

Jesús, S. R., Javier, C. F., Carlos, S. J., Paloma, L., Rocío, M. C., Pablo, B., del Mar, C. M., Marta, P. A., José Manuel, V. P., Belén, R., Jesús, C., Raquel, B., Antonio, Z. G., & Franciso Javier, M. P. (2022). Humoral and Cellular Response after mRNA Vaccination in Nursing Homes: Influence of Age and of History of COVID-19. *Vaccines*, *10*(3), 1–8. https://doi.org/10.3390/vaccines10030383

Jones, I., & Roy, P. (2021). Sputnik V COVID-19 vaccine candidate appears safe and effective. In *The Lancet* (Vol. 397, Issue 10275, pp. 642–643). Elsevier B.V. https://doi.org/10.1016/S0140-6736(21)00191-4

Joshi, G., Borah, P., Thakur, S., Sharma, P., Mayank, & Poduri, R. (2021). Exploring the COVID-19 vaccine candidates against SARS-CoV-2 and its variants: where do we stand and where do we go? In *Human Vaccines and Immunotherapeutics* (Vol. 17, Issue 12, pp. 4714–4740). Taylor and Francis Ltd. https://doi.org/10.1080/21645515.2021.1995283

Kak, G., Raza, M., & Tiwari, B. K. (2018). Interferon-gamma (IFN-γ): Exploring its implications in infectious diseases. In *Biomolecular Concepts* (Vol. 9, Issue 1, pp. 64–79). Walter de Gruyter GmbH. https://doi.org/10.1515/bmc-2018-0007

Kampf, G., Todt, D., Pfaender, S., & Steinmann, E. (2020). Persistence of coronaviruses on inanimate surfaces and their inactivation with biocidal agents. In *Journal of Hospital Infection* (Vol. 104, Issue 3, pp. 246–251). W.B. Saunders Ltd. https://doi.org/10.1016/j.jhin.2020.01.022

Kasuga, Y., Zhu, B., Jang, K. J., & Yoo, J. S. (2021). Innate immune sensing of coronavirus and viral evasion strategies. In *Experimental and Molecular Medicine* (Vol. 53, Issue 5, pp. 723–736). Springer Nature. https://doi.org/10.1038/s12276-021-00602-1

Kevadiya, B. D., Machhi, J., Herskovitz, J., Oleynikov, M. D., Blomberg, W. R., Bajwa, N., Soni, D., Das, S., Hasan, M., Patel, M., Senan, A. M., Gorantla, S., McMillan, J. E., Edagwa, B., Eisenberg, R., Gurumurthy, C. B., Reid, S. P. M., Punyadeera, C., Chang, L., & Gendelman, H. E. (2021). Diagnostics for SARS-CoV-2 infections. In *Nature Materials* (Vol. 20, Issue 5, pp. 593–605). Nature Research. https://doi.org/10.1038/s41563-020-00906-z

Kim, C. H. (2020). Sars-cov-2 evolutionary adaptation toward host entry and recognition of receptor o-acetyl sialylation in virus–host interaction. *International Journal of Molecular Sciences*, *21*(12), 1–34. https://doi.org/10.3390/ijms21124549

Kipshidze, N., Kipshidze, N., & Fried, M. (2021). *COVID-19 Vaccines: Special Considerations for the Obese Population*. https://doi.org/10.1007/s11695-021-05404-y/Published

Klein, S. L., & Flanagan, K. L. (2016). Sex differences in immune responses. In *Nature Reviews Immunology* (Vol. 16, Issue 10, pp. 626–638). Nature Publishing Group. https://doi.org/10.1038/nri.2016.90

Kooistra, E. J., de Nooijer, A. H., Claassen, W. J., Grondman, I., Janssen, N. A. F., Netea, M. G., van de Veerdonk, F. L., van der Hoeven, J. G., Kox, M., Pickkers, P., Hemelaar, P., Beunders, R., Frenzel, T., Schouten, J., van der Velde, S., van der Eng, H., Roovers, N., Klop-Riehl, M., Gerretsen, J., … Joosten, I. (2021). A higher BMI is not associated with a different immune response and disease course in critically ill COVID-19 patients. *International Journal of Obesity*, *45*(3), 687–694. https://doi.org/10.1038/s41366-021-00747-z

Kurteva, E., Vasilev, G., Tumangelova-Yuzeir, K., Ivanova, I., Ivanova-Todorova, E., Velikova, T., & Kyurkchiev, D. (2022). Interferon-gamma release assays outcomes in healthy subjects following BNT162b2 mRNA COVID-19 vaccination. *Rheumatology International*, *42*(3), 449–456. https://doi.org/10.1007/s00296-022-05091-7

Lee, C. Y. P., Lin, R. T. P., Renia, L., & Ng, L. F. P. (2020). Serological Approaches for COVID-19: Epidemiologic Perspective on Surveillance and Control. In *Frontiers in Immunology* (Vol. 11). Frontiers Media S.A. https://doi.org/10.3389/fimmu.2020.00879

Li, G., Fan, Y., Lai, Y., Han, T., Li, Z., Zhou, P., Pan, P., Wang, W., Hu, D., Liu, X., Zhang, Q., & Wu, J. (2020). Coronavirus infections and immune responses. In *Journal of Medical Virology* (Vol. 92, Issue 4, pp. 424–432). John Wiley and Sons Inc. https://doi.org/10.1002/jmv.25685

Li, X., Geng, M., Peng, Y., Meng, L., & Lu, S. (2020). Molecular immune pathogenesis and diagnosis of COVID-19. In *Journal of Pharmaceutical*

Analysis (Vol. 10, Issue 2, pp. 102–108). Xi'an Jiaotong University. https://doi.org/10.1016/j.jpha.2020.03.001

Liu, D. X., Liang, J. Q., & Fung, T. S. (2021). Human Coronavirus-229E, -OC43, -NL63, and -HKU1 (Coronaviridae). In *Encyclopedia of Virology* (pp. 428–440). Elsevier. https://doi.org/10.1016/b978-0-12-809633-8.21501-x

Luo, X., Zhou, G. Z., Zhang, Y., Peng, L. H., Zou, L. P., & Yang, Y. S. (2020). Coronaviruses and gastrointestinal diseases. In *Military Medical Research* (Vol. 7, Issue 1). BioMed Central Ltd. https://doi.org/10.1186/s40779-020-00279-z

Mahdy, M. A. A., Younis, W., & Ewaida, Z. (2020). An Overview of SARS-CoV-2 and Animal Infection. In *Frontiers in Veterinary Science* (Vol. 7). Frontiers Media S.A. https://doi.org/10.3389/fvets.2020.596391

Malik, Y. A. (2020). *Properties of Coronavirus and SARS-CoV-2*.

Mascellino, M. T., di Timoteo, F., de Angelis, M., & Oliva, A. (2021). Overview of the main anti-sars-cov-2 vaccines: Mechanism of action, efficacy and safety. In *Infection and Drug Resistance* (Vol. 14, pp. 3459–3476). Dove Medical Press Ltd. https://doi.org/10.2147/IDR.S315727

Mazzini, L., Martinuzzi, D., Hyseni, I., Benincasa, L., Molesti, E., Casa, E., Lapini, G., Piu, P., Trombetta, C. M., Marchi, S., Razzano, I., Manenti, A., & Montomoli, E. (2021). Comparative analyses of SARS-CoV-2 binding (IgG, IgM, IgA) and neutralizing antibodies from human serum samples. *Journal of Immunological Methods*, *489*. https://doi.org/10.1016/j.jim.2020.112937

Mendonça, S. A., Lorincz, R., Boucher, P., & Curiel, D. T. (2021). Adenoviral vector vaccine platforms in the SARS-CoV-2 pandemic. In *npj Vaccines* (Vol. 6, Issue 1). Nature Research. https://doi.org/10.1038/s41541-021-00356-x

Mishra, S. S., Ranjan, S., Sharma, C. S., Singh, H. P., Kalra, S., & Kumar, N. (2020). Computational investigation of potential inhibitors of novel coronavirus 2019 through structure-based virtual screening, molecular dynamics and density functional theory studies. *Journal of Biomolecular Structure and Dynamics*, 1–13. https://doi.org/10.1080/07391102.2020.1791957

Misumi, I., Starmer, J., Uchimura, T., Beck, M. A., Magnuson, T., & Whitmire, J. K. (2019). Obesity Expands a Distinct Population of T Cells in Adipose

Tissue and Increases Vulnerability to Infection. *Cell Reports*, *27*(2), 514-524.e5. https://doi.org/10.1016/j.celrep.2019.03.030

Moss, P. (2022). The T cell immune response against SARS-CoV-2. In *Nature Immunology* (Vol. 23, Issue 2, pp. 186–193). Nature Research. https://doi.org/10.1038/s41590-021-01122-w

Naaber, P., Tserel, L., Kangro, K., Sepp, E., Jürjenson, V., Adamson, A., Haljasmägi, L., Rumm, A. P., Maruste, R., Kärner, J., Gerhold, J. M., Planken, A., Ustav, M., Kisand, K., & Peterson, P. (2021). Dynamics of antibody response to BNT162b2 vaccine after six months: a longitudinal prospective study. *The Lancet Regional Health - Europe*, *10*. https://doi.org/10.1016/j.lanepe.2021.100208

Neumann-Podczaska, A., Al-Saad, S. R., Karbowski, L. M., Chojnicki, M., Tobis, S., & Wieczorowska-Tobis, K. (2020). COVID 19 - Clinical picture in the elderly population: A qualitative systematic review. In *Aging and Disease* (Vol. 11, Issue 4, pp. 988–1008). International Society on Aging and Disease. https://doi.org/10.14336/AD.2020.0620

Ni, W., Yang, X., Yang, D., Bao, J., Li, R., Xiao, Y., Hou, C., Wang, H., Liu, J., Yang, D., Xu, Y., Cao, Z., & Gao, Z. (2020). Role of angiotensin-converting enzyme 2 (ACE2) in COVID-19. In *Critical Care* (Vol. 24, Issue 1). BioMed Central. https://doi.org/10.1186/s13054-020-03120-0

Nimptsch, K., Konigorski, S., & Pischon, T. (2019). Diagnosis of obesity and use of obesity biomarkers in science and clinical medicine. In *Metabolism: Clinical and Experimental* (Vol. 92, pp. 61–70). W.B. Saunders. https://doi.org/10.1016/j.metabol.2018.12.006

Nordström, P., Ballin, M., & Nordström, A. (2022). Risk of SARS-CoV-2 reinfection and COVID-19 hospitalisation in individuals with natural and hybrid immunity: a retrospective, total population cohort study in Sweden. *The Lancet Infectious Diseases*, *22*(6), 781–790. https://doi.org/10.1016/S1473-3099(22)00143-8

Nyankerh, C. N. A., Boateng, A. K., & Appah, M. (2022). Ocular Complications after COVID-19 Vaccination, Vaccine Adverse Event Reporting System. *Vaccines*, *10*(6), 941. https://doi.org/10.3390/vaccines10060941

Odih, E. E., Afolayan, A. O., Akintayo, I. O., & Okeke, I. N. (2020). Could water and sanitation shortfalls exacerbate SARS-CoV-2 transmission risks?

In *American Journal of Tropical Medicine and Hygiene* (Vol. 103, Issue 2, pp. 554–557). American Society of Tropical Medicine and Hygiene. https://doi.org/10.4269/ajtmh.20-0462

Oran, D. P., & Topol, E. J. (2020). Prevalence of asymptomatic SARS-CoV-2 infection. A narrative review. *Annals of Internal Medicine*, *173*(5), 362–368. https://doi.org/10.7326/M20-3012

Örd, M., Faustova, I., & Loog, M. (2020). The sequence at Spike S1/S2 site enables cleavage by furin and phospho-regulation in SARS-CoV2 but not in SARS-CoV1 or MERS-CoV. *Scientific Reports*, *10*(1). https://doi.org/10.1038/s41598-020-74101-0

Pai, M., Grill, A., Ivers, N., Maltsev, A., Miller, K. J., Razak, F., Schull, M., Schwartz, B., Stall, N. M., Steiner, R., Wilson, S., Niel Zax, U., Juni, P., & Morris, A. M. (2021). *Vaccine Induced Prothrombotic Immune Thrombocytopenia (VIPIT) Following AstraZeneca COVID-19 Vaccination*. https://doi.org/10.47326/ocsat.2021.02.17.1.0

Pal, M., Berhanu, G., Desalegn, C., & Kandi, V. (2020a). Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2): An Update. *Cureus*. https://doi.org/10.7759/cureus.7423

Pal, M., Berhanu, G., Desalegn, C., & Kandi, V. (2020b). Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2): An Update. *Cureus*. https://doi.org/10.7759/cureus.7423

Pal, M., Berhanu, G., Desalegn, C., & Kandi, V. (2020c). Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2): An Update. *Cureus*. https://doi.org/10.7759/cureus.7423

Pal, M., Berhanu, G., Desalegn, C., & Kandi, V. (2020d). Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2): An Update. *Cureus*. https://doi.org/10.7759/cureus.7423

Pardi, N., Hogan, M. J., Porter, F. W., & Weissman, D. (2018). mRNA vaccines-a new era in vaccinology. In *Nature Reviews Drug Discovery* (Vol. 17, Issue 4, pp. 261–279). Nature Publishing Group. https://doi.org/10.1038/nrd.2017.243

Park, S. E. (2020). Epidemiology, virology, and clinical features of severe acute respiratory syndrome-coronavirus-2 (SARS-COV-2; Coronavirus Disease-

19). In *Korean Journal of Pediatrics* (Vol. 63, Issue 4, pp. 119–124). Korean Pediatric Society. https://doi.org/10.3345/cep.2020.00493

Patel, M., Chaubey, A. K., Pittman, C. U., Mlsna, T., & Mohan, D. (2021). Coronavirus (SARS-CoV-2) in the environment: Occurrence, persistence, analysis in aquatic systems and possible management. In *Science of the Total Environment* (Vol. 765). Elsevier B.V. https://doi.org/10.1016/j.scitotenv.2020.142698

Pellini, R., Venuti, A., Pimpinelli, F., Abril, E., Blandino, G., Campo, F., Conti, L., de Virgilio, A., de Marco, F., di Domenico, E. G., di Bella, O., di Martino, S., Ensoli, F., Giannarelli, D., Mandoj, C., Manciocco, V., Marchesi, P., Mazzola, F., Moretto, S., … Ciliberto, G. (2021). Initial observations on age, gender, BMI and hypertension in antibody responses to SARS-CoV-2 BNT162b2 vaccine. *EClinicalMedicine*. https://doi.org/10.1016/j.eclinm.2021.100928

Piernas, C., Patone, M., Astbury, N. M., Gao, M., Sheikh, A., Khunti, K., Shankar-Hari, M., Dixon, S., Coupland, C., Aveyard, P., Hippisley-Cox, J., & Jebb, S. A. (2022). Associations of BMI with COVID-19 vaccine uptake, vaccine effectiveness, and risk of severe COVID-19 outcomes after vaccination in England: a population-based cohort study. *The Lancet Diabetes & Endocrinology*. https://doi.org/10.1016/S2213-8587(22)00158-9

Pouwels, K. B., Pritchard, E., Matthews, P. C., Stoesser, N., Eyre, D. W., Vihta, K. D., House, T., Hay, J., Bell, J. I., Newton, J. N., Farrar, J., Crook, D., Cook, D., Rourke, E., Studley, R., Peto, T. E. A., Diamond, I., & Walker, A. S. (2021). Effect of Delta variant on viral burden and vaccine effectiveness against new SARS-CoV-2 infections in the UK. *Nature Medicine*, *27*(12), 2127–2135. https://doi.org/10.1038/s41591-021-01548-7

Precit, M. R., Yee, R., Anand, V., Mongkolrattanothai, K., Pandey, U., & Bard, J. D. (2020). A case report of neonatal acute respiratory failure due to severe acute respiratory syndrome coronavirus-2. *Journal of the Pediatric Infectious Diseases Society*, *9*(3), 390–392. https://doi.org/10.1093/JPIDS/PIAA064

Pugliese, G., Liccardi, A., Graziadio, C., Barrea, L., Muscogiuri, G., & Colao, A. (2022). Obesity and infectious diseases: pathophysiology and epidemiology of a double pandemic condition. In *International Journal of Obesity* (Vol. 46, Issue 3, pp. 449–465). Springer Nature. https://doi.org/10.1038/s41366-021-01035-6

Pušnik, J., Richter, E., Schulte, B., Dolscheid-Pommerich, R., Bode, C., Putensen, C., Hartmann, G., Alter, G., & Streeck, H. (2021). Memory B cells targeting SARS-CoV-2 spike protein and their dependence on CD4+ T cell help. *Cell Reports*, *35*(13). https://doi.org/10.1016/j.celrep.2021.109320

Ramasamy, M. N., Minassian, A. M., Ewer, K. J., Flaxman, A. L., Folegatti, P. M., Owens, D. R., Voysey, M., Aley, P. K., Angus, B., Babbage, G., Belij-Rammerstorfer, S., Berry, L., Bibi, S., Bittaye, M., Cathie, K., Chappell, H., Charlton, S., Cicconi, P., Clutterbuck, E. A., … Zizi, D. (2020). Safety and immunogenicity of ChAdOx1 nCoV-19 vaccine administered in a prime-boost regimen in young and old adults (COV002): a single-blind, randomised, controlled, phase 2/3 trial. *The Lancet*, *396*(10267), 1979–1993. https://doi.org/10.1016/S0140-6736(20)32466-1

Raza, H. A., Sen, P., Bhatti, O. A., & Gupta, L. (2021). Sex hormones, autoimmunity and gender disparity in COVID-19. In *Rheumatology International* (Vol. 41, Issue 8, pp. 1375–1386). Springer Science and Business Media Deutschland GmbH. https://doi.org/10.1007/s00296-021-04873-9

Redondo, N., Zaldívar-López, S., Garrido, J. J., & Montoya, M. (2021). SARS-CoV-2 Accessory Proteins in Viral Pathogenesis: Knowns and Unknowns. In *Frontiers in Immunology* (Vol. 12). Frontiers Media S.A. https://doi.org/10.3389/fimmu.2021.708264

Ricke, D. O. (2021). Two Different Antibody-Dependent Enhancement (ADE) Risks for SARS-CoV-2 Antibodies. *Frontiers in Immunology*, *12*. https://doi.org/10.3389/fimmu.2021.640093

Riddell, S., Goldie, S., Hill, A., Eagles, D., & Drew, T. W. (2020). The effect of temperature on persistence of SARS-CoV-2 on common surfaces. *Virology Journal*, *17*(1). https://doi.org/10.1186/s12985-020-01418-7

Ridha Guedjati, M., Lachekhab, K., & Adjali, A. (2022). *Acta Scientific Orthopaedics (ISSN: 2581-8635) Relationship Between Body Composition and Body Mass Index in Obese Women*. https://biostatgv.

Romero-Pinedo, S., Quesada, M., Horndler, L., Álvarez-Fernández, S., Olmo, A., Abia, D., Alarcón, B., & Delgado, P. (2022a). Vaccine Type-, Age- and Past Infection-Dependence of the Humoral Response to SARS-CoV-2 Spike

S Protein. *Frontiers in Immunology*, *13*. https://doi.org/10.3389/fimmu.2022.809285

Romero-Pinedo, S., Quesada, M., Horndler, L., Álvarez-Fernández, S., Olmo, A., Abia, D., Alarcón, B., & Delgado, P. (2022b). Vaccine Type-, Age- and Past Infection-Dependence of the Humoral Response to SARS-CoV-2 Spike S Protein. *Frontiers in Immunology*, *13*. https://doi.org/10.3389/fimmu.2022.809285

Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine. (2021). *New England Journal of Medicine*, *384*(16), 1576–1578. https://doi.org/10.1056/nejmc2036242

Samanovic, M. I., Cornelius, A. R., Gray-Gaillard, S. L., Richard Allen, J., Karmacharya, T., Wilson, J. P., Wesley Hyman, S., Tuen, M., Koralov, S. B., Mulligan, M. J., & Sedaghat Herati, R. (2022). Robust immune responses are observed after one dose of BNT162b2 mRNA vaccine dose in SARS-CoV-2-experienced individuals. In *Sci. Transl. Med* (Vol. 14). https://www.science.org

Sarhan, A. R., Flaih, M. H., Hussein, T. A., & Hussein, K. R. (2020). *Novel coronavirus (COVID-19) Outbreak in Iraq: The First Wave and Future Scenario*. https://doi.org/10.1101/2020.06.23.20138370

Schiffner, J., Backhaus, I., Rimmele, J., Schulz, S., Möhlenkamp, T., Klemens, J. M., Zapf, D., Solbach, W., & Mischnik, A. (2021). Long-Term Course of Humoral and Cellular Immune Responses in Outpatients After SARS-CoV-2 Infection. *Frontiers in Public Health*, *9*. https://doi.org/10.3389/fpubh.2021.732787

Schilling-Loeffler, K., Falkenhagen, A., & Johne, R. (2022). Coronaviruses are stable on glass, but are eliminated by manual dishwashing procedures. *Food Microbiology*, *106*. https://doi.org/10.1016/j.fm.2022.104036

Shah, V. K., Firmal, P., Alam, A., Ganguly, D., & Chattopadhyay, S. (2020a). Overview of Immune Response During SARS-CoV-2 Infection: Lessons From the Past. In *Frontiers in Immunology* (Vol. 11). Frontiers Media S.A. https://doi.org/10.3389/fimmu.2020.01949

Shah, V. K., Firmal, P., Alam, A., Ganguly, D., & Chattopadhyay, S. (2020b). Overview of Immune Response During SARS-CoV-2 Infection: Lessons

From the Past. In *Frontiers in Immunology* (Vol. 11). Frontiers Media S.A. https://doi.org/10.3389/fimmu.2020.01949

Shan, C., Yao, Y. F., Yang, X. lou, Zhou, Y. W., Gao, G., Peng, Y., Yang, L., Hu, X., Xiong, J., Jiang, R. di, Zhang, H. J., Gao, X. X., Peng, C., Min, J., Chen, Y., Si, H. R., Wu, J., Zhou, P., Wang, Y. Y., … Yuan, Z. M. (2020). Infection with novel coronavirus (SARS-CoV-2) causes pneumonia in Rhesus macaques. *Cell Research*, *30*(8), 670–677. https://doi.org/10.1038/s41422-020-0364-z

Siddique, S., & Ahmed, S. (2021). COVID-19 Vaccines in Pakistan: Efficacy, Adverse Effects and Availability. *Journal of Islamabad Medical & Dental College*, *10*(2), 125–130. https://doi.org/10.35787/jimdc.v10i2.723

Simões, R. S. de Q., & Rodríguez-Lázaro, D. (2022). Classical and Next-Generation Vaccine Platforms to SARS-CoV-2: Biotechnological Strategies and Genomic Variants. In *International Journal of Environmental Research and Public Health* (Vol. 19, Issue 4). MDPI. https://doi.org/10.3390/ijerph19042392

Simon, A. K., Hollander, G. A., & McMichael, A. (2015). Evolution of the immune system in humans from infancy to old age. In *Proceedings of the Royal Society B: Biological Sciences* (Vol. 282, Issue 1821). Royal Society of London. https://doi.org/10.1098/rspb.2014.3085

Singh, L., Bajaj, S., Gadewar, M., Verma, N., Ansari, M. N., Saeedan, A. S., Kaithwas, G., & Singh, M. (2021). Modulation of Host Immune Response Is an Alternative Strategy to Combat SARS-CoV-2 Pathogenesis. In *Frontiers in Immunology* (Vol. 12). Frontiers Media S.A. https://doi.org/10.3389/fimmu.2021.660632

Singhal, T. (2020a). A Review of Coronavirus Disease-2019 (COVID-19). In *Indian Journal of Pediatrics* (Vol. 87, Issue 4, pp. 281–286). Springer. https://doi.org/10.1007/s12098-020-03263-6

Singhal, T. (2020b). A Review of Coronavirus Disease-2019 (COVID-19). In *Indian Journal of Pediatrics* (Vol. 87, Issue 4, pp. 281–286). Springer. https://doi.org/10.1007/s12098-020-03263-6

Sughayer, M. A., Souan, L., Abu Alhowr, M. M., al Rimawi, D., Siag, M., Albadr, S., Owdeh, M., & al Atrash, T. (2022). Comparison of the effectiveness and duration of anti-RBD SARS-CoV-2 IgG antibody response

between different types of vaccines: Implications for vaccine strategies. *Vaccine*, *40*(20), 2841–2847. https://doi.org/10.1016/j.vaccine.2022.03.069

Takahashi, T., Ellingson, M. K., Wong, P., Israelow, B., Lucas, C., Klein, J., Silva, J., Mao, T., Oh, J. E., Tokuyama, M., Lu, P., Venkataraman, A., Park, A., Liu, F., Meir, A., Sun, J., Wang, E. Y., Casanovas-Massana, A., Wyllie, A. L., … Iwasaki, A. (2020). Sex differences in immune responses that underlie COVID-19 disease outcomes. *Nature*, *588*(7837), 315–320. https://doi.org/10.1038/s41586-020-2700-3

Tay, M. Z., Poh, C. M., Rénia, L., MacAry, P. A., & Ng, L. F. P. (2020). The trinity of COVID-19: immunity, inflammation and intervention. In *Nature Reviews Immunology* (Vol. 20, Issue 6, pp. 363–374). Nature Research. https://doi.org/10.1038/s41577-020-0311-8

Teijaro, J. R., & Farber, D. L. (2021). COVID-19 vaccines: modes of immune activation and future challenges. In *Nature Reviews Immunology* (Vol. 21, Issue 4, pp. 195–197). Nature Research. https://doi.org/10.1038/s41577-021-00526-x

Thomas, S. (2020). The structure of the membrane protein of sars-cov-2 resembles the sugar transporter semisweet. *Pathogens and Immunity*, *5*(1), 342–363. https://doi.org/10.20411/pai.v5i1.377

Toor, S. M., Saleh, R., Sasidharan Nair, V., Taha, R. Z., & Elkord, E. (2021). T-cell responses and therapies against SARS-CoV-2 infection. In *Immunology* (Vol. 162, Issue 1, pp. 30–43). Blackwell Publishing Ltd. https://doi.org/10.1111/imm.13262

Tormo, N., Navalpotro, D., Martínez-Serrano, M., Moreno, M., Grosson, F., Tur, I., Guna, M. R., Soriano, P., Tornero, A., & Gimeno, C. (2022a). Commercial Interferon-gamma release assay to assess the immune response to first and second doses of mRNA vaccine in previously COVID-19 infected versus uninfected individuals. *Diagnostic Microbiology and Infectious Disease*, *102*(4). https://doi.org/10.1016/j.diagmicrobio.2021.115573

Tormo, N., Navalpotro, D., Martínez-Serrano, M., Moreno, M., Grosson, F., Tur, I., Guna, M. R., Soriano, P., Tornero, A., & Gimeno, C. (2022b). Commercial Interferon-gamma release assay to assess the immune response to first and second doses of mRNA vaccine in previously COVID-19 infected

versus uninfected individuals. *Diagnostic Microbiology and Infectious Disease*, *102*(4). https://doi.org/10.1016/j.diagmicrobio.2021.115573

Tormo, N., Navalpotro, D., Martínez-Serrano, M., Moreno, M., Grosson, F., Tur, I., Guna, M. R., Soriano, P., Tornero, A., & Gimeno, C. (2022c). Commercial Interferon-gamma release assay to assess the immune response to first and second doses of mRNA vaccine in previously COVID-19 infected versus uninfected individuals. *Diagnostic Microbiology and Infectious Disease*, *102*(4). https://doi.org/10.1016/j.diagmicrobio.2021.115573

Tretyn, A., Szczepanek, J., Skorupa, M., Jarkiewicz-Tretyn, J., Sandomierz, D., Dejewska, J., Ciechanowska, K., Jarkiewicz-Tretyn, A., Koper, W., & Pałgan, K. (2021). Differences in the concentration of anti-sars-cov-2 igg antibodies post-covid-19 recovery or post-vaccination. *Cells*, *10*(8). https://doi.org/10.3390/cells10081952

Turner, J. S., O'Halloran, J. A., Kalaidina, E., Kim, W., Schmitz, A. J., Zhou, J. Q., Lei, T., Thapa, M., Chen, R. E., Case, J. B., Amanat, F., Rauseo, A. M., Haile, A., Xie, X., Klebert, M. K., Suessen, T., Middleton, W. D., Shi, P. Y., Krammer, F., … Ellebedy, A. H. (2021). SARS-CoV-2 mRNA vaccines induce persistent human germinal centre responses. *Nature*, *596*(7870), 109–113. https://doi.org/10.1038/s41586-021-03738-2

Upasani, V., Rodenhuis-Zybert, I., & Cantaert, T. (2021). Antibody-independent functions of B cells during viral infections. In *PLoS Pathogens* (Vol. 17, Issue 7). Public Library of Science. https://doi.org/10.1371/journal.ppat.1009708

Walsh, E. E., Frenck, R. W., Falsey, A. R., Kitchin, N., Absalon, J., Gurtman, A., Lockhart, S., Neuzil, K., Mulligan, M. J., Bailey, R., Swanson, K. A., Li, P., Koury, K., Kalina, W., Cooper, D., Fontes-Garfias, C., Shi, P.-Y., Türeci, Ö., Tompkins, K. R., … Gruber, W. C. (2020). Safety and Immunogenicity of Two RNA-Based Covid-19 Vaccine Candidates. *New England Journal of Medicine*, *383*(25), 2439–2450. https://doi.org/10.1056/nejmoa2027906

Wang, Y., Wang, Y., Chen, Y., & Qin, Q. (2020). Unique epidemiological and clinical features of the emerging 2019 novel coronavirus pneumonia (COVID-19) implicate special control measures. In *Journal of Medical Virology* (Vol. 92, Issue 6, pp. 568–576). John Wiley and Sons Inc. https://doi.org/10.1002/jmv.25748

Wang, Z., Muecksch, F., Schaefer-Babajew, D., Finkin, S., Viant, C., Gaebler, C., Hoffmann, H. H., Barnes, C. O., Cipolla, M., Ramos, V., Oliveira, T. Y., Cho, A., Schmidt, F., da Silva, J., Bednarski, E., Aguado, L., Yee, J., Daga, M., Turroja, M., … Nussenzweig, M. C. (2021). Naturally enhanced neutralizing breadth against SARS-CoV-2 one year after infection. *Nature*, *595*(7867), 426–431. https://doi.org/10.1038/s41586-021-03696-9

Wei, J., Pouwels, K. B., Stoesser, N., Matthews, P. C., Diamond, I., Studley, R., Rourke, E., Cook, D., Bell, J. I., Newton, J. N., Farrar, J., Howarth, A., Marsden, B. D., Hoosdally, S., Jones, E. Y., Stuart, D. I., Crook, D. W., Peto, T. E. A., Walker, A. S., … Cunningham, C. (2022). Antibody responses and correlates of protection in the general population after two doses of the ChAdOx1 or BNT162b2 vaccines. *Nature Medicine*. https://doi.org/10.1038/s41591-022-01721-6

Weinberger, B., Herndler-Brandstetter, D., Schwanninger, A., Weiskopf, D., & Grubeck-Loebenstein, B. (2008). Biology of immune responses to vaccines in elderly persons. In *Clinical Infectious Diseases* (Vol. 46, Issue 7, pp. 1078–1084). https://doi.org/10.1086/529197

WHO, C. O. F. (2020). World health organization. *Responding to Community Spread of COVID-19. Reference WHO/COVID-19/Community_Transmission/2020.1*.

Wölfel, R., Corman, V. M., Guggemos, W., Seilmaier, M., Zange, S., Müller, M. A., Niemeyer, D., Jones, T. C., Vollmar, P., Rothe, C., Hoelscher, M., Bleicker, T., Brünink, S., Schneider, J., Ehmann, R., Zwirglmaier, K., Drosten, C., & Wendtner, C. (2020). Virological assessment of hospitalized patients with COVID-2019. *Nature*, *581*(7809), 465–469. https://doi.org/10.1038/s41586-020-2196-x

Yao, Y., Jeyanathan, M., Haddadi, S., Barra, N. G., Vaseghi-Shanjani, M., Damjanovic, D., Lai, R., Afkhami, S., Chen, Y., Dvorkin-Gheva, A., Robbins, C. S., Schertzer, J. D., & Xing, Z. (2018). Induction of Autonomous Memory Alveolar Macrophages Requires T Cell Help and Is Critical to Trained Immunity. *Cell*, *175*(6), 1634-1650.e17. https://doi.org/10.1016/j.cell.2018.09.042

Zahid, M. N., Moosa, M. S., Perna, S., & Buti, E. bin. (2021). A review on COVID-19 vaccines: stages of clinical trials, mode of actions and efficacy. In

Arab Journal of Basic and Applied Sciences (Vol. 28, Issue 1, pp. 225–233). Taylor and Francis Ltd. https://doi.org/10.1080/25765299.2021.1903144

Zhang, S., Huang, W., Ren, L., Ju, X., Gong, M., Rao, J., Sun, L., Li, P., Ding, Q., Wang, J., & Zhang, Q. C. (2022). Comparison of viral RNA–host protein interactomes across pathogenic RNA viruses informs rapid antiviral drug discovery for SARS-CoV-2. *Cell Research*, *32*(1), 9–23. https://doi.org/10.1038/s41422-021-00581-y.

Zhu, Z., Lian, X., Su, X., Wu, W., Marraro, G. A., & Zeng, Y. (2020). From SARS and MERS to COVID-19: A brief summary and comparison of severe acute respiratory infections caused by three highly pathogenic human coronaviruses. In *Respiratory Research* (Vol. 21, Issue 1). BioMed Central Ltd. https://doi.org/10.1186/s12931-020-01479-w

Appendices

Appendix

الاسم : الجنس:

العمر : الوزن : الطول:

رقم الهاتف :

نوع اللقاح :

الجرعة :

تاريخ اخذ الجرعة : تاريخ سحب العينة :

مصاب سابقاً : تاريخ الإصابة:

الامراض المزمنة :

سكر:

ضغط :

امراض القلب :

مدخن :

Cancer

Pregnant

الخلاصة

مرض فيروس كورونا 2019 هو مرض تنفسي حاد وبائي سببه فيروس شديد العدوى وممرض ظهر في الصين في اواخر 2019 ولايزال ينتشر بسرعه في جميع أنحاء العالم مما تسبب في مخاوف خطيرة ادت الى الإعلان عن حالة طوارئ صحية عامة في جميع أنحاء العالم من قبل منظمة الصحة العالمية . أثبتت الأدلة المتزايدة أن انتقال العدوى من إنسان إلى آخر يؤثر في المقام الأول على الجهاز التنفسي العلوي يليه تلف الجهاز التنفسي السفلي مما يؤدي إلى التهاب رئوي وخيم. حيث إنه يهدد صحة الإنسان والسلامة العامة، قتل أكثر من 6. 37 مليون شخص في جميع أنحاء العالم.

حيث أجريت دراسة (من النوع المقطع العرضي) لمدة 6 أشهر ابتداء من نوفمبر / 2021 إلى أبريل / 2022 في كلية العلوم الطبية التطبيقية وبلغ العدد الإجمالي للمشاركين 174 مشاركا ؛ تم تقسيمهم إلى ثلاث مجموعات ، 105 شخصًا منهم تلقى لقاح فايزر ، وتلقى 59 شخصًا منهم لقاح سينوفارم ، وتلقى 10 أشخاص منهم لقاح الأسترازينيكا ، كان بينهم تسعون شخصًا (51.7٪) من الذكور وثمانية واربعين شخصا (48.2٪) من الاناث . تراوحت اعمارهم بين 18 و 70 عامًا وتم اجراء الاختبارات المناعية لهم ومقارنه النتائج (القياس الكمي للأجسام المضادة لفيروس كورونا وقياس الانترفيرون كاما)

عند مقارنه تركيز الاجسام المضادة بين انواع اللقاحات كان هناك فرق معنوي بينهم حيث ان لقاحا الاسترازنيكا والسينوفارم هم الاقل تركيزا مقارنه بلقاح الفايزر وعلى الرغم من عدم وجود فروقات معنويه بين الفئات العمرية المقسمة والتي تشمل فئه عمري اقل من 25 عاما وفئه عمريه اكبر من 25 عاما الى انه الفئة العمرية الاصغر اظهرت استجابة اكبر للقاح بتراكيز مناعيه اعلى . كذلك اظهر الذكور استجابة مناعية اعلى من الاناث وبالأخص المشاركين المطعمين بلقاح الفايزر وكذلك لم تخلو النتائج من تفاوتات واختلافات بين الذكور مع بعضهم والاناث مع بعضهن .اما بالنسبة الى الجرع فهناك اختلاف معنوي بين الجرعة الاولى والثانية بالنسبة لتركيز الاجسام المضادة للفيروس وكذلك فروقات بين كل جرعة بالنسبة للأنواع الثلاثة و كذلك لوحظ ان تركيز الاجسام المضادة يختلف باختلاف الاسابيع حيث يصل الى اعلى تركيز في الاسبوع السادس والسابع واقل تركيز كان في الاسبوع العاشر .كذلك لم نلاحظ هناك فرق معنوي بين الاشخاص الملقحين والمصابين سابقا والاشخاص الملقحين وغير المصابين اما في الاشخاص الذين يعانون من السمنة والذين لديهم وزن زائد هناك فرق معنوي بين الاستجابة المناعية لا نواع اللقاحات فيهم وفي النهاية نستنتج من هذه

الدراسة أن المشاركين الذين تم تطعيمهم بلقاح فايزر ينتجون أعلى تركيزمن الاجسام المضاده والانترفيرونات ، وكان المشاركون الأصغر سنًا الذين تقل أعمارهم عن 25 عامًا لديهم أجسام مضادة أعلى وتركيزات IFN-أعلى من المشاركين الأكبر سنًا ، ولا يوجد فرق بين الذكور والإناث في الاستجابة المناعية لـلقاح ، لا فرق بين الجرعة الأولى والثانية بعد التطعيم وليس هناك تأثير للعدوى السابقة في تحسين الاستجابة للقاح.

جامعة كربلاء

تقييم مستويات IFN-γ, IgG المتكونة لأنواع لقاحات كوفيد المختلفة

رساله مقدمه

الى مجلس كلية العلوم الطبية التطبيقية - جامعة كربلاء

وهي جزء من متطلبات نيل شهادة الماجستير في التحليلات المرضية

بواسطة

رواق طالب حسن هادي

بكالوريوس تحليلات مرضية/ 2017 كلية العلوم الطبية التطبيقية – جامعة كربلاء

بأشراف

أ.د. سهاد هادي محمد
دكتوراه احياء مجهرية

(ذو الحجه)1444 **(تموز) 2022**