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Genetic Polymorphism of Some Genes in Vaccinated and Infected Patients with Covid 19 in Babylon province

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BY

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بِسْمِ اللهِ الرَّحْمنِ الرَّحِيمِ

(يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنْكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ وَاللَّهُ بِمَا تَعْمَلُونَ خَبِيرٌ).

صدق الله العلي العظيم

سورة المجادلة (الآية 11)

Dedication

To whom ..... Who bore my responsibilities for my happiness, and he was yes the jam's, my ideal, my dear father.

To whom ..... Who taught me that life was its guiding light, and that I should live for it and gain from it.....I stayed up all nights..... my tender mother.

To ..... the candles that accompany me for the duration of life, the fountain of love and the coolness of the eyes, my brother..... and my sisters .

To..... everyone for whom my heart overflows with love, tenderness, sacrifice and sincerity, I dedicate the product of this humble effort.

MAHA

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My heartfelt gratitude also goes out to my family, friends, and anybody else who has assisted and supported me by praying for my professional success.

MAHA

Summary:

The COVID-19 pandemic is caused by Severe Acute Respiratory Syndrome Coronavirus-2 and it has shown significant geographical heterogeneity in frequency and fatality. Total 40 blood samples were collected from covid-19 patients in Marjan Teaching Hospital , Al-Hilla Teaching hospital , Imam sadiq hospital and al-mahaweel hospital, and forty blood sample from vaccinated people The study was conducted in the laboratories of the Department of Biology / University of Babylon / College of Science for woman for the period from October 2021 to February 2022 with the aim of identifying the genetic polymorphism of the *IL-6* gene using amplification-refractory mutation system (ARMS-PCR) technique and *CD147* gene, *FGA* gene analysis by genomic sequencing technique .

The majority of the samples in this study came from Female, indicating that female were more likely than men to be admitted to the hospital and to be at high risk. The majority of patients (52.5%) were females, while (47.5%) percent were males , and there are statistically significant differences in this category for patients (0.7518) , while the majority of vaccinated equal in both females and males (50%) and there are statistically significant differences in this category for vaccinated (1.00) . In both genders, the adult age range was(30-50) years old with (20%),while (51–71) years old with (80%) respectively for patients , we also note that there are high differences in ($P \leq 0.05$) in these age groups (0.0001), while the adult age range was (30-50) years old with (47.5%),while (51–71) years old with (52.5%) respectively for vaccinated and there are statistically significant differences in this category for vaccinated, with a difference in case distribution giving a higher percentage than others. we notice a significant difference ($P \leq 0.05$) between the vaccinated and the patients, the age group (30-51), while the statistical differences between the vaccinated and the patients, the age group (51-71). There were statistical differences between the vaccinated and the infected, between men and women.

Moreover, the study IL-6 gene polymorphisms (by using ARMS-PCR) in two study group (patients and Vaccinated), The results of the study, The GG genotype is wild type ,while CC is mutant type , and allele frequencies C, and G were 0.2125, and 0.7875 respectively, and odd ratio 34.82 . Furthermore, From the results, genotype GG, and the allele G was the highest frequency and genotype CC and the allele C was the less frequency. For infected people CC, GG, and GC were 1, 22, and 17 respectively, allele frequencies C, G were 0.2375, and 0.7625 respectively, and odd ratio 0.947 . Furthermore, From the results, genotype GG, and the allele G was the highest frequency and genotype CC and the allele C was the less frequency , there is a high significant variance in the rates of homozygote and heterozygote for vaccinated people and infected people.

The findings of the ELISA test revealed a rise in interleukin-6 levels in infected samples showed the Concentration level Mean ± SD (36.36 ± 13.74) when compared to vaccinated samples showed the Mean ± SD (11.30 ± 2.26) .

Also this study included the determined the gene polymorphisms for the CD147 gene according to the sequence technique from the 229 bp fragments that compared with NCBI reference sequences, showed that there are two different distributions in the samples , the presence of an substation mutations in the eighth exon in position (151),and the presence of an insertion mutations in the seventh intron in position(220-221) in both vaccinated and infected samples, and showed to the sequence identity of the NCBI homolog of the CD147 gene there are two different distributions in the samples .they are missense mutations at site (151),and insertion mutations at site (220-221). CD147 transmembrane protein, may be a novel route for SARS-CoV-2 entry.

Sequencing study of the investigated section revealed 392 bp of the FGA gene, the presence of an insertion mutation in the fifth exon in position (315-316) resulting a change in the amino acid sequence .This may be the reason for blood clots and elevated level D-dimer in covid patients .

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List of Abbreviations

Symbol	Definition
A	Adenine
ACE2	Angiotensin- Converting Enzyme-2
ALI	Acute lung injury
Anti-PD-1	Antibodies programmed cell death protein1
ARDS	Acute respiratory distress syndrome severity
ARMS-PCR	Amplification Refractory Mutation System-PCR
BCOV	Bovine Corona Virus
BHK-21	Baby hamster kidney -21cells
BP	Base pair
BSG	Basigin
C	Cytosine
CD4	Clusters of differentiation
CD147	Clusters of differentiation 147
CEPI	Coalition for Epidemic Preparedness Innovations
Conc.	Concentration
COG-UK	COVID-19 Genomics UK
COVID-19	coronavirus disease-2019
COVs	Coronaviruses
CRP	C-reactive protein
CRS	Cytokine Release Syndrome
°C	Degrees Celsius
EC1	E-Cadherin domain an IgC2-type
EC2	E-Cadherin domain an IgI-type
E	Envelope
EDTA	Ethylene di amine tetra acetic
ELISA	Enzyme-linked Immunosorbent assay
EMMPRIN	Extracellular matrix metalloproteinase inducer
FABG	Favor prep Blood Genomic DNA
FGA	Fibrinogen alpha chain
FGB	Fibrinogen beta chain
FGG	Fibrinogen gamma chain
F primer	Forword

G	Guanine
GAVI	Global Vaccine Alliance
gpIIb/IIIa	glycoprotein IIb/IIIa receptors
HBV	Hepatitis B virus
HCoV-229E	Human coronavirus -229E
HCoV-OC43	Human coronavirus –OC43
HCoV-NL63	Human coronavirus –NL63
HCoV-HKU1	Human coronavirus –HKUI
HCV	Hepatitis C virus
HCOVs	Human COVs
HE	Hemagglutinin-esterase
HEK293	Human embryonic kidney 293 cells
HRP	Horseradish Peroxidase
IgA	Immunoglobulin A
IgE	Immunoglobulin E
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IL-1	Interleukin-1
IL6	Interleukin-6
IL6R	Interleukin-6 receptor
IQR	Interquartile Range
Kb	Kilo base pair
KDa	Kilo Daltons
LAG-3	Lymphocyte-activating gene-3
M	Membrane
MERS-COV	Middle East respiratory syndrome coronavirus
Min.	Minute
MMP	matrix metalloproteinase
N	Nucleocapsid
NAT	Nucleic acid test
NS	Non-Significant
NSP1	Non-structural protein 1
OD	Optical Density
ORFs	open reading frames
PBS	Phosphate-Buffered Saline

PCR	Polymerase chain reaction
RBD	receptor-binding domain
RdRp	RNA-dependent RNA polymerase
Rpm	Revolutions per minute
R primer	Revers
SARS	Severe acute respiratory syndrome
SARS-COV	Severe acute respiratory syndrome coronavirus
SARS-CoV-1	Severe acute respiratory syndrome coronavirus - 1
SARS-CoV-2	severe acute respiratory syndrome coronavirus-2
Sec.	Second
SNPs	Single Nucleotides Polymorphism
T	Thymine
TBE	Tris Borate EDTA Buffer
Th17	T-helper 17 cells
TLRs	Toll like receptors
TNF-α	Tumor necrosis factor-alpha
UTR	Untranslated region
VOC-202012/01	Variant of Concern 202102/02
WHO	World Health Organization
X	Horizontal axis
Y	Vertical axis
Aα	Alpha
2019-nCoV	2019 novel coronavirus

Chapter one
Introduction

1.1 Introduction:

In March 2020, the World Health Organization (WHO) declared a pandemic caused by an emerging coronavirus named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The pathogen rapidly surged across the globe, causing havoc on all continents and severely destabilizing healthcare systems(WHO,2020). An infection with this virus can cause a variety of mild to severe symptoms, which are collectively named coronavirus disease-2019 (COVID-19) (WHO,2020). SARS-CoV-2 is a member of the genus Beta coronavirus,family Coronaviridae , SARS-CoV-2 is primarily transmitted through respiratory droplets and contact routes(Harapan *et al.*,2020 ; Pourkarim *et al.*,2020).

Infections with the novel coronavirus SARS-CoV-2 resulting in COVID-19 development represent the major medical and scientific challenges of our time, knowledge on SARS-CoV-2 infection pathways and mechanisms associated with immune defense or immunopathology is growing exponentially, as it is indispensable to design the proper diagnostic and therapeutic strategies(Lutfiye and Nezih, 2021), the host genetic variation may (partially) influence COVID-19 prevalence and mortality, it has been hypothesized that spatial variation in human polymorphisms can explain some of the diversity in infection prevalence also, Genetic polymorphisms in the regulatory regions of cytokine genes may be associated with differential cytokine production in COVID-19 patients, the interleukin-6 (*IL-6*) is an important cytokine with pleiotropic functions such as metabolic regulation to inflammation, auto-immunity and acute-phase response, and COVID-19 patients had high levels of *IL-6* that were associated with pulmonary inflammation and extensive lung damage(Hafizae seda Vatansever and Eda Becer ,2020). Also, COVID-19 infection has an aggressive inflammatory response with a large amount of pro-inflammatory cytokines, known as the ‘cytokine

storm(Hafizae seda Vatansever and Eda Becer ,2020). *IL-6* can be produced by almost all stromal cells and by immune system cells, such as B lymphocytes, T lymphocytes, macrophages, dendritic cells, monocytes, mast cells and many non-lymphocytes, such as fibroblast and endothelial cells (Jones and Jenkins,2018). In additional SARS-CoV-2 activates the innate and adaptive immune systems, leading to the release of several cytokines, including *IL-6*, this gives rise to a systemic inflammatory response called cytokine release syndrome (CRS) in lots of patients diagnosed with severe COVID-19, which accounts for the high mortality rates (Zhang *et al*.,2020).

CD147, also known as Basigin or EMMPRIN, is a transmembrane glycoprotein that belongs to the immunoglobulin superfamily (Cui J *et al.*,2018).Which is involved in tumor development, plasmodium invasion and virus infection (Chen *et al.*,2005 ; Huang *et al.*,2014).*CD147* plays a functional role in facilitating SARS-CoV invasion for host cells, and *CD147*-antagonistic peptide-9 has a high binding rate to human embryonic kidney (HEK293) cells and an inhibitory effect on SARS-CoV (Chen *et al.*,2005),and the importance of *CD147* in virus invasion for host cells, owing to the similar characteristics of SARS-CoV and SARS-CoV-2(Chen *et al.*,2005) .

Fibrinogen alpha chain (*FGA*) gene is protein encoded and is the alpha component of fibrinogen, a blood-borne glycoprotein composed of three pairs of non - identical polypeptide chains. Following vascular injury, fibrinogen is cleaved by thrombin to form fibrin, which is the most abundant component of blood clots (Ro *et al.*, 2013). Interleukins like *IL-6* and *IL-1* contribute to an increasing fibrinogen levels and thereby may be indicative of an impending cytokine storm (Aggarwal *et al.*, 2020), though its interaction with fibrinogen genes (*FGA*, *FGB*, *FGG*).The role of fibrinogen in acute COVID-19 cases and clot formation has been

considered in researches, so that fibrinogen which serves as an important coagulation factor and an acute phase reactant; for its versatile role relating to coagulation, inflammation, blood viscosity and the related implications in SARS-Cov-2 (COVID 19) management, the fibrinogen is a glycoprotein that is produced in liver as an anti-infective organ(Guan *et al.*,2019 and Wang *et al.*,2020).Severe acute respiratory syndrome coronavirus 2 (SARS-Cov-2)-induced infection, the cause of coronavirus disease 2019 (COVID-19), is characterized by unprecedented clinical pathologies. Phenotypic vascular characteristics are strongly associated with various coagulopathies that may result in either bleeding and thrombocytopenia or hyper coagulation and thrombosis (Gupta *et al.*,2020; Perico *et al.*,2021).

1.2 The Aim of Study:

This study aimed to evaluate some genes associated with developing covid-19 in comparing with vaccinated people against the Coronavirus. To achieve this aim the following steps were suggested:

- Blood samples Collected from Covid -19 patients after diagnosis by real time pcr with positive result and also blood Samples collected from Vaccinated Peoples.
- Estimation the level concentration of cytokines in serum patients and the relation of level with mutation.
- Detection SNPs for certain genes susceptibility to Coronavirus infection: *IL6*, *CD147*, and *FGA* gene.

Chapter TWO

Literatures Review

2. Literatures Review:

2.1 Definitions of Coronavirus:

Coronaviruses(subfamily Orthocoronavirinae, family Coronaviridae ,order Nidovirales) are enveloped, single-strand, positive-sense RNA viruses. Currently, four different genera exist, Alpha coronavirus , Beta coronavirus,Gamma coronavirus and Delta coronavirus, whose reservoirs are bats and rodents for alpha- and beta coronaviruses or birds for gamma- and delta coronaviruses. From their natural reservoirs , CoVs may jump to other animals, including humans, with the transmission to humans usually requiring an intermediate host (Lorusso *et al.*, 2020).

Coronaviruses (CoVs) are RNA viruses that cause disease in humans and other vertebrates. They can infect humans, pets, birds, bats, mice, and a variety of other wild animal's respiratory, gastrointestinal, hepatic, and central nervous systems (Chan *et al*., 2020).

SARS-CoV-2 is a novel positive-sense RNA virus with 79% homology with severe acute respiratory syndrome coronavirus (SARS-CoV) and 50% homology with Middle East respiratory syndrome coronavirus (MERS-CoV), however it is far more infectious than others (Seyed *et.al.*, 2021).

Coronaviruses are RNA viruses with a genome made up of a single stranded, positive - sense RNA with a size ranging from 26 to 31 kilo bases, it is the largest of all RNA viruses in terms of morphology and genetics, the coronavirus genome is sequenced in the following order 5′-leader – untranslated region (UTR) –then replicate / transcriptase - spike (S) - finally, the 3′UTR - poly (A) tail is attached to the envelope (E)-membrane (M)-nucleocapsid (N). It has multiple open reading frames (ORFs) 1a and 1b that overlap, and the other ORFs encode the main structural proteins spike, envelope, membrane, and nucleocapsid (Peng

Zhou *et al.*,2020), the S protein mediates viral attachment to specific cell receptors and fusion between the envelope and plasma membrane and it is the main inducer of virus neutralising antibodies, the small membrane (E) protein plays an important role in viral envelope assembly, but it is not essential for virus propagation, the membrane (M) protein, the most abundant structural component, is a type III glycoprotein consisting of a short amino-terminal ectodomain, a triple-spanning transmembrane domain, and a long carboxyl-terminal inner domain, the nucleocapsid (N) protein is a highly basic phosphoprotein that in addition to its function in the virion also modulates viral RNA synthesis (Peng Zhou *et al.*,2020). In addition to the common set of proteins, CoVs related to bovine coronavirus (BCOV), included in the subgenus Embecovirus (genus Beta coronavirus), possess an additional structural protein, the haemagglutinin-esterase (HE) is closely related to the haemagglutinin-esterase fusion protein of influenza C virus, (Brian and Baric, 2005; Decaro and Buonavoglia, 2008).

Coronavirus was first used to describe a virus with spike - like projections, and the word (crown), which is derived from the Greek korn and means (crown) or (wreath), and originally used to describe certain characteristics of the infective type (virion) as seen through an electron microscope, which has a distinctive bulbous form projection (spike). Most notably these projections were later discovered to be proteins molecules anchored on the lipid bilayer membrane's surface (Guo *et al.,* 2020) .

The pathogen of the now ongoing outbreak of new pneumonia is COVID -19 (SARS-CoV-2), the seventh known virus that can infect humans, the remaining six are HCoV-229E, HCoV-OC43, HCoV-NL63, HCoV-HKU1, SARS-COV and MERS-COV (Corman *et al*., 2019). At the 24 the May 2020, a total of 5 103 006 people were confirmed to

have COVID-19 and ~333 401 deaths have occurred, and thus, COVID-19 (SARS-CoV-2) has become a pandemic (Lutfiye and Nezih, 2021).

2.2. History of SARS-CoV-2:

Eighteen years after the emergence of severe acute respiratory syndrome (SARS) in China and 8 years after the emergence of Middle East respiratory syndrome (MERS) in Saudi Arabia, a novel coronavirus (CoV) epidemic, recently classified as pandemic by the WHO, is threatening the human population worldwide (Zhou *et al.*, 2020). The disease now is referred to as coronavirus disease 2019 (COVID-19), and is caused by a novel human CoV, which was initially denominated 2019 novel coronavirus (2019-nCoV) and later renamed as SARS coronavirus 2 (SARS-CoV-2) by Coronavirus Study Group of the International Committee on Taxonomy of Viruses (Gorbalenya *et al.*, 2020).

COVID- 19 emerged in December 2019 in Wuhan City, Hubei Province, China, in humans exposed to wildlife at the Huanan seafood wholesale market, which is the largest seafood market in central China, and where different species of farm and wild animals are commonly sold (Lorusso *et al*., 2020).

The epidemic has then expanded not only to neighbouring Asian countries, but also to other continents . Only two human COVs (HCOVs) had been known before the SARS emergence, namely HCoV-229E, an alpha coronavirus originated in bats and transmitted to humans through alpacas, and HCoV-OC43, a beta coronavirus which had passed from rodents to humans through cattle (Corman *et al*., 2015, 2018). After 2002–2003 SARS epidemic, the renovated interest in HCOVs allowed the discovery of two additional viruses, the alpha coronavirus HCoV-NL63 and the beta coronavirus HCoV-HKU1, derived from bats and rodents, respectively(Tao *et al*., 2017).All these four viruses are usually responsible for mild respiratory symptoms in immunocompetent patients,

SARS-COV and MERS-COV are two unrelated beta coronaviruses originated in bats and transmitted to humans by wild carnivores and dromedary camels in contrast to other HCOVs , these two viruses displayed an increased virulence causing severe pneumonia and even the death of affected people with mortality rates of about 10 % and 30 %, respectively (Guarner , 2020). The occurrence of three highly pathogenic CoVs with a zoonotic origin in less than two decades highlights the role of animals in generating COVs with increased virulence that can adapt to humans causing epidemics with high impact on human health(Guarner , 2020). Indeed, COV infections of veterinary interest have been known since almost a century, so that animal COVs are paradigmatic of how this large family of viruses evolves generating strains with different biological properties (Cavanagh, 2007; Pedersen, 2014 and Decaro, *et al.,* 2020).

2.3 Variants of SARS-CoV-2:

2.3.1 SARS-CoV-2 Alpha variant:

The Alpha variant (B.1.1.7) is a SARS-CoV-2 variant of concern. It is estimated to be 40–80% more transmissible than the wild-type SARS-CoV-2, it was first detected in November 2020 from a sample taken in September in the United Kingdom, and began to spread quickly by mid-December, around the same time as infections surged, this increase is thought to be at least partly because of one or more mutations in the virus' spike protein, the variant is also notable for having more mutations than normally seen (Peacock, 2020). Through January 2021, more than half of all genomic sequencing of SARS-CoV-2 was carried out in the UK (Donnelly, 2021) . The new Alpha variant caused alarm because it involved 23 separate mutations, 17 of which were linked to the building blocks of proteins that form the virus. It is unusual for so many

mutations to appear all at once that makes it less likely they have happened by chance and more likely they give the virus some kind of evolutionary advantage (Rachel, 2020 ; Sugden, 2021). On 2nd February 2021, Public Health England (PHE) reported that they had detected " a limited number of B.1.1.7 VOC-202012/01 genomes with E484K mutations" (PHE,2021), which they dubbed Variant of Concern 202102/02 (VOC-202102/02). (PHE,2021). One of the mutations (N501Y) is also present in Beta variant and Gamma variant. On 31st May 2021, the World Health Organization announced that the Variant of Concern would be labelled "Alpha" for use in public communications (WHO, 2021).

Mutations in SARS-CoV-2 are common over 4,000 mutations have been detected in its spike protein alone, according to the COVID-19 Genomics UK (COG-UK) Consortium (Wise, 2020). VOC-202012/01 is defined by 23 mutations 14 non-synonymous mutations, 3 deletions, and 6 synonymous mutations (Chand *et al*., 2020), i.e., there are 17 mutations that change proteins and six that do not (Peacock, 2020).

2.3.2 SARS-COV- 2 Beta variant:

The Beta variant (B.1.351), is a variant of SARS-CoV-2, the virus that causes COVID-19, it was initially discovered in the Nelson Mandela Bay metropolitan region of the Eastern Cape province of South Africa in October 2020, which was announced by the country's health service on December 18, 2020, this SARS-CoV-2 strain is one of several thought to be of particular relevance, Phylogeographic analysis suggests this variant emerged in the Nelson Mandela Bay area in July or August 2020(Tegally *et al*., 2020). The World Health Organization labelled the variant as Beta variant, not to replace the scientific name but as a name for the public to commonly refer to the WHO considers it to be a variant of concern (WHO, 2020).

There are three mutations of particular interest in the spike region of the lineage B.1.351 genome K417N , E484K, and N501Y, and a further five spike mutations which have so far generated less concern L18F, D80A, D215G, R246I, and A701V (Corum and Zimmer , 2021).

Two mutations found in the Beta variant, E484K and K417N, are not found in Alpha variant. Moreover , Beta does not have the 69-70del mutation found in the other variant (Abdool Karim, 2020).

2.3.3 SARS-CoV-2 Omicron variant:

The Omicron variant (B.1.1.529) is a variant of SARS-CoV-2, It was first detected on 22nd November 2021 in laboratories in Botswana and South Africa based on samples collected on 11–16 November (Schrieber, 2021). The first known sample was collected in South Africa on 8th November. The first known case, outside of South Africa, was a person arriving in Hong Kong from South Africa via Qatar on 11th November, and another person who arrived in Belgium from Egypt via Turkey on the same date. (Lambrecht, 2021; Philip, 2021).

On 26 November 2021, WHO designated it as a variant of concern and named it "Omicron" after the fifteenth letter in the Greek alphabet (WHO, 2021).

On 7th January 2022, the variant has been confirmed in 135 countries (WHO, 2021). Omicron has an unusually large number of mutations compared to previous variants (Torjesen , 2021 ; William, 2021; Wei, 2021 and Gowrisankar *et al.,* 2022). Several of the mutations are novel and involve changes to the spike protein reducing the ability for COVID-19 vaccines to prevent symptomatic disease (Al Jurdi *et al*., 2022). Omicron speading around 70 times faster than the Delta variant in the bronchi (lung airways) but evidence suggests that it is less severe than previous strains, especially compared to the Delta variant, omicron might be less able to penetrate deep lung tissue, overall, the extremely high rate

of spread, combined with its ability to evade both double vaccination and the body's immune system, means the total number of patients requiring hospital care at any given time is still of great concern (Al Jurdi *et al*.,2022).

The Omicron variant has a total of 60 mutations compared to the reference / ancestral variant: 50 nonsynonymous mutations, 8 synonymous mutations, and 2 non-coding mutations (William, 2021). Thirty-two mutations affect the spike protein, the main antigenic target of antibodies generated by infections and of many vaccines widely administered. Many of those mutations had not been observed in other strains (Cookson and Barnes, 2021 ; Callaway, 2021). The variant is characterized by 30 amino acid changes, three small deletions, and one small insertion in the spike protein compared with the original virus, of which 15 are located in the receptor-binding domain(Zimmer, 2021). it also carries a number of changes and deletions in other genomic regions. Additionally, the variant has three mutations at the furin cleavage site (Zimmer, 2021). The furin cleavage site increases SARS-CoV-2 infectivity (Zhang *et al*., 2021).

Other variants of either interest or concern: Gamma, Delta, Epsilon, Zeta, Eta, Theta, Iota, Kappa, Lambda, Mu. (WHO,2020).

2.4 Immunological genes and Covid-19:

2.4.1 The interleukin-6 (*IL-6*):

In the 1970s, *IL-6* was originally identified by Kishimoto's group as a soluble protein produced by T cells that activates the differentiation of B cells into antibody producing cells. Accordingly, it was initially known as B cell stimulatory factor 2, *IL-6* is secreted from several type of cells such as T cells, macrophages, endothelial cells, fibroblasts and monocytes (Narazaki and Kishimoto, 2018).

IL-6 is an important cytokine with pleiotropic functions such as metabolic regulation to inflammation, auto-immunity and acute-phase response, the Acute increase in circulating levels of pro-inflammatory cytokines including *IL-6*, *IL-1*, TNF-α and interferon is the reason for the cytokine storm, and *IL-6* may be a therapeutic target for inhibiting the cytokine storm and cytokine storm-associated organ damage (Hafize seda Vatansever and Eda Becer ,2020).

The targets of the *IL-6* are B cells, T cells, basophils, eosinophils and neutrophils, the functions of *IL-6* on B cells are differentiation of the B cells as well as immunoglobulin M(IgM) , immunoglobulin E (IgE) and immunoglobulin A(IgA) production, in addition, *IL-6* also controls the activation, differentiation and survival of the T cells, and it trigger the activation of leukocytes(Qian *et al*.2019). Increased levels of *IL-6* are demonstrated in low prognoses or metastatic cancers, *IL-6* secretion causes antibody production from B cells and it enhances auto-antibody hyper gamma globulinemia and addition, *IL-6* causes auto-immunity, chronic inflammation and either *CD4* positive T-cell activation which triggers Th17 differentiation, or *CD4* positive T-cell inhibition which inhibits Treg differentiation (Qian *et al*.2019).

SARS-CoV-2 activates the innate and adaptive immune systems, leading to the release of several cytokines, including *IL-6*, this gives rise to a systemic inflammatory response called cytokine release syndrome (CRS) in lots of patients diagnosed with severe COVID-19, which accounts for the high mortality rates (Zhang *et al*.,2020). In addition, the polymorphisms in the *IL-6* gene are associated with specific viral infections including influenza virus, hepatitis C (HCV), and hepatitis B virus (HBV)(Riazalhosseini *et.al*.,2018). A recent meta-analysis report an association between a polymorphism in the *IL-6* gene and predisposition and disease severity of pneumonia, suggests that the *IL-6*

allele carries a status of higher *IL-6* production and pneumonia severity (Ulhaq and Soraya ,2020).

2.4.1.1 A role for *IL-6* in the pathology of SARS-CoV-2:

IL-6 is a pleiotropic cytokine with broad-ranging effects within the integrated immune response. One of the roles of *IL-6* is to support immune competence, defined as the ability of a host to respond to infections (Rose-John *et al.*, 2017).In the early stage of the infectious inflammation, *IL-6* is produced by monocytes and macrophages stimulated by the TLRs (Zhang *et al*.,2020).

High levels of interleukin 6 (*IL-6*) and Interleukin 8 (*IL 8*) are found in the acute stage associated with lung lesions in SARS-CoV-1 patients(Wang *et al*, 2004). Especially, *IL-6* can induce the hyper-innate inflammatory response due to the SARS - CoV-1 invasion of the respiratory tract (Wang *et al*, 2004). Interestingly, in human epithelial cells, SARS-CoV-1 is able to induce greater *IL-6* when it is compared to influenza A virus (Okabayashi *et al*., 2006). This happens also with SARS-CoV-2 in COVID-19 patients: some retrospective and meta-analysis studies show how elevated *IL-6* and C-reactive protein (CRP) correlate with mortality and severe disease in comparison to moderate disease (Zhou *et al.*, 2020 and Giamarellos – Bourboulis *et al*.,2020). Extra evidence suggests that critically ill patients with severe respiratory failure and SARS-CoV-2 have either immune dysregulation or macrophage- activation syndrome both of which are characterized by pro inflammatory cytokines(Giamarellos – Bourboulis *et al*., 2020).The immune dysregulation, in particular, is driven by the Interleukin-6 (*IL- 6*) and not by Interleukin-1beta (*IL - 1*beta) and two key features of this immune dysregulation are: overproduction of pro-inflammatory cytokines by monocytes and lymphocyte dysregulation with *CD4* lymphopenia (Giamarellos – Bourboulis *et al*., 2020).

A relevant studies show how *IL-6* plays a major role in acute lung injury (ALI), proof that is obtained in a murine model, while loss of *IL-6* shows to alleviate the severity of acute lung injury (ALI) in response to acid respiration (Song *et al.,* 2007; Imai *et al.,* 2008).

The loss of front line anti-viral defence mechanism may be responsible for this second wave activation, thus prolonging *IL-6* secretion(McGonagle *et al.,* 2020).Furthermore, Sustained *IL-6* secretion has also been correlated to serum viral RNA load in critically patients, and viral RNA load is in turn correlated to acute respiratory distress syndrome (ARDS) severity (Chen *et al.*, 2020). All evidences pointing to a possible detrimental role of *IL-6* in SARS-CoV-2 infection. Patients in the acute and later stages of the disease should be monitored because high levels of *IL-6* are linked to SARS-CoV-1 and SARS-COV-2 infections, and because lung lesions in SARS-CoV-2 have been linked to high serum levels of *IL-6* (Giamarellos – Bourboulis *et al.,* 2020; Zhou *et al.,* 2020).

2.4.2 Cluster of differentiation 147 (*CD147*):

CD147 is a cell-surface glycoprotein belonging to the immunoglobulin superfamily that plays a role in intercellular recognition(Muramatsu , 2012) .The *CD147* gene encompasses a stretch of 7500 bp on chromosome 19p13.3 and encodes a protein from eight exons (Iacono *et al.*,2007). It is expressed by a variety of cell types, including endothelial cells, epithelial cells, and lymphocytes (Agrawal *et al.*,2012).

Structure of human *CD147* consists of 269 amino acids (Miyauchi *et al.,* 1991), comprising a signal peptide (21 amino acids), an extracellular domain (185 amino acids), a transmembrane domain (24 amino acids) and a cytoplasmic tail (39 amino acids) (Biswas *et al.,* 1995). The extracellular part consists of two extracellular domains, an

IgC2-type (EC1) domain at the N-terminal part of the extracellular domain and an IgI-type (EC2) domain at the C-terminal domain of the extracellular domain (Yu *et al.,* 2008).

2.4.2.1 Role *CD147* in SARS-CoV-2 infection (COVID-19):

SARS-CoV-2 has spread much faster than SARS because of the high affinity of SARS-CoV-2 spike proteins to angiotensin- converting enzyme (ACE2) and its viral load (Seif *et al*,2020)**.** Multiple investigations have portrayed the significant role of *CD147* in mediating SARS-CoV-2 infection, and anti-viral effect of *CD147* antagonist peptide-9, as a consequence (Chen *et al.,* 2005). *CD147* has been found to be involved in the indirect interaction between cyclophilin A and viral spike protein, while it binds directly to the viral S protein (Shilts *et al.*, 2021)**.**

At the end of 2020, Wang , *et al.* reported for the first time the interaction between SARS-CoV-2 Spike protein and the host cell receptor *CD147*, and showed that modulation of the receptor levels affected the ability of the virus to infect target cells. Moreover, they showed that *CD147* receptor was involved in SARS-CoV-2 infection of immune cells, which do not express ACE2, and proposed this pathway as a novel entry route (Wang *et al* .,2020).

The host-cell-expressed basigin (*CD147*) may bind spike protein of SARS-CoV-2 and possibly be involved in host cell invasion (Wang *et al.,* 2020).

These affirm the importance of *CD147* in virus infection for host cells, the primary determinant of coronavirus tropism is spike protein, which mediates the viral infection by binding to membrane receptors on the host cells(Hulswit *et al.*,2016).

Neuropilin-1 is identified as a co-factor involved in ACE2-mediated SARS-CoV-2infection (Cantuti-Castelvetr *et al.*,2020; Daly *et al.*,2020). The membrane fusion and endocytosis are two main entry modes for virus infection(Harrison ,2015; Slonska *et al.*,2016), and enter of the *CD147* to the cells through clathrin-independent endocytosis (Eyster *et al.*, 2009; Maldonado-Baez *et al.*,2013). Rab5 is a crucial regulator of endocytosis and locates at early endosome, the co-localization of *CD147*, spike, and Rab5 was detected in baby hamster kidney-21(BHK-21-*CD147*) cells and lung tissues from patient with COVID-19 indicating that the receptor *CD147* and virions were endocytosed and located at the early endosome, SARS-CoV 2 enters the host cells through *CD147*-mediated endocytosis (Saitoh *et al.*,2017) .

More recent studies suggests *CD147* as SARS-CoV-2 entry receptor of platelets and megakaryocytes, leading to hyper activation and thrombosis, that differs from common cold coronavirus CoV-OC43 (Barrett *et al.*, 2021). Furthermore, another study states that platelets challenged with SARS-CoV-2 undergo activation, dependent on the *CD147* receptor, SARS-CoV-2 does not replicate in human platelets and other routes for SARS-CoV-2 infection remain unclear (Maugeri *et al.*, 2021).

2.4.3 Fibrinogen alpha chain gene(*FGA*) :

FGA gene is protein encoded and is the alpha component of fibrinogen, a blood-borne glycoprotein composed of three pairs of non - identical polypeptide chains(Ro *et al.*, 2013). Fibrinogen (coagulation factor I), as a large glycoprotein (340 kDa) is essential for the clot formation, thrombin mediated proteolysis converts the soluble fibrinogen to insoluble fibrin which provides a structural integrity to the clots, so that fibrinogen which serves as an important coagulation factor and an

acute phase reactant for its versatile role relating to coagulation, inflammation, blood viscosity and the related implications in COVID 19 management (Ro *et al.*, 2013) . Further, it stimulates platelet aggregation by binding to the gpIIb/IIIa receptors, ahexameric homodimer composed of 2 Aα, 2Bβ and 2γ is encoded by a gene on long arm of chromosome 4 and is shown to have heritable changes (Eljilany and Elzouki , 2020). The Bβ and γ polypeptide chains of fibrinogen are encoded by a three gene cluster on human chromosome four, the fibrinogen genes (*FGB-FGA-FGG*).The normal adult range of fibrinogen is 2-5 mg/mL with a circulating half-life of about 4 days. In addition to its procoagulant role, fibrinogen affects the inflammatory response by modulating the leukocyte migration, and hence serves as an acute inflammatory reactant (Hajsadeghi *et al.*, 2012).

Up-regulation of fibrinogen mRNA is also observed in peripheral blood mononuclear cells infected by SARS-COV in labs (Ng *et al.*, 2004). During an acute-phase response to infection, injury, or neoplasia, the production of fibrinogen by the liver increases to restore homeostasis (Bini *et al.*, 2000). The production of fibrinogen is increased at exhepatic tissues, as in the lung (Rybarczyk *et al.*,2003 ; Lawrence and Simpson-Haidaris, 2004), also helps in this process. However, the excessive production of fibrinogen and formation of fibrin at the site of injury may enhance cytokines production or imbalance procoagulant and/or fibrinolytic activities (Ware and Matthay , 2000 ; Idell , 2002). In addition, most SARS patients have thrombocytopenia, elevated D-dimers, and prolonged activated partial thromboplastin time, which suggest dysregulation of the coagulation and fibrin polymerization pathways (Lee *et al.,* 2003). Taken together with the fibrinogen up-regulation in both infected peripheral blood mononuclear cells and Vero E6 cells (Ng *et al.*, 2004), it seems that increased fibrinogen expression and fibrin

degradation products can play an important role in SARS pathogenesis (Ng *et al.,* 2004).

2.4.3.1 Role *FGA* in SARS-CoV-2 infection (COVID-19):

The acute respiratory syndrome coronavirus 2 (SARS-Cov-2) induced infection, the cause of coronavirus disease 2019 (COVID-19), is characterized by various coagulopathies that may result in either thrombosis (Gupta *et al.,* 2020 ; Perico *et al.,*2021), the fibrinogen chains of *FGA*, *FGG* and *FGB* are top hub proteins related to COVID-19 fatalities. On the other hand, neighbor proteins related to fibrinogen chains are apolipoprotein A2(APOA2), orosomucoid 2,1 (ORM2, ORM1) and complement factor protein (CFP) . Considering molecular pathways of the coagulation process, in which fibrinogen chains are involved, may open a window to help in treatment of disease. Liver overreacts during acute inflammatory phase in hospitalized COVID-19 patients and secretes several reactants such as fibrinogen, C reactive protein (CRP), ferritin and plenty of cytokines (Guan *et al.,* 2019;Wang *et al.,* 2020).The function of fibrinogen is important, as it regulates antimicrobial activity of the immune cells and clot formation. D-dimer is a product of fibrin degradation in blood after clot fibrinolysis. Increasing in D-dimer is accompanied by reduction of fibrinogen release from the platelets (Tang *et al.,* 2020), and in patients with COVID-19 and other infectious diseases, increasing in D-dimer and decreasing in the secretion of fibrinogen lead to activation of immune responses. On the other hand, decreasing in D-dimer and increasing in the secretion of fibrinogen, activates the coagulation mechanism and thrombi formation (Thachil, 2020).

Among the key genes that interact with D-dimer, fibrinogen level, and coagulation process, *FGA* is prominent a lthough additional gene clusters help govern D-dimer and fibrinogen count and thrombosis in

COVID-19 patients (Abu-Farha *et al.,* 2020). High circulating level of fibrinogen has been linked to COVID-19 coagulopathy. However, it is believed that in COVID-19, patients fibrinogen is probably increased to protect the host (Thachil , 2020).C reactive protein (CRP) released by liver is anti-infective and increases in acute phase of COVID-19, along with ferritin and fibrinogen, as a defense mechanism against pathogens (Wang *et al.,* 2020). CPR forms a complex with histones to protect them from endothelial damage resulting from edema and thrombosis in hosts suffering from COVID-19 (Abrams *et al.*, 2013). During the progression of the various stages of the COVID-19, markers of viral replication, as well as the fibrinogen depletion with increased D-dimer levels followed by a cytokine storm, are levels , kely to be indicative of a poor prognosis (Grobler *et al.*,2020 and Roberts *et al.*,2020).

This poor prognosis is further worsened as together with a substantial deposition of micro clots in the lungs (Renzi *et al.*, 2020 ; Bobrova *et al.,* 2020) . And there were also numerous reports of damage to erythrocytes , platelets, and dysregulation of inflammatory biomarkers (Grobler *et al.*,2020; RobertsI *et al.*,2020 ; Lam *et al.*, 2020 and Akhter *et al.,* 2020).

2.5 Immunological diagnosis of SARS-CoV-2 infection:

Polymerase chain reaction (PCR) tests are useful for detecting SARS-CoV-2 RNA in an upper respiratory (preferably a nasopharyngeal) specimens, and a number of diagnostic procedures to assess immunity built against SARS-CoV-2 are still being developed validated and optimized(Vogel, 2020). In addition, to that antibody testing is evolving, and the market is flooded with test kits (both ELISA and rapid tests in the form of lateral flow immunoassays) . However, only a small number of these kits are certified, and the results need to be interpreted with caution(Vogel, 2020). Preliminary data indicated that COVID-19 presents

with a classical antibody response consisting of early induction of IgM, followed by IgA and IgG antibodies, 128 IgG seems to appear early in the course of clinical presentation probably due to the relatively long incubation period(Vogel, 2020). There is not yet enough evidence with regard to the development of long-term protective immunity. However, antibody testing is so far more valuable in mapping the situation in individual populations, as planned by the WHO in the Solidarity II project (Vogel, 2020). Additionally, clinical trials with type I and III interferons in COVID-19 are currently conducted. Targeting T-cell exhaustion to reverse the dysfunctional state and restore immune responses can be achieved by anti-PD-1 and LAG-3 therapies, revealing novel therapeutic opportunities for persisting infections (Barber *et al*., 2006 ; Nguyen *et al* ., 2015 ; George *et al.*,2020 and Spagnolo *et al.*,2020).

2.5.1 Immune Responses to Natural Infection Versus Vaccination:

There is currently still only little understanding of the relationships between SARS-CoV-2 infection, antibody responses and protection. A central issue is to determine whether vaccine candidates induce immunity, and there are serious ethical and technical limitations to challenging vaccinated volunteers with live wild - type viruses with the aim of determining vaccine effectiveness(Liu *et al.,* 2006). So, trials should include volunteers with a high risk of natural infection, such as populations in highly affected regions and healthcare workers, and based on the current evidence,SARS-CoV-2 infection induces at least some immunity in some people possibly even the majority and that a prior SARS-CoV-2 infection offers significant protection against reinfections. (Liu *et al.,* 2006; Stefan *et al.*,2022).

2.6 Vaccines:

The SARS-CoV-2 is enhanced by the fact that asymptomatic and pre-symptomatic individuals can transmit the virus, in contrast to SARS-COV-1 and MERS-COV, which are transmitted by symptomatic patients and could be contained more efficiently (Hoehl *et al.*,2020 ; Li *et al.*,2020). At present, many investigations aim at defining optimal strategies to limit viral transmission while simultaneously permitting business and social activities (Anderson *et al.*,2020), as well as, to limit the damage of COVID-19, primary efforts focus on confinement, with physical distancing and multiple further measures preventing infection (Salathe *et al.*,2020 ; Cheng *et al*.,2020).

Tan *et al*.(2020) explained of in infected individuals, the results are only positive for a relatively short time window, on average until the 14^{th} day after symptom onset. Furthermore, a positive nucleic acid test (NAT) result did not allow scientists to conclude whether the affected person is or will become immune (Tan *et al*.,2020). Therefore, serological tests are needed, as they can detect the various types of antibodies in the blood that persist for months or even years. Also, the world cannot allow the majority of citizens to become infected with SARS-CoV-2, because the overall burden would be enormous (Tan *et al*.,2020).

Current data indicate that the outbreak of the COVID-19 pandemic only affects low proportions (usually in the single-digit range) of the population, at least in countries that are taking effective measures against the spread of the virus (Vogel, 2020). To avoid pandemic propagation, the reproduction number Ro (viral transmission) must remain below 1, meaning that each infected person transmits on average to <1 additional individual, a key aim for continuously reducing case numbers. In

contrast, a reproduction number Ro of >1 means a highly unfavorable exponential increase of new infections. Spontaneous disappearance of the virus is unlikely (Thevarajan *et al.*,2020).

Most notably, protection from viral infection is mainly achieved by virus neutralizing antibodies, a principle that applies to the vast majority of viral infections to which humans acquire robust immune protection due to infection or vaccination, it is urgent to develop vaccines aiming at the induction of protective immune responses, primarily through virus neutralizing antibodies specific for SARS-CoV-2, although at least 1-2 years are required to make effective vaccines available globally. Vaccination may still be the most rapid and economical strategy to achieve widespread immune protection (Gates, 2020). Most notably, so-called (Herd immunity) is reached when a critical percentage of the population has become immune, leaving the virus only limited local chances to circulate, and this may be the case when >90% of persons are immune. However, broad immunization is already very helpful as soon as only approximately 60–70% have become immune because relatively simple measures against viral spread will then be sufficient to contain the virus, and in case of future outbreaks of newly emerging microbes belonging to well-studied pathogen families (Gates, 2020). Previously established experience will accelerate the development and use of vaccines, allowing to reach herd immunity more rapidly. Several organizations, including WHO, the Coalition for Epidemic Preparedness Innovations (CEPI) and the global Vaccine Alliance GAVI, are increasing preparation efforts for future pandemics. The lessons learned from SARS-CoV-2 will certainly support this development still remains very challenging (Gates, 2020).

The basic and applied knowledge acquired during the last few decades is highly useful to design promising vaccines with increased

likelihood of inducing protective immune responses and avoiding adverse effects and harmful disease enhancement. Now, probably more than ever, the world depends on rational effective strategies built on robust scientific evidence. Vaccine development has started at a strongly accelerated pace already, shortly after the beginning of the SARS-CoV-2 outbreak, and the WHO is publishing a regularly updated list of the vaccines in development (Lurie *et al.,* 2020 ; Le *et al.,* 2020 and WHO, 2020).

Most notably, vaccines can be based on whole viruses (live-attenuated or inactivated), viral vectors, nanoparticles or virus-like particles, subunit components, proteins/peptides, RNA, DNA or live cells. The first vaccine trial against COVID-19 was started in China on February 15th , 2020 ,using dendritic cells that are genetically modified with structural and enzymatic proteins of SARS-CoV-2. A second trial, also in China, was done with a similar vaccine, complemented by the infusion of antigen-specific T cells, while both of these vaccines are tested therapeutically in COVID-19 patients, most other vaccines are tested in healthy volunteers (The National Institute of Health ,2020). In the US, the first trial was launched in March 2020, using lipid nanoparticle encapsulated mRNA encoding the spike (S) protein (The National Institute of Health, 2020). Furthermore, in early April 2020, a DNA vaccine trial was initiated with a plasmid encoding the S protein, sponsored by Inovio Pharmaceuticals and CEPI, since mid-April 2020, several vaccines consisting of inactivated SARS-CoV-2 virus have been tested in China (Tseng *et al.,* 2012) . The first viral vector COVID-19 vaccine is developed at the University of Oxford, UK, and it is based on a chimpanzee adenovirus and encodes the S protein (Van Doremalen *et al.,* 2020), and it is now in phase 2/3 testing. A similar vaccine is based on adenovirus, after promising results in phase 1 in Wuhan, China, this vaccine has also moved forward, to a phase 2 trial (Zhu *et al*., 2020).

Hence, COVID-19 vaccines is designed to optimally expose the receptor-binding domain (RBD) to the immune system for the efficient induction of neutralizing antibody responses could potentially exert un unprecedented pressure on the virus, resulting in a halt of viral spread (Plotkin,2020).

Vaccines continue to provide strong protection against severe disease and hospitalization especially after a third dose of an mRNA vaccine is given. However, it is still only partially possible to predict vaccine efficacy and safety (Ahmed *et al.,* 2022 ; Al Jurdi *et al.*,2022).

2.7 Single Nucleotides Polymorphism(SNP):

The single-nucleotide polymorphisms (SNPs) are single genetic code variations, represents genetic variation for a single nucleotide (SNP) containing only two alleles (Hajihosseinlo *et al.,* 2012). DNA sequence variations are sometimes described as mutations and sometimes as polymorphisms, a mutation is defined as any change in a DNA sequence away from normal, this implies there is a normal allele that is prevalent in the population and that the mutation changes this to a rare and abnormal variant (Twyman, R., 2020).

In contrast , a polymorphism is a DNA sequence variation that is common in the population . In this case no single allele is regarded as the standard sequence , instead there are two or more equally acceptable alternatives , the arbitrary cut - off point between a mutation and a polymorphism is 1 per cent , that is to be classed as a polymorphism , the least common allele must have a frequency of 1per cent or more in the population ,if the frequency is lower that this , the allele is regarded as a mutation (Twyman, R., 2020) . Single nucleotide polymorphisms (SNPs) can cover the entire genome at different sequences in different species, it may cause phenotypic diversity between individuals (Huq *et al*., 2016). As it is a site within the sequence of the nitrogenous bases of DNA that

can be occupied by one of two nitrogenous bases, so it becomes a specific allele. When the second is resolved, it becomes the other allele, and such a site of nitrogenous bases in which there are likely to be two mutual bases in each allele in the genome can be described as a SNP. It occurs in at least 1% of the population, and the SNP is very stable and does not change much from generation to generation, which can be tracked in population studies. It is estimated that about 10 million SNPs are located within the 3.2 billion nucleotides that make up the human genome (Al-Miqdad, 2010 ; Zhao *et al*., 2018). Two out of three SNPs occur as a result of the substitution of the cytosine base (cytosine C) for the base (thymine T), only about 3-5% of the DNA sequence encodes protein synthesis, and therefore most of the SNPs are found outside the coding sequences, so the SNPs that are within the coding sequence are of interest to researchers because they often alter the vital function of the protein (Hoskins *et al.*, 2001 ; Ismail and Essawi, 2012).

It can be said that genetic differences represented by SNPs are one of the main factors for the existence of differences between individuals, which distinguishes them from each other, the importance of SNPs arises as individual genetic sensors that play a role in explaining the susceptibility of individuals to disease, or their susceptibility to respond to a particular drug, or to a particular virus. They are also signals that help determine the genetic sequence, and a single nucleotide polymorphism occurs when a single nucleotide is replaced by another, such as replacing a nucleotide A with a C, G or T nucleotide (AL-Miqdad,2010) (Figure 2-1). Vignal *et al*. (2008) and Zhao *et al.* (2018) indicate that the SNP is a bi-allelic, which is either homozygous in both alleles for the dominant or recessive form, or different (heterozygote) in each base allele and a different one within the same gene.

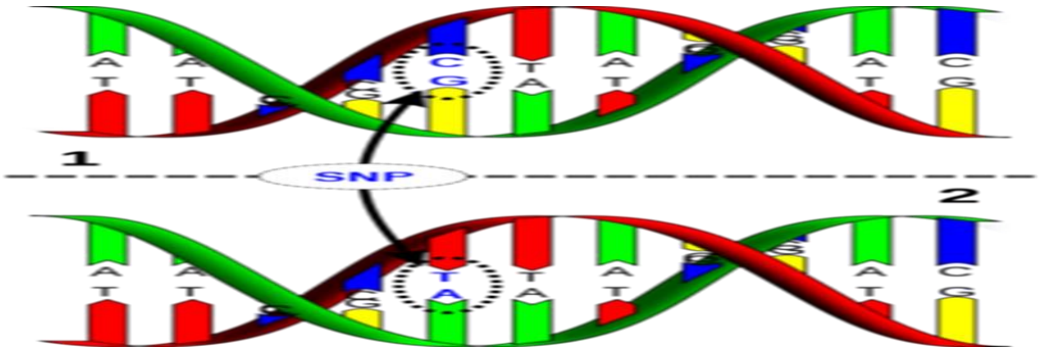


Figure 2-1: Single nucleotide polymorphism (SNP) method (AL-Miqdad,2010).

Surprisingly, researchers notice several hundreds of point mutations or SNPs among the different isolates from all over the world with different sequence data sets, and 47 key point mutations or SNPs are located along the entire genome in the sequence just in 12 different countries (single sequence comparison). These mutations involved in the different protein-protein recognition, point mutations or SNPs have great implications for the target drug binding and receptor binding (Puty *et al.*, 2019). The overall mutations phylogeny shows the 3 groups of mutations which are evolved in these 3 months. Predominantly, the mutations are also found in the different vital proteins of SARS-CoV-2 (spike glycoprotein, Nsp1, RdRp and others) and warrants epidemiologists and medical fraternity for the use of drug treatment options, this also suggests that SARS-CoV-2 is highly venerable to have quick changes and mutate even during the person-to-person transmission.

This also helps to overcome the previous misconception of SARS-CoV-2 may not get mutated during person-to-person transmission (Andersen *et al.*, 2020), and the rate and number of SNPs or mutations in SARS-CoV-2 within three months of outbreak underlines the complexity of virus to handle and corroborate the quick evolution of

SARS-CoV-2(Narayanan *et al.,* 2008 ; Vankadari and Wilce, 2020; Wrapp *et al.,* 2020 and Miller *et al.,* 2020).

Since SNP frequency and existence vary among population groups, groups with gene expression-related SNPs may show higher vulnerability to Sars-COV-2(COVID-19) infection (Mollica *et al.,* 2020). However, the genes and SNPs could be ordered in terms of their relative contributions, the strength of the contribution of genetics could be challenging to quantify, several SNPs affect the genes' action via indirect path; therefore, it may not always apply to all individuals, the SNPs may not necessarily affect COVID-19 infection, as the COVID-19 pandemic developed recently and is ongoing(Siyeon *et al.,* 2022) .

Liu and Cordess (2004) and More *et al*. (2019) indicate that the SNP has an important role in the evolution of genetic markers as it is the most abundant source of genetic polymorphism in any organism, and its light can reveal phenotypic polymorphism that cannot be determined or is not clear by application other marker technologies. It is also a modern concept in the world of markers, which arose mainly from the need for more accurate genetic markers to study diseases that arise from several interfering factors, and in order to provide for the needs of the great progress in techniques for determining genetic shapes and patterns. With the rapid development of writing and improving relevant databases, the SNP has been widely employed in genetic studies, such as genetic mapping, structural analysis of population genetics, genetic association studies, phylogeny, individual identification and product traceability (Zhao *et al*., 2018). SNP detection and analysis methods have become more simple, mefficient and fast, and measurement methods are constantly being developed by researchers (Xu *et al*., 2015).

Chapter Three

Materials and Methods

3. Materials and Methods:

3.1 Materials:

3.1.1 Devices:

The devices that used in the present study were shown in Table (3-1).

Table 3-1: The devices which were used in the study.

Devices	Origin
Autoclave	Hirayama – Japan
Balance (two digits)	Kern – Germany
Biological safety cabinet	Labogene – Denmark
Centrifuge	Gemmy –Taiwan
E-graph –UV (Gel documentation)	ATTA – Japan
ELISA instrument system	BioTek-USA
Eppendrof Centrifuge 5418	Eppendrof
Horizontal gel electrophoresis unit	Mupid – Japan
Incubator	Memmert- Germany
Microwave Oven	Shownic
Nano drop 2000	Biodrop – USA
PCR Workstation	Jeiotech – Korea
Refrigerator (4°C and -20°C)	Concord – Lebanon
Sequencing system	Macrogen – South Korea
Thermal cycle PCR system	Techne – UK
Water bath	GFL – Germany
Vortex	Heidolph – Germany

3.1.2 Equipments:

The equipments that used in the present study were shown in Table (3-2).

Table 3-2: The equipments which were used in the study.

Equipment	Origin
Aerosol resistant pipette tips	Promega – USA
Barrier tips (0.1 – 10)µl	Bio Basic – Canada Inc.
Blue tips	Asco – Jordan
Cylinders of different sizes	Bio Basic – Canada Inc.
Disposable Syringe (5 ml)	Asco – Jordan
EDTA tubes	Asco – Jordan
Gel loading tips	Bio Basic – Canada Inc.
Gel Tube	Asco – Jordan
Glassware different sizes	Bio Basic – Canada Inc.
Micropipettes (adjustable from to 10), (from10 to 100) µl and (from 100 to 1000) µl	Eppendorf-USA
Gloves	China
Yellow tips	Asco – Jordan

3.1.3 Chemicals:

The Chemical and Biological materials that used in the present study were shown in Table (3-3).

Table 3-3: Chemical and Biological materials.

Materials	Origin
Agarose	Bio Basic – Canada Inc.
Ethidium Bromide stain	Biotech
Ethanol	Merck – Germany
Nuclease Free Water	Promega-USA
Tris Borate EDTA Buffer 10X (TBE)	Promega-USA
Primers	Macogene-Korea
1Kb DNA ladder	Promega

3.1.4 Kits:

The kits that used in the present study were shown in Table(3-4).

Table 3-4: Kits and contents which were used in the study.

Kit	Origin
ELISA kits (IL-6)	Elabscience –USA
DNA Extraction kit (from blood)	Favorgen – Taiwan
PCR preMix 20 µl reaction	Promega-USA

3.1.5 Primers:

The primers that used in the present study were shown in Table (3-5).

Table 3-5: Primers which were used in the study.
All primers manufactured by Macrogen Corporation- South Korea.

Primer		Sequence 5'→3'	Product Size(bp)	Ref.
IL-6 G allele & C allele	F	GCACTTTTCCCCCTAGTTGTGTCTTCCG	206bp	All Primers Designed by this study
	R	ATTGTGCAATGTGACGTCCTTTAGCTTG	152bp	
	F	GACTTCAGCTTTACTCTTTGTCAAGACA	302bp	
	R	GAATGAGCCTCAGACATCTCCAGTCCTA		
CD147	F	GCTCTGCACCCCTGTAAGTT	229bp	
	R	CCTTTGTCATTCTGGTGCTG		
FGA	F	GTTGTTAGGCCTCGCGTTC	392bp	
	R	ATGGAACCGGATCAGAGACG		

3.6 PCR pre Mix:

The PCR pre Mix that used in the present study were shown in Table (3-6).

Table 3-6 : PCR pre Mix 20 µl reaction (Promega).

Components	20 µl reaction
Top DNA polymerase (Taq)	1 U
Each: dNTPs (dATP, dGTP, dCTP, dTTP)	250µM
Tris-Hcl (pH 9.0)	10 mM
KCl	30 Mm
MgCl2	2 mM

3.1.7 DNA ladder:

The DNA ladder (Promega) that used in the present study was shown in Table (3-7).

Table 3-7 : DNA ladder 100-1500bp (Promega).

Material
1- Ladder consist of 11\double-stranded DNA with size (100-1500bp). 2- Loading dye has a Composition: 3- [15% Fic ll, 0.03% br0mophenol blue,0.03% xylene cyanol, o.4% orange G, 10mMTris-HCl(pH 7.5) and 50mM EDTA].

3.2 Methods:

3.2.1 Study Design :- Study design was illustrated in Figure (3-1).

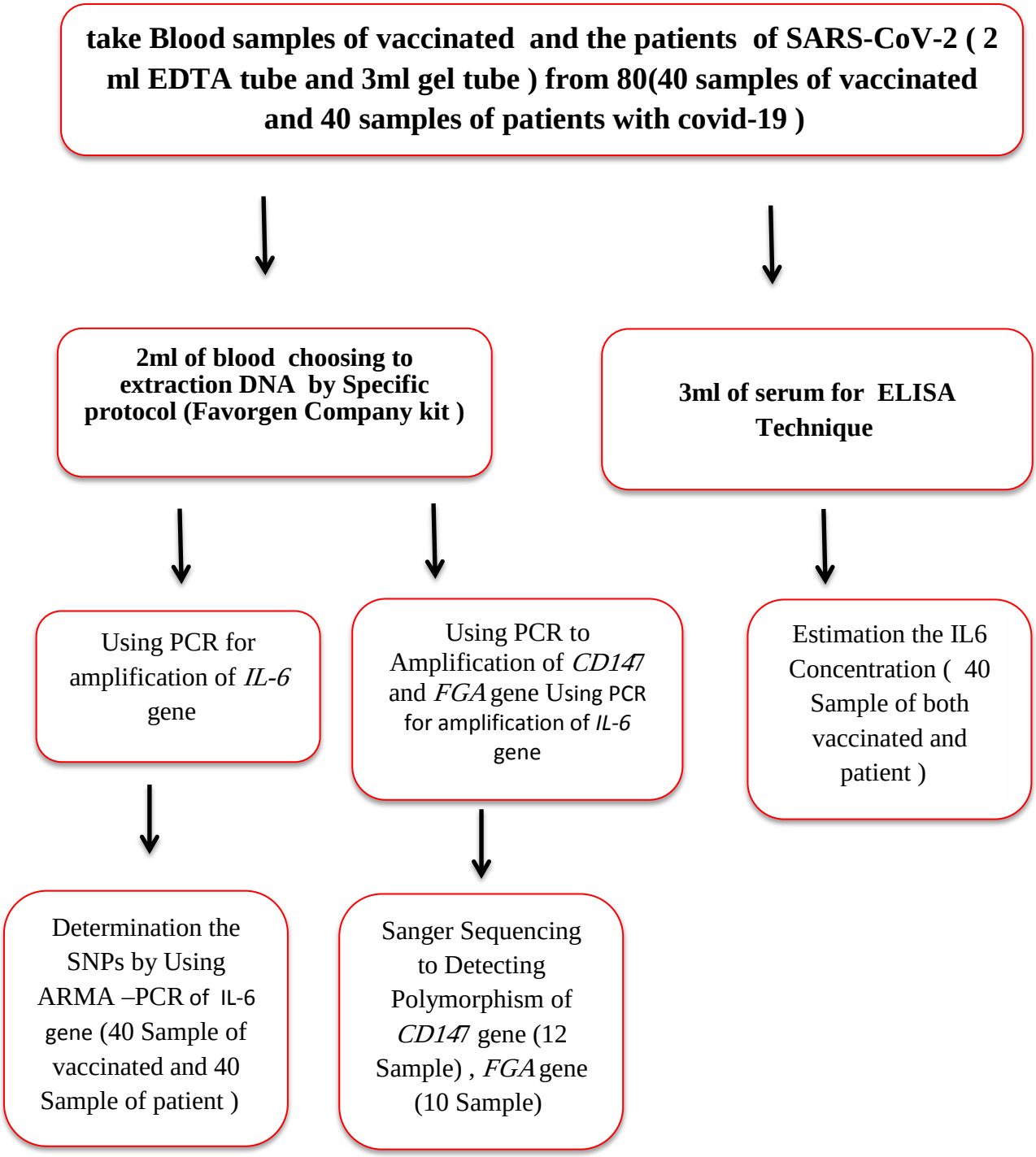


Figure 3-1 : The study design.

3.2.2 Samples Collection:

Forty blood and serum sample from a people that probably infected with SARS CoV-2 (Covid -19) and forty blood and serum sample from a people vaccinated thus, the total sumtion of samples eighty sample. Provided from the Covid-19 patients of Marjan Teaching Hospital and Al-Hilla Teaching hospital and Imam sadiq hospital and al-mahaweel hospital from (October 2021) to (February 2022). All the involved samples were from different areas in Babylon province. The personal data of patients were provided (Name, Age, Gender, Result (+ve) Real time PCR, X ray). The samples were labeled with numbers because they could not be mixed up of other . Transport and storage of the sample using a special container for transport, containing ice, in order to preserve the sample during transportation**.** As for the vaccinated people samples were provided from the centers vaccinated of Marjan Teaching Hospital and Al-Hilla Teaching hospital and Imam sadiq hospital and al-mahaweel hospital, and all the involved samples were from different areas in Babylon province. Their samples were taken two month after taking the second dose of the vaccine and the personal data (name, age, sex, and type of vaccine) were provided to the vaccinated. Samples were marked with numbers because they cannot be mixed with other samples. Transport and storage of the sample using a special container for transport that contains ice in order to save the sample during transport.

3.2.3 Tris Borate EDTA Buffer (TBE Buffer) preparation:

Tris Borate EDTA Buffer was prepared by diluting 100 ml Tris Borate EDTA (10x) in 900 mL distilled water to make Tris Borate EDTA (1x), which was then used to prepare Agarose for the Gel electrolysis (Sambrook and Russell , 2001)**.**

3.2.4 Agarose preparation:

The Gel electrophoresis was done with Agarose, which has been made through melting 2 g of Agarose in 100 ml of diluted Tris Borate EDTA solution (1x) (Sambrook and Russell , 2001) .

3.2.5. DNA extraction Favorgen Kit :

3.2.5.1 Intended Use :

The deoxygenated DNA (DNA) was extracted from the blood using a (kit) supplied by Favorgen Company - Taiwan, based on the steps indicated by the manufacturer.

3.2.5.2 Protocol:

1. A total of 200 ul of frozen blood was transferred to 1.5 ml microcenterfuge tube and phosphate buffer saline (PBS) way added if the sample less than 200 ul.
2. Added 30 ul Proteinase k (10 mg/mi, not provided) to the sample and briefly mix. Then incubate for 15 min at 60 °C.
3. Cell Lysis:
    - Added 200 ul FABG Buffer to the sample and mix by overtaxing .
    - Incubated in a 70°C water bath for 1S min to lyse the sample, During incubation, invert the sample every 3 min.
    - Preheated required an Elution Buffer in a 70 °C water bath for DNA Elution.
    - Follow the General Protocol starting from Step 4 (DNA Binding).
4. DNA Binding:
    - Added 250 ul of ethanol (96-100%) to the sample and vortex for 10 sec. Pipette the sample to mix well if there is any precipitate formed.

- Placed a FABG Column to a Collection Tube. Transferred the sample mixture carefully to FABG Column Centrifuge at speed 16:000 rpm for I min. Discard the Collection Tube and placed the FABG Column to a new Collection Tube.
- Follow the General Protocol starting from Step 13 (Column Washing).

5. Column Washing:
    - Added 400 ul of W1 Buffer to the FABG Column and centrifuge for 30 sec at speed 14,000 rpm. Discard the flow-through and placed the FABG Column back to the Collection Tube.
    - Added 600 ul of Wash Buffer to the FABG Column and centrifuge for 30 sec at speed 14,000 rpm. Discard the flow-through and placed the FABG Column back to the Collection Tube. -- Make sure that ethanol has been added to Wash Buffer when first open.
    - Centrifuge for an additional 3 min at speed 14,000 rpm to dry the column. -- Important Step! This step will avoid the subsequent enzymatic reactions from being inhibited by residual liquid.
6. Elution:
    - Placed the dry FABG Column to a new 1.5 ml micro centrifuge tube**.**
    - Added 100 ul of Preheated Elution Buffer or TE to the membrane center of FABG Column.
    - Incubated the FAGB Column at 37 °C for 10 min in an incubator.

- Centrifuge for 1 minute at full speed 14,000 rpm to elute the DNA. -- Standard volume for elution is 100 ul. Store the DNA fragment at -20°C.

3.2.6 Conventional Polymerase Chain Reaction (PCR):

3.2.6.1 Primers Preparation:

All primers used in the enzyme polymerase reaction were prepared for amplification studied genes, and that by dissolved the primers (Forward and Reverse) in 300 µl of Nuclease Free Water according to the supplied company instructions (**Humanizing Genomics Macrogene**) to obtained final concentration of solution 100 picomole/microliter as stock solution. The tubes were shaken, then the working solution was prepared by diluted 10 µl of primers (Forward and Reverse) of stock solution in 90 µl of Nuclease Free Water to obtain the work solution with 10 picomole/microliter and storage of -20°C .

3.2.6.2 Polymerase Chain Reaction Mixture:

The mixture of polymerase chain reaction was prepared to amplify the study genes by mixing components master mix with the forward and reverse primers, and the DNA template and Nuclease free water as shown in Table (3-8).

Table 3-8 : Polymerase Chain Reaction Mixture.

NO.	Materials	Volume
1	Master Mix	12.5µl
2	DNA sample	4 µl
3	Primer Forward (10µl)	1µl
4	Primer Reverse (10µl)	1µl
5	Nuclease free water	6.5µl
	Total	25 µl

3.2.6.3 Amplification Conditions:

Polymerase Chain Reaction-thermocycler was used to amplified the study genes, the amplification conditions and the thermal cycles for each gene have been controlled as shown in a Table (3-9).

Table 3-9 : Amplification Conditions for Polymerase Chain Reaction.

***IL-6* gene**			
Step Type	**Temperature**	**Time**	**Cycling**
Initial Denaturation	**92°C**	**3 min.**	**1**
Denaturation	**92°C**	**30 Sec.**	**32**
Annealing	**61°C**	**35 Sec.**	**32**
Extension	**72°C**	**35 Sec.**	**32**
Final Extension	**72°C**	**3 min.**	**1**
Hold	**4°C**	**α**	**1**
***CD147* gene**			
Step Type	**Temperature**	**Time**	**Cycling**
Initial Denaturation	**92°C**	**2 min.**	**1**
Denaturation	**92°C**	**30 Sec.**	**30**
Annealing	**57.5°C**	**30 Sec.**	**30**
Extension	**72°C**	**30 Sec.**	**30**
Final Extension	**72°C**	**3 min.**	**1**
Hold	**4°C**	**α**	**1**
***FGA* gene**			
Step Type	**Temperature**	**Time**	**Cycling**
Initial Denaturation	**92°C**	**3 min.**	**1**
Denaturation	**92°C**	**30 Sec.**	**32**
Annealing	**55°C**	**30 Sec.**	**32**
Extension	**72°C**	**30 Sec.**	**32**
Final Extension	**72°C**	**3 min.**	**1**
Hold	**4°C**	**α**	**1**

3.2.6.4 Electrophoresis:

The gene amplification products from the polymerase chain reaction (PCR) were loaded electrically using the Agarose gel prepared in paragraph (3.2.4 Agarose preparation). The gel was then fully dissolved and cooled at room temperature. The gel has poured in casting tray that combs were fixed on it and then was lifted to harden at room temperature.

The gene amplification products (Amplicons) were loaded into the pits that resulted after lifting the combs and the DNA ladder was putted in a first and last pits in first line of pits and only in a first pit in second line for the purpose of comparing the molecular size of the resulting bands and the relay tank was filled with a buffer (1X TBE) and the electrophoresis of study genes amplification products were performed with a voltage of 100 V for a duration of 45 minutes (Sambrook and Russell , 2001) .

3.2.6.5 DNA concentration and purity measurement:

Measuring the concentration and purity of DNA with a Nanodrop device is an easy and quick process that reveals to us the error rate that may exist in the sample, so the standard reading of DNA is ~1.8. As for the readings that differ from this percentage, it is an indication of the presence of contamination in the sample, meaning that the sample still contains Protein or some other material, and the work is done in the device depending on the steps indicated by the processing company, which are summarized as follows:

1- Turn on the device (Nanodrop) .
2- Choose the icon for the Nanodrop device on the desktop.
3- Choose nucleic acid from the list.
4- Choose the type of nucleic acid (DNA).
5- Choose the standard unit of nucleic acid, which is ng/ µl.
6- Choose the appropriate wavelength for the solution to be examined (260-280) nm, and since it is a DNA test, we choose 260 nm.
7- Choose the icon Add to the report to add all the measurements of all samples and save them until you return to them when needed.
8- Calibrate (beep) the device by Blank, placing 1-2 microliter of Blank solution on the device lens, then lowering the device arm,

then pressing the word Blank, after which the lens was cleaned with special cleaning paper.

9- Put the sample in the designated place and press the word "Measure" to start the measurement.

3.2.7 Human IL-6 (Interleukin-6) ELISA Kit(Elabscience):

3.2.7.1 Intended Use :

This sandwich kit was for the accurate quantitative detection of human Interleukin-6 (also known as IL-6) in serum, plasma, cell culture super nates, cell lysates, tissue homogenates.

3.2.7.2 Assay Principle:

An Enzyme-Linked Immunosorbent Assay (ELISA) was included in the micro ELISA plate provided in this kit has been pre-coated with an antibody specific to Human IL-6. Samples were added to the micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for Human IL-6 and Avidin-Horseradish Peroxidase (HRP) conjugate are added successively to each micro plate well and incubated. Free components were washed away. The substrate solution was added to each well. Only those wells that contain Human IL-6, 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) was measured spectrophotometrically at a wavelength of 450 ± 2 nm.

3.2.7.3 Contents:

Standard Solution (4800U/ml), Pre-coated micro ELISA plate, Standard Diluent, Avidin-HRP, Concentrated HRP Conjugate (100 x),Stop Solution, Substrate Reagent, Reference Standard and sample

Diluent, HRP Conjugate Diluent , Wash Buffer Concentrate (25x), Biotinylated Detection AB Diluent, User instruction, Certificate of Analysis , Plate Sealer and Zipper bag.

3.2.7.4 Reagent Preparation :

- Bring all reagents to room temperature (18-25℃) before use. If the kit will not be used up in one assay, please only take out the necessary strips and reagents for present experiment, and store the remaining strips and reagents at required condition.
- **Wash Buffer:** Diluted 30 mL of Concentrated Wash Buffer with 720 mL of deionized or distilled water to prepare 750 mL of Wash Buffer. Note: if crystals have formed in the concentrate, warm it in a 40℃ water bath and mix it gently until the crystals have completely dissolved.
- **Standard working solution:** Centrifuge the standard at 10,000×g for 1 min. Added 1mL of Reference Standard &Sample Diluent, let it stand for 10 min and invert it gently several times. After it dissolved fully, mix it thoroughly with a pipette. This reconstitution produced a working solution of 100 pg/mL(or added 1 mL of Reference Standard &Sample Diluent, let it stand for 1-2 min and then mix it thoroughly with a vortex meter of low speed. Bubbles generated during vortex could be removed by centrifuging at a relatively low speed). Then make serial dilutions as needed. The recommended dilution gradient was as follows: 100,50,25, 12.5,6.25, 3.13 ,1.56 , 0 pg/mL Dilution method: Take 7 EP tubes, add 500 μL of Reference Standard & Sample Diluent to each tube. Pipette 500 μL of the 100 pg/mL working solution to the first tube and mix up to produce a 50 pg/mL working solution. Pipette 500 μL of the solution from the former tube into the latter one

according to this step. The illustration below is for reference. Note: the last tube is regarded as a blank. Don't pipette solution into it from the former tube, as seen in figure (3-2).

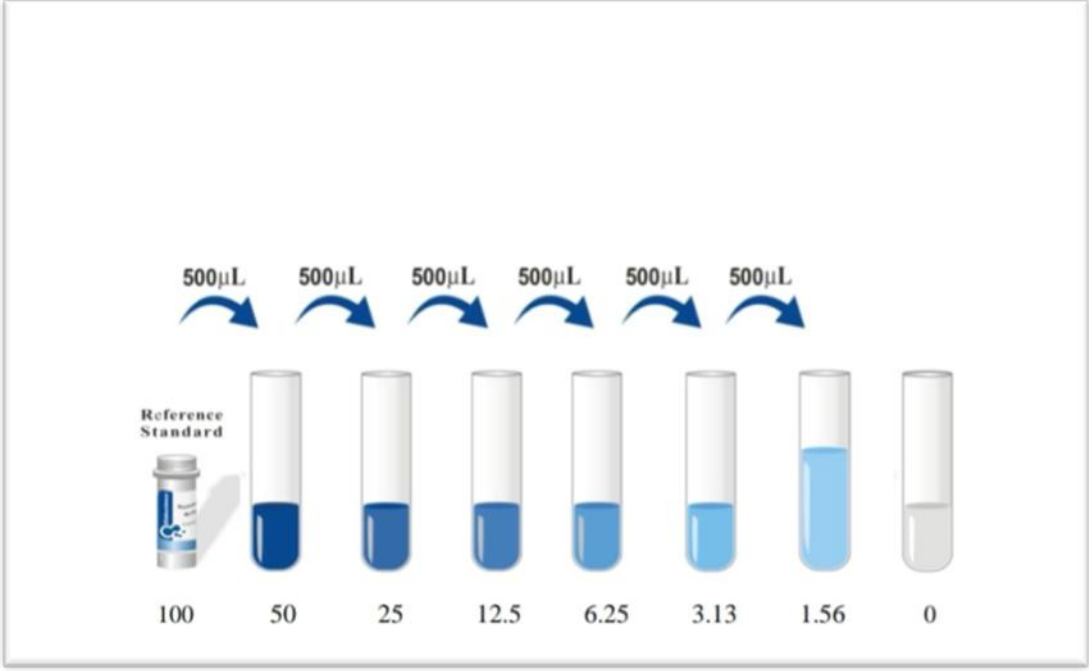


Figure 3-2 : Dilution of Standard Solutions for IL-6.

- **Biotinylated Detection Ab working solution:** Calculate the required amount before the experiment (100 µ L/well). In preparation, slightly more than calculated should be prepared. Centrifuge the Concentrated Biotinylated Detection Ab at 800×g for 1 min, then dilute the 100× Concentrated Biotinylated Detection Ab to 1× working solution with Biotinylated Detection Ab: Diluent(Concentrated Biotinylated Detection Ab: Biotinylated Detection Ab Diluent= 1: 99).
- **HRP Conjugate working solution:** Calculate the required amount before the experiment (100 µL/well). In preparation, slightly more than calculated should be prepared. Centrifuge the Concentrated HRP Conjugate at 800×g for 1 min, then dilute the 100× Concentrated HRP Conjugate to 1× working solution with HRP

Conjugate Diluent(Concentrated HRP Conjugate: HRP Conjugate Diluent=1:99).

3.2.7.5 Assay Procedures:

1. Determine wells for diluted standard, blank and sample. Added 100 μL each dilution of standard, blank and sample into the appropriate wells (It is recommended that all samples and standards be assayed in duplicate). Cover the plate with the sealer provided in the kit. Incubate for 90 min at 37℃. Note: solutions should be added to the bottom of the micro ELISA plate well, avoid touching the inside wall and causing foaming as much as possible.
2. Decanted the liquid from each well, do not wash. Immediately added 100 μ L of Biotinylated Detection Ab working solution to each well. Cover the plate with a new sealer. Incubated for 1 hour at 37°C.
3. Decanted the solution from each well , added 350 μL of wash buffer to each well. Soak for 1min and aspirate or decant the solution from each well and pat it dry against clean absorbent paper. Repeat this wash step 3times. Note: a microplate washer can be used in this step and other wash steps. Make the tested strips in use immediately after the wash step. Do not allow wells to be dry.
4. Added 100 μL of HRP Conjugate working solution to each well. Covered the plate with a new sealer. Incubate for 30 min at 37°C.
5. Decanted the solution from each well, repeat the wash process for 5 times as conducted in step 3.
6. Added 90 μ L of Substrate Reagent to each well. Covered the plate with a new sealer. Incubated for about 15 min at 37°C. Protect the plate from light. Note: the reaction time can be shortened or extended according to the actual color change, but not more than30

min. Preheat the Microplate Reader for about 15 min before OD measurement.

7. Added 50 µl of Stopped Solution to each well. Note: adding the stop solution should be done in the same order as the substrate solution, as seen in figure (3-3).

Figure 3-3 : IL-6 post-stop solution addition.

8. Determined the optical density (OD value) of each well at once with a micro-plate reader set to 450 nm.

3.2.7.6. Calculation of Result:

Drawn a best fit curve across the points on the graph by estimating the average OD for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis.

The measurements were better done using computer-based curve-fitting applications, also the best fit line found using regression analysis, as seen in Figure (3-4).

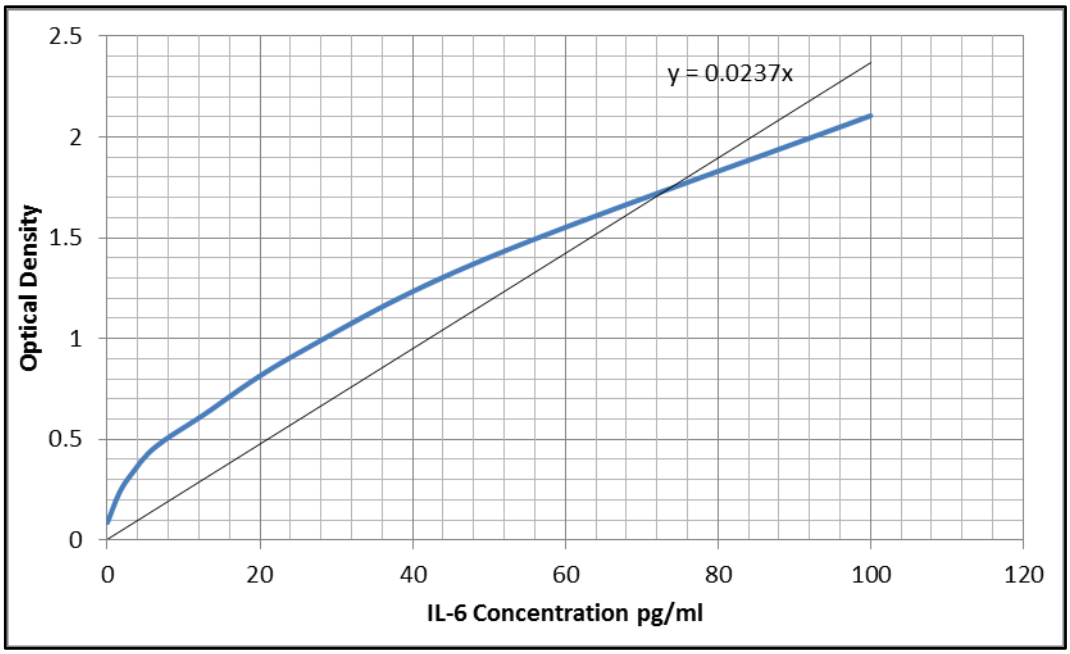


Figure 3-4 : Curve the average of optical density of standard (450 nm) with the IL-6 concentration U/ml.

3.2.8 Tetra–ARMS-PCR technique for *IL-6* gene Polymorphisms :

3.2.8.1 Principle :

The Amplification Refractory Mutation System (ARMS) is an application of PCR in which DNA is amplified by allele specific primers, in PCR mismatch at the 3' end of the primer can dramatically reduce the annealing and hence the amplification, and this is due to the absence of 3' to 5' exonuclease proofreading activity of Taq polymerase, high fidelity DNA polymerases, that have this activity , cannot be used in ARMS it is an extremely useful method for identification of point mutations or polymorphisms, since the ARMS PCR is mostly done to identify a mutation or a polymorphism it is also important that it should be able to identify whether the change in DNA is heterozygous or homozygous, as heterozygous or homozygous is differentiated by using ARMS primers

for the mutant/polymorphic and the normal (wild type) alleles, the reactions for the mutant and the normal alleles are usually carried out in separate tubes, but these may be done in the same tube after labeling the two primers with different fluorescent dyes (Kwok *et al.*, 1989; Newton *et al.*, 1989 and Old *et al.*, 1990) .

3.2.8.2 Protocol:

Added 1 ul of each primer {F,R (G allele) and F,R (C allele)} prepared by (Microgene Company), 3 ul of DNA sample, 12.5 ul of Master Mix (Promega) and 5.5 u l of nuclease water free and sum of 25 ul , after the preparation is completed, it is mixed by a vortex for the purpose of mixing for a few seconds. The following program was applied in PCR thermocycler, version; master cycler-nexus (Eppendorf - Germany) show to table (3- 10).

Table 3-10 : Amplification Conditions for Tetra –ARM-PCR Technical of *IL-6*.

Gene	Initial Denaturation	Cycle conditions				Final Extension	Final hold
		Denaturation	Annealing	Extension	Cycles number		
***IL-6* gene**	**92°C, 3 min**	**92°C, 30 sec**	**61 °C, 35 sec**	**72°C, 35 sec**	**32**	**72°C, 3 min**	**4°C**

The PCR amplified products were confirmed by resolving on 1.5 percent agarose in parallel with 100 bp DNA ladder. Gel electrophoresis was carried out at a constant voltage of 100 V for 60 min in 1X TBE buffer. It was made sure that all PCR resolved bands were specific and consisted of band 302 bp (GC) , 206 bp(GG) , 152 bp(CC) fragment each (Kwok *et al.*, 1989; Newton *et al.*, 1989 and Old *et al.*, 1990) .

3.2.9 Standard Sequencing:

PCR product sent for Sanger sequencing by Macrogen Corporation –Korea using ABI3730XL, an automated DNA sequences. Results received by email and the steps to analyzed as follows :

PCR , DNA sequence , DNA sequence analysis , DNA Alignment using blast , Homology search . and the programs used in this study as follows :

1. Bio Edit : Software Bio Edit Sequence Alignment Editor software Version 7.1. they matched the appropriate sequences in the reference database (DNASTAR, Madison , WI, USA).
2. Jalviews : NCBI homology sequence identity of CD147 gene of local samples against Ref Seq. CD147 gene (accession No. NG_007468.1).
3. Blast: NCBI's nucleotide blast (http://blast.ncbi.nlm.nih.gov./blast/Blast.cgi).
4. MSA viewer : NCBI Multiple Sequence Alignment Viewer, Version 1.22.0.

3.2.10 Statistical Analysis:

The Statistical Analysis System- SAS (2012) program was used to detect the effect of difference factors in study percentage. Chi-square test was used to significant compare between percentage (0.05 and 0.01 probability) in this study.

Chapter Four

Results and Discussion

4. Results and Discussion:

4.1 Gender and Age Distribution of COVID -19 patient :

COVID-19 is a genetically complex disease with numerous genetic and environmental associations. The majority of the samples in this study came from Female, indicating that female were more likely than men to be admitted to the hospital and to be at high risk. As shown in Table(4-1), the majority of patients (52.5%) were females, while (47.5%) percent were males , and there are statistically significant differences in this category for patients (0.7518) , while the majority of vaccinated equal in both females and males (50%) and there are statistically significant differences in this category for vaccinated (1.00). In both genders, the adult age range was(30-50) years old with (20%) ,while (51–71) years old with (80%) respectively for patients , we also note that there are high differences in ($P \leq 0.05$) in these age groups (0.0001), while the adult age range was(30-50) years old with (47.5%) ,while (51–71) years old with (52.5%) respectively for vaccinated and there are statistically significant differences in this category for vaccinated , with a difference in case distribution giving a higher percentage than others. As shown in Table (4-1). As shown in Table (4-2), we notice a significant difference ($P \leq 0.05$) between the vaccinated and the patients, the age group (30_50), while the statistical differences between the vaccinated and the patients, the age group (51_71). There were statistical differences between the vaccinated and the infected, between men and women. Females had much higher serological levels than males, and females reported more adverse effects than males, according to Chiara *et al.* (2021). This was consistent with study findings, which showed that females were more affected than males in

terms of illness and vaccination response, indicating a natural immune response to SARS (Baggio *et al.,* 2013). It's worth mentioning that immune responses to viral infections vary by gender, and that the immunological and endocrine systems change dramatically as people age, increasing susceptibility to infectious diseases and decreasing vaccine efficiency. In both females and males, immunological senescence affects both the innate and adaptive immune systems, as shown in China and Europe, where women make up the majority of healthcare personnel, these dynamics are based on gender assumptions (women are seen more suitable for care and assistance) affecting behavior (WHO ,2021). In other study , the average age of 1161 COVID-19 patients in India was 38 years (IQR, 27-52) according to the study Sjoerd Euser *et al.* (2021), with 20-39 year old males being the most affected group. Among those who were impacted, men outnumbered women. This contradicts the findings of our study in terms of gender and age. According to Zijian *et al.(*2020) the investigation covered 72,314 patient records, which included confirmed instances, suspected cases, clinically diagnosed cases (only in Hubei Province), clinically diagnosed cases, and asymptomatic cases. The majority of confirmed cases (86.6%) were between the ages of 30 and 79, were found in Hubei (74.7%), and were classified as moderate (80.9 percent). This corresponds to the age range of people infected with SARS in this study.

Table 4-1: Criteria and Demographic Distribution of SARS – COV 2 infected patients and vaccinated people.

Parameters		Patients (N: 40)	χ^2 P(value) Patient	Vaccinated (N : 40)	χ^2 P(value) Vaccinated
Age	30-50 y	8(20%)	14.40* (0.0001)	19(47.5%)	0.1000 (0.7518)
	51-71 y	32(80%)		21(52.5%)	
Gender	Male	19 (47.5%)	0.1000 (0.7518)	20(50%)	0.000 (1.00)
	Female	21(52.5%)		20(50%)	
(P≤ 0.05)					

Table 4-2: Demographic Distribution of SARS – COV 2 between infected patients and vaccinated people according to age and gender.

Parameters		Patients (N: 40)	Vaccinated (N : 40)	χ^2 P(value)
Age	30-50 y	8(20%)	19(47.5%)	4.4815* (0.0343)
	51-71 y	32(80%)	21(52.5%)	2.2830 (0.1308)
Gender	Male	19(47.5%)	20(50%)	0.0250 (0.8728)
	Female	21(52.5%)	20(50%)	0.0244 (0.8759)
(P≤ 0.05)				

4.2 Study the polymorphism of *IL-6* by ARMS-PCR technique :

SARSCoV2 causes the secretion of many cytokines, including *IL-6*, by activating the innate and adaptive immune systems. Many people with severe COVID19 (CRS), SARSCoV2, and polymorphisms in the *IL-6* gene were connected to specific viral infections, such as influenza virus, hepatitis C (HCV), and hepatitis B virus. This results in a systemic inflammatory reaction known as cytokine release syndrome (HBV) (Zhang *et al.*,2020) .

Through the results of the study, genotypes were defined based on the visualization of various band patterns. Depending on the product size of primers of ARM-PCR (302bp,206bp,152bp) a section of *IL-6* gene and GG is wild type ,while CC is mutant type (Figure 4-1) .

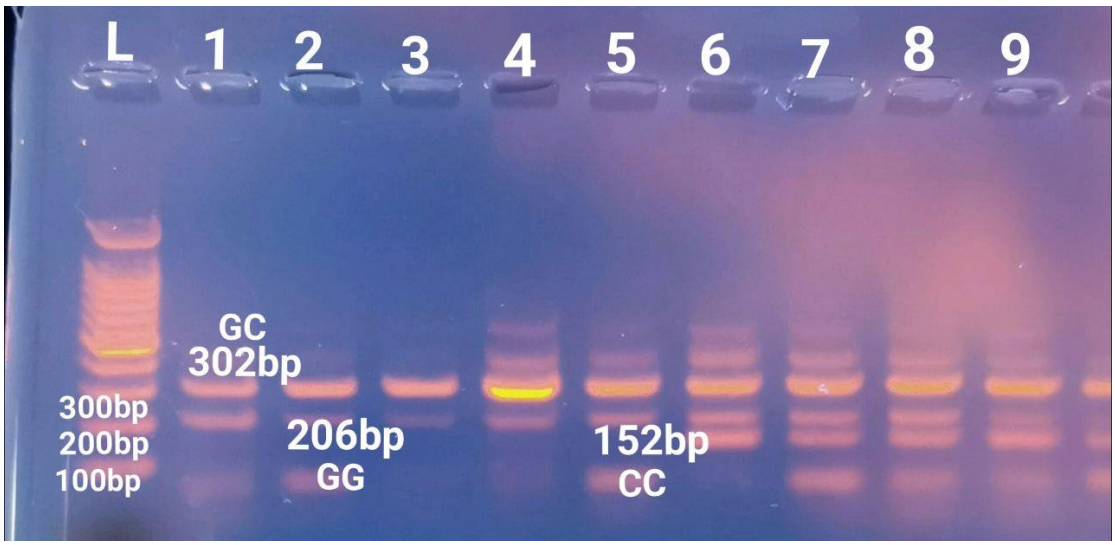


Figure 4-1: Gel electrophoresis image of monoplex PCR amplified of *IL-6* (302bp fragment) by ARMS- PCR technique in 1.5% agarose gel, 100bp DNA ladder. At 100 V .for 45mim. visualized under U.V light after staining with 1µl of ethidium bromide at the concentration of 0.5mg/ml.

The genotype and allele frequencies for *IL6* are presented in Table (4-3) which showed the recurrence of genotype patterns for vaccinated people CC, GG, and GC were 2, 25, and 13 respectively, and allele frequencies C, and G were 0.2125, and 0.7875 respectively, and odd ratio 34.82 . Furthermore, From the results, genotype GG, and the allele G was the highest frequency and genotype CC and the allele C was the less frequency. For infected people CC, GG, and GC were 1, 22, and 17 respectively, allele frequencies C, G were 0.2375, and 0.7625 respectively, and odd ratio 0.947 . Furthermore, From the results, genotype GG, and the allele G was the highest frequency and genotype CC and the allele C was the less frequency as the shown in the results, there is a high significant variance in the rates of homozygote and heterozygote for vaccinated people and infected people .

Table (4-3): Genotype and allele frequencies for *IL-6*.

Group	Genotypes	No.	Genotype frequencies (%)	Allelic frequencies(%)		Odd Ratio	χ^2 (P-value)
				C	G		
Vaccinated (40)	CC	2	5	0.2125	0.7875	34.82	**0.000**
	GC	13	32.5				
	GG	25	26.5				
Infected (40)	CC	1	2.5	0.2375	0.7625	0.947	**0.100**
	GC	17	42.5				
	GG	22	55				

These results agreed with the findings of the WHO, which indicated Population diversities of *IL-6* gene polymorphisms at rs1800796/ rs1800795 loci showed that the populations of India, Mexico,

Turkey, Brazil, Russia, Italy, South Africa, Netherland, Greece frequently have the GG genotype while the populations of China, Spain , Sweden, Poland, Germany, and the UK frequently have GC genotype .Only the Japanese population frequently showed the CC genotype for rs1800796 polymorphism(WHO, 2020). And these results agreed with the findings of (Falahi *et al*., 2022) indicated there were no appreciable variations in the genotype or allele distribution of a few selected SNPs in the promoter region of the *IL-6* gene between patients with severe COVID-19 and patients with mild COVID-19.Another local Study by Iman S.H.(2022) explained that G allele may represent a significant risk factor for COVID-19 in the Iraqi population and there is an association between *IL-6*-174 G/C polymorphism and COVID-19 patients.

4.2.1 Estimation of the IL-6 Concentration By ELISA Technique :

The findings of the ELISA test revealed a rise in interleukin-6 levels in infected samples showed the Concentration level Mean ± SD (36.36 ± 13.74) when compared to vaccinated samples showed the Mean ± SD (11.30 ± 2.26) as indicated in the table below (4-4)

Table (4-4): Interleukin-6 Concentration in Covid patients and Vaccinated person .

Group Statistics	Variables	N	Mean	Std. Deviation	P .Value
ELISA	Vaccinated	40	11.30	2 . 26	0.000
	Infected	40	36.36	13 .74	

This is consistent with the findings of studies conducted by Chen *et al.* 2020; Del Valle *et al.* 2020; Zhang *et al.* (2020) and Hopfer *et al.*, 2021). They discovered an increase in *IL-6* and proposed that IL-6 could be an independent predictor of COVID-19 disease severity. Furthermore , Merza *et al.* (2021) indicated that, the changes in serum cytokines with SARS-CoV-2 indicated the host's immune responses against the coronavirus inflammation seem to be different from what has been seen with other viral pathogens, where the interleukin-1, IL-6 ,IL10 ,IL4 and tumor necrosis factor-alpha (TNF-) serum concentrations were determined using enzyme-linked immunosorbent assays (ELISA), the concentrations of IL-1 and TNF- were not substantially different across groups. However, the level of IL-6 was much higher. When compared to control and recovered groups, it was higher in intermediate COVID-19 and severe COVID-19 cases, demonstrating that it is an independent predictor in the coronavirus disease , and the serum concentration of IL-6 might be considered as a reflective sign of the COVID-19 severity, besides, these findings indicate different immune regulatory events during and after SARS-CoV-2 infection, which may contribute to our understanding of the pathogenesis of this disease (Merza *et al.*,2021) .

In addition, when the polymorphism was compared to the ELISA test of the *IL-6*, the mean of Concentration the GG samples of infected patients are higher (38.6862) than those of the vaccinated people the mean of Concentration the GG (12.0169) and the mean of Concentration the CC samples of infected patients are higher (28.5232) than those of the vaccinated people the mean of Concentration the CC (8.6498), and the mean of Concentration the GC samples for the infected are higher (31.9087) than for the vaccinated people the mean of Concentration (10.3311) . As seen in table (4-5). This is consistent with

the findings of a research by Kerget and Kerget (2021), which found that people with the GG genotype had greater serum IL-6 levels than those with the GC genotype.

Table (4-5): Distribution of IL-6 levels according to its gene polymorphism.

Genotypes	Infected (40) sample		Vaccinated (40) sample	
	N. (%)	Mean Conc.	N. (%)	Mean Conc.
GG	22 (55%)	38.6862	25 (62.5%)	12.0169
CC	1 (2.5%)	28.5232	2 (5%)	8.6498
GC	17 (42.5%)	31.9087	13 (32.5%)	10.3311
Conc. :Concentration				

4.3 Cluster of differentiation 147 (*CD147*):

Cluster of differentiation 147 (*CD147*) has been identified as the binding receptor for the viral S protein, with functional importance in viral entry, among the important claims for the existence of another receptor, mediating severe acute respiratory syndrome corona virus-2 (SARS-CoV-2) infection (Chan *et al.*, 2016 and Chu *et al.*, 2018). Cluster of differentiation 147 (*CD147*), also known as basigin or

extracellular matrix metalloproteinase inducer (EMMPRIN), is a transmembrane protein that plays a role in tumor formation, plasmodium invasion, and bacterial or viral infection (Lu *et al.*, 2018). As a result of the important function of *CD147* in mediating SARS-CoV-2 , infection and the antiviral impact of *CD147* antagonist peptide-9, multiple studies have been published (Chen *et al.*, 2005). Furthermore, in the case of middle-aged and elderly patients, the increased sensitivity to failing respiratory systems and inadequate detection worsens the situation and SARS-CoV-2 also causes cardiovascular damage, which is mediated by *CD147* (Gorbalenya *et al.*, 2020).

Genotypes were defined based on the visualization of various band patterns. Using the primers used for amplification, the PCR-derived products belonging to Different designs were sequenced in a unique way.

Using Bio Edit Sequence Alignment Editor Software Version 7.1, the sequencing results of various PCR products were edited, aligned, and assessed as long as they matched the appropriate sequences in the reference database (DNA STAR, Madison, WI, USA). Each sequenced sample's detected differences were numbered in PCR amplicons and their matching positions within the referring genome. The PCR amplified sequence of *CD147* for sequence homology searches in public databases, the query gene was run through NCBI's nucleotide blast (http://blast.ncbi.nlm.nih.gov./blast/Blast.cgi).In this study, *CD147* gene was studied for the Homo sapiens. PCR was utilized to boost a 229bp a section of *CD147* genes of the part from exons 7 and 8, as well as the intervening intron (Figure 4-2).

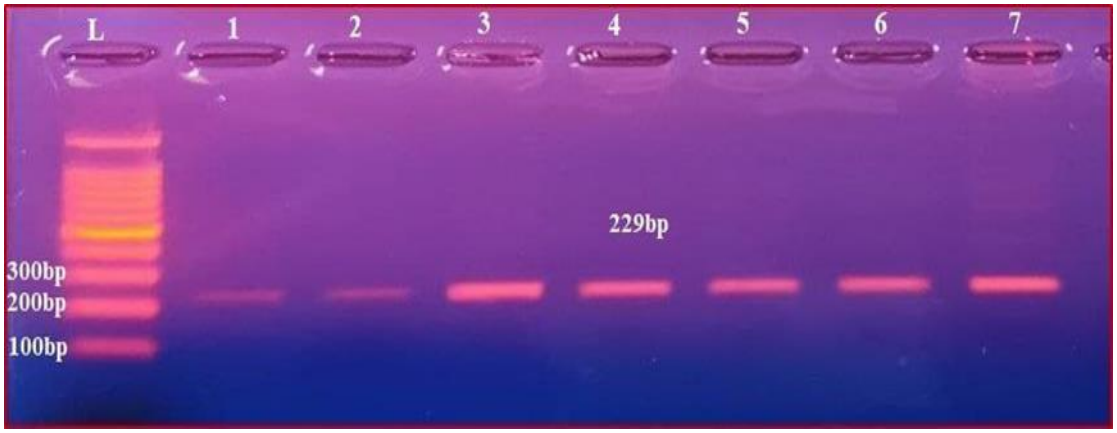


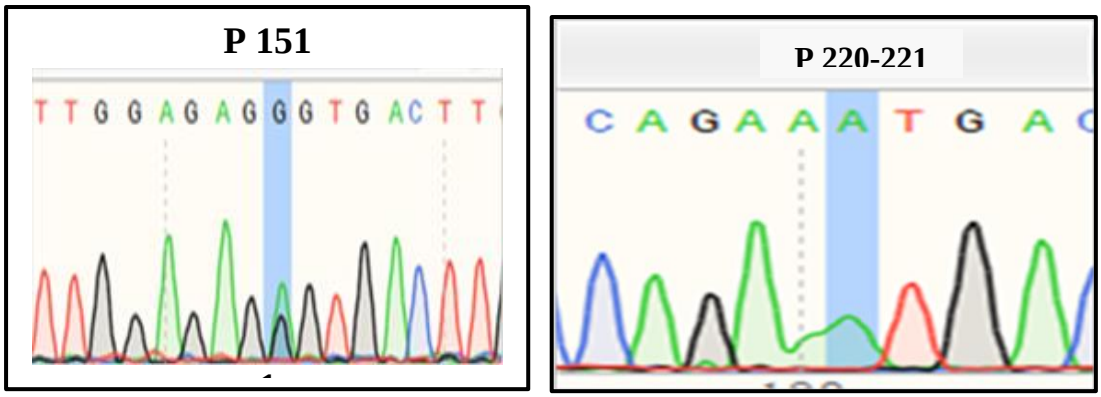
Figure 4-2: Gel electrophoresis image of monoplex PCR amplified *CD147* (229 bp fragment) by conventional PCR in 1.5% agarose gel, 100 bp DNA ladder. at 100 V. for 45 min. visualized under U.V light after staining with 1µl of ethidium bromide at the concentration of 0.5mg/ml .

The current study comprised twelve samples (four sample of vaccinated and eight sample of patients) from this locus that had previously been demonstrated to amplify *CD147* gene sequences in human chromosome 19. After running these PCR amplicons through NCBI blast (https://blast.ncbi.nlm.nih.gov/Blast.cgi), the sequencing reactions revealed the exact identity. The estimated locations as well as features a obtained by comparing the observed DNA sequences of these local samples with the returned DNA sequences, PCR fragments were found(Gen Bank acc.no. NG-007468.1). (Index 1) .

The alignment results of the (229 bp) of *CD147* gene samples revealed the presence of several SNPs that were variably distributed in the investigated human samples in comparison with their corresponding referring DNA sequences (Figure 4-3) (Index 2, 3).

Figure 4-3: Multiple sequence alignment of *CD147* of the intron7 and exon 8 with Ref. Seq. from NCBI. Substation and insertion mutation highlighted.(S1-S12) indicated to selected sample of *CD147*. The program used way BLASTN.

Detection of substitution and insertion SNPs sequencing chromatograms, additionally their extensive annotation's, were reported, as well as the observed chromatogram detail's SNPs were displayed based on their positions in the PCR amplicons. (Figure 4-4).



A>G **A ins.**

Figure 4-4: shows the substitution pattern mutations found in the targeted target's DNA chromatogram 229 bp amplicons in the human *CD147* gene. The sites of the detected substitution and insertion mutations in the PCR products were highlighted. S1-S12 refer to the studied no. 1 to no. 12 samples. The symbol">" refers to the mutation event, while the phrase "ins" refers to insertion mutation.

To summarize all of the findings from the 229 bp fragments that were sequenced in the NCBI reference sequences, the specific sites of the detected changes were given to sample vaccinated people showed to the (4) substation mutation (A>G) of the P (151) and (3) insertion mutation (A ins.) of the P (220-221) and the infected people samples showed to the (4) substation mutation (A>G) of the P (151) and (2) insertion mutation (A ins.) of the P (220-221) (Table 4-6).

Table 4-6: The observed a pattern SNPs in the 229 bp amplicons of *Human* (vaccinated and infected population) in comparison with their corresponding Referring sequences from the NCBI (Gen Bank accession number NG - 007468.1). The letter "S" stands for sample number.

Population	Sample no.	Native	Allele	Position
Vaccinated	4	A	G	151
	3	A ins.	-	220-221
Infected	4	A	G	151
	2	A ins.	-	220-221

The observed variants had demonstrated two in the examined samples, distinct distributions in terms of the targeted 229 bp amplicons, happened in the substation (A>G) Of the seventht interon and insertion (Ains.) of the eighth exon .

This genetic difference is present in the non-coding DNA (the seventh intron) at site 151 and the change was from UCA Serine (AGT) to Proline CCA (GGT). The genetic difference in the eighth exon at the position 220-221 changes the amino acid sequence in this region of the peptide, since this mutation was an insertion mutation and the change was from Tyrosine UAC (ATG) to Leucine UUA (AAT) and thus led to a change in the amino acid sequence causing the amino acid sequence of CD147 protein a replacement effect.

Showed to the sequence identity of the NCBI homolog of the *CD147* gene for local samples versus Ref seq. *CD147* gene (accession number NG-007468.1). A non-conservative missense mutation is a type of missense mutation where the alteration in nucleotide causes the formation of a completely different kind of amino acid in the chain.

Below (Table 4-7), for vaccinated people, the nucleotide polymorphisms and amino acid polymorphisms and type of polymorphism to the missense mutation (GAG > GGG) Glutamic acid > Glycine in p.(151) , the insertion mutation (AAA) Lysine in p.(220-221) for the samples (S.9, S.10, S.11, S .12) , For the injured the nucleotide polymorphism and Amino acid polymorphism and type of polymorphism to the missense mutation (GAG > GGG) Glutamic acid > Glycine in p.(151) ,the insertion mutation (AAA) Lysine in p.(220-221) to the samples (S.1, S.2, S.3, S.4,S.5,S.6,S.7,S.8).As a result, the amino acid sequence of CD147 protein was replaced. See Figures (4-5) and (4-6) .

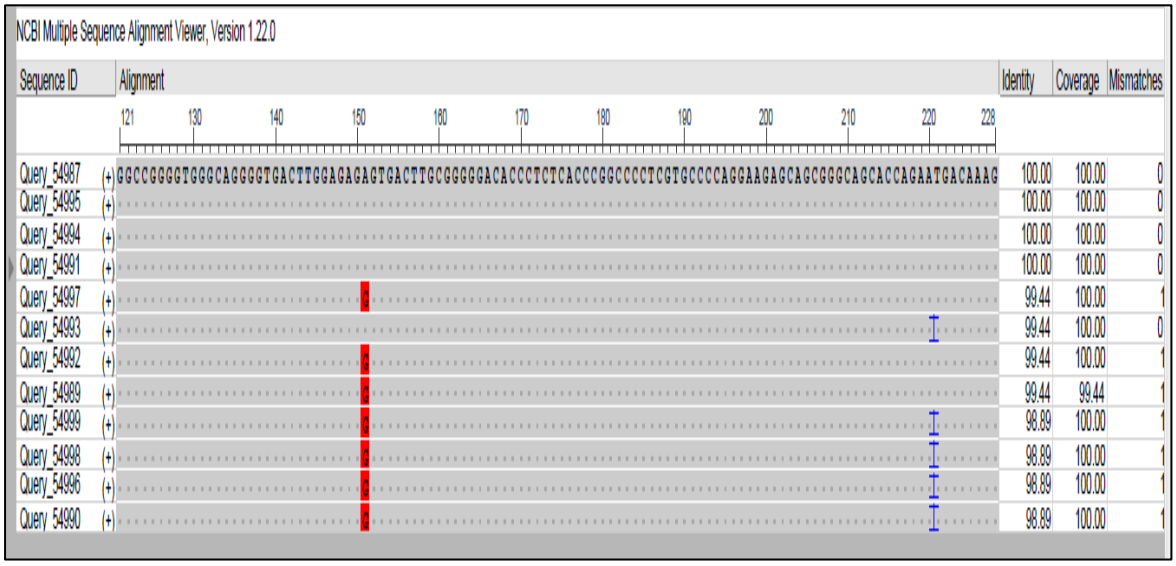


Figure 4-5: NCBI Multiple Sequence Alignment Viewer, Version 1.22.0.

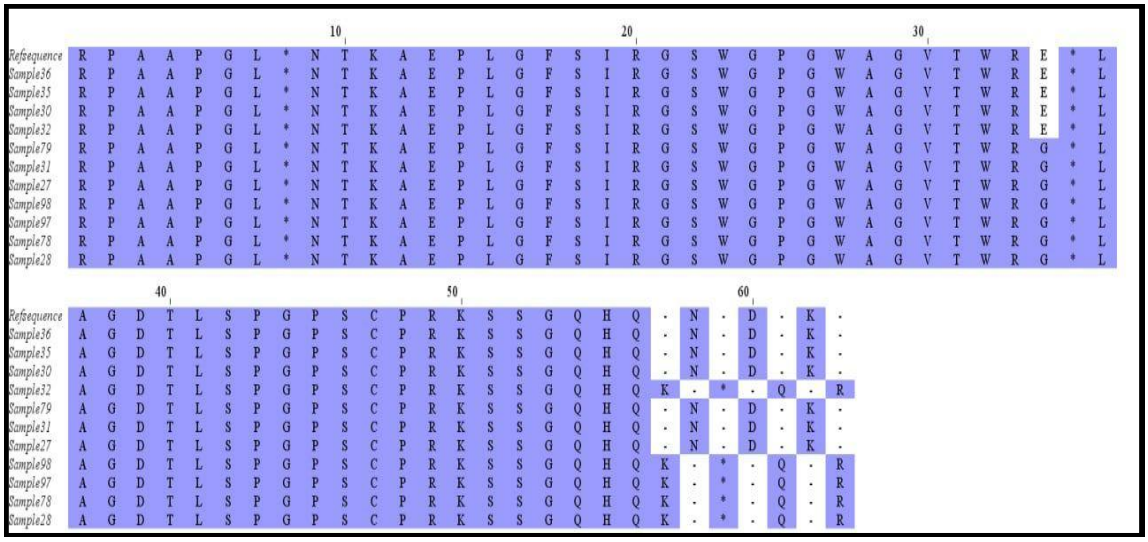


Figure A : (G= Glycine, E= Glutamic acid, K= Lysine).

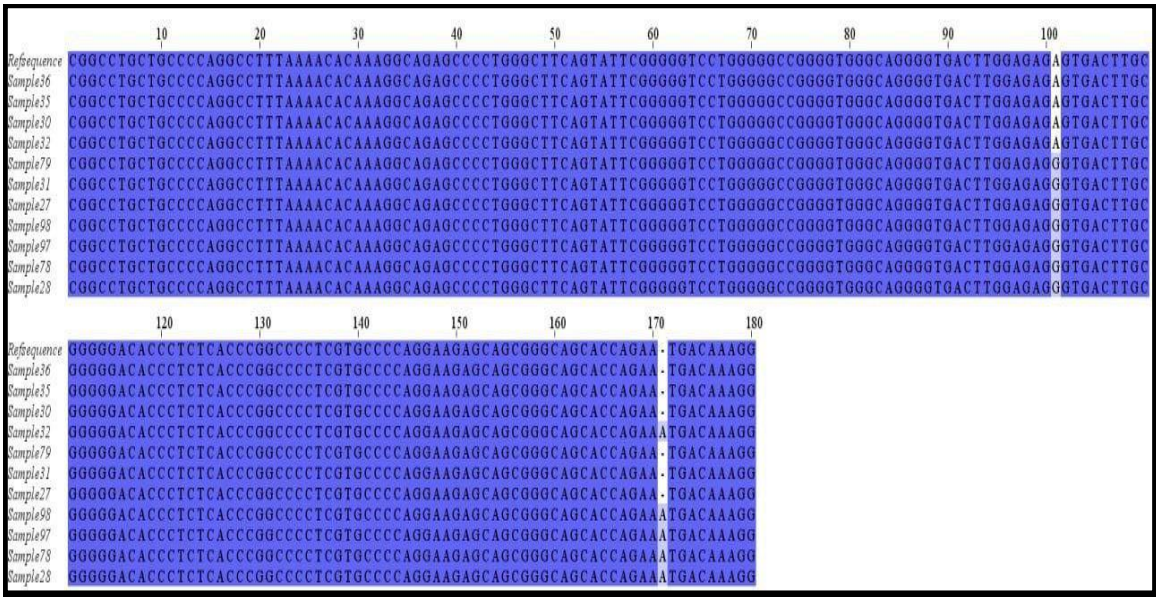


Figure B : (G= Guanine, C= Cytosine, T= Thymine, A= Adenine).

Figure 4-6: Multiple Sequence alignment of *CD147* of local Sample against Ref. Seq. *CD147* gene (accession No. NG_007468.1).By using Jalview Softwere .

Table 4-7: NCBI homology sequence identity of *CD147* gene of local samples against Ref Seq. CD147 gene (accession No. NG_007468.1).

Sample No.	NCBI homology sequence identity			
	*Nucleotide polymorphism	**Amino acid polymorphism	Type of polymorphism	Position of polymorphism
S.1	GAG>GGG	E>G	Missense	151
S.2	GAG>GGG AAA	E>G K	Missense Insertion	151 220-221
S.3	GAG>GGG	E>G	Missense	151
S.4	Non	Non	Non	-
S.5	GAG>GGG	E>G	Missense	151
S.6	AAA	K	Insertion	220-221
S.7	Non	Non	Non	-
S.8	Non	Non	Non	-
S.9	GAG>GGG AAA	E>G K	Missense Insertion	151 220-221
S.10	GAG>GGG	E>G	Missense	151
S.11	GAG>GGG AAA	E>G K	Missense Insertion	151 220-221
S.12	GAG>GGG AAA	E>G K	Missense Insertion	151 220-221

*(G= Guanine, C= Cytosine, T= Thymine, A= Adenine)

**(G= Glycine, E= Glutamic acid, K= Lysine).

The findings support those of William *et al.* (2021), who found that the antigenicity of the SARS-CoV-2 spike protein is changing, and that amino acid changes that affect antibody neutralization, as well as spike amino acid substitutions that affect neutralizing antibodies, are present at significant frequencies in the global virus population . Furthermore, as SARS-CoV-2 mutations contribute to increased viral transmissibility and immune escape, it is identified *CD147* as a universal receptor for SARS-CoV-2 and its variants, and *CD147* antibody exhibits universal inhibition against SARS-CoV-2 and variants, and found that infection was markedly suppressed by *CD147* knockout, as reported by Jiejie *et al.(*2021).

4.4 Study the genetic polymorphisms of *FGA* gene:

The COVID-19 has mostly respiratory manifestations, although it can also harm extra pulmonary systems, such as the heart and systemic vasculature (Huang *et al.*, 2020; Klok *et al.*, 2020 ; Marone and Rinaldi, (2020); and Snell, 2021). Indeed, SARS-CoV-2 infection has been connected to cardiovascular changes (arrhythmias, ischemic heart disease, or cardiomyopathies), which are mostly caused by coagulation irregularities and endothelial damage, which can lead to thrombosis (Alvarado-Moreno *et al.*, 2021 ; Thachil *et al.*, 2020). The *FGA* gene in humans encodes the protein fibrinogen alpha chain. The alpha subunit of the coagulation factor fibrinogen, which is a component of blood clots, is encoded by this gene. Because the encoded preproprotein is proteolytic ally digested by thrombin during the conversion of fibrinogen to fibrin following vascular damage, mutations in this gene are harmful.COVID-19 individuals with severe conditions have afibrinogenemia and renal amyloidosis, which is caused by acute respiratory syndrome (Huang *et al.*,2020) and leads to cytokine storm development Coagulopathy

(Haematology, 2020), which is usually detected among patients and is associated by severe thromboembolic disorders, is one of the primary distinguishing aspects of COVID-19 (Tang *et al.*,2020).

Ten samples have been included in the locus, with 392bp amplicons of the *FGA* locus being designed. These amplicons are located on Chromosome 4 provide instructions for making a protein called the fibrinogen A alpha (Aα) chain, one piece (subunit) of the fibrinogen protein. All amplified *FGA* amplicons were evaluated for specific, and clean bands before being sent to sequencing assays. The sequencing reactions revealed the inveterate identity of the amplified products, through using NCBI blasts to determine their identity .

The sequencing results of the PCR products of different samples were edited, aligned, and analyzed as long as with the respective sequences in the reference database using Bio Edit Sequence Alignment Editor Software Version 7.1 (DNA STAR, Madison, WI, USA). The observed variations in each sequenced sample were numbered in PCR amplicons as well as in its corresponding position within the referring genome. The PCR amplified sequence of *FGA* for sequence homology searches in public databases, the gene was used as a query and subjected to nucleotide blast at NCBI (http://blast.ncbi.nlm.nih.gov/blast/Blast.cgi). In this study, *FGA* gene was studied for the Homo sapiens. PCR was used to amplify a 392 bp segment of the *FGA* genes of the exons 5. (Figure 4-7).

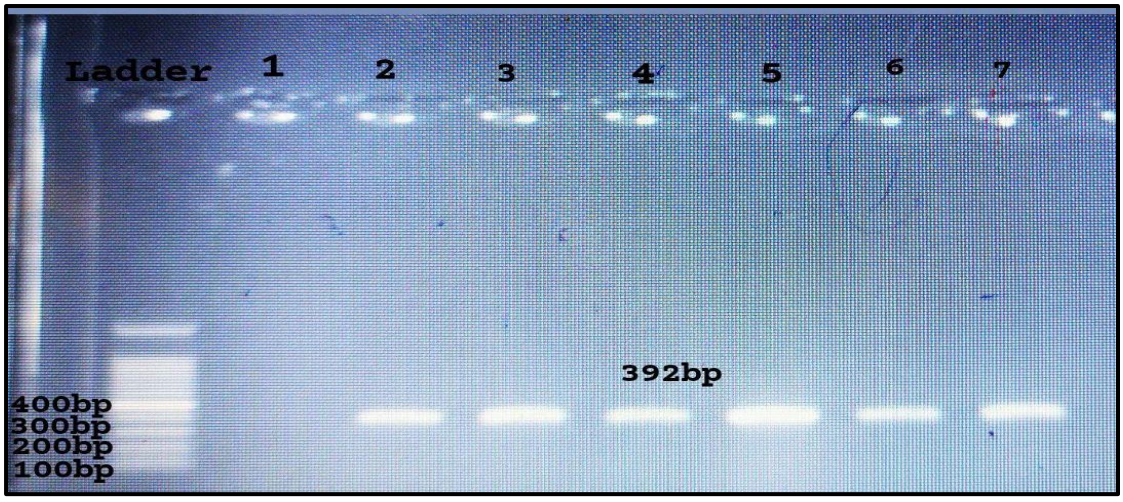


Figure 4-7: Gel electrophoresis image of monoplex PCR amplified of *FGA* (392 bp fragment) by PCR in 1.5% agarose gel, 100 b p DNA ladder. Volt:100 for 45 min. visualized under U.V light after staining with 1µl of ethidium bromide at the concentration of 0.5mg/ml .

The current study comprised ten samples from this locus that had previously been demonstrated to amplify *FGA* gene sequences in human chromosome 4. After performing NCBI BLAST for these PCR amplicons (https://blast.ncbi.nlm.nih.gov/Blast.cgi), the sequencing reactions revealed the exact identity. The approximate locations and other features of the obtained PCR fragments were identified by comparing the observed DNA sequences of these local samples with the retrieved DNA sequences (Gen Bank acc.NC- 000004.12). (Index 4)

Following the placement of the 392bp amplicons' sequences within chromosome no. 4, the sequences of the forward primer of the 392bp amplified amplicons were highlighted in detail (Index 5 , 6).

In contrast to their corresponding reference DNA sequences, the alignment results of the 392 bp samples revealed the presence of little SNPs that were variously distributed in the analyzed human samples. Figure (4-8).

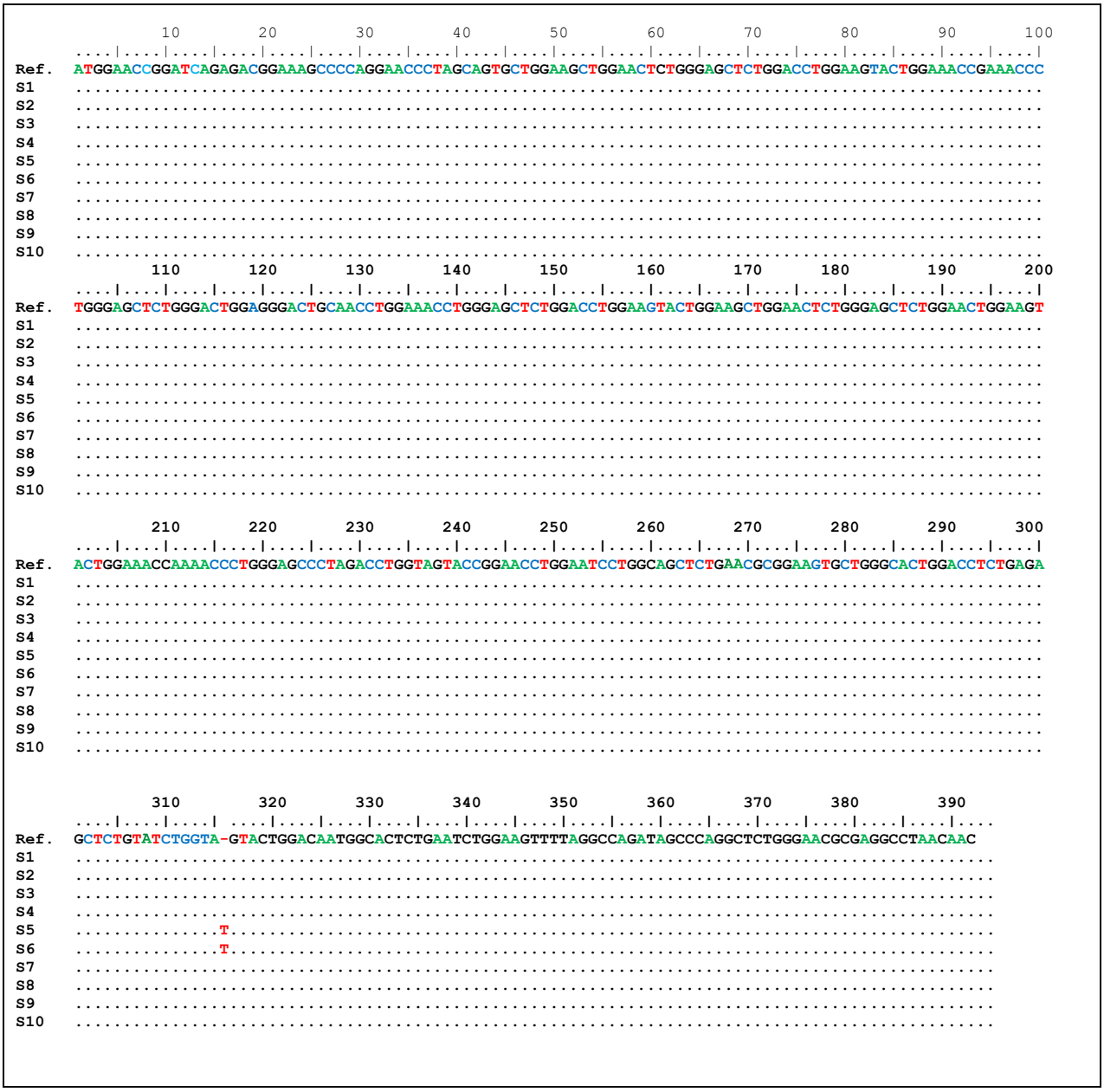


Figure 4-8: DNA sequences alignment of ten genetic groups of human with their corresponding reference sequences of the 392 bp amplicons of the highlighted according to its position in the PCR products. The symbol "ref" refers to the NCBI referring sequence, "S1-S10" refer to (the samples 1 to 6 of infected and the samples 7 to 10 of vaccinated) , respectively.

Detection of insertion SNPs sequencing chromatograms, as well as their extensive annotations, were documented, and the chromatogram details of the observed SNPs were displayed based on their positions in the PCR amplicons. Figure (4-9).

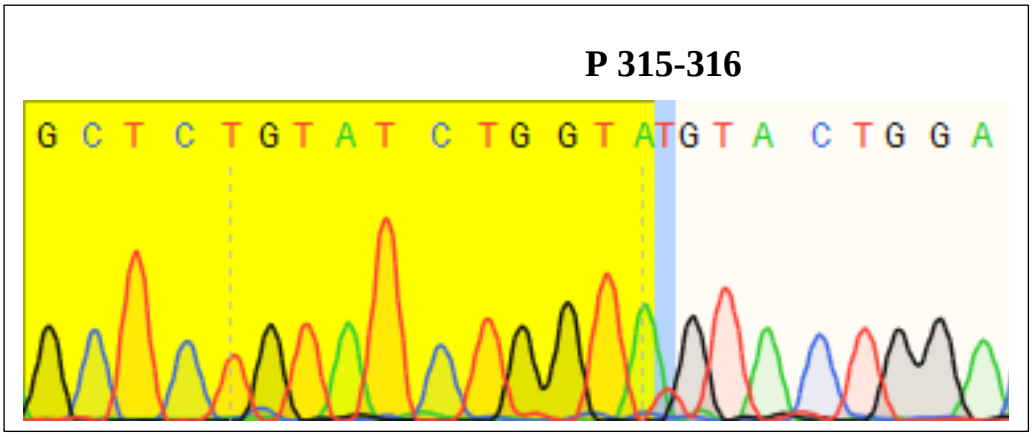


Figure 4-9: The pattern of insertion mutations found in the DNA chromatogram of the targeted human *FGA* gene. The sites of the detected insertion d 392 bp amplicons within the mutations were highlighted according to their positions in the PCR products. S1 – S10 refer to the studied no. 1 to no. 10 samples. The phrase "ins" refers to insertion mutation.

To summarize all of the findings from the 392 bp fragments that were sequenced, in the NCBI reference sequences, the specific sites of the detected changes were given the infected people samples (S5,S6) showed to the (2) insertion (Tins.) of the P (315-316) .

In terms of the targeted 392 bp amplicons, the identified variations had one different distribution in the examined samples.This genetic difference in the fifth exon at position 315-316 changes the amino acid sequence in this region of the peptide, because this SNP is an insertion mutation, and the change was from Histidine CAU (GTA) to Threonine ACA (TGT), resulting a change in the amino acid sequence and causing

the next nucleotid bases to change as a result of the insertion mutation. This mutation was only found in a few samples of sars-cov2 infected patients and did not arise in vaccinated people. However, no study has been published yet that shows a direct link between Fibrinogen alpha chain (*FGA*) and SARS-Cov-2.

5. Conclusion and Recommendations :

5.1 Conclusion:

1- Real time PCR has a better sensitivity technique for identification SARS-CoV-2 nucleic acids or genes, is the most popular and precise approach for detecting SARS-CoV-2 nucleic acids or genes.

2- The Covid-19 infected patients have high levels of *IL-6* comparing with vaccinated person this mean *IL-6* is a reliable indicator of severe illness in COVID-19-infected people.

3- Significant results were seen in *CD147* gene polymorphism between covid patients and vaccinated persons at ($P \leq 0.05$).Tis may be role of *CD147* in mediating SARS-CoV-2 infection as a novel route for SARS-CoV-2 entry.

4- Significant results were seen in *FGA* gene polymorphism between covid patients and vaccinated persons at ($P \leq 0.05$). This may be the reason for blood clots and elevated level D-dimer in covid patients .

5- Sequencing analysis of the gene *CD147* and *FGA* showed the presence of substitution (A>G) and insertion (A ins.) mutations of *CD147* gene and only insertion (T ins.) mutations of *FGA* gene that lead to Change in amino acid in position (151) of (A>G) , P.(220-221) of (A ins.) and P.(315-316) of (T ins.) .

5.2 Recommendations:

1- Investigating another gene polymorphisms and mutation to show their role in COVID-19 development .

2- Estimating the role of another Cytokines markers in the development of COVID-19.

3- Study the large group of Covid patients with different condition, location and Sars -Cov-2 Variants.

4- Study the Spike protein expression and Characterization before and after vaccination.

5- Estimating the vaccinated person comparing with Pfizer ,AstraZeneca and Sino pharm vaccine .

6- Further study to monitor the more critical cases , mortality rate and duration after onset of infection and relationship of proteins .

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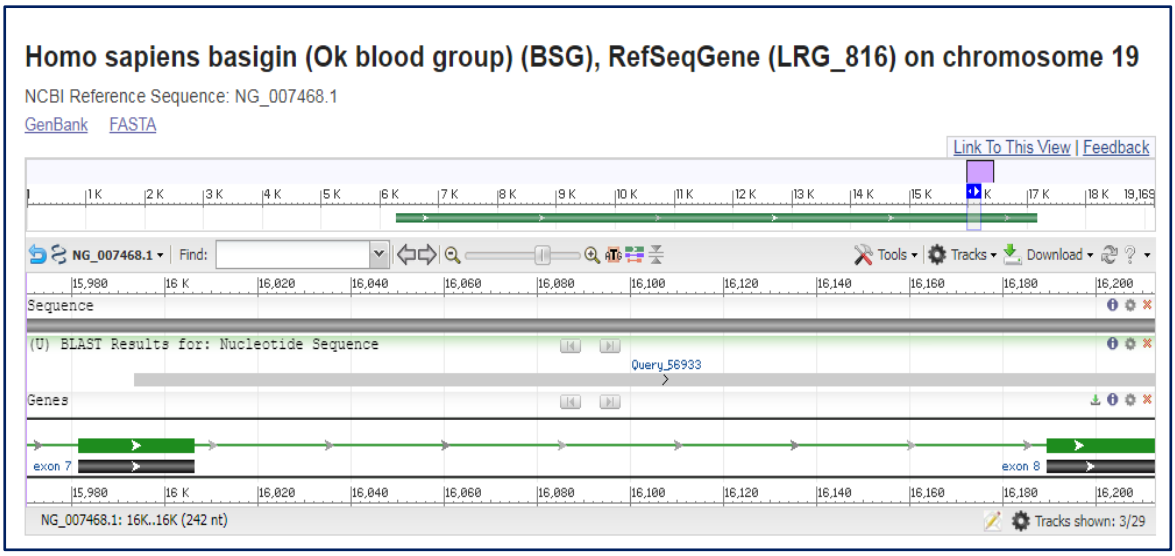
Index :

Index 1: The precise location of the 229 bp amplicon that partially encompassed a region of the genome that was a part of the cost *CD147* gene on chromosome 19 (Gen Bank accession number NG- 007468.1).



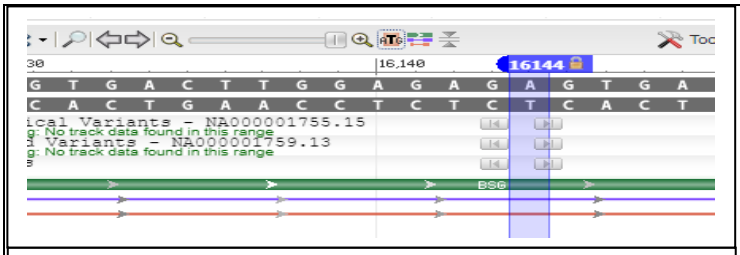
Index 2: The position and length of the 229 bp PCR amplicons used to amplify a portion of the *CD147* gene within chromosome no. 19 (Gen Bank acc. no. NG_007468.1). The grey colored sequences referred to the position of the forward and reverse primers respectively.

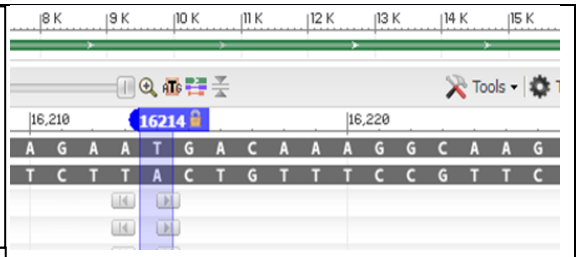
Amplicon	Referring locus sequences (5′ - 3′)	Length
DNA sequences within the *CD147* gene	*GCTCTGCACCCCTGTAAGTTCCAGCTGTGGGGGTCGGGGGTCCCAAGGAGCG GCCTGCTGCCCCAGGCCTTTAAAACACAAAGGCAGAGCCCCTGGGCTTCAGTA TTCGGGGGTCCTGGGGGCCGGGGTGGGCAGGGGTGACTTGGAGAGAGTGACTT GCGGGGGACACCCTCTCACCCGGCCCCTCGTGCCCCAGGAAGAGCAGCGGGCA GCACCAGAATGACAAAGG**	229 bp

* refers to the forward primer sequences.

** refers to the reverse primer sequences.

Index 3: *CD147* SNPs validity on Chromosome 19 sites.





SNPs validity on Chromosome 19 sites :

consensus Identity

[<-1.gene CD147 ….

[<-2.gene CD147 ….

[<-3.gene CD147 ….

[<-4.gene CD147 ….

[<-5.gene CD147 ….

[<-6.gene CD147 ….

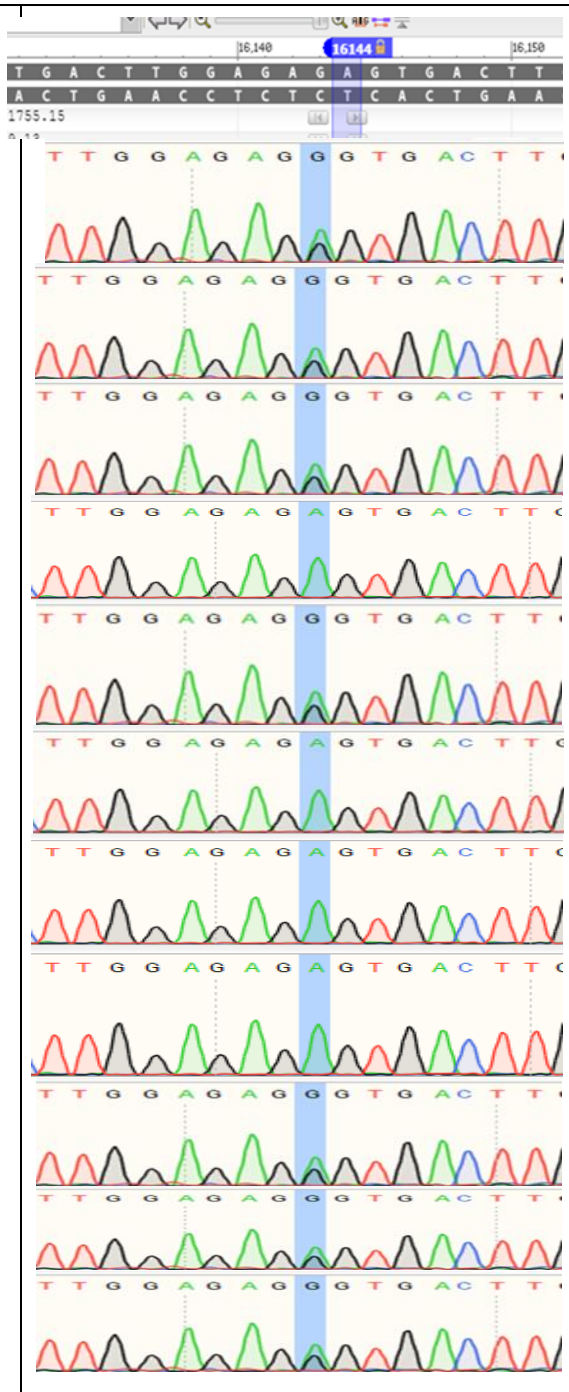
[<-7.gene CD147 ….

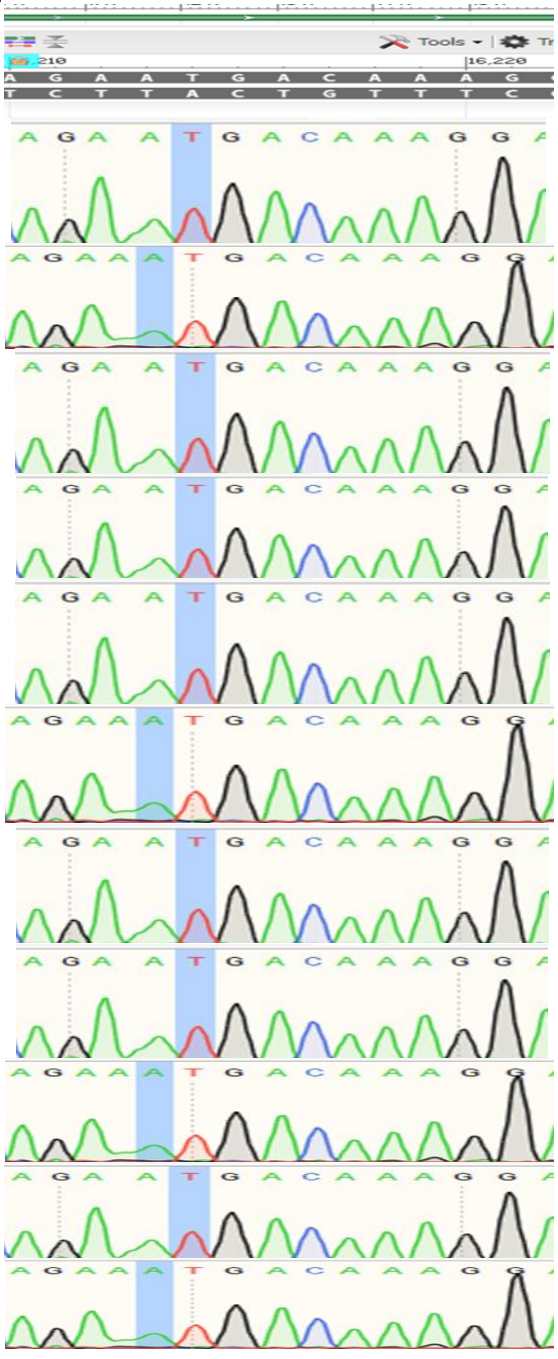
[<-8.gene CD147 ….

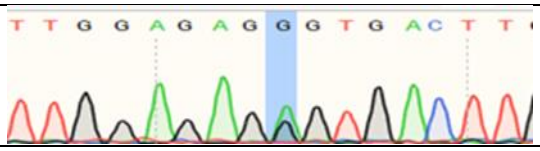
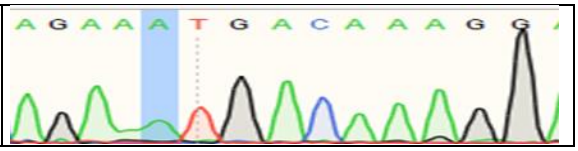
[<-9.gene CD147 ….

[<-10.gene CD147 ….

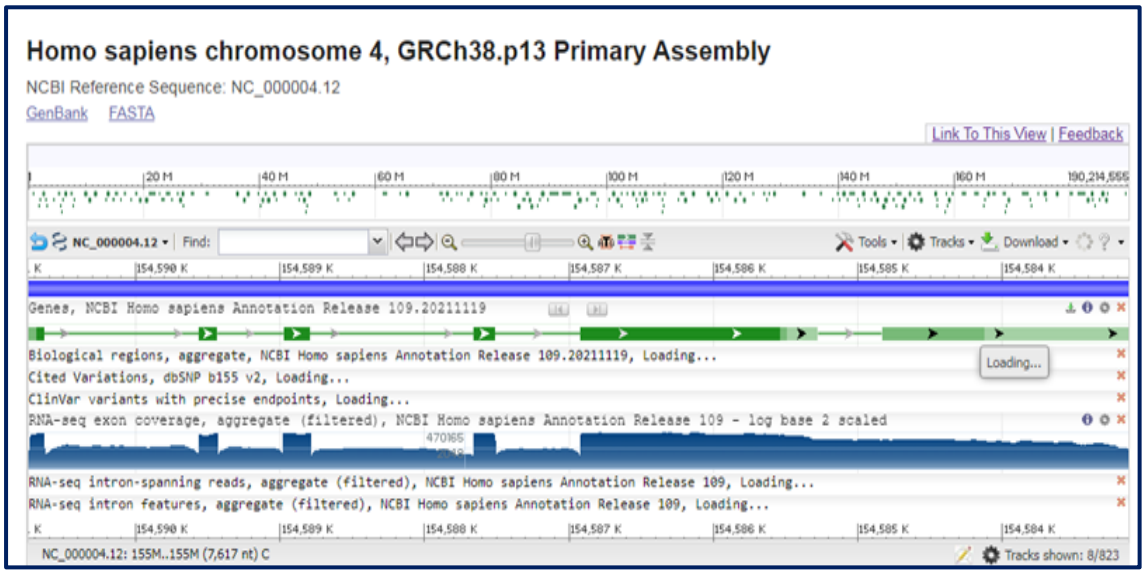
[<-11.gene CD147 ….





[<-12.gene CD147 ….		

Index 4: The precise location of the 392 bp amplicon that partially encompassed a region of the genome that was covered a portion the *FGA* gene within chromosome no. 4 (Gen Bank acc. no. NC-000004.12).



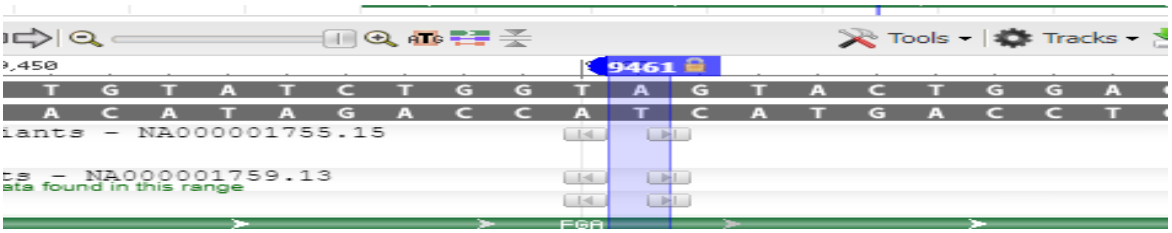
Index 5 : The location and length of the 392 bp PCR amplicons that were used to amplify a part of the *FGA* gene on chromosome no. 4 (Gen Bank acc. no. NC-000004.12). The forward and reverse primer positions were represented by the grey colored sequences .

Amplicon	Referring locus sequences (5′ - 3′)	Length
DNA sequences within the *FGA* gene	*ATGGAACCGGATCAGAGACGGAAAGCCCCAGGAACCCTAGCAGTG CTGGAAGCTGGAACTCTGGGAGCTCTGGACCTGGAAGTACTGGAAA CCGAAACCCTGGGAGCTCTGGGACTGGAGGGACTGCAACCTGGAAA CCTGGGAGCTCTGGACCTGGAAGTACTGGAAGCTGGAACTCTGGGA GCTCTGGAACTGGAAGTACTGGAAACCAAAACCCTGGGAGCCCTAG ACCTGGTAGTACCGGAACCTGGAATCCTGGCAGCTCTGAACGCGGA AGTGCTGGGCACTGGACCTCTGAGAGCTCTGTATCTGGTAGTACTGG ACAATGGCACTCTGAATCTGGAAGTTTTAGGCCAGATAGCCCAGGCT CTGGGAACGCGAGGCCTAACAAC**	392 bp

* refers to the forward primer sequences.

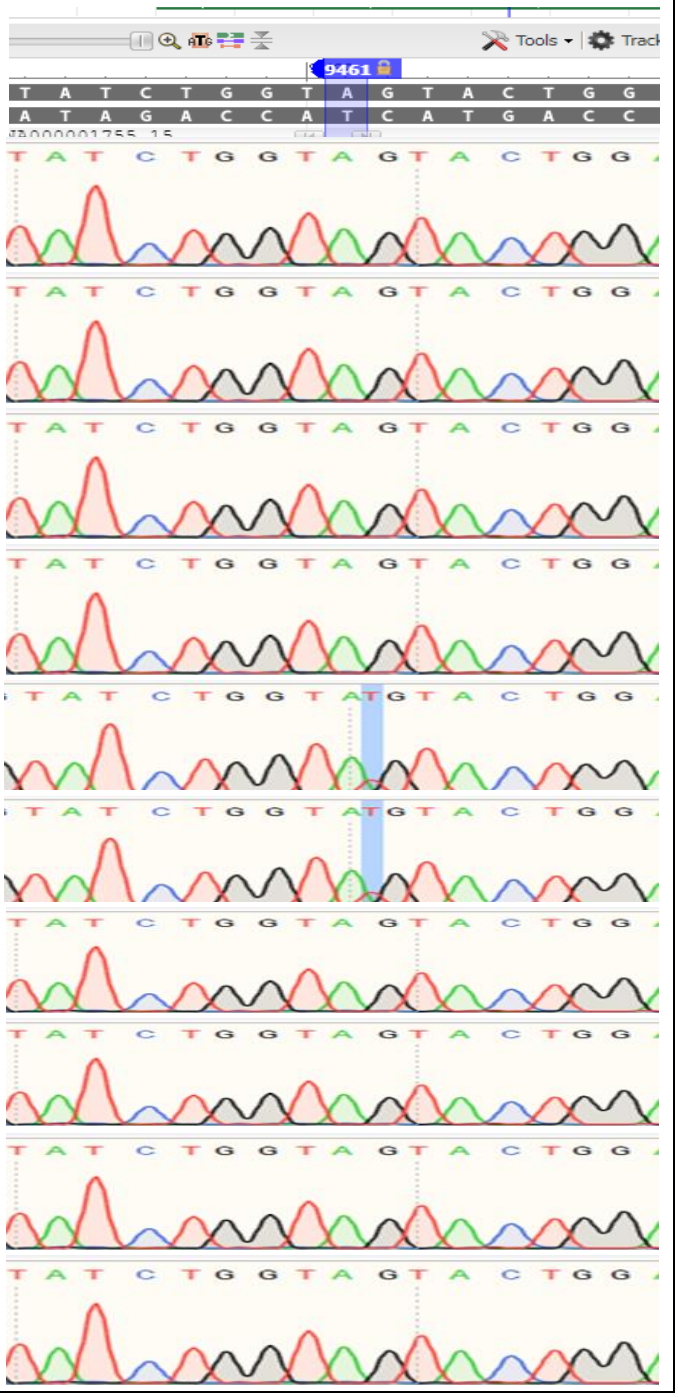
** refers to the reverse primer sequences.

Index 6: *FGA* SNPs validity on Chromosome 4 sites.



SNPs validity on Chromosome 4 sites :

Consensus Identity



[<- 1.gene FGA…

[<- 2.gene FGA…

[<- 3.gene FGA…

[<- 4.gene FGA…

[<- 5.gene FGA …

[<- 6.gene FGA…

[<- 7.gene FGA…

[<- 8.gene FGA…

[<- 9.gene FGA…

[<- 10.gene FGA…

الخلاصة:

ان سبب جائحة COVID- 19 هوفيروس متلازمة الجهاز التنفسي الحادة الوخيمة Coronavirus-2 وقد أظهر تباينًا جغرافيًا كبيرًا في التكرار والوفيات. تم جمع 40 عينة دم من مرضى كوفيد -19 في مستشفى مرجان التعليمي ومستشفى الحلة التعليمي ومستشفى الإمام الصادق ومستشفى المحاويل، وأربعين عينة دم من الاشخاص الملقحين. أجريت الدراسة في مختبرات قسم الأحياء / بجامعة بابل / كلية العلوم للبنات للفترة من تشرين الأول 2021 إلى شباط 2022 بهدف التعرف على تعدد الأشكال الجيني (Polymorphism) لجين *IL-6* باستخدام تقنية نظام الطفرة الحرارية التضخمية (ARMS-PCR) و تحليل الجين *CD147*، وجين *FGA* بتقنية تحليل التسلسل الجيني (Sequencing).

اغلب عينات المرضى في هذه الدراسة كانت من الأناث ، مما يشير إلى أن الإناث أكثر عرضة من الرجال عرضة للاصابة ودخول المستشفى. غالبية المرضى (52.5٪) من الإناث ، بينما (47.5٪) من الذكور ، وتوجد فروق ذات دلالة إحصائية في هذه الفئة للمرضى (0.7518) ، بينما غالبية الملقحين متساوون في كل من الإناث والذكور (50٪)، وتوجد فروق إحصائية في هذه الفئة للملقحين (1.00). في كلا الجنسين ، كان النطاق العمري للبالغين(30-50) سنة بنسبة (20٪)، بينما (50-71) سنة بنسبة (80٪) على التوالي للمرضى ، نلاحظ أيضًا أن هناك اختلافات معنوية تحت مستوى ($P \leq 0.05$) في هذه الفئات العمرية (0.0001) ، بينما كان عمر البالغين (30-50) سنة بنسبة (47.5٪) ، بينما (51-71) سنة بنسبة (52.5٪) على التوالي للملقحين وهناك فروق ذات دلالة إحصائية في هذه الفئة للملقحين ، مع وجود اختلاف في توزيع الحالات يعطي نسبة أعلى من غيره. ونلاحظ فرق معنوي تحت مستوى ($P \leq 0.05$) بين الملقحين والمرضى للفئة العمرية (30-50) ، بينما الفروق ذات دلالة إحصائية بين الملقحين والمرضى للفئة العمرية (51-71). توجد فروق ذات دلالة إحصائية بين الملقحين والمصابين بين الرجال والنساء.

علاوة على ذلك ، فإن دراسة الأشكال الجينية لـ *IL-6* (باستخدام ARMS-PCR) في مجموعتين دراسيتين (المرضى والملقحين) ، إذ أظهرت نتائج الدراسة ان النمط الجيني GG من النوع البري ، بينما CC هو نوع متحور ، وترددات الأليل C و G كانت 0.2125 و 0.7875 على التوالي بنسبة ارجحية 34.82.علاوة على ذلك بينت النتائج ان النمط الجيني GG والأليل G هو أعلى تكراراً والنمط االجيني CC و الأليل C كان أقل تكراراً ، أما للأشخاص

المصابين كانت CC و GG و GC (1 ، 22 ، 17)على التوالي ، وكان تكرار الأليل C و G (0.2375 , 0.7625)على التوالي بنسبة أرجحية 0.947، بالإضافة الى ذلك كان النمط الجيني GG و الأليل G هو الأعلى تكراراً والنمط الجيني CC والأليل C الأقل تكرارًا ، أظهرت النتائج ان هناك تباين كبير في معدلات الزيجوت متجانسة الزيجوت ومتغايرة الزيجوت للأشخاص الملقحين والمصابين.

كشفت نتائج اختبار الاليزا عن ارتفاع في مستويات *IL-6* في العينات المصابة وان متوسط التركيز ± SD (13.74 ± 36.36) بالمقارنة مع العينات الملقحة ± SD (11.30) ± 2.26 .

تضمنت هذه الدراسة أيضًا تحديد تعدد الأشكال الجيني للجين *CD147* وفقًا لتقنية تحليل التسلسل الجيني لحجم القطعة (229) زوج قاعدي بالمقارنة مع التسلسل المرجعي لـ NCBI ، وأظهرت أن هناك توزيعين مختلفين في العينات ، وجود طفرات استبدال في الاكسون الثامن في الموقع (151) ، ووجود طفرات إدخال في الإنترون السابع في الموقع (220-221) في كل من العينات الملقحة والمصابة ، وأظهرت هوية التسلسل المتماثل NCBI للجين *CD147* هناك توزيعان مختلفان في العينات ، هما طفرات مغلوطة في الموقع (151) ، وطفرات إدخال في الموقع (220-221). قد يكون بروتين الغشاء لـ *CD147* طريقًا جديدًا لدخول SARS-CoV-2.

كشفت دراسة التسلسل للجزء الذي تم فحصه عن (392) زوج قاعدي من جين *FGA* وجود طفرة إدخال في الإكسون الخامس في الموضع (315-316) مما أدى إلى تغيير في تسلسل الأحماض الأمينية ، وقد يكون هذا هو سبب تجلط الدم وارتفاع مستوى D-dimer في مرضى كوفيد.

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة بابل
كلية العلوم للبنات
قسم علوم الحياة

تعدد الاشكال الوراثية لبعض الجينات للملقحين والمصابين بكوفيد 19 في محافظة بابل

رسالة مقدمة الى
مجلس كلية العلوم للبنات – جامعة بابل
كجزء من متطلبات نيل درجة الماجستير في العلوم/علوم الحياة

من قبل

مها عادل حسين راضي المرشدي

بإشراف

الأستاذ الدكتور حسنين خليل ابراهيم شريف

2022 م 1444 هـ