Ministry of Higher Education and Scientific Research
University of Babylon
College of Medicine
Department of Medical Microbiology

Study of some gene polymorphism and related biomarkers in patients with SARS-COV2 in Babylon province

A Thesis

Submitted to Council of the College of Medicine University of Babylon in a Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Science/ Medical Microbiology

By

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2022 A.D. **1444 A.H.**

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

﴿نَرْفَعُ دَرَجَاتٍ مَنْ نَشَاءُ وَفَوْقَ

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Dedication

To the bearer of God's message .....Mohammad

To the leaders .....my twelve imams

To the motivators.....my father

To the Compassionate heart.....my mother

To whom depends on them in my affairs...my sister

To the love.....my husband

To the my heart.....my son

Shahad 2022

Acknowledgements

Praise to Allah who gave me power & motivation to finish this work. It is pleasure to express my deep appreciation to my supervisors **Dr. Ifad Kerim Al-shibly** and **Dr.Ahmed Hussein Jasim** for their invaluable advice, assistance, cooperation, and support throughout the course of preparing my thesis. Faithful thanks to the dean of the College of Medicine University of Babylon **Dr. Mohend A. N. Al-Shalah** and the Head of Department of Medical Microbiology **Dr. Hayam Khalis Al-Masoudi** for their help and great cooperation.

I would like to thank all my lecturers throughout the study.

I would like to thank **Dr. Shakir H. Mohammed Al-Alwany** & **Dr. Rasha Kadhim Mahdi** for their scientific guidance.

Also, I would like to thank for their cooperation during the sample collecting time.

I should never forget to extend my gratefulness and appreciation to the staff of Intensive care Unit of Marjan Teaching Hospital for their kind cooperation.

Lastly, I address my thanks to the participant patients and my friends who cooperated with me during this study.

Shahad 2022

Summary

This is a case-control study design conducted for a period from February, 2021 to October 2021 to evaluate the role of some selected serological, hematological, and genetic factors among patients with SARA-COV2. In this study, one hundred-seventy subjects were enrolled as two comparison case-control groups, the case group included 70 patients suffered from SARS-COV2, all were selected from those who were admitted to the Intensive Care Unite(ICU) , and were diagnosed by a specialist physician with sever acute respiratory syndrome due to SARS-COV2 documented by Real-Time Polymerase Chain Reaction(RT-PCR) beside other clinical and laboratory criteria at Marjan Medical City.

The control group in this study included 100 apparently healthy subjects all were confirmed free from covid-19 by Rapid Test (SARS-COV2 antibody test negative).

The demographic characteristics of patients and control subjects in this study showed that the mean age of patients was 47.17±11.72 and that of control subjects was 31.77±8.84 years. The frequency distribution of patients and control subjects according to age groups found that the highest percentage of patients with SARS-COV2{29 (41.4%)} fall in the age group (≥ 50 years), while the lowest percentage was 2 (2.9%) of them those aged less than 30 years. Regarding control' group included 47 (47.0%) of the age group <30 year, 32 (32.0%) for 30-39 year, 14(14.0%) for 40-49 year, and only 7(7.0%) for ≥ 50 year .

Regarding sex distribution in the current study, patients' group included 38 (54.3 %) males and 32 (45.7 %) females, whereas, control group included 33 (33.0 %) males and 67 (67.0 %) females. Results of the Univariate Logistic regression analysis to identify variables independently associated with

hospitalization for SARS-COV2 found that those in who age group (≥ 50 year) were significantly more likely to be hospitalized for SARS-COV2 compared with those in who age group less than <30 year (OR 97.357; 95% CI, 18.91-500.99, *p<0.001*). while males were significantly more likely to be hospitalized for SARS-COV2 compared with females (OR 2.411; 95% CI, 1.286 -4.520, *p=0.006*).

Evaluation of the frequency distribution of patients with covid-19 according to body mass index and smoking established in this study found that patients group included 36 (51.4%) patients with SARS-COV2 were obese (BMI ≥ 30 kg/m2), whereas 34 (48.6%) of them were non-obesity. The control group included 73 (73.0%) of the participants who had no SARS-COV2 were non- obesity, and only 27 (27.0%) of them were obese.

Regarding smoking status, the current study shows that the Patients group included 28 (40.0%) patients with SARS-COV2 were smokers, whereas 42 (60.0%) of them were non-smokers. The control group included 87 (87.0%) of the participants who had no SARS-COV2 were non-smoker , and smoker only 13 (13.0%) of them were smoker. Again, Univariate Logistic regression analysis to identify variables independently associated with hospitalization for SARS-COV2. The results found that those who were obese were significantly more likely to be hospitalized for SARS-COV2 compared with those who were non-obese (OR 2.863; 95% CI, 1.504-5.450, *p=0.001*).

Where as those smokers were significantly more likely to be hospitalized for SARS-COV2 compared with those in who non-smokers (OR 4.462; 95% CI, 2.099-9.482, *p=<0.001*). During the time of the current study, some selected biochemical and heamatological parameters were evaluated{ including WBCs, Ferritin level, D-Dimer, CRP, Lymphocytes (%),Neutrophil (%)}, and results reveals that all parameters for the case (with SARS-COV2) group was significantly higher than that for the control group(*P value <0.001*) , except for WBCs were non-significant difference between patients and control groups (*P.*

value=0.293). The current study shows that very high percentages of laboratory tests for the control group fell within normal values. While there are patients with SARS-COV2 who have a marked increase from the normal values, and some of them decrease for some patients in laboratory tests.

The immunological evaluation to clarify the theory of cytokines storm carried in the present study revealed that (TNF-α, IL33, GM-CSF) for the case (with SARS-COV2) group was significantly higher than that for the control group.

The correlations between some serum Immunological markers levels and demographic characteristics and some markers in patients with SARS-COV2 showed that, all parameters had a positive significant relationship with each other except for lymphocytes, which had a negative relationship for all parameters (WBCs/ r= -0.480, *P <0.001*), (Ferritin/ r= -0.510, *P <0.001*), (D-Dimer/ r= -0.543, *P <0.001*), (CRP/ r= -0.506, *P <0.001*), (Neutrophil / r= -0.692, *P <0.001*), (IL33 / r= -430, *P <0.001*), (TNF-a / r= -423, *P <0.001*), (GM-CSF / r= -0.308, *P <0.001*), and (Age / r= -0.448, *P <0.001*). While there was no significant association between WBCs and TNF-a (*P. value=0.142*).

The genetic aspect of this study was illustrated through the assessment of some selected genetic polymorphisms in some immune genes among patients with SARS-COV2 and healthy controls. The distribution of *TNFa* (*rs1800629*) Polymorphism, *IL-13* (*rs20541*) Polymorphism, and *TLR6* (*rs5743789*) genes Polymorphism were detected by Amplification Refractory Mutation System – Polymeres Chain Reachtion (ARMS-PCR) technique. The genotype distribution had no deviation from Hardy-Weinberg equilibrium. Results of genotype and allele frequency distribution showed non-significant differences between patients with SARS-COV2 and healthy controls *P value* were all fairly above the lower acceptable limits (>0.05).

الخلاصة:

هذا هو تصميم دراسة الحالة الضابطة التي أجريت لفترة من شباط 2021 إلى تشرين الاول 2021 لتقييم دور بعض العوامل المصلية والدموية والوراثية المختارة بين مرضى المتلازمة التنفسية الحادة الشديدة النوع 2.في هذه الدراسة ، تم تسجيل مائة وسبعين شخصًا كمجموعتين مقارنة بمجموعة الحالات والشواهد ، وشملت مجموعة الحالات سبعين مريضًا يعانون من فيروس كورونا ، تم اختيارهم جميعًا من أولئك الذين تم دخولهم في وحدة العناية المركزة (ICU) ، وتم تشخيصهم من قبل الطبيب المتخصص بالمتلازمة التنفسية الحادة الوخيمة بسبب SARS-COV2 الموثق بواسطة تفاعل البلمرة المتسلسل (RT-PCR) بجانب المعايير السريرية والمخبرية الأخرى في مدينة مرجان الطبية في محافظة بابل.

تضمنت مجموعة السيطرة في هذه الدراسة مائة من الأشخاص الذين يبدو أنهم يتمتعون بصحة جيدة ، وقد تم تأكيد خلوهم من المتلازمة التنفسية الحادة الشديدة النوع 2 عن طريق الاختبار السريع (اختبار الأجسام المضادة لفيروس كوفيد -١٩ سلبي).

أظهرت الخصائص الديموغرافية للمرضى والأشخاص الاصحاء في هذه الدراسة أن متوسط عمر المرضى كان 11.72 ± 47.17 وأن متوسط عمر الأشخاص الاصحاء كان 8.84± 31.77 سنة. وجد التوزيع التكراري للمرضى والأشخاص الاصحاء حسب الفئات العمرية أن أعلى نسبة للمرضى المصابين بـ المتلازمة التنفسية الحادة الشديدة النوع٢ {(41.4%) 29} تقع في الفئة العمرية (≤ 50 عامًا) ، بينما كانت أقل نسبة (2.9%) 2 منهم من تقل أعمارهم عن 30عامًا. فيما يتعلق بمجموعة السيطرة تضمنت (47.0%) 47من الفئة العمرية أقل من 30 سنة (32.0%) 32 , 30-39سنة ، (14.0%)14 40-49 سنة ، و فقط (7.0%)7 لـ 50 ≤ عام.

أما فيما يتعلق بالتوزيع الجنسي في الدراسة الحالية ، فقد اشتملت مجموعة المرضى على (54.3 %) 38 من الذكور و (45.7 %) 32 من الاناث ، بينما ضمت المجموعة السيطرة (33.0%)33 من الذكور و (67.0 %) 67من الإناث. وجدت نتائج تحليل الانحدار اللوجستي أحادي المتغير لتحديد المتغيرات المرتبطة بشكل مستقل بالاستشفاء لـ مرضى المتلازمة التنفسية الحادة الشديدة النوع 2 ان أولئك الذين في الفئة العمرية (50 ≤ عامًا) كانوا أكثر عرضة بشكل كبير للدخول إلى المستشفى لـ SARS-COV2 مقارنة مع أولئك الذين في الفئة العمرية أقل من 30> سنة (OR 97.357; 95% CI, 18.91-500.99, *p<0.001*). بينما كان الذكور أكثر عرضة بشكل ملحوظ

للدخول إلى المستشفى من أجل SARS-COV2مقارنة بالإناث - (OR 2.411; 95% CI, 1.286 .
4.520, *p=0.006*)

وجد تقييم التوزيع التكراري لمرضى المتلازمة التنفسية الحادة الشديدة النوع 2وفقًا لمؤشر كتلة الجسم والتدخين المحدد في هذه الدراسة أن مجموعة المرضى تضمنت (51.4%) 36 من مرضى المتلازمة التنفسية الحادة الشديدة النوع 2 يعانون من السمنة المفرطة ,(BMI ≥ 30 kg/m2) ، بينما كان (48.6%) 34 منهم غير مصابين بالسمنة. ضمت مجموعة السيطرة (73.0%) 73من المشاركين الذين لم يكن لديهم المتلازمة التنفسية الحادة الشديدة النوع 2يعانون من السمنة ، و (27.0%) 27 منهم فقط يعانون من السمنة.

فيما يتعلق بحالة التدخين ، أظهرت الدراسة الحالية أن مجموعة المرضى تضمنت 28 (40.0%) من المرضى المصابين بـ المتلازمة التنفسية الحادة الشديدة النوع 2من المدخنين ، بينما كان (60.0%) 42 منهم من غير المدخنين. ضمت المجموعة الضابطة (87.0%) 87من المشاركين الذين ليس لديهم SARS-COV2 من غير المدخنين ، وكان (13.0%) 13 فقط منهم مدخنين. مرة أخرى ، تحليل الانحدار اللوجستي أحادي المتغير لتحديد المتغيرات المرتبطة بشكل مستقل بالاستشفاء لـ المتلازمة التنفسية الحادة الشديدة النوع 2. ووجدت النتائج أن أولئك الذين يعانون من السمنة كانوا أكثر عرضة بشكل كبير للدخول إلى المستشفى بسبب المتلازمة التنفسية الحادة الشديدة النوع 2مقارنة مع غير البدينين(*p=0.001* 1.504-5.45 ,95% CI ;OR 2.863)

في حين أن هؤلاء المدخنين كانوا أكثر عرضة بشكل ملحوظ للدخول إلى المستشفى من أجل المتلازمة التنفسية الحادة الشديدة النوع 2مقارنة مع غير المدخنين -(OR 4.462; 95% CI, 2.099 9.482, *p=<0.001*) خلال فترة الدراسة الحالية ، تم تقييم بعض المعلمات الكيميائية والدموية المختارة {بما في ذلك كريات الدم البيضاء ، مستوى الفيريتين ، D-Dimer، مستوى البروتين المتفاعل ، الخلايا الليمفاوية (٪) ، العدلات (٪)} ، والنتائج تكشف أن جميع المعلمات للحالة (مع-SARS COV2) كانت المجموعة أعلى بكثير من مجموعة السيطرة (قيمة P <0.001) ، باستثناء كريات الدم البيضاء كانت هناك فروق غير معنوية بين المرضى ومجموعات السيطرة (قيمة P. = 0.293). تظهر الدراسة الحالية أن النسب العالية جدًا من الاختبارات المعملية للمجموعة السيطرة تقع ضمن القيم الطبيعية. بينما يوجد مرضى مصابين بـ المتلازمة التنفسية الحادة الشديدة النوع 2لديهم زيادة ملحوظة عن القيم الطبيعية ، وبعضهم ينخفض لبعض المرضى في الفحوصات المخبرية.

أظهر التقييم المناعي لتوضيح نظرية عاصفة السيتوكينات التي أجريت في هذه الدراسة أن مجموعة (TNF-a و IL33 و GM-CSF) للحالة (معSARS-COV2) كانت أعلى بكثير من مجموعة السيطرة. لتقييم قيمة قطع TNF-α وكذلك للتنبؤ بـ SARS-COV2 كاختبارات تشخيصية أو اختبارات تشخيصية مساعدة

أظهرت الارتباطات بين بعض مستويات الواسمات المناعية في الدم والخصائص الديموغرافية وبعض الواسمات في المتلازمة التنفسية الحادة الشديدة النوع 2 ، أن جميع المعلمات لها علاقة معنوية إيجابية مع بعضها البعض باستثناء الخلايا الليمفاوية ، والتي كانت لها علاقة سلبية لجميع المعلمات (WBCs / r = -0.480 ، P <0.001) ، (Ferritin / r = -0.510 ، P <0.001) ، (D-Dimer / r = -0.543 ، P <0.001) ، (CRP / r = -0.506 ، P <0.001) ، (العدلة / r = -0.692 ، P <0.001) ، (IL33 / r = -430 ، P <0.001) ، (TNF-a / r = -423 ، P <0.001) ، (GM-CSF / r = -0.308 ، P <0.001) ، و (العمر / r = -0.448 ، P <0.001). بينما لم يكن هناك ارتباط معنوي بين كريات الدم البيضاء و TNF-a (قيمة P. = 0.142).

تم توضيح الجانب الجيني لهذه الدراسة من خلال تقييم بعض الأشكال الجينية المختارة في بعض الجينات المناعية بين مرضى المتلازمة التنفسية الحادة الشديدة النوع 2ومجموعة الاصحاء. تم الكشف عن تعدد الأشكال في تعدد الأشكال *rs1800629*) *TNFa*) ، وتعدد الأشكال *rs20541*) *IL-13*) ، وتعدد الأشكال *rs5743789*) *TLR6*) عن طريق تقنية تضخيم الطفرة الحرارية - تقنية الوصول إلى سلسلة البوليمرات (ARMS-PCR). لم يكن لتوزيع النمط الجيني أي انحراف عن توازن هاردي واينبرغ. أظهرت نتائج التركيب الوراثي وتوزيع تردد الأليل اختلافات غير معنوية بين المرضى الذين يعانون من المتلازمة التنفسية الحادة الشديدة النوع 2 وقيمة مجموعة الاصحاء P كانت جميعها أعلى من الحدود الدنيا المقبولة .(0.05<).

وزارة التعليم العالي والبحث العلمي

جامعة بابل/ كلية الطب

فرع الأحياء المجهرية الطبية

دراسة بعض تعدد الاشكال الجينية والمعلمات الحيوية في المرضى المصابين بفايروس كورونا المستحدث في جامعة بابل

أطروحة

مقدمة الى مجلس كلية الطب/ جامعة بابل وهي جزء من متطلبات نيل

درجة الدكتوراه فلسفة في العلوم/ الأحياء المجهرية الطبية

من قبل

شهد سعد محمد عبد الامير

بكالوريوس احياء مجهرية/ جامعة بابل (٢٠١٤)

ماجستير احياء مجهرية طبية/ جامعة بابل (٢٠١٨)

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2019-nCoV	2019 novel coronavirus
ACE2	angiotensin-converting enzyme 2
ADCC	antibody-dependent cellular cytotoxicity
AHC	Apprantly Healthy Control.
AIBV	Avian Infectious Bronchitis Virus
ARDS	Acute Respiratory Syndrome
ARDS	Acute Respiratory Distress Syndrome
ARMS	Amplification Refractory Mutation System
BCoV	Bovine Coronavirus
BMI	Body Mass Index
CBC	Complet Blood Count
CCL	CC Chemokine Ligands
CCoV	Canine Coronavirus
CD	Cluster of Differentiation.
CDC	Centers for Disease Control and Prevention.
COPD	Chronic Obstructive Pulmonary Disease
COVID-19	Coronavirus 2019 disease
CRP	C-reactive protein
CRS	Cytokines Release Syndrome
CT	Computerized tomography scan.
CXCL	Chemokine (C-X-C motif) ligand
DC	Dendritic Cell
DENV	Biomarker of Severe forms of Dengue virus

DNA	Deoxyribonucleic acid
dNTPs	Deoxynucleotide triphosphates.
dsRNA	double-stranded RNA
E-protein	Envelope protein
ECoV	Equine Coronavirus
EDTA	Ethylene diamine tetra acetic acid.
ELISA	Enzyme Linked Immunosorbent Assay
FECoV	Feline Corona Virus
FGF	Fibroblast Growth Factor
FIA	Fluorescence Immunoassay
FIPV	Feline Infectious Peritonitis Virus
G-CSF	Granulocyte-Stimulating Colony Factor
GGO	Ground glass opacity
GM-CSF	Granulocyte-macrophage colony-stimulating factor
GvHD	Graft versus host disease
HCl	Hydrochloric acid
HCOV	Human coronavirus.
HIV	Human Immunodeficiency virus
HLA-DR	Human leukocyte antigen DR
HRP	Avidin-Horseradish Peroxidase
IBD	Inflammatory bowel disease
ICTV	International Committee on Taxonomy of Viruses
ICUs	Intensive care units
IFNs	Interferons
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IL	Interleukin
ILC2s	type 2 innate lymphoid cells
IP10	Interferon gamma-induced protein-10.

IQR	interquartile range
IRF	IFN regulatory factor
kg	kilogram
LRRs	leucine-rich repetitions
m	Meter
M-protein	Membrane protein
MAC	Complement Membrane Attack complex
MAS	Macrophage-Activation Syndrome
MASP-2	MBL-associated serine protease 2
MBL	Mannose-binding lectin
MCP1	Monocyte Chemoattractant Protein-1
M-CSF	Macrophage-stimulating colony factor
mDCs	Myeloid Dendritic Cell
MERS-CoV	Middle East Respiratory Syndrome Coronavirus
MHC I	Major Histocompatibility Complex Class I
MHC II	Major Histocompatibility Complex ClassII
MHV	Murine Hepatitis Virus
min	Minute
MIP	Macrophage Inflammatory Proteins.
mRNA	Messenger Ribonucleic acid.
mTNF-α	Transmembrane form of TNF-α
NF-κB	Nuclear factor kappa-B.
NK	Natural killer
nm	nanomete
N-protein	Nucleo-capsid protein
OD	Optical Density
ORF	Open Reading Frame
PAMPs	Pathogen-Associated Molecular Patterns
PBMCs	Peripheral Blood Mononuclear Cells.

PCR	Polymerase Chain Reaction
PD-1	Programmed Death-1.
PDGF	Platelet-Derived Growth Factor.
PEDV	Porcine Epidemic Diarrhoea Virus
PHEV	Porcine Hemagglutinating Encephalomyelitis
PRCV	Porcine Respiratory Corona Virus
PRR	Pattern Recognition Receptor
RNA	Ribonucleic Acid
RT-PCR	Real Time Polymerase Chain Reaction
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
SDAV	Sialodacryoadenitis CoV
S-ferriten	Serum ferriten
SNPs	Single-nucleotide polymorphisms
SNVs	Single Nucleotide Variants
SOFA	Sequential Organ Failure Assessment
S-proteins	Spike- Proteins
SPSS	Statistical Package for Social Sciences
ssRNA	Single-Stranded Ribonucleic Acid
ST2	Suppression of Tumorigenicity 2
sTNF-α	Soluble form of TNF-α
STRPs	short tandem repeat polymorphisms
TACE	TNFα-converting enzyme
TACE	TNF-α-converting enzyme
TCoV	Turkey Coronavirus
TEGV	Transmissible Gastroenteritis Virus
Th2	T-helper 2
TLR	Toll-like receptor
TNF	Tumor necrosis factor
TNF-α	Tumor necrosis factor-α

Tregs	T-regulatory cells
UK	United Kingdom
USA	United States of America
VEGF	Vascular endothelial growth factor.
VNTRs	Variable Number of Tandem Repeats
WHO	World Health Organization
µl	Microliter

CHAPTER ONE

Introduction and Literatures Review

1.1. INTRODECTION

Coronavirus Disease 2019 (Covid-19) outbreak emerged in Wuhan, China, whose spreading dynamics is not fully understood (Huang *et al*., 2020; Zeng *et al*., 2020).

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV2) is a novel lineage B beta-coronavirus in the phylogenetic tree. The genome of SARS-CoV2 is 29891 nucleotides in size, encoding 9860 amino acids, and has 89% nucleotide identity with bat SARS-like-CoVZXC21 and 82% with that of human SARS-CoV (Chan *et al*., 2020).

SARS-CoV-2 is transmitted between humans via respiratory droplets which are produced when an infected individual talks, sneezes, or coughs ,Droplet transmission can occur within 1–4 m (Guo *et al*., 2020; WHO, 2020).

SARS-CoV2 has been shown to survive in aerosolized form for more than 3 hours under experimental conditions, but this mechanical generation of aerosols is unlikely to mimic the true clinical scenario (van Doremalen, 2020).

Signs and symptoms are used in the initial diagnosis of suspected SARS-CoV-2 infection and to help identify those requiring tests (Struyf, 2021).

To reduce the overall mortality rate, identifying the risk factors related to disease severity and mortality in SARS-COV2 patients is urgently required. Previous studies have shown that older age, underlying comorbidities, high D-dimer level, and abnormalities of several biochemical variables were closely associated with disease severity or even death of SARS-COV2 patients (Zhang *et al*.,2020; Wu *et al*., 2020; Zhou *et al*., 2020; Du *et al*.,2020).

Cytokine storm is the main mechanism of systemic inflammation-mediated immunopathology in severe SARS-COV2 cases. In most of the

severe SARS-COV2 cases, infiltration of innate immune cells, such as neutrophils and macrophages, is increased in the lungs, leading to massive production of pro-inflammatory cytokines and chemokines, including IL-6, IL-1β, IL-2, IL-8, IL-17, IL-10, G-CSF, GM-CSF, IFN-γ, IP10, MCP1, MIP1A, and TNF-α. Furthermore, prominent acute inflammatory markers, such as acute phase reactants, including C-reactive protein, lactate dehydrogenase, and D-dimer, are also increased (Meng *et al*.,2021).

Prolonged high level of cytokines, characterized as the cytokine storm, may exacerbate systemic immune disorder (Chen *et al*.,2020). Patients with SARS-COV2 may develop severe complications due to cytokine storm (Xu *et al*.,2020). thus immunosuppression has been proposed as an essential means to manage severe SARS-COV2 cases (Moore and June,2020).

In response to SARS-CoV2 infection, various cytokines, such as IL-6, TNF-α, IL-8, IL-1, IL-21, and monocyte chemoattractant protein-1 (MCP-1), are upregulated in macrophages and/or monocytes to promote pathogen elimination and tissue repair (Huang *et al*.,2020).

TNF-α is one of the most potent proinflammatory cytokines with broad spectrum of actions. Marked elevations reported in many inflammatory conditions including cytokine release syndrome. Serum TNF-α levels found elevated in SARS-COV2 patients with being more pronounced in more severe patients (Huang, *et al*.,2020).

GM-CSF is a myelopoietic growth factor and pro-inflammatory cytokine that plays a central role in a broad range of immune-mediated diseases. GM-CSF, which is secreted by macrophages, T cells, mast cells, natural killer cells, endothelial cells, and fibroblasts, regulates macrophage number and function. It acts as a pro-inflammatory signal, prompting macrophages to launch an immune cascade that ultimately results in tissue damage (Mehta *et al*.,2020; Thwaites *et al*.,2021).

The IL-33 is a regulator of inflammation, and can induce T-helper 2 (Th2)-mediated innate and adaptive immune responses (Kakkar and Lee, 2008; Kotsiou *et al*., 2018). Additionally, IL-33 participates in the pathogenesis of renal, neurological, hepatic, pulmonary, and eye diseases. It is a cell cytokine that promotes inflammatory responses and has a characteristic of alarm (Moussion *et al*., 2008; Li *et al*., 2019; Wang *et al*., 2020).

Interleukin 13 (IL-13) is a protein that in humans is encoded by the *IL13* gene(Minty *et al*.,1993).IL-13 genes are located in a region of 140 kb on chromosome 5q31-33 that codes for a cluster of Th2 cytokines(Quirico-Santos *et al*.,2007).

The secondary structural features of IL-13 are similar to that of Interleukin 4 (IL-4) however it only has 25% sequence identity to IL-4 and is capable of IL-4 independent signaling(Bieber,2020).

TLR6 is a transmembrane protein, member of toll-like receptor family, which belongs to the pattern recognition receptor (PRR) family. TLR6 acts in a heterodimer form with toll-like receptor 2 (TLR2). Its ligands include multiple diacyl lipopeptides derived from gram-positive bacteria and mycoplasma and several fungal cell wall saccharides. After dimerizing with TLR2, the NF-κB intracellular signalling pathway is activated, leading to an pro-inflammatory cytokine production and activation of innate immune response(Weiss *et al*.,2005).

Single-nucleotide polymorphisms (SNPs) are the most common type of polymorphism found in the human genome, and represent the main reason for 90% of all types of genetic variations among individuals(Hetherington *et al*.,2002).

Aim of the study:

To evalute some selected immunological parameters associated with cytokin storm and study the gentic polymorphism *IL13 (rs20541)* , *TLR6(rs5743789)* and *TNFa (rs 1800629)* genes among patients with SARS-CoV2 on Iraq.

The objectives of this work are:

1.Study the demographic data on patients with SARS-COV2 .

2.Evaluate serm level of some selected immune Markers and parameters such as (CRP, S-ferriten, D-dimer)by FIA , CBC by autamatic hematology analyzer (GM-CSF, IL33, TNF-a) by ELISA test for patients and control.

3-Genetic polymorphism of *IL13 (rs20541)* , *TLR6(rs5743789)* and *TNFa (rs 1800629)* genes by ARMS method for patients and control.

4-Statistical analysis of results.

1.2. LITERATURES REVIEW

1.2.1. Historical Preview of SARS-COV2

Coronavirus 2019 disease (COVID-19) has become pandemic since being first reported in China (Ebrahim *et al*.,2020). In December 2019, several Health Centers in Wuhan, in the Hubei Province of China, reported a cluster of patients with pneumonia of unknown etiology (Chan *et al*., 2020).

Their clinical presentations were similar to those of SARS outbreak that occurred in 2003(Chan-Yeung and Xu 2003; Lee *et al*., 2003).

Coronavirus Disease 2019 (Covid-19) outbreak emerged in Wuhan, China, whose spreading dynamics is not fully understood (Huang *et al*., 2020; Zeng *et al*., 2020), increased in a very short time and was declared as a pandemic by the World Health Organization (WHO) on March 11, 2020. It is reported that with serious measures taken, the outbreak in China has decreased (Gautam and Hens, 2020).

However, as of April 22, worldwide, 2.546.527 confirmed cases and 175.621 confirmed deaths were reported by WHO (2020a). The most prominent feature of the virus is its rapid spread and long incubation period (Linton *et al*., 2020; Qian *et al*., 2020; Wilder-Smith and Freedman, 2020).

However, the first confirmed case in Hubei of a resident aged 55 could be traced back to November 17, 2019 (South China Morning Post, March 13, 2020) or earlier to November 4 or even to mid-October as predicted by a coalescent framework modeling (Pekar *et al*.,2021).

Deep sequencing analysis from lower respiratory tract samples indicated a novel coronavirus with > 75% sequence homology to SARS-CoV in the submitted clinical samples, which was named 2019 novel coronavirus (2019-nCoV). By January 5 of 2020, the whole genome sequence of 2019-nCoV was completed by Wuhan Institute of Virology, China CDC and Shanghai Public Health Clinical Center of Fudan University

(zhou *et al*.,2020; Wu *et al*., 2020; Lu *et al*.,2020). and deposited immediately to the GenBank (Wu *et al.,* 2020).

By January 7, 2020, a new coronavirus of probable bat origin using a host receptor ACE2 for human cell infection was isolated and characterized as an etiological agent of the 2019-nCoV (zhou *et al*.,2020; Zhu *et al*., 2020).Subsequently, WHO named this mysterious pneumonia as coronavirus disease 2019 or COVID-19 and the ICTV named its etiological agent the SARS-CoV-2 (Wu *et al*., 2020).

Wuhan, with a population of over 11 million people, was locked down on January 23, 2020 for quarantine to stop the arising respiratory tract transmission of SARS-CoV2 from person to person. Rapid spread of SARS-CoV2 to its neighboring cities, provinces and other countries in a short period of time caused a worldwide pandemic(Huang *et al*.,2020; Khan *et al*., 2020).

The term "Coronavirus" was coined in 1968, due to presence of crown-like morphology based electron microscopic studies. Then, it was characterized by international committee on the taxonomy of virus and it belongs to the family "Coronaviridiae" under the order "Nidovirales" in 1975. It has three different genera such as group I, II and III based on serological cross-reactivity (Fig. 1.1). Porcine Respiratory Corona Virus (PRCV), Feline Corona Virus (FECoV), Porcine Epidemic Diarrhoea Virus (PEDV), Transmissible Gastroenteritis Virus (TEGV), Canine Coronavirus (CCoV), Feline Infectious Peritonitis Virus (FIPV), Porcine Epidemic Diarrhoea Virus (PEDV), Human Coronavirus HCoV-229E and HCoVNL63, are classified under group I and cause respiratory diseases. Group II viruses includes, HCoV-OC43, HCoV-HKU1 and Murine Hepatitis Virus (MHV) causes respiratory infection in human (Woods and Wesley, 1988).

MHV is a prototype of Coronavirus cause several diseases like hepatitis, respiratory diseases, enteric disease, encephalitis and chronic demyelination. On the other hand, viruses like Bovine Coronavirus (BCoV), Porcine Hemagglutinating Encephalomyelitis Virus (PHEV), Equine Coronavirus (ECoV), Sialodacryoadenitis CoV (SDAV) and Severe acute respiratory syndrome (SARS CoV) causes infection in animals are also classified under this group. However, classification of SARS-CoV under group II is still debatable, whether it should be allocated to group II or a new group IV. Moreover, Avian Infectious Bronchitis Virus (IBV) and Turkey Coronavirus (TCoV) causes infection in birds are classified under Group III. Experimental and *in silico* analysis revealed that, these viruses are different from the virus causing infection in animals based on replicase and nucleocapsid (N) sequences(Li, 2015).

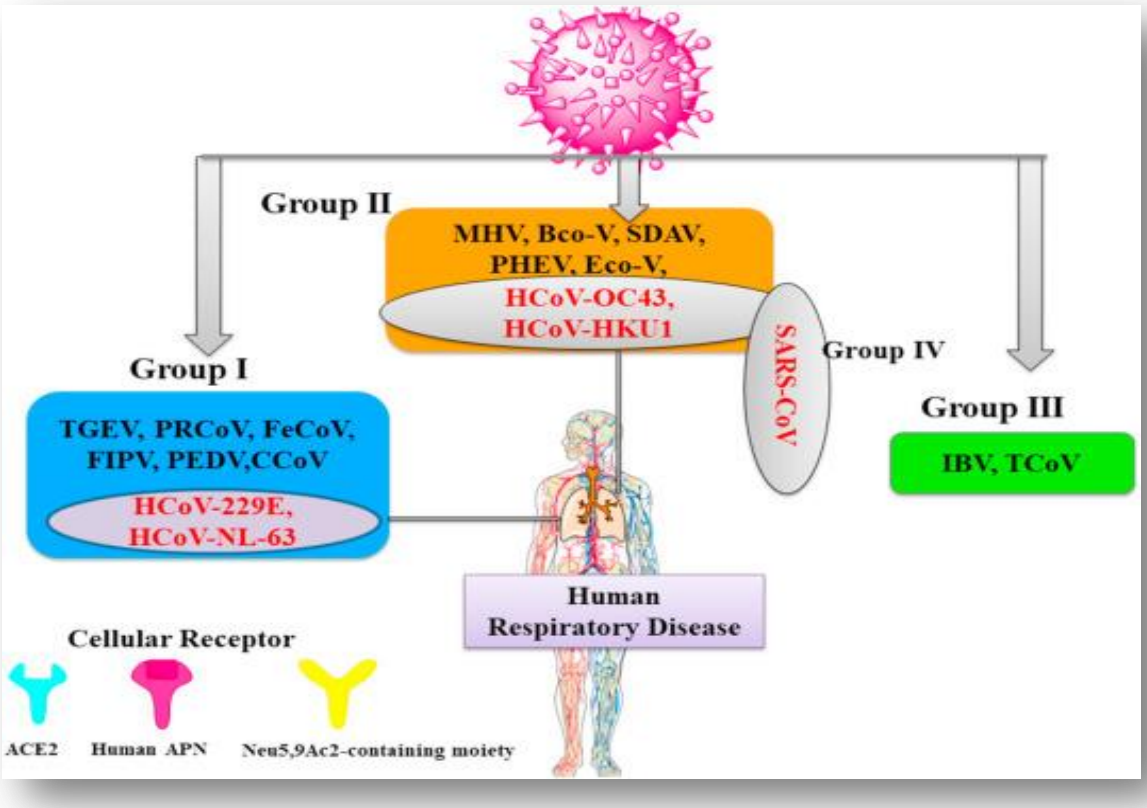


Figure (1.1) Diverse group of Coronavirus and their mode of entry to human through major receptors (Agrahari *et al*.,2021).

1.2.2. Definition and Structure of SARA-COV2

The spike proteins of SARS-CoV-2 associated with the host receptor named (ACE2) of sensitive cells and tissues can result in infection of target cells. Angiotensin-converting enzyme 2 (ACE2) is also the receptor for SARS-CoV, and both types of coronavirus can cause a severe acute respiratory disease and possess high human-to-human transmissibility (Benvenuto *et al*., 2020).

The expression of ACE2 has been identified in multiple tissues in human body, including lung alveolar mucosa, oral mucosa, gastrointestinal duct, kidney and conjunctiva, indicating potential infection routes of SARS-CoV-2 via these tissues (Hamming *et al*., 2004; Xu *et al*., 2020).

The Coronavirus belongs to the family of viruses that are very common in mammals. In total , 07 different Corona viruses such as α-Coronavirus (229E & NL63), β-Coronavirus (OC43, HKU1, MERS-CoV & SARS-CoV) including the novel SARS CoV-2 has been reported so far (Berger, 2020).

Coronaviruses are a family of enveloped RNA viruses broadly distributed in humans and animals that cause acute and chronic diseases. Of the six coronavirus species previously known to cause human diseases, four typically cause common cold symptoms and two — SARS-CoV and Middle East respiratory syndrome coronavirus (MERS-CoV) — can cause fatal respiratory disease(Peiris *et al*.,2003; Graham *et al*.,2013).

Novel Coronavirus having different protein structure than others. It comprises non-segmented enveloped and positive sense single stranded RNA with no DNA stage. Linear and helical capsids are present on the surface of Coronavirus, however the nucleocapsid is present within the envelope of the virion (Ferner and Aronson, 2020).

This virus harbours pleomorphic RNA or spike proteins (S-proteins) peplomers of 80–160 nM in size with 27–32 kb positive polarity (Sahin, *et*

al.,2020). The most prominent feature of Coronaviruses is the club-shaped spike projections emanating from the surface of the virion. These spikes are defining features of the virion and appear as a solar corona, prompting the name, Coronavirus. The major structural components of these viruses are Spike protein (S), Membrane protein (M) 16s Nucleo-capsid protein (N) and Envelope protein (E) are encoded from 30 end of viral genome (Fig. 1.2) (Mousavizadeh and Ghasemi, 2021).

The N-linked glycocylated trimeric spike protein (S) of ~150 kDa present on the surface of virus used N-terminal signals to gateway towards the host endoplasmic reticulum. The S protein has two subunits S1 acts as receptor binding domain and S2 forms the stalk that provides spike structure. The M protein near about 25–30 kDa with lesser N-terminal glycosylated ectodomain & greater C-terminal endodomain, which is responsible for shape of the virion (Jaimes and Whittaker, 2018).

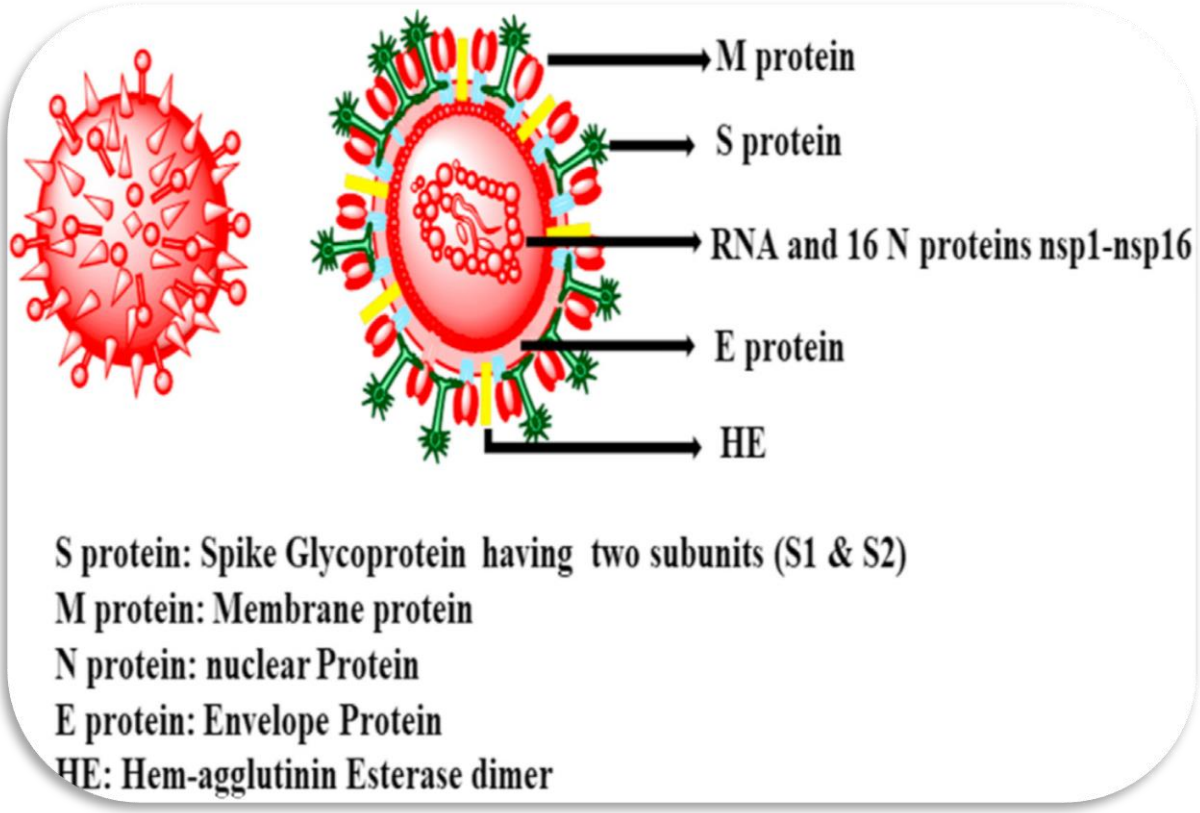


Figure (1.2)The major structural components of coronavirus (Agrahari *et al*.,2021).

1.2.3. Pathogenesis & Replicative Cycle of SARS-Cov2

Coronavirus has an unusual two step replication mechanism. Usually RNA viruses contain only one open reading frame (ORF) which will encode for single polyprotein. Then, the polyprotein is catalytically cleaved into various small functional viral proteins, although the virus can accommodate up to ten distinct ORFs. Replicase are the biggest ORFs of Corona virus translated by most of the ribosome, it could be twice in the size compared to the other RNA viral genome. A series of enzymes encoded by replicase gene that uses the leftover genome as template and then transcribe to a group of smaller, overlapping mRNA molecules, which further translated for structural protein. Then the viral proteins and ve ssRNA assembled to construct new viral particles (Fehr and Perlman, 2015)

SARS-CoV-2 is an enveloped β-coronavirus, with a genetic sequence very similar to SARS-CoV-1 (80%) and bat coronavirus RaTG13

(96.2%)(Yan , *et al*., 2020).The viral envelope is coated by spike (S) glycoprotein, envelope (E), and membrane (M) proteins (fig 2). Host cell binding and entry are mediated by the S protein. The first step in infection is virus binding to a host cell through its target receptor. The S1 sub-unit of the S protein contains the receptor binding domain that binds to the peptidase domain of angiotensin-converting enzyme 2 (ACE 2). In SARS-CoV-2 the S2 sub-unit is highly preserved and is considered a potential antiviral target (Millet and Whittaker ,2015).

Coronaviruses have the capacity for proofreading during replication, and therefore mutation rates are lower than in other RNA viruses. As SARS-CoV-2 has spread globally it has, like other viruses, accumulated some mutations in the viral genome, which contains geographic signatures. Researchers have examined these mutations to study virus characterisation and understand epidemiology and transmission patterns. In general, the mutations have not been attributed to phenotypic changes affecting viral transmissibility or pathogenicity. The G614 variant in the S protein has been postulated to increase infectivity and transmissibility of the virus (Korber *et al*., 2020).

SARS-CoV-2 binds to ACE 2, the host target cell receptor(Cevik *et al*., 2020).

In an experimental hamster model, the virus causes transient damage to the cells in the olfactory epithelium, leading to olfactory dysfunction, which may explain temporary loss of taste and smell commonly seen in SARS-CoV2 (Sia *et al*.,2020).

Active replication and release of the virus in the lung cells lead to non-specific symptoms such as fever, myalgia, headache, and respiratory symptoms (Cevik *et al*., 2020).

The distribution of ACE 2 receptors in different tissues may explain the sites of infection and patient symptoms. For example, the ACE 2

receptor is found on the epithelium of other organs such as the intestine and endothelial cells in the kidney and blood vessels, which may explain gastrointestinal symptoms and cardiovascular complications (Monteil *et al*., 2020).

Lymphocytic endotheliitis has been observed in postmortem pathology examination of the lung, heart, kidney, and liver as well as liver cell necrosis and myocardial infarction in patients who died of SARS-CoV2 (Cevik *et al*., 2020; Varga *et al*., 2020).

1.2.4.Transmission of SARS-COV-2

Like other coronaviruses, the primary mechanism of transmission of SARS-CoV-2 is via infected respiratory droplets, with viral infection occurring by direct or indirect contact with nasal, conjunctival, or oral mucosa, when respiratory particles are inhaled or deposited on these mucous membranes (Hui *et al*., 2020).

Target host receptors are found mainly in the human respiratory tract epithelium, including the oropharynx and upper airway. The conjunctiva and gastrointestinal tracts are also susceptible to infection and may serve as transmission portals(Hui *et al*., 2020).

Most transmission occurs through close range contact (such as 15 minutes face to face and within 2 m) and spread is especially efficient within households and through gatherings of family and friends (Cevik *et al*., 2021).

Aerosol transmission can still factor during prolonged stay in crowded, poorly ventilated indoor settings (meaning transmission could occur at a distance >2 m) (Yu *et al*., 2004; Cevik *et al*., 2021; Klompas *et al*., 2020).

The role of faecal shedding in SARS-CoV-2 transmission and the extent of fomite (through inanimate surfaces) transmission also remain to be fully understood. Both SARS-CoV-2 and SARS-CoV-1 remain viable for many days on smooth surfaces (stainless steel, plastic, glass) and at lower

temperature and humidity (eg, air conditioned environments)(Chin *et al*., 2020; van Doremalen *et al*.,2020).

Certain clinical procedures involving the upper airway such as obtaining a nose or throat swab, endotracheal intubation, manual ventilation, or nebulization are capable of generating particles < 5 μm, allowing for airborne transmission in healthcare settings. (WHO, 2020) In particular, intensive care units (ICUs) have been associated with a higher risk of infection (Guo *et al*, 2020).

Fomite transmission, transmission from contact with contaminated surfaces, is possible with high rates of contamination of floors and the soles of healthcare staff as well as computer mice, doorknobs, and trash cans, The virus is viable for up to 72 hours on plastic and stainless steel, 24 hours on cardboard, and 4 hours on copper. These survival times appear to be longer than those of SARS-CoV-2 under similar conditions and may contribute to the broader spread of SARS-CoV-2 in comparison(van Doremalen *et al*., 2020).

Infection from direct contact with body fluids from infected individuals is likely to be another possible route of transmission. SARS-CoV-2 has been detected in saliva, blood, urine, tears, feces, and cerebrospinal fluid samples(Zhang *et al*., 2020; Sun and Guan, 2020).

Asymptomatic SARS-CoV-2 transmission also occurs because of individuals with infection who are never symptomatic (or who experience very mild or almost unrecognizable symptoms). The proportion of individuals with infection who never have apparent symptoms is difficult to quantify because it requires intensive prospective clinical sampling and symptom screening from a representative sample of individuals with and without infection. Nonetheless, evidence from household contact studies indicates that asymptomatic or very mild symptomatic infections occur (Byrne *et al*., 2020; Buitrago-Garcia *et al*., 2020).

Severe acute respiratory syndrome coronavirus-2's (SARS-CoV-2) environmental persistence suggests transmission may also result from hand contact with contaminated surfaces (van Doremalen *et al*., 2020).

1.2.5. SARS-CoV-2 Infection and Pathogensis

SARS-CoV-2 has structural differences in its surface proteins that enable stronger binding to the ACE 2 receptor**(** Wrapp *et al*., 2020). and greater efficiency at invading host cells(Cevik *et al*., 2020).

SARS-CoV-2 also has greater affinity (or bonding) for the upper respiratory tract and conjunctiva (Wölfel *et al*., 2020). thus can infect the upper respiratory tract and can conduct airways more easily (Hui *et al*., 2020).

Severely ill or immune-compromised patients may have relatively prolonged virus shedding, and some patients may have intermittent RNA shedding; however, low level results close to the detection limit may not constitute infectious viral particles. While asymptomatic individuals (those with no symptoms throughout the infection) can transmit the infection, their relative degree of infectiousness seems to be limited (Qiu *et al*., 2020; Buitrago-Garcia *et al*.,2020; Meyerowitz *et al*.,2021;).

People with mild symptoms (paucisymptomatic) and those whose symptom have not yet appeared still carry large amounts of virus in the upper respiratory tract, which might contribute to the easy and rapid spread of SARS-CoV-2 (Cevik *et al*.,2021).

Symptomatic and pre-symptomatic transmission (one to two days before symptom onset) is likely to play a greater role in the spread of SARS-CoV-2 (Qiu *et al*., 2020).

1.2.6.Clinical and Epidemiological Features of SARS-Cov2

It appears that all ages of the population are susceptible to SARS-CoV-2 infection, and the median age of infection is around 50 years (Chen *et al*.,2020; Huang *et al*.,2020; Wang *et al*.,2020; Guan *et al*.,2020) .

However, clinical manifestations differ with age. In general, older men (>60 years old) with co-morbidities are more likely to develop severe respiratory disease that requires hospitalization or even die, whereas most young people and children have only mild diseases (non-pneumonia or mild pneumonia) or are asymptomatic (Wang *et al*.,2020; Lu *et al*.,2020).

Self-reported olfactory and taste disorders were also reported by patients in Italy (Giacomelli *et al*.,2020). Most people showed signs of diseases after an incubation period of 1–14 days (most commonly around 5 days), and dyspnoea and pneumonia developed within a median time of 8 days from illness onset(Wu and McGoogan, 2020) .

In a report of 72,314 cases in China, 81% of the cases were classified as mild, 14% were severe cases that required ventilation in an intensive care unit (ICU) and a 5% were critical (that is, the patients had respiratory failure, septic shock and/or multiple organ dysfunction or failure) (Chen *et al*.,2020).

On admission, ground-glass opacity was the most common radiologic finding on chest computed tomography (CT) (Chen *et al*.,2020 ;Huang *et al*.,2020; Wang *et al*.,2020; Guan *et al*.,2020) . Most patients also developed marked lymphopenia, similar to what was observed in patients with SARS and MERS, and non-survivors developed severer lymphopenia over time (Chen *et al*.,2020 ;Huang *et al*.,2020; Wang *et al*.,2020; Guan *et al*.,2020).

Compared with non-ICU patients, ICU patients had higher levels of plasma cytokines, which suggests an immunopathological process caused by a cytokine storm (Huang *et al*.,2020; Yang *et al*.,2020). In this cohort of patient, around 2.3% people died within a median time of 16 days from disease onset (Wu and McGoogan, 2020) .

Men older than 68 years had a higher risk of respiratory failure, acute cardiac injury and heart failure that led to death, regardless of a history of cardiovascular disease (Chen *et al*.,2020).

Most patients recovered enough to be released from hospital in 2 weeks(Guan *et al.*,2020)

An estimated R0 of 2.5 (ranging from 1.8 to 3.6) has been proposed for SARS-CoV-2 recently, compared with 2.0–3.0 for SARS-CoV90. Notably, most of the SARS-CoV-2 human-to-human transmission early in China occurred in family clusters, and in other countries large outbreaks also happened in other settings, such as migrant worker communities, slaughterhouses and meat packing plants, indicating the necessity of isolating infected people (Yu *et al*.,2020; Middleton *et al*.,2020; Joob and Wiwanitkit, 2020).

Nosocomial transmission was not the main source of transmission in China because of the implementation of infection control measures in clinical settings (Wu and McGoogan, 2020) .

By contrast, a high risk of nosocomial transmission was reported in some other areas. For example, a cohort study in London revealed 44% of the frontline health-care workers from a hospital were infected with SARS-CoV-2 (Houlihan *et al*., 2020).

The high transmissibility of SARS-CoV-2 may be attributed to the unique virological features of SARS-CoV-2. Transmission of SARS-CoV occurred mainly after illness onset and peaked following disease severity, However, the SARS-CoV-2 viral load in upper respiratory tract samples was already highest during the first week of symptoms, and thus the risk of pharyngeal virus shedding was very high at the beginning of infection (Zou *et al*.,2020; Wolfel *et al*.,2020).

A patient with SARS-CoV2 spreads viruses in liquid droplets during speech. However, smaller and much more numerous particles known as aerosol particles can also be visualized, which could linger in the air for a long time and then penetrate deep into the lungs when inhaled by someone else (Stadnytskyi *et al*.,2020; Meselson, 2020; van Doremalen *et al*.,2020).

Airborne transmission was also observed in the ferret experiments mentioned above. SARS-CoV-2-infected ferrets shed viruses in nasal washes, saliva, urine and faeces for up to 8 days after infection, and a few naive ferrets with only indirect contact were positive for viral RNA, suggesting airborne transmission (Kim *et al*.,2020).

In addition, transmission of the virus through the ocular surface and prolonged presence of SARS-CoV-2 viral RNA in faecal samples were also documented (Wu *et al*.,2020; Lu *et al*.,2020).

Coronaviruses can persist on inanimate surfaces for days, which could also be the case for SARS-CoV-2 and could pose a prolonged risk of infection (Kampf *et al*.,2020).

These findings explain the rapid geographic spread of SARS-CoV2, and public health interventions to reduce transmission will provide benefit to mitigate the epidemic, as has proved successful in China and several other countries, such as South Korea(Li *et al*., 2020;Chinazzi *et al*.,2020; Lu *et al*.,2020).

1.2.7. Clinical Invastigation

The SARS-COV2 is diagnosed based on asking questions about contacts and travel of the patient during past two weeks and accurate tests like molecular methods, serology and viral culture. RT-PCR (Real Time Polymerase Chain Reaction) is a molecular method that is commonly used for diagnosis. Lower respiratory tract samples are better than upper ones because they have higher viral load. The other methods have some defects: Antibody detection has the less sensitivity and viral culture take more time ,National health and health commission of china recommend Computed Tomography Scan (CT scan) as the main way for diagnosis because RT-PCR may have some errors in samples. Radiological tests are important for early detection of the disease. Chest CTs imaged of SARS-CoV2 patients are broad-spectrum, but the regular chest CT results is bilateral distribution of

patchy shadows and ground glass opacity (GGO) (Wang *et al*.,2020; Zu *et al*, 2020).

1.2.8.Clinical Features of SARS -CoV2

SARS-CoV2 is the global concern as it causes the largest outbreak till date that teaches us the virulence of SARS-CoV-2. Most of the infected population show the symptoms of highly increased rate of fever, dry cough then bilateral pneumonia (83%, 82%, and 75% among 99 patients in Wuhan). Some infected person also struggles for respiration and dumpiness (around 14 and 31%). Along with the above symptoms few patients from Wuhan also felt muscle ache, confusion, headache, sore throat, rhinorrhoea, chest pain, diarrhoea, nausea and vomiting. It has been also observed that low immunity and continuous exposer towards that virus can lead to infection of SARS-CoV2. Further it drives to serious disease, organ failure and then death. Thus, if such symptoms are present, urgent medical attention is recommended. However, in some cases asymptomatic carriers are being reported thus it's an alarm for the world to maintain the most hygienic processes. Mostly people with medical history and old age person are more prone towards the infection as their immune system is too weak to fight. Most of the time persons having preceding medical records with perpetual medication cannot be recommend for different types of drugs simultaneously for different therapy. Hence it's always recommend by various health agency to prevent the disease as much as possible by maintaining the hygienic condition (Fung and Liu, 2019; Kannan *et al*., 2020; Chang *et al*., 2020).

On infection, the most common symptoms are fever, fatigue and dry cough (Chenm*et al*.,2020 ;Huang *et al*.,2020;Wang *et al*.,2020; Guan *et al*.,2020) . Less common symptoms include sputum production, headache, haemoptysis, diarrhoea, anorexia, sore throat, chest pain, chills and nausea

and vomiting in studies of patients in China (Chen *et al*.,2020 ;Huang *et al*.,2020; Wang *et al*.,2020; Guan *et al*.,2020).

Signs and symptoms are used in the initial diagnosis of suspected SARS-CoV-2 infection and to help identify those requiring tests. A number of key symptoms have been suggested as indicators of mild to moderate SARS-CoV2, including: cough, fever greater than 37.8 °C, headache, breathlessness, muscle pain, fatigue, and loss of sense of smell and taste (Struyf, 2021)**.**

The fact that a symptomatic reinfection with SARS-CoV-2 can occur already 3 months after the first infection is not unexpected. Symptomatic reinfections with human non-SARS coronaviruses are common and not atypical within 1 year after initial infection, despite the presence of antibodies. Reinfections with human non-SARS coronaviruses are, however, typically milder as was the case in our patient (Schmidt *et al.*,1986 ;Galanti and Shaman, 2020).

SARS-COV2 can present along a clinical spectrum from asymptomatic, mild symptoms (e.g. cold-like) (Scott *et al*.,2020; Song *et al*., 2020), influenza-like (e.g. fever, malaise and myalgia) to severe lower respiratory disease with dyspnea and pneumonia (Cao *et al*., 2020).

Among patients with SARS-COV2, fever was the most common symptom, concomitant with dry cough, dyspnoea, fatigue and pneumonia. However, several non-respiratory clinical features were also reported, such as gastrointestinal symptoms (typically diarrhoea) (Gu *et al*., 2020).

General clinical presentation including severity of lung disease, medical history, exposure history, measures taken for personal protection, smoking history, comorbidities, allergies, thyroid diseases and rheumatic diseases were extracted from the hospital electronic medical record system(Chan *et al*., 2020).

It is essential to be aware of clinical manifestations of SARS-CoV2, even though the symptoms are nonspecific. Common symptoms are fever, nonproductive cough and myalgia or fatigue, normal or decreased leukocyte counts and radiographic evidence of pneumonia. At first people may complain of diarrhea and nausea. A few days later, they develop fever. Fever is usually detected in patients but it is not the main symptom. Headache, dizziness, abdominal pain, diarrhea, nausea, and vomiting are some of the less common symptoms. ICU care is required for aged patients or patients likely to have comorbidities including hypertension, diabetes, cardiovascular diseases and cerebrovascular disorders. Subsequent problems during hospitalization are mostly acute respiratory syndrome (ARDS), arrhythmia, and shock. According to scientific observations, as the status of patient gets worse, urea and creatinine blood levels gradually rised (Wang *et al*.,2020; Zu *et al*., 2020).

1.2.9. Risk Factors for SARS-Cov2

Risk factors for poorer outcomes that have been identified in the general population include older age, male sex, having specific comorbid conditions, and select race/ethnicity (Guan *et al*., 2020). Among adults aged more than 65 years approximately 89 % suffer from one or more underlying comorbidities, including obesity (48 %), cardiovascular disease (28 %), hypertension (50 %) and diabetes mellitus (28 %) as well as chronic lung disease (35 %) (Garg *et al*., 2020).

To reduce the overall mortality rate, identifying the risk factors related to disease severity and mortality in SARS-CoV2 patients is urgently required. Previous studies have shown that older age, underlying comorbidities, high D-dimer level, and abnormalities of several biochemical variables were closely associated with disease severity or even death of SARS-CoV2 patients (Zhang *et al*.,2020; Wu *et al*., 2020; Zhou *et al*., 2020; Du *et al*.,2020).

Most people infected with SARS-CoV2 have mild disease and recover. Fever and dry cough are the dominant symptoms. Severe and critical illness occurred in approximately 20% of the patients after admission to the hospital. Currently available evidence suggests that elder age and coexisting medical condition is as- sociated with a higher risk of poor outcome.(Ruan *et al*., 2020;Yang *et al*.,2020).

The high Sequential Organ Failure Assessment (SOFA) score and increased D-dimer levels were also identified as risk factors for SARS-CoV2 mortality (Zhou *et al*.,2020).

Systematic reviews have shown that people with SARS-CoV2 who have chronic comorbidities such as hypertension, diabetes, and cardiovascular disease are at high risk of progressing to severe SARS-CoV2 disease (Yang *et al.*, 2020).

Risk factors for SARS-CoV2 infection could be different, and we found no evidence of an association between these conditions and a positive SARS-CoV2 test. We found that chronic kidney disease and obesity were associated with testing positive for SARS-CoV2. And associated with increased risk of other respiratory infections (Zammit *et al*.,2010;McDonald *et al*., 2015).

Previous study have reported that smoking is associated with increased risk of intensive care unit admission or death among people with SARS-COV2 (Vardavas and Nikitara, 2020).

Sex-related differences in immune response have been reported, revealing that men had higher plasma innate immune cytokines and chemokines at baseline than women(Takahashi *et al.*,2020). In contrast, women had notably more robust T cell activation than men, and among male participants T cell activation declined with age, which was sustained among female patients. These findings suggest that adaptive immune response may be important in defining the clinical outcome as older age and male sex is

associated with increased risk of severe disease and mortality.Increased levels of pro-inflammatory cytokines correlate with severe pneumonia and increased ground glass opacities within the lungs(Wu *et al*., 2020; Liu *et al*., 2020).

1.2.10. Immune response and SARS-CoV-2

1.2.10.1. Innate immune response to SARS-CoV-2

The components of the innate immune system act as first responders for the detection and clearance of viral infections. Innate immune cells secrete proinflammatory cytokines that inhibit viral replication, stimulate the adaptive immune response, and recruit other immune cells to the site of infection. Granulocytes degranulate in response to extracellular pathogens, releasing enzymes and toxic proteins. Monocytes traffic to tissues and differentiate into monocyte-derived macrophages and dendritic cells (mDCs). Macrophages and neutrophils phagocytose and destroy pathogens as well as infected cells. Activated DCs present pathogen-derived antigens to naive helper T cells to initiate the adaptive immune response. Natural killer (NK) cells kill virally infected cells via degranulation, receptor-mediated apoptosis, and antibody-dependent cell-mediated cytotoxicity. Finally, the complement system plays a role in immune cell recruitment, activation, and destruction of pathogens. In spite of these critical antiviral functions, an overactive innate immune response can contribute to disease pathogenesis. This Minireview will cover what is currently known about the innate immune response to SARS-CoV-2 infection, how this relates to the pathogenesis of SARS-CoV2, and the implications for development of therapeutics. Although lung epithelial cells also express innate immune receptors and produce inflammatory cytokines in response to SARS-CoV-2 infection (Blanco-Melo *et al.*, 2020), this antiviral response has been discussed previously (Vareille *et al*., 2011).

The role of peripheral monocytes in the proinflammatory response is unclear. Guo et al. identified a unique subpopulation of monocytes present in the severe phase of SARS-CoV2 (Guo *et al*., 2020).

Thus far, very little has been published on the role of DCs in SARS-CoV-2 infection. A significant decrease in conventional DCs and plasmacytoid DCs has been reported in SARS-CoV2 patients in comparison with healthy controls (Wilk *et al*., 2020)

There is a potential role for complement during SARS-CoV-2 infection. Similar to what has been reported in SARS (Huang *et al*., 2005), serum levels of complement proteins are increased in severe SARS-CoV2 patients in comparison with mild cases and healthy controls (Gao *et al*., 2020)

Additionally, gene functional enrichment analysis of differentially regulated genes in the PBMCs of SARS-CoV2 patients showed an enrichment in genes associated with complement activation and the classical pathway (Xiong *et al*., 2020)

Mannose-binding lectin (MBL) is a pattern-recognition protein present in the serum that, together with MBL-associated serine protease 2 (MASP-2), mediates activation of the lectin pathway by binding to sugars expressed by a variety of pathogens. Interestingly, Gao *et al*. found that SARS-CoV-2 nucleocapsid (N) protein interacts with MASP-2, inducing MASP-2 auto-activation and cleavage of complement protein, C4 (Gao *et al*., 2020).

1.2.10.2 The Cytokine Response to SARS-CoV-2 Infection

The term "cytokine storm," first proposed to characterize the uncontrollable inflammatory states in graft versus host disease (GvHD) in 1993, has now been broadly used to define a situation in which inflammatory cytokines are overly secreted in response to certain diseases (Ferrara *et al*.,1993).

Inflammatory cytokines, a large group of proteins or peptides secreted by immune cells, play vital roles in inflammatory processes by promoting pathogen recognition, immune cell recruitment, threat elimination, and systemic homeostasis (Speyer and Ward ,2011).

Inflammatory cytokines classified into chemokines, interleukins, and growth factors, among which the tumor necrosis factor and interleukin families have been well investigated for their roles in multiple inflammatory responses ,for example, tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) increase vascular permeability subsequently easing leukocyte infiltration, while interleukin-6 (IL-6) can elicit complement expression, which are essential for innate immune response (Medzhitov,2007).

Yet investigating individual cytokines and their corresponding receptor functions in certain inflammatory conditions remains challenging. Previous study have illustrated that some chemokine receptors are able to bind multiple ligands, demonstrating considerable redundancy within the chemokine network (Zlotnik *et al*.,2006).

Cytokine release is a tightly controlled immune process in response to pathogen exposure,with only a few pathogens, such as SARS-CoV-2, which can routinely initiate a cytokine storm and consequently lead to organ damage and even death (Vaninov,2020).

A variety of cell types and factors are involved in the initiation and progression of cytokine storm. At the early phase of SARS-CoV-2 infection, rapid viral replication triggers a delayed secretion of antiviral interferons (IFNs), meanwhile promoting the release of proinflammatory cytokines, including IL-6, IL-1β, and TNF-α (Mehta *et al*.,2020).

Stimulated by IFN signaling, lung macrophages secrete certain chemokines, which recruit other inflammatory immune cells, such as neutrophils, monocytes, and dendritic cells, to the infection sites (Rao *et al*.,2017).The activated immune cells secret more cytokines, further

worsening injury to the lung (Vaninov,2020). SARS-CoV2 provides a compelling example of cytokine storm (Moore and June,2020).

In response to SARS-CoV-2 infection, various cytokines, such as IL-6, TNF-α, IL-8, IL-1, IL-21, and monocyte chemoattractant protein-1 (MCP-1), are upregulated in macrophages and/or monocytes to promote pathogen elimination and tissue repair (Huang *et al*.,2020).

Prolonged high level of cytokines, characterized as the cytokine storm, may exacerbate systemic immune disorder(Chen *et al*.,2020).Patients with SARS-CoV2 may develop severe complications due to cytokine storm(Xu *et al*.,2020). thus immunosuppression has been proposed as an essential means to manage severe SARS-CoV2 cases (Moore and June,2020).

Cytokine storm is the main mechanism of systemic inflammation-mediated immunopathology in severe SARS-CoV2 cases. In most of the severe SARS-CoV2 cases, infiltration of innate immune cells, such as neutrophils and macrophages, is increased in the lungs, leading to massive production of pro-inflammatory cytokines and chemokines, including IL-6, IL-1β, IL-2, IL-8, IL-17, IL-10, G-CSF, GM-CSF, IFN-γ, IP10, MCP1, MIP1A, and TNF-α. Furthermore, prominent acute inflammatory markers, such as acute phase reactants, including C-reactive protein, lactate dehydrogenase, and D-dimer, are also increased (Meng *et al*.,2021).

Pervious study have reported an association between progression to severe SARS-CoV2 and dysregulated secretion of proinflammatory cytokines. Both intensive care unit (ICU) and non-ICU SARS-CoV2 patients in Wuhan, China had increased plasma concentrations of IL-1β, IL-1Ra, IL-7, IL-8, IL-9, IL-10, basic FGF, GCSF, GM-CSF, IFN-ɣ, CXCL10, CCL2, CCL3, CCL4, PDGF, TNFα, and VEGF in comparison with levels seem in healthy controls (Huang *et al*., 2020).

Further, in comparison with non-ICU patients, ICU patients had higher concentrations of IL-2, IL-7, IL-10, GCSF, CXCL10, CCL2, CCL3,

and TNFα. Similar results were reported in a USA cohort where increases in circulating IL-6, IL-1Ra, CCL2, CCL8, CXCL2, CXCL8, CXCL9, and CXCL16 were observed in SARS-CoV-2^{+} patients in comparison with patients with non- SARS-CoV2 -related respiratory issues (Blanco-Melo *et al*., 2020).

Further analysis of the SARS-CoV2 cytokine response was performed by classifying critically ill patients according to criteria used in sepsis, which are categorized as (1) macrophage-activation syndrome (MAS), (2) immune dysregulation characterized by low human leukocyte antigen DR (HLA-DR) expression on CD14^{+} monocytes without elevated ferritin, and (3) an intermediate state lacking obvious dysregulation (Giamarellos-Bourboulis *et al.*, 2020).

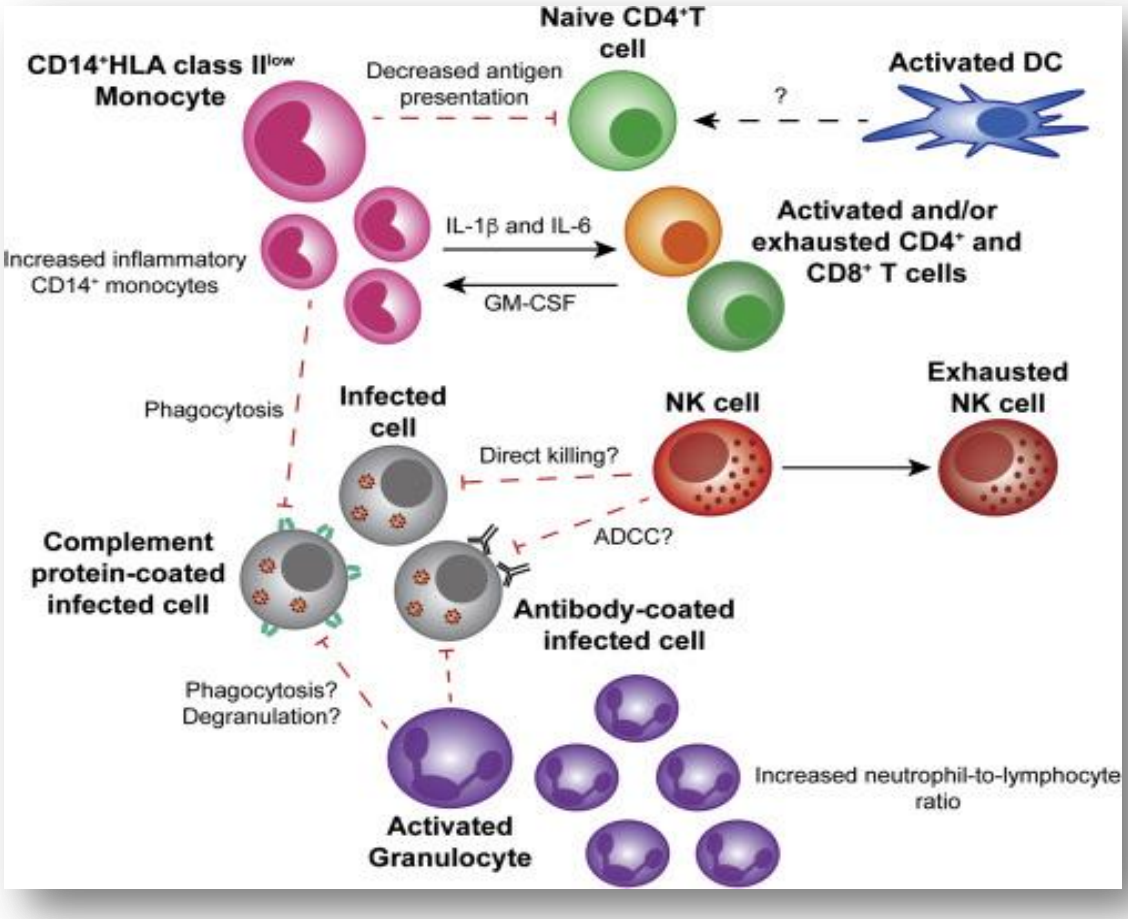


Figure (1.3)The Peripheral Innate Immune Response to Severe SARS-CoV2 Infection (McKechnie and Blish,2020) .

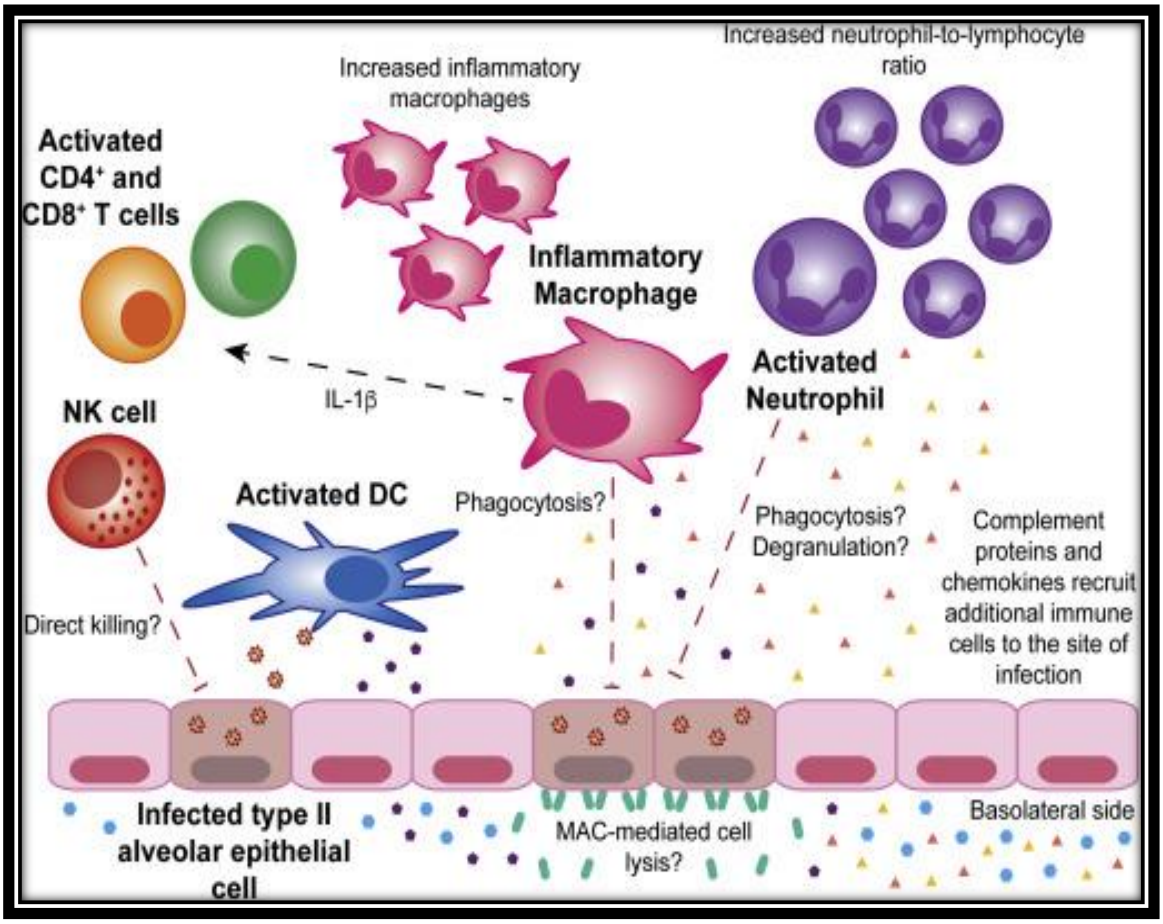


Figure (1.4) The Innate Immune Response to Severe SARS-CoV-2 Infection of the Lung . (McKechnie and Blish,2020) .

1.2.10.3. Immunopathology Related to Innate Immune Response in SARS-COV2

After viral entry, the initial inflammatory response attracts virus-specific T cells to the site of infection, where the infected cells are eliminated before the virus spreads, leading to recovery in most people (Mangalmurti and Hunter, 2020).

In patients who develop severe disease, SARS-CoV-2 elicits an aberrant host immune response (Mangalmurti and Hunter, 2020; Blanco-Melo, *et al*., 2020).

For example, postmortem histology of lung tissues of patients who died of SARS-CoV2 have confirmed the inflammatory nature of the injury, with features of bilateral diffuse alveolar damage, hyaline-membrane formation, interstitial mononuclear inflammatory infiltrates, and desquamation consistent with acute respiratory distress syndrome (ARDS), and is similar to the lung pathology seen in severe Middle East respiratory syndrome (MERS) and severe acute respiratory syndrome (SARS)(Xu, *et al*., 2020; Carsana, *et al*., 2020).

A distinctive feature of SARS-CoV2 is the presence of mucus plugs with fibrinous exudate in the respiratory tract, which may explain the severity of SARS-CoV2 even in young adults(Wang, *et al*., 2020).

This is potentially caused by the overproduction of pro-inflammatory cytokines that accumulate in the lungs, eventually damaging the lung parenchyma .Some patients also experience septic shock and multi-organ dysfunction (Mangalmurti and Hunter, 2020).

For example, the cardiovascular system is often involved early in SARS-CoV2 disease and is reflected in the release of highly sensitive troponin and natriuretic peptides(Liu, *et al*., 2020).

Consistent with the clinical context of coagulopathy, focal intra-alveolar haemorrhage and presence of platelet-fibrin thrombi in small arterial vessels is also seen(Carsana, *et al*., 2020).

Cytokines normally mediate and regulate immunity, inflammation, and haematopoiesis; however, further exacerbation of immune reaction and accumulation of cytokines in other organs in some patients may cause extensive tissue damage, or a cytokine release syndrome (cytokine storm), resulting in capillary leak, thrombus formation, and organ dysfunction (Mangalmurti and Hunter, 2020; Wu, *et al*., 2020).

1.2.10.4. Adaptive Immunity to SARS-Cov2

The adaptive immunity, encompassing humoral and cellular immune responses, is a key to clear a wide variety of viral infections, rendering patients recovered from viral diseases(Ni *et al*.,2020).

The adaptive immune system responds to pathogens in an antigen-specific manner to develop protective immunity. The adaptive immune system consists of three major lymphocyte types: B cells (antibody producing cells), $CD4^+$ T cells (helper T cells), and $CD8^+$ T cells (cytotoxic, or killer, T cells) (Murphy and Weaver, 2016).

In SARS-CoV-2 infection, both virus-specific B-cell mediated humor immunity and T-cell mediated cellular immunity have been implicated in recovered SARS-CoV2 patients.(Ni *et al*.,2020; Grifoni *et al*.,2020; Le Bert *et al*.,2020; Cao *et al*.,2020; Woodruff *et al*.,2020).

Notably, in asymptomatic patients, SARS-CoV-2-specific IgGs were minimally produced and poorly maintained (Ibarrondo *et al*.,2020).in contrast, patients of severe disease mounted potent virus-specific IgG responses(Long *et al*.,2020; Lee *et al*.,2020; Zhang *et al*.,2021).

Data regarding the adaptive immune responses in SARS-CoV2 are limited. Both cellular and humoral responses were identified and further investigated in SARS-CoV2 (Prompetchara *et al*., 2020).

It is presumed that SARS-CoV2 induces a similar Th1 type immune response as other viral infections (Russel *et al*., 2020).

The count of CD8+ T cells was reported to be decreased during SARS-CoV2 infection, and, in severe cases, memory CD4+ T cell and T regulatory cell count was significantly reduced ,These findings were accompanied by a decreased number of CD4+ and CD8+ T cells in lymph nodes. Lymph nodes and spleen in SARS-CoV2 patients were described as atrophic, which highlights the role of SARS-CoV2 in potentiating cell degeneration (Zhang *et al*., 2021).

In mild stages and/or in patients presenting with mild symptoms solely, the lymphocyte count was found to be significantly higher as compared to patients with severe disease. This also applied to both T cell (CD3+ cells) and CD8+ T cell (CD3+ /CD8+ cells) populations (Cao *et al.* 2020).

In both mild and severe cases of SARS-CoV2, the CD8+ T cell counts were decreased as compared to healthy donors. Moreover, CD8+ T cells presented in SARS-CoV2 patients were found to less degranulate (decreased CD107a externalization) and to produce lower levels of IL-2, IFN γ, and granzyme B as compared to healthy donors (Zheng *et al*., 2020).

In peripheral blood T cells isolated from patients in intensive care units (ICUs), the expression of PD-1 was significantly higher as compared to T cells isolated from patients with mild disease or from healthy donors (Moon 2020).

Study further revealed that as the disease severity progresses, the serum levels of pro-inflammatory cytokines increase as well (Shi *et al*. 2020).

Different immune responses are associated with mild and severe stages of SARS-CoV2. These findings led to conclusions that stimulating the immunity in the non-severe (mild) stages of the disease can be beneficial. In contrast, however, once a severe impairment of lung functions had already

occurred, further damage is potentiated by the immune system, and, therefore, immunosuppression is required instead (Shi *et al*. 2020).

SARS-CoV-2, similarly to other coronaviruses, restrains antigen presentation by downregulating MHC class I and II molecules, which inhibits the T cellmediated immune responses (Zheng *et al*., 2020). Nevertheless, humoral immune responses also play a substantial role in SARS-CoV2 infections, even though antibodies may not be sufficient to neutralize the virus (Guo *et al*., 2020).

The most concerning evidence regarding the development of antibodies is that each patient has completely different kinetics of humoral responses (Guo *et al*. 2020). Most of the patients develop antibodies after 7 days since the disease onset. During the first seven days of the disease, the detectability of the virus-specific antibodies in SARS-CoV2 patients was less than 40 % and, therefore, the use of serology testing is of limited value (Zhao *et al*., 2020).

It has been demonstrated, that SARS-CoV2 plasma has a crossreactivity to SARS-CoV, but does not show crossreactivity to other coronaviruses (Guo *et al*., 2020). In the early phase of SARS-CoV2 infection, RT-qPCR should be the dominant diagnostic tool (Zhao *et al*., 2020).

It has also been shown that 22 % of patients with RT-qPCRconfirmed positivity were IgM negative (Guo *et al*., 2020).

Since the day 15 after the disease onset, IgM and IgG antibodies were detected in 94.3 % and 79.8 % of the patients, respectively ,in some patients, the detection of IgM antibodies was observed at the same time as the detection of IgG ,also, the IgM and IgG antibodies showed similar dynamics in selected patients (Zhao *et al*., 2020).

The duration of IgG antibodies still remains unknown. The only estimation could be done from the immunology memory studies performed

on SARS-CoV, where SARS-specific antibodies were maintained for an average of 2 years (Wu *et al*., 2020).

The titer of the virus-specific antibodies was correlated with the disease severity, and it has been shown that a high titer of SARS-CoV2 antibodies serves as an independent risk factor for critical manifestation of SARS-CoV2 (Cao, 2020).

1.2.11. Tumor Necrosis Factor Alpha (TNF a) with SARS-COV2

IFNs are a subgroup of cytokines that have a fundamental role in the immune response against both viral and microbial pathogens(Fensterl and Sen, 2009). Three major types of IFNs (IFN-I, IFN -II and IFN -III) are characterized based on their receptor specificity (Darbeheshti *et al*.,2021).

TNF-α is produced primarily as a transmembrane precursor by monocytes/macrophages, but a number of other immune and structural cell types, such as T and B lymphocytes, mast cells, neutrophils, fibroblasts and airway epithelial cells also secrete this cytokine (Tay *et al*.,2020).

The TNF-α precursor is cleaved by TNFα-converting enzyme (TACE) to liberate TNF-α that acts by binding to two distinct membrane receptors on target cells: TNFR1 and TNFR2 (Heir and Stellwagen, 2020; Gough and Myles, 2020).

TNFR1 is constituitively expressed within the lymphoid system and nearly all cells of the body, which likely accounts for TNF's wide-ranging functions. TNFR2 expression is limited to certain lymphocyte populations including T-regulatory cells (T_{regs}) (Horiuchi *et al*.,2010; Heir and Stellwagen, 2020; Gough and Myles, 2020).

TNFR1 signaling tends to be pro-inflammatory and apoptotic, whereas TNFR2 signaling is anti-inflammatory and promotes cell proliferation(Heir and Stellwagen, 2020; Gough and Myles, 2020).

Suppression of TNFR1 signaling has been important for treatment of autoimmune disease (Rolski and Błyszczuk,2020). whereas TNFR2 signaling promotes wound healing(Gough and Myles, 2020).

TNF-α exists as a transmembrane form (mTNF-α) and as a soluble form (sTNF-α) sTNF-α results from enzymatic cleavage of mTNF-α (Qu and Li, 2017). by a process called substrate presentation. mTNF-α is mainly found on monocytes/macrophages where it interacts with tissue receptors by cell-to-cell contact (Qu and Li, 2017).

sTNF-α selectively binds to TNFR1, whereas mTNF-α binds to both TNFR1 and TNFR2(Probert, 2015).TNF-α binding to TNFR1 is irreversible, whereas binding to TNFR2 is reversible (Szondy and Pallai, 2017).

The primary role of TNF is in the regulation of immune cells. TNF, as an endogenous pyrogen, is able to induce fever, apoptotic cell death, cachexia, and inflammation, inhibit tumorigenesis and viral replication, and respond to sepsis via IL-1 and IL-6-producing cells. Dysregulation of TNF production has been implicated in a variety of human diseases including Alzheimer's disease (Swardfager *et al*.,2010). cancer, major depression, psoriasis, and inflammatory bowel disease (IBD) ,Though controversial, study have linked depression and IBD to increased levels of TNF (Bobińska *et al*.,2017).

TNF-α, a major pleiotropic mediator of acute and chronic systemic inflammatory responses, can simultaneously regulate cells apoptosis and proliferation while promoting the production of other chemokines and cytokines. It is also involved in a series of physiological processes such as anti-tumor responses, control inflammation, and immune system homeostasis (Aggarwal ,2003;Croft ,2009). TNF-α is also one of the most important pro-inflammatory cytokines of the innate immune response,

dysregulated TNF-α signaling can trigger (CRS) cytokines release syndrome (Ragab,2020).

TNF-α is one of the most potent proinflammatory cytokines with broad spectrum of actions. Marked elevations reported in many inflammatory conditions including cytokine release syndrome. Serum TNF-α levels found elevated in SARS-CoV2 patients with being more pronounced in more severe patients (Huang, *et al*.,2020).

A rise in TNF-α could result in the facilitation of viral infection and organ damage (Deng and Pei,2020). SARS-CoV viral spike protein is able to modulate TNF-α-converting enzyme (TACE)-dependent shedding of the ACE2 ectodomain, required for the viral entry which is coupled to TNF-α production (Haga *et al.*,2008).

Therefore, it is hypothesized that the use of TNF inhibitors might be effective in blocking viral entry and detrimental effects of exuberant TNF-α, as shown in preclinical studies on severe respiratory syncytial virus and influenza infections (Feldmann *et al*.,2020).

1.2.12. Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF) with SARS-COV2

Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a complex cytokine and a member of the colony-stimulating superfamily. While it does have a role as a growth factor for myeloid cells, this is considered a lesser function compared with other cytokines such a granulocyte-stimulating colony factor (G-CSF) and macrophage-stimulating colony factor (M-CSF), and GM-CSF is now considered a central player in the integrated immune response and a central mediator of tissue inflammation (Lang *et al*.,2020).

GM-CSF is a myelopoietic growth factor and pro-inflammatory cytokine that plays a central role in a broad range of immune-mediated

diseases. GM-CSF, which is secreted by macrophages, T cells, mast cells, natural killer cells, endothelial cells, and fibroblasts, regulates macrophage number and function. It acts as a pro-inflammatory signal, prompting macrophages to launch an immune cascade that ultimately results in tissue damage (Mehta *et al*.,2020; Thwaites *et al*.,2021).

GM-CSF is believed to be a key driver of lung inflammation in severe and critical SARS-CoV2 pneumonia, operating upstream of other pro-inflammatory cytokines and chemokines (Mehta *et al*.,2020; Zhou *et al*.,2020).

GM-CSF in SARS-COV2 disease is its complex role in lung homeostasis and inflammation. In the healthy lung, GM-CSF has a critical role for maintaining the maturation and function of alveolar macrophages and surfactant metabolism and is required to maintain pulmonary function, as well as contributing to lung sentinel cell-mediated immunity. GM-CSF also appears central in driving inflammation locally and systemically (Lang *et al*.,2020)

And experimental models of acute lung injury support this hypothesis, demonstrating that resident alveolar macrophages secrete a variety of inflammatory cytokines that lead to the influx of innate cells including neutrophils, further amplifying the activation of alveolar epithelial cells and tissue damage (Mehta *et al*.,2020).

The capacity of GM-CSF to amplify inflammatory response within the lung and its systemic effects, mediated by linking the release of upstream inflammatory cytokines such as IL-1, IL-6, and TNF across monocytes and macrophages and activated T cells in a positive feedback loop, highlight its potential importance in driving systemic inflammation and disease. However, as noted above, adding to the complexity is that GM-CSF is also a critical cytokine for healthy pulmonary function and is necessary for the maturation and maintenance of alveolar macrophages; and in some

experimental models, it confers resistance to viral respiratory balance, underscoring that all putative inflammatory cytokines also play roles in integrated host defense (Becher *et al*.,2016).

The central role of GM-CSF in the inflammatory response and because GM-CSF appears to be upstream of other key inflammatory cytokines invites targeting strategies against it in effort to down-modulate states of hypercytokinemia. GM-CSF levels are generally extremely low or undetectable in healthy individuals and are detectable in blood of patients with inflammatory manifestations of SARS-CoV2,(Huang *et al*.,2020).

And CD14+CD16+ monocytes, a rich source of GM-CSF, are expanded in such patients as well (Zhou *et al*.,2020). GM-CSF targeting is actively being investigated in a variety of autoimmune diseases and has been successfully studied in a mouse model of cytokine release from CAR-T cells (Sterner *et al*.,2019).

1.2.13. Interleukin 33 (IL-33) with SARS-COV2

Interleukin 33 (IL-33), the gene for which is located on chromosome 9, is a newly discovered member of the IL-1 cytokine family and a pivotal regulator of inflammatory and immune responses (Molofsky *et al*., 2015; Di Salvo *et al*., 2018; Chen *et al*., 2020).

It functions in association with the suppression of tumorigenicity 2 (ST2) receptor. In fact, the IL-33/ST2 pathway is involved in causing an imbalance between widespread inflammation and tissue regeneration in several organs, such as the lungs, liver, skin, and gastrointestinal system, ultimately leading to fibrosis (Rankin *et al*., 2010; Tan *et al*., 2018; Wu *et al*., 2018; Imai *et al*., 2019).

The IL-33 is a regulator of inflammation, and can induce T-helper 2 (Th2)-mediated innate and adaptive immune responses (Kakkar and Lee, 2008; Kotsiou *et al*., 2018). Additionally, IL-33 participates in the pathogenesis of renal, neurological, hepatic, pulmonary, and eye

diseases. It is a cell cytokine that promotes inflammatory responses and has a characteristic of alarm (Moussion *et al*., 2008; Li *et al*., 2019; Wang *et al*., 2020).

IL-33 is a cytokine of the IL-1 family that is expressed in barrier tissues and exerts pleiotropic functions. In the lungs, IL-33 is promptly released, mainly by injured epithelial alveolar cells, following infection and cellular damage(Gabryelska *et al*.,2019).

Previous researches have reported that IL-33/ST2 axis is involved in the inflammatory responses to several viral infections, including bronchiolitis, influenza, herpes simplex virus, coxsackie B virus, lymphocytic choriomeningitis virus and metapneumovirus (Zeng *et al*.,2015; Lay *et al*.,2015).

During different infections, the deleterious or protective role of IL-33/ST2 axis depends on the type and invasiveness of infectious agent, the involved organs, whether the infection is acute or chronic, the host immune compartment and microenvironments (Rostan *et al*.,2015).

Clinical studies have found higher sST2 levels with no difference in IL-33 levels in patients with hepatitis B virus infection, and the increased sST2 levels were found to correlate with disease severity and predicted poor survival (Mehraj *et al*.,2016).

In sera of patients with HIV infection, high levels of sST2 and low levels of IL-33 have been detected (Miyagaki *et al*.,2010). Moreover, it has been reported that persistently high sST2 concentrations were associated with severity of viral acute lower respiratory infection (Portugal *et al*.,2020).

As an important biomarker of severe forms of dengue virus (DENV) infection, serial changes of sST2 levels may be a more reliable predictor for dengue fatality (Hsieh *et al.*,2019). For the novel SARS-CoV-2 infection in SARS-CoV2 patients, the role and change of sST2 and IL-33 is still unknown(Zeng *et al*.,2020).

1.2.14. Interleukin 13 (IL-13) with SARS-COV2

Interleukin 13 (IL-13) is a protein that in humans is encoded by the *IL13* gene(Minty *et al*.,1993).IL-13 genes are located in a region of 140 kb on chromosome 5q31-33 that codes for a cluster of Th2 cytokines(Quirico-Santos *et al*.,2007).

The secondary structural features of IL-13 are similar to that of Interleukin 4 (IL-4) however it only has 25% sequence identity to IL-4 and is capable of IL-4 independent signaling (Bieber,2020).IL-13 is a cytokine secreted by T helper type 2 (Th2) cells, CD4 cells, natural killer T cell, mast cells, basophils, eosinophils and monocytes (Corren ,2013).

The anti-inflammatory properties of IL-13 are probably based on their ability to induce regulatory T-cell expression (Skapenko *et al*.,2005)

IL-13 is a central regulator in IgE synthesis, goblet cell hyperplasia, mucus hypersecretion, airway hyperresponsiveness, fibrosis and chitinase up-regulation(Moriyama *et al*.,2014). It is a mediator of allergic inflammation and different diseases including asthma(Corren ,2013).

IL-13 is implicated in numerous processes, including (i) recruitment of eosinophils and M2 macrophages to the lung, (ii) induction of mucus secretion into the airways and goblet cell metaplasia, (iii) proliferation of smooth muscle cells, and (iv) fibrosis via fibroblast activation and subsequent collagen deposition (Marone *et al*.,2019).

Interleukin (IL)-13 is critical in maintaining mucosal homeostasis, being implicated in allergy, parasitic and viral infection, as well as vaccine-specific immunity (Chang *et al*.,2020).

At the lung mucosae, IL-13 is expressed by a range of innate immune cell types, particularly type 2 innate lymphoid cells (ILC2s), whose rapid response to external stimuli (pathogens, toxins, and allergens) acts to

facilitate barrier tissue responses and condition downstream immune outcomes(Mindt *et al*.,2018).

IL-13 activity at the lung triggers smooth muscle contraction, mucus secretion, and the recruitment/activation of inflammatory immune cells. However, overexpression of IL-13 is associated with allergic lung hyperinflammation, airway tissue remodeling, and hyperresponsiveness (Zhu *et al*.,2002).

Interestingly, IL-13 dysregulation is known to be a hallmark of several disease conditions, including allergic pulmonary diseases, atopic dermatitis, and also some cancers (Kioi *et al*.,2006).

Since coronavirus disease 2019 (COVID-19) is fundamentally characterized by dysregulation of the lung mucosae, we postulate that IL-13 is associated with destructive lung hyperinflammation/immune activity that underpins severe SARS-CoV2 disease progression (Deimel *et al*.,2021).

IL-13, as an integral orchestrat or of pathogenic responses in the lung, was of particular interest to us. Plasma levels of IL-13 were significantly higher in SARS-CoV2 positive patients compared to uninfected patients (Huang *et al*., 2020; Petrey *et al*., 2021; Yang *et al*., 2020)

Th2-high asthmatics producing high levels of IL-13 in their lungs appear to have reduced susceptibility to severe SARS-CoV2 (Zhu *et al*.,2020; Ramakrishnan *et al*.,2021).

1.2.15. Toll-like receptor 6 (TLR6) with SARS-COV2

Toll-like receptors are expressed on all innate immunity cells including macrophages, basophils, neutrophils, natural killer cells, and dendritic cells as well as on adaptive immune cell lymphocytes (T and B cells) (El-Zayat *et al*., 2019).

The cellular repair mechanism is activated by activation of TLRs that stimulate the signalling cascade of host immune system which helps the release of immune modulators and cytokines (Wang *et al*.,2016).

TLR6 is a transmembrane protein, member of toll-like receptor family, which belongs to the pattern recognition receptor (PRR) family. TLR6 acts in a heterodimer form with toll-like receptor 2 (TLR2). Its ligands include multiple diacyl lipopeptides derived from gram-positive bacteria and mycoplasma and several fungal cell wall saccharides. After dimerizing with TLR2, the NF-κB intracellular signalling pathway is activated, leading to an pro-inflammatory cytokine production and activation of innate immune response(Weiss et al.,2005).

The protein encoded by this gene is a member of the toll-like receptor (TLR) family which plays a fundamental role in pathogen recognition and activation of innate immunity. TLRs are highly conserved from Drosophila to humans and share structural and functional similarities. They recognize pathogen-associated molecular patterns (PAMPs) that are expressed on infectious agents, and mediate the production of cytokines necessary for the development of effective immunity. The various TLRs exhibit different patterns of expression(Medzhitov ,2007).

This receptor functionally interacts with toll-like receptor 2 (TLR2) to mediate cellular response to gram-positive bacteria, mycoplasma, fungi, some viruses and even protozoa(de Almeida *et al.*,2013).

TLRs serve a critical part inside this detection of infectious components as well as the stimulation of the body's immune response. Pro-inflammatory cytokines like interleukin-1 (IL-1), IL-6, and tumor necrotic factor (TNF), and also type 1 interferon, are produced if TLR circuits are engaged. TLRs including TLR2, TLR3, TLR4, TLR6, TLR7, TLR8, and TLR9 could be involved in SARS-CoV2 illnesses(Ismael ,2022).

Every TLR is made up of an ectodomain with leucine-rich repetitions (LRRs), a single transmembrane, as well as a cytoplasmic site that modulates signaling pathway. Peoplehave 11

distinct types of TLRs, with several of them being surface but some being intra cellular sensors. TLR3, TLR7, TLR8, TLR9, and TLR10 were all endosomal receptors(Horova *et al.*,2021).

TLR2, TLR4, or TLR6 are limited towards themembrane of a cell, while these are present in pulmonary or bronchi epithelial cells. TLRs may also be classed depending on their ability to recognize PAMP. TLR4 recognizes glycoprotein, TLR7 or TLR8 recognize viral single-stranded ribonucleic acid (ssRNA), TLR3 recognizes infectious double-stranded RNA (dsRNA), and TLR9 recognizes highly contagious deoxyribonucleic acid (dsRNA) (DNA).TLR4, TLR7, or TLR8 are reported to recognize the genetic information of SARS-COV-2(i.e., ssRNA). Whenever TLRs interact using PAMP, NF-B or IFN regulatory factor (IRF) are activated, resulting through the generation of Type I IFN, that triggers adaptive immunity, that are regarded the major antimicrobial reaction. Among humans, ten TLR group members have been discovered (Ziegler *et al.*,2020; Li and Wu .2021).

TLRs are now split into 2 groups depending on where they are found inside the cell: "TLR1, TLR2, TLR4, TLR5, TLR6, and TLR10 were cell surface TLRs that identify bacterial elements such as proteins or lipids originating from pathogenic organisms, while TLR3, TLR7, TLR8, as well as TLR9 are nucleic acid ligand-sensing TLRs". TLR2 is a membrane receptor that identifies a variety of ligands, including those produced by viruses, bacteria, fungus, or parasites. TLR2 produces heterodimers involving TLR1 or TLR6, which use MyD88 to transmit signals. Whereas the role of TLR2 in immune function to coronavirus infections has yet to be determined(Ismael ,2022).

TLRs' pathways, as a component of innate immunity, could be involved in the pathogenesis of SARS-CoV-2 because several studies have shown that

TLRs play an important role in the pathogenesis of SARS-CoV and the Middle East respiratory syndrome (MERS)(Birra *et al*.,2020).

1.2.16. Genetic Polymorphisms Susceptibility to SARS-COV2

DNA polymorphisms exhibit significant variation between individuals, typically at frequencies of greater than 1%. Human DNA sequence variants include single nucleotide polymorphisms (SNPs) or single nucleotide variants (SNVs), and insertions/deletions (indels). Other polymorphisms include short tandem repeat polymorphisms (STRPs) and variable number of tandem repeats (VNTRs) – highly polymorphic markers used in human linkage mapping, forensics, and paternity testing.

Genetic variation occurs within and among populations, leading to polymorphisms that could be associated with genetic trait or also a phenotype in the presence of an environmental stimulus (Hirschhorn and Daly, 2005)

Single-nucleotide polymorphisms (SNPs) are the most common type of polymorphism found in the human genome, and represent the main reason for 90% of all types of genetic variations among individuals(Hetherington *et al*.,2002).

The SNPs are the most common form of variation in the genome and they are extensively used to study genetic differences between individuals and populations. These SNPs may contribute to changes in the genomic sequence, either in the coding (exons), intergenic, or noncoding (introns) region (Van Dijk *et al*., 2014; Ahmad *et al*., 2018).

The TNF gene (*TNF*; OMIMg 191160) is located within the highly polymorphic major histocompatibility complex region of chromosome 6. It exerts a range of inflammatory and immunomodulatory activities that are important in host defense(Otto and Wenzel, 2008).

The genetic polymorphisms named single nucleotide polymorphisms (SNPs) are responsible for 90% of the variations that take place in the human genome. They may be located in the coding region, or in the gene regulatory region; generating alterations in the amino acid sequence of the coded protein, or at its production rate. Among the SNPs chosen for the scope of this word, the -308 G/A (rs1800629), in the promoter region, is believed to increase the genetic expression and consequently the level of production of TNF-α, due to the transition of the wild allele G to the variant allele A. This fact offers three possibilities :The wild genotype G-308/G-308 is the most common genetic profile of TNF-α, categorizing the individuals as low producers, and therefore are expected to express a less intense inflammatory reaction when compared to the intermediate profile, G-308/A-308. The high producer profile is the rare mutant A-308/A-308, which produces higher amounts of TNF-α, and therefore the individuals with this genotype are expected to react intensely to inflammation. These genetic polymorphisms may play pertinent roles in the outcome of SARS-CoV2 outcome in high producers or intermediate producers of this cytokine(Silva *et al*.,2022).

Recently, numerous polymorphisms in the gene for IL-13 have been identified and have been associated with IgE levels and/or allergic diseases(Vercelli,2002). The *IL13* rs20541 polymorphism which is located in exon 4 with an amino acid change from arginine to glutamine at position 130 was suggested to influence the serum IgE level (Graves *et al*., 2000).

The onset of single nucleotide polymorphisms (SNPs) in TLR6 encoding gene or protein leads towards alteration in the functioning of this PRR. The polymorphic residues on the amino acid sequence of TLRs result in SNPs that alter the inflammatory signalling cascade associated with different disorders (Mukherjee *et al*.,2019).

CHAPTER TWO
MATERIALS & METHODS

2. Materials and Methods

2.1. Materials

2.1.1. Instruments and Equipment

Instruments and equipment used in this study are listed in table (2-1 A and B).

Table 2.1.A: Instruments used in this study with their Manufacturer Company and origin

Instruments	Manufactured Company (Origin)
Autoclave	Wsid / Korea
CBC XT2000i	Sysmex / JAPAN
Centrifuge	Hettichzentrifuge, Sigma / Germany
Deep freeze (-20 C)	Bosch / Germany
Electrophoresis system	Germany
ELISA reader & Washer	Bio Tech (USA)
Fume hood	UK
Incubator (model IB-909)	Memmert / Germany
Micro centrifuge	Hettichzentrifuge, Sigma / Germany
PCR Hood	Analytikjena /China
Refrigerator	Arcelik / Turkey
Sensitive balance	Sartoris / Germany
Ultraviolet imaging device	Motic / USA
Vortex	My Fu gene /China
Water bath (1-A)	Memmert / Germany
PCR-Thermocycler	Germany

Table 2.1.B: Equipment used in this study with their Manufacturer Company and origin.

Equipment	Manufactured Company (Origin)
clear Microtubes 1.5ml	Extragene / Taiwan
Eppendorf tubes with different size 2ml, 1.5ml, 0.5 ml	
Flask with different size 25cm^2 , 75cm^2	China
Gloves	Bio Basic – Canada
Medical cotton	Kardelen /Turkey
Medical injection syringes	MEDECO /UAE
Micropipettes various sizes(1000,100,10) µl	Extragene/Taiwan
PCR Tubes 0.2 ml	Extragene/Taiwanio
Tourniquet for blood	Xinle /China

2.1.2 Reagents and Buffers:

Reagents and buffers used in this study are listed in table (2-2)

Table 2.2: Reagents and buffers used in this study with their Manufacturer Company and origin.

Reagents	Manufactured Company (Origin)
Absolute Ethanol alcohol	Merck – Germany
Agarose E	Conda / Spain
DNA Ladder(100-1500bp)	Promega /USA
Loading dye	Promega /USA
Proteinase K	Intron /S. Korea

Rad Safe Nucleic Acid staining solution	Zymo Research / USA
TBE DNA Sequencing Grade(10X)	Intron /S. Korea
Tris EDTA buffer (TE)	Bio basic /Canada

Table 2.3: DNA ladder 100-1500bp (Promega)

Material
Ladder consist of 11 double – stranded DNA with size (100-1500bp) Loading dye has composition: [15% ficoll, 0.03% bromophenol blue, 0.03% xylene cyanol, 0.4% orange G, 10Mm Tris-HCL (PH7.5) 50mm EDTA].

2.1.3 Kits and Marker

Kits and marker used in this study with their Manufacturer Company and origin are listed in table (2-4).

Table 2.4: Kits and markers used in this study with their Manufacturer Company and origin

Kits	Manufacturer Company/ Origin
G-Spin Total DNA Extraction Kit	Intron / Korea
Human GM_CSF ELISA kit	Elabscience / China
Human IL33 ELISA kit	Elabscience / China
Human TNF-a ELISA kit	Elabscience / China
Rapid Test Cassette(COVID-19 IgG/IgM)	Biozek /Holenda

2.1.4 Primes of *IL13 (rs20541)* , *TLR6(rs5743789)* and *TNFa (rs 1800629)* SNPs

Primers sets used in this study to detect the three SNPs (rs20541) (rs5743789) and (rs 1800629)of *IL13*, *TLR6* and *TNFa* gene polymorphism ,respectively with their product size and source as well as origin are listed in table (2- 5).

Table 2.5: Primers sets that used for detection of *IL13* (rs20541) , *TLR6*(rs5743789) and *TNFa* (*rs 1800629*) genes polymorphism.

Gene	Sequence (5′-3′)	Product size (bp)	Source/origin
IL13 (*rs20541)* IF1	GAAACTTTTTCGCGAGGGCCA	A allele: 214 G allele: 251	IDT / USA
IL13 (*rs20541*) IR1	GATGCTTTCGAAGTTTCAGTTGACCC	TWo outer primers: 418	IDT / USA
IL13 (*rs20541*) OF1	CTAACAGTACCCACCTCATGGGGACTT		IDT / USA
IL13 (*rs20541*) OR1	GAAGGCTGAGGTCGGCTAGGCT		IDT / USA
TLR6 (*rs5743789*) IF	AAAATTTTTGCTTTGCAAAAGTCA	A allele: 202 T allele: 153	IDT / USA
TLR6 (*rs5743789*) IR	TTTGTCTTTTTCACTCTCTTGCTGA	two outer primers: 306	IDT / USA
TLR6 (*rs5743789*) OF	AGGAGAAAATCTAGGTGACTTTGGAT		IDT / USA
TLR6 (*rs5743789*) OR	TCTTCTTTGGTGAGGTGTCTGTTT		IDT / USA
Forward G allele Common Reverse	ATAGGTTTTGAGGGGC ATCG AAGAATCATTCAACC AGCGG	290bp	

Primer *TNFa* (*rs 1800629)*			Ahmed *et al*.,2020
Forward A allele primer Common Reverse Primer *TNFa* (*rs 1800629*)	ATAGGTTTTGAGGGGC ATCA AAGAATCATTCAACC AGCGG	290bp	

F= forward, R= reverse, bp= base pair

2.2.Methods

2.2.1. Study Design and Dating of the study

This is a case-control study design conducted for a period from february, 2021 to october 2021 to evaluate the role of some selected serological, heamatological, and genetic factors among patients with SARS-COV2. As shown in the study scheme bellow.

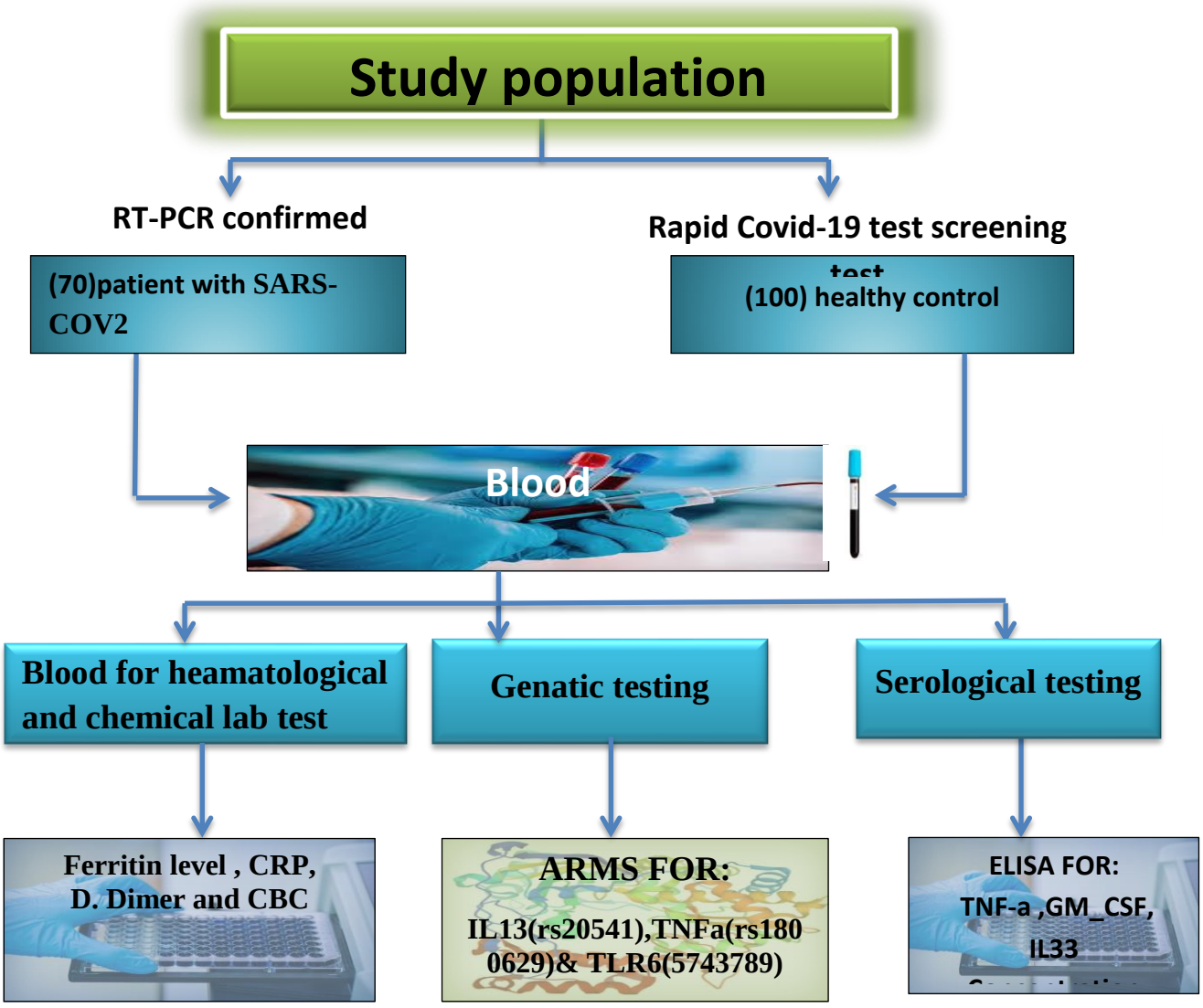


Figure (2.1) Study scheme

2.2.2 Study size and population

In this study, one hundred-seventy subjects were enrolled as two comparison case-control groups, the case group included seventy patients suffered from SARS-COV2, all were selected from those who were admitted to the ICU, and were diagnosed by a specialist physician with sever acute respiratory syndrome due to SARS-COV2documented by RT-PCR beside other clinical criteria and laboratory tests in Marjan Medical City in Babylon province. The control group in this study included one hundred apparently healthy subjects all were confirmed free from SARS-COV2 by Rapid Test (covid-19 antibody test negative).

2.2.3. Inclusion and Exclusion criteria :

Inclusion:

All ages and both sex are accepted in this study. The inclusion criteria for the patients group included any patient who had recently diagnosed with sever acute respiratory syndrome due to SARS-COV2and admitted to the ICU, by a specialist physician according to positive RT-PCR, clinical and laboratory measures. The inclusion criteria for the control group included those persons who had negative history of SARS-COV2in the past two months and confirmed by covid-19 rapid test.

Exclusion:

1-All patient with negative RT-PCR.

2-Patients with Comorbidities (cancer, Kidney disease, liver disease).

3-Patients and control with a known immunological disorders (Autoimmune disease or chronic immunosuppression).

4-Patients and control on chronic intake of steroid or immune-modulating treatment.

5-Pregnant women.

2.2.4. Specimen collection:

2.2.4.1. Blood Sample:

Five ml of fresh venous blood were collected from each participant 2ml of which was kept in EDTA tube at 4°C and use within 24 hours for extraction of human DNA .

2.2.4.2 Serum samples:

The other 3ml of blood kept in the jelly tube without anticoagulant let on laboratory table at 37 °C (up to 1 hour) until be clotting. The latter was undergone centrifugation at 2500 rpm for 15 minutes then the serum was collected and preserved at -20°C until being used to avoid loss of bioactive human IL-33,TNF-a,GM-CSF (Elabs. Kit).

2.2.5. Ethical Issues

All samples of the present study were collected after obtaining ethical clearance from the Ethics Committees of Babylon health office and agreement of doctor management of Intensive care unit in Babylon province. Moreover, this study was approved by the ethical research committee in the college of medicine, Babylon University, and Babylon health directorate. All of the patients and the healthy control subjects were counseled and vocally agreed on the inclusion in the study before participation in the study.This study was performed & being facilitated with permission from Babylon

university, College of Medicine , and the General Health Directorate of Babylon province.

2.2.6. Study methodology:

All the participants were asked for acceptance to participation in this study after full explanation of the study aim and procedures. Full questionnaire were filled contained within information about name, age, gender, smoking habit, past medical history, drug history, occupation, residence and Body Mass Index (BMI). All the patients were subjected for chemical testing regarding CRP, CBC, D-dimer, and s-ferritin. Serological testing regarding IL-33, GM-CSF, and TNF-α were conducted for those control subjects selected according to their past medical and family history.The genetic study included 70 patients with the highest serological parameters records and twenty healthy control for *IL13 (rs20541)* , *TLR6(rs5743789) and TNFa (rs 1800629)* polymorphism evaluation by ARMS technology.

2.2.7.Anthropometric assessments

The anthropometric techniques used to measure weight and height were recommended by Lohman *et al*.(1988). All anthropometric measurements were taken with stress on body height and weight that were measured in light clothes using a portable stadiometer.**Height** was determined using a wall mounted, non-extendable measuring tape with subjects standing in an erect barefoot position, arms by side, and feet together. **Weight measurements** were taken with each subject standing at the center of the weighing scale in light clothing without shoes and socks.

Body mass index (BMI) was calculated using the formula

BMI = weight (kg)/ height2 (m)2 and classifying under weight (BMI<18), normal (BMI 18 - 24.9), overweight (BMI 25 - 29.9), obesity (BMI 30-39.9) and morbid obesity (BMI > 40) (Sturm,2007).

2.3. The Genetic Study:

2.3.1. Extraction of Total DNA from Clinical Samples:

The G-spin™ Total DNA Extraction Mini Kit is suitable for use with fresh or frozen whole blood and blood which has been treated with citrate, heparin, or EDTA. Pre-separation of leukocytes is not necessary • Purification does not require phenol/chloroform extraction or EtOH precipitation, and provides the simplest protocols. DNA is eluted in Buffer CE , TE (10:1), 10mM Tris (pH 7.5 ~ 8) or water, is prepared for direct addition to PCR or other enzymatic reactions. Alternatively, it can be safely stored at (–20°C) for later use. The purified DNA is protein-free, nucleases-free and does not include other contaminants or inhibitors. G-spin™ Total DNA Extraction Mini Kit is optimized for extraction of (20-30kb) DNA fragments and able to extract up to 50 kb fragments.

All reagents required for the total DNA extraction were provided with DNA extraction kit (G-Spin total DNA Extraction kit, Cat .No. 14001 Intron / Korea).

2.3.2. Kit Contents :

The contents of G-Spin total DNA extraction kit are listed in table (2-6).

Table 2.6 : List of reagents and buffers of G-Spin total DNA extraction kit used in this study.

Label	Contents 50 Columns	Contents 200 Columns
Buffer CL	25 ml	90 ml
Buffer BL 1	25 ml	90 ml
Buffer WA1	40 ml	160 ml
Buffer WB 2	14 ml	56 ml
Buffer CE 3	20 ml	40 ml
Spin Column4 / Collection Tube5	50 ea	200 ea
RNase A (Lyophilized powder)6	3 mg x 1 vial	3 mg x 4 vials
Proteinase K (Lyophilized powder)6	22 mg x 1 vial	22 mg x 4 vials

2.3.3.Assay Procedure:

The procedure was carried out in accordance with the manufacturer's instruction as following:

Extraction of total DNA from Blood Samples

1. A 200 µl of whole blood was pipeted into a (1.5 ml) microcentrifuge tube.
2. 40 µl proteinase K Solution and 5 µl of RNase A solution was added into sample tube and gently mixed.
3. A 200 µl of Buffer BL was added into upper sample tube and mixed thoroughly. This step is important in order to ensure effective decomposition, and it is important that the BL sample and buffer blood are thoroughly mixed to give a dissolution solution.
4. The mixture was placed at Room Temperature for (2minutes).
5. The lysate was incubated at 56°C for 10 min. For complete lysis, mixed 3 or 4 times during incubation by inverting tube. If it lysis perfectly, the red color of lysate became the dark green.

6. The 1.5 ml tube was centrifuged briefly to remove drops from the inside of the lid.
7. A 200 µl of absolute ethanol was added into the lysate, and then mixed well by pulse vortex. After mixing, briefly centrifuged the (1.5 ml) tube to remove drops from inside of the lid. This step is an equilibration step for binding genomic DNA to column membrane. Also, this step conduces to pass efficiently cell lysate through a column.
8. The mixture from step 7 was applied carefully to the spin column (in a 2 ml collection tube) without wetting the rim, close the cap, and centrifuged at 13,000 g for 1 min . Discarded the filtrate and placed the spin column in a new (2 ml) collection tube (additionally supplied).
9. Seven hundred µl of Buffer WA was added to the spin column without wetting the rim, and centrifuged for 1 min at 13,000 g. Discarded the flow-through and reused the collection tube.
10. Seven hundred µl of Buffer WB was added to the spin column without wetting the rim, and centrifuged for 1 min at 13,000 g. Discarded the flow-through and placed the column into a new (2.0 ml) collection tube (additionally supplied),.Then was centrifuged again for additional 1 min to dry the membrane. Discarded the flow-through and collection tube altogether.
11. The spin column was placed into a new (1.5 ml) tube (not supplied), and added (50 µl) of Buffer CE directly onto the membrane. Then incubated for (1 min) at room temperature and then centrifuged for (1 min) at (13,000 g) to elute.

2.3.4. Measurement of concentration and purity of extracted DNA

The extracted genomic DNA was by using Nanodrop spectrophotometer (THERMO. USA), that check and measurement the purity of DNA through reading the absorbance in at (260 /280 nm) as following steps:

1. After opening up the Nanodrop software, chosen the appropriate application (Nucleic acid, DNA).
2. A dry wipe was taken and cleaned the measurement pedestals several times. Then carefully pipette 2µl of free nuclease water onto the surface of the lower measurement pedestals for blank the system.
3. The sampling arm was lowered and clicking OK to initialized the Nanodrop, then cleaning off the pedestals and 1µl of DNA was added to measurement.

2.4. Polymerase Chain Reaction (PCR) Mixture

PCR Mixture was prepared according to type and target region of DNA as in table(2-7)and (2-8).

2.4.1 PCR Mixture for *IL13* (rs20541), *TLR6*(rs5743789) SNP Detection

The PCR mixture components, volumes and their concentrations for amplification *IL13* (rs20541) and *TLR6* (rs5743789) for SNP detection by ARMS-PCR were reported in table(2-7).

Table 2.7: Mixture of ARMS-PCR for *IL13* (rs20541) and, *TLR6*(rs5743789) SNP Detection

No.	Content of PCR Reaction Mixture	Volume/ µl
1	Master mix	**10µl**
2	Outer Forward primers	1µl
3	Outer Reverse primers	1µl
4	Inner Forward primers	1µl
5	Inner Reverse primers	1µl
6	Template DNA	2 µl
7	Nuclease free water	4µl
8	Total	20 µl

2.4.2. PCR Mixture for *TNFa* (rs 1800629) SNP Detection

The PCR mixture components, volumes and their concentrations for amplification for *TNFa* (rs 1800629) SNP detection by ARMS-PCR were reported in table(2-8).

Table 2.8: Mixture of ARMS-PCR for *TNFa*(rs 1800629) SNP Detection

No.	Content of PCR Reaction Mixture	Volume/ µl
1	Master mix	12.5 µl
2	Common primer	1.5µl
3	Normal or Mutant Primer	1.5µl
4	DNA template	5µl
5	Nuclease free water	4.5µl
6	Total volume	25µl

2.4.3. PCR Programs

The programs were determined by gradient PCR and listed in table (2-9) and (2-10).

2.4.3.1. PCR Program for *IL13* (rs20541) and *TLR6*(rs5743789) SNP Detection

The steps, temperatures, times and number of cycles for detection of *IL13* (rs20541) and *TLR6*(rs5743789) SNP were detailed in table (2-9)

Table 2.9:Amplification conditions of *IL13* (rs20541) and *TLR6*(rs5743789) SNP

Gene	Initial denaturation	Denaturation	Annealing	Extension	Final extension	No. of cycles
***IL13* (rs20541)**	$94C^0$ /4 min	$94C^0$ / 1 min	$63C^0$ /50 sec	72 C^0 /50 sec	72 C^0 /4min	35
***TLR6*(rs5743789)**	$94C^0$ /5 min	$94C^0$ / 1min	58 C^0 /50sec	72 C^0 /1min	72 C^0 /4min	35

2.4.3.2. PCR Program for *TNFa*(*rs 1800629*) SNP Detection

The steps, temperatures, times and number of cycles for detection of *TNFa*(rs 1800629) SNP were detailed in table (2-10)

Table 2.10: **The steps, temperatures, times and number of cycles for detection of *TNFa*(rs 1800629) SNP**

Gene	Initial denaturation	Denaturation	Annealing	Extension	Final extension	No. of cycles
TNFa(rs 1800629)	$95C^0$ /4 min	$95C^0$ / 30 sec	$56C^0$ /30 sec	72 C^0 /30 sec	72 C^0 /5min	40

2.4.4. Agarose Gel Electrophoresis Technique

The PCR products were analyzed by agarose gel electrophoresis according to the following steps:

2.4.4.1. Preparation of TBE Buffer (1X)

To prepare 500 ml of 1X TBE buffer, 50 ml of TBE (10X) stock solution was mixed with 450 ml of dH_2O. The pH value was adjested to 8 with concentrated HCl or 0.5 M tris base solution. Then the volume was completed to 500ml with dH_2O.

2.4.4.2. Gel electrophoresis protocol

1. Device setup: The casting gates were sited on the ends of the gel tray and locked in place firmly against casting tray. This was done by engaging the "claws" of the gate in the recess of the side wall of the tray. The comb was sited into the slots of the gel tray, (1.0 mm above the base of gel casting tray) so that the sample wells are near the cathode.

2. Gel dissolving: A1g of agarose was dissolved in 100ml of 0.5 X TBE solution by mealting to 100°C to prepare 1% agarose gel for migrated genomic DNA extracts. Whereas, 1% or/and 2% agarose gel was prepared in 1X TBE buffer for migrated PCR products or digested DNA by restriction enzyme respectively.

3. Gel casting: After agarose gel dissolving completely, it let to cooling to approximately 60°C and 2-3 µl of the safe stain stock solution was added, then slowly pour the agarose into the gel- casting tray, and any air bubbles were removed. The comb was positioned at approximately 1.5 cm from one edge of the gel. The agarose was allowed to solidify at room temperature at least 30 min. After that, the claws were disengaged from the gel tray and the comb was separated gently. Then the gel was placed in the gel tank in such a way that the wells should be on end with the cathode. 1X TBE buffer (depending the purpose) was added to the buffer tank until it was about 5 mm above the top of the gel.

4. Loading the samples: Each 5µl of the genomic DNA sample was mixed with 3µl loading dye briefly and loaded into the wells. Whereas, the PCR

products were loaded without loading dye because of the PCR master mix contained loading dye.

5. Gel electrophoresis conditions: After sample loading the electric field was turned on at 5 V/cm (75V) for 60-120 min until bromophenol blue dye reached at the end edge of the gel.

6. The gel was photographed using gel documentation system (Cleaver Scientific - UK).

2.5. The Immunological Study

2.5.1. Human IL-33(Interleukin 33), Human TNF-a(Tumer Necrosis Factor-a) & Human GM-CSF (Granulocyte-macrophage colony-stimulating factor) ELISA Kit

The concentration of IL33 in the serum of patients with respiratory infections was evaluated by enzyme linked immunosorbent assay (ELISA) to determine total IL33,TNFa &GM-CSF in the serum. The ELISA kit designed by Elabscience which contain the following solutions and materials in the table below.

Table 2.11 : ELISA kit of IL33, TNFa &GM-CSF

Item	Company	Origin
1-Biotinylated Detection Ab Diluent 2-Certificate of Analysis 3- Concentrated Biotinylated Detection Ab (100×) 4- Concentrated HRP Conjugate (100×) 5- Concentrated Wash Buffer (25×) 6- HRP Conjugate Diluent 7- Micro ELISA Plate (Dismountable) 8- Plate Sealer 5 pieces 9- Product Description 10- Reference Standard 11- Reference Standard & Sample Diluent 12- Stop Solution 13- Substrate Reagent	Elabscience	China

2.5.2. ELISA Reagents Preparation:

i. Washing buffer solution:

This solution was prepared by diluted the stander wash buffer that provides with kit in a ratio 1:25, where the total volume 30ml of wash buffer with kit were diluted in 750 ml of deionized or distilled water D.W and mix gently. Wash buffer was used for washing the wells in the micro-titer plates

ii. Standard solution preparation:

These solutions were used to prepare the standard curve that is used for calculate the concentration of these factors that mentioned above. Through 15 minute these solution must be prepared. Preparation was completed by added one ml of standard and sample diluent solution that provides with kits, this gave a high concentration and the transfer 0.5 ml of it into the tube number 2 that contain 0.5ml of standard and sample diluent to obtain the concentration half the previous concentration and so on to obtain seven serial dilutions were putted in the wells from (B-H), while in A well putted zero concentration (diluted only).

iii. Biotinylated detection antibody:

This solution is present with kit in 120µL amount. It was prepared by dilution with specialized solution in a ratio 1:100.

iv. Conjugated horseradish peroxidase HRP:

It was prepared by diluted in a ratio 1:100 with solution that provide with kit

2.5.3. Principles of Assay

The enzyme-linked immunosorbent assay is a test that uses antibodies/ antigens and color change to identify a substance and used widely used as a medical diagnostic tool. According to Elabscience® manufactured company, USA, The IL33**,** TNFa &GM-CSF

test principle uses the Sandwich-ELISA principle. The micro ELISA plate has been pre-coated with an antibody specific to Human IL33, TNFa &GM-CSF. Standards or samples are added to the micro ELISA plate wells and combined with the specific antibody. Then a Biotinylated detection antibody specific for Human (IL33**,** TNFa &GM-CSF**)** and Avidin-Horseradish Peroxidase (HRP) conjugate are added successively to each microplate well and incubated. The enzyme-substrate reaction is terminated by the addition of stop solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The OD value is proportional to the concentration of Human IL33 . The concentration of Human IL33, TNFa &GM-CSF in the samples calculated by comparing the OD of the samples to the standard curve by using trend line equation applied for all sample in Excel Microsoft office 2016 program.

2.5.4. Assay procedure according to Elabscience ELISA procedures manual:

1- A volume of 100 µl of standard working solution was added to the first two columns of wells. Each concentration of the solution was added in duplicate, to one well each, side by side.

2. A volume of 100 µl of samples serum was added to the other wells.

3. The plate was covered with the sealer provided in the kit and incubated for 90 min at 37°C.

4. The liquid was Removed out of each well, without washing and immediately 100 µl of Biotinylated Detection Ab working solution was added to each well.

5. The plates were Covered with the plate sealer and mix up gently and incubated for 1 hour at 37°C.

6. The solution from each well was removed and 350 µl of wash buffer was added to each well. Soaked for 1~2 min and the solution from each well was removed and plate dries against the clean absorbent paper. This wash step repeated 3 times.

7. A volume of 100 µl of HRP Conjugate working solution was added to each well.

8. The plate was covered with the plate sealer and incubated for 30 min at 37°C.

9. The solution was removed from each well, washed with 350 µl of wash buffer five times as conducted in the previous step.

10. A volume of 90 µl of Substrate Reagent was added to each well.

11. The plate was covered with a new plate sealer and incubated for about 15 min at 37°C immediately to protect the plate from light.

12. A volume of 50 µl of Stop Solution was added to each well.

13. The optical density (OD value) of each well at once with a microplate reader set to 450 nm and the results calculated by using Excel Microsoft office 2016 program

Table 2.12: IL33 standards arrangement in the plate

tube	1	2	3	4	5	6	7
Pg/ml	1000	500	250	125	62.5	31.25	15.63
O.D	2.78	2.203	1.779	1.005	0.691	0.39	0.312

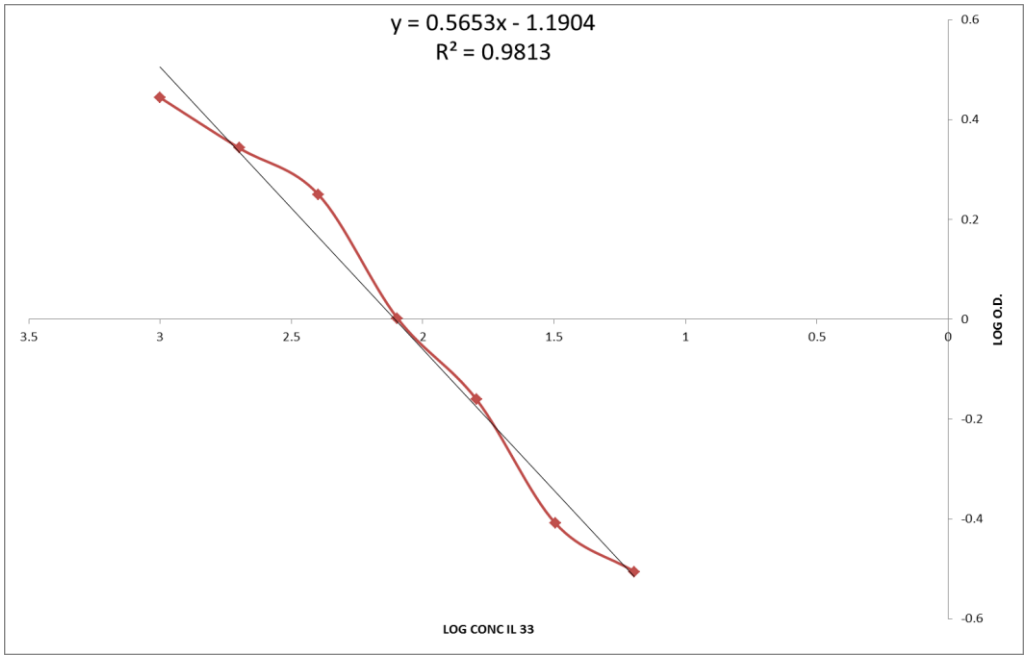


Fig (2.2) IL33 standards curve

Table 2.13: TNFa standards arrangement in the plate

Tube	1	2	3	4	5	6	7
Pg/ml	500	250	125	62.5	31.25	15.63	7.81
O.D	1.836	1.072	0.84	0.427	0.3	0.186	0.103

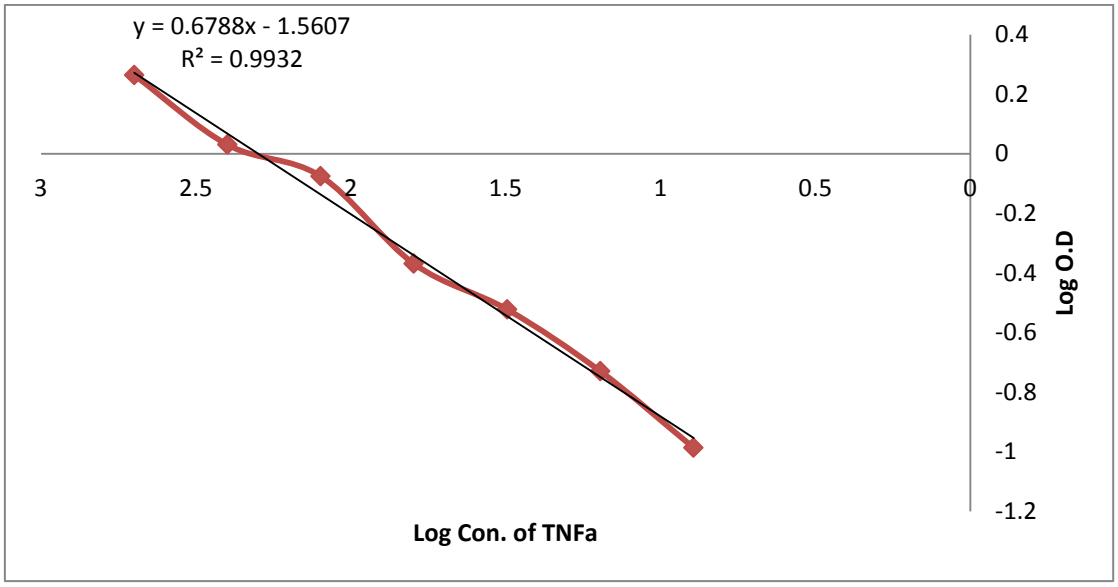


Fig (2.3) TNF a standards curve

Table 2.14: GM-CSF standards arrangement in the plate

Tube	1	2	3	4	5	6	7
Pg/ml	500	250	125	62.5	31.15	15.63	7.81
O.D	3.143	2.537	2.084	1.122	0.573	0.267	0.128

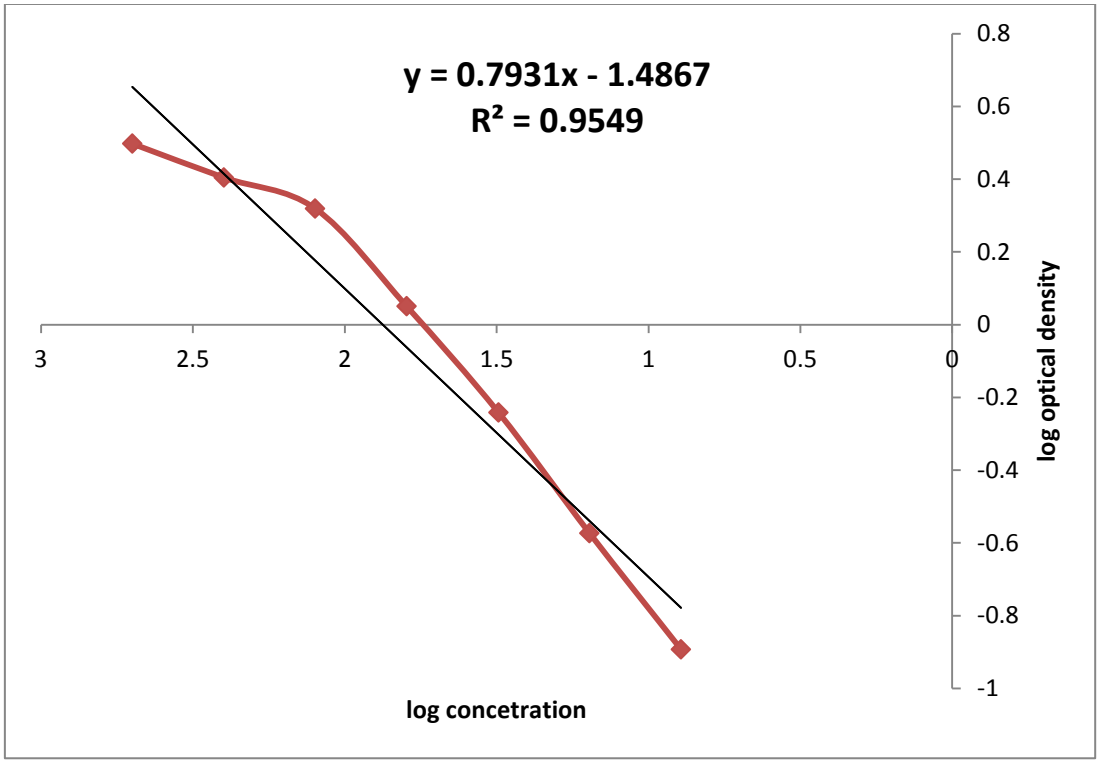


Fig (2.4) GM-CSF standards curve

2.6. Determination of C-Reactive Protein CRP Concentrations

2.6.1. Intended Use:

AFIAS CRP is a fluorescence immunoassay (FIA) for measuring C-Reactive Protein (CRP) in whole blood, serum, and plasma in humans. It's helpful for managing and tracking autoimmune disorders and viral mechanisms like rheumatoid arthritis(Pepys and Hirschfield.,2003).

2.6.2. Principle.

The test employs a sandwich immunodetection process, in which the detector antibody in the buffer attaches to antigen in the sample, forming antigen-antibody complexes, which then move to the nitrocellulose matrix, where they are captured by the other immobilized-antibody on the test strip. The further antigen in a sample, the more antigen-antibody complexes form, resulting in a stronger fluorescence signal on the detector antibody, which is processed by the instrument for AFIAS tests to determine CRP concentration (Pepys and Hirschfield.,2003).

2.6.3. Test Procedure.

Methodology in General (with pipette tip)

1) For AFIAS checks, choosed “General Mode” in the instrument.
2) 100 µl of serum was placed into the sample well on the cartridge.
3) The cartridge was Placed inside the cartridge holder.
4) A tip was inserted into the cartridge's tip opening.
5) The ‘START' button was tap.
6) After 3 minutes, the test result will be shown on the screen.

2.6.4. Interpretation Of Test Result.

The AFIAS test instrument automatically calculates the test outcome and shows the CRP concentration of the test sample in mg/L.

The reference value (cut-off) is 10 mg/L.

The AFIAS CRP has a working range of 0.5-200 mg/L.

2.7. Determination of D-Dimer Concentrations

2.7.1. Intended Use

AFIAS D-Dimer is a fluorescence immunoassay (FIA) for determining D-Dimer levels in human whole blood and plasma. It can help in the treatment and control of thromboembolic condition patients' clinical evaluations.

Only for use in in vitro diagnostics.

2.7.2. Principle

The test employs a sandwich immunodetection process, in which the detector antibody in the buffer attaches to antigen in the sample, forming antigen-antibody complexes, which then move to the nitrocellulose matrix, where they are captured by the other immobilized-antibody on the test strip. The more antigen in the sample, the more antigen-antibody complexes form, resulting in a greater fluorescence signal on the detector antibody, which is processed by the instrument for AFIAS tests to determine D-Dimer concentration(Pepys and Hirschfield.,2003).

2.7.3. Test Procedure

1) For AFIAS checks, choosed “General Mode” in the instrument.
2) 100 µl of serum was placed into the sample well on the cartridge.
3) the cartridge was Placed inside the cartridge holder.
4) A tip was inserted into the cartridge's tip opening.
5) The ‘START' button was tap.
6) After 3 minutes, the test result will be shown on the screen.

2.7.4. Interpretation Of Test Result

The AFIAS test instrument automatically calculates the test outcome and shows the D-Dimer concentration of the test sample in mg/L. (FEU, Fibrinogen equivalent units).

The reference value (cut-off) is 500 mg/L.

The AFIAS D-Dimer has a working range of 50-10,000 mg/L.

2.8. Determination of Ferritin Concentrations

2.8.1. Intended Use

AFIAS is an acronym for the acronym for the acronym for

Ferritin is a fluorescence immunoassay (FIA) for measuring ferritin levels in human serum and plasma.

It's useful for determining the amount of human ferritin in the body.

2.8.2.Principle

The detector recombinant protein in the buffer attaches to antibody in the sample, producing recombinant protein-antibody complexes, which move onto the nitrocellulose matrix and are caught by the other immobilized-antigen on the test strip. The further antibody in the sample, the more recombinant protein-antibody complex forms, resulting in a greater fluorescence signal on the detector recombinant protein, which is then analyzed by the instrument for AFIAS tests to determine ferritin concentration in the sample(Mary Ann Knovich .,2009)

2.8.3. Test Procedure

1)100 µl of sample was added into the sample well on the cartridge.

2) The cartridge was Placed inside the cartridge holder.

3) Inserted a tip into the cartridge's tip opening.

4) On the phone, tap the ‘START' button.

5) After 10 minutes, the test result will be shown on the screen.

2.8.4. Interpretation Of Test Result

The AFIAS test instrument automatically calculates the test outcome and shows the ferritin concentration of the test sample in ng/mL.

Women's reference range is 20-250 ng/mL, while men's range is 30-350 ng/mL.The AFIAS Ferritin has a working range of 10-1,000 ng/mL.

2.9. Determination of SARS-COV2 Kit (Rapid test).

2.9.1. Principle:

The test detects the presence of patient-generated antibodies against SARS-CoV-2, according to company biozek The test can detect two types of antibody isotypes: IgG and IgM.

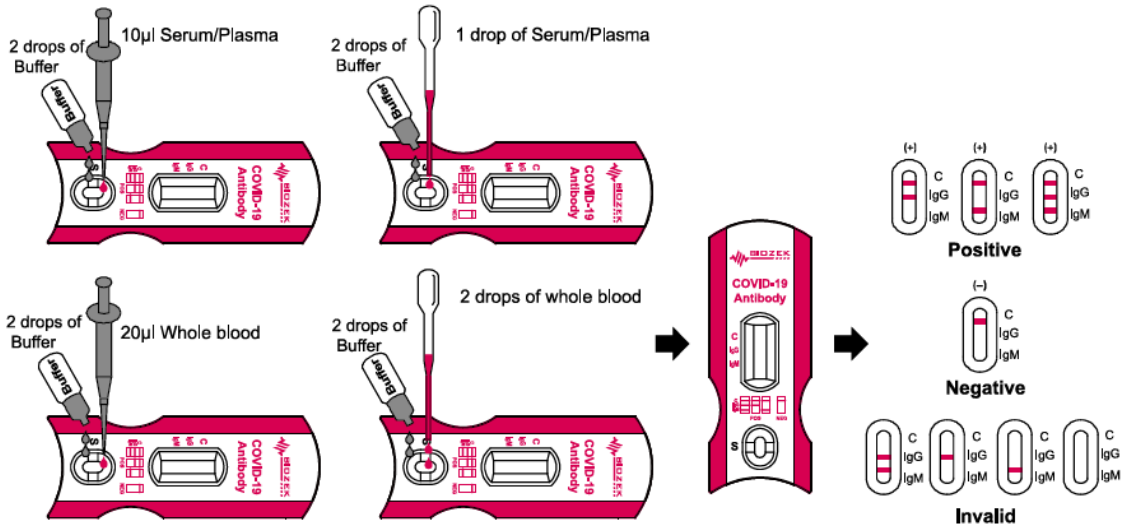
2.9.2. Procedure:

1- 20 uL of whole blood is squeezed to ascite, then released to discharge

2-(2)drops of Buffer.

2.9.3.Interpretation of Rapid test result:

A sample can be positive if there are IgM, IgG, or both IgM and IgG antibodies present.



Fireg(2.5):SARS-COV2 rapid test Procedure (biozek Kit)

2.10.Determination of WBC,Lymphocyte & neutrophile by Complete Blood Count

Hematological parameters were performed on EDTA blood using (SYSMEX/ JAPAN) in hematology laboratory Marjan Medical City in Babylon province. five par to estimate numbers and percentages of white blood cells. Whole blood was collected via an EDTA tube. An automatic hematology analyzer (Sysmex Corp., Japan) was used to measure, Total WBCs, Lymphocytes Neutrophils(Abdel-Hamid *et al*.,2019).

2.11. Statistical Analysis

Data were collected, summarized, analyzed and presented using statistical package for social sciences (SPSS) version 23 and Microsoft Office Excel 2010. Numeric data were presented as mean, standard deviation, range, median and interquartile range (IQR) after performance of Kolmogorov- Smirnov normality test and making decision about normally and non-normally distributed variables. **Mann Whitney U test** was used to study differences in mean rank between any two groups provided that the variable was non-parametric. On the other hand independent samples t-test was used to study difference in mean between any two groups provided that the variable is normally distributed.

Chi-square test was used to study association between any two categorical variables. Odds ratio and 95% confidence interval was estimated to measure risk. Pearson's correlation coefficient was used to study correlation between numeric variables. Receiver operator characteristic curve analysis was used to find out the proper cutoff values and further analysis of sensitivity and specificity was carried out accordingly. The level of significance was considered at P-value of 0.05 or less and highly significant level at 0.01 or less (Daniel 2009).

CHAPTER THREE

Results and Discussion

3. Results and Discussion

3.1. Demographic characteristics of patients with SARS-CoV2 and control subjects

The demographic characteristics of patients and control subjects are shown in table (3-1).

Table 3.1: Demographic characteristics of patients with SARS-CoV2 and control subjects

Age groups		Control (N0.=100)	Patients (No.=70)
<30 year	No	47	2
	%	47.0%	2.9%
30-39 year	No	32	23
	%	32.0%	32.9%
40-49 year	No	14	16
	%	14.0%	22.9%
≥ 50 year	No	7	29
	%	7.0%	41.4%
	Mean± SD (Range)	31.77±8.84 (21-53 year)	47.17±11.72 (28-69 year)
Gender		**Control (N0.=100)**	**Patients (No.=70)**
Male	No	33	38
	%	33.0%	54.3%
Female	No	67	32
	%	67.0%	45.7%

***N*: number of cases; SD: standard deviation**

The present study included 170 subjects, 70 SARS-COV2 patients enrolled from those patients who were admitted to the intensive care units in Merjan Medical city in Babylon province, Iraq from February, 2021 to October 2021, and 100 apparently healthy subjects.The demographic characteristics of patients and control subjects are shown in table (3-1). The mean age of patients was 47.17±11.72 and that of control subjects was 31.77±8.84 years. These results correspond with the previous studies Mohsin *et al*.,(2021), which found that the mean age of the patients with SARS-COV2 was 41.17±15.11 years, while Liu *et al*., (2020), revealed the mean age of the 101 hospitalized patients with confirmed SARS-COV2 was 45 ± 18.01 years (range, 11 months to 80 years).

The frequency distribution of patients and control subjects according to age was also shown in the table (3-1). These results found that the highest percentage of 29 (41.4%) of patients with SARS-COV2 fall in the age group (≥ 50 years), while the lowest percentage was 2 (2.9%) of them those aged less than 30 years. Regarding control' group included 47 (47.0%) of the age group <30 year, 32 (32.0%) for 30-39 year, 14(14.0%) for 40-49 year, and only 7(7.0%) for ≥ 50 year. These results agreed with the study findings done by Boddington *et al*., (2020), which revealed that the highest percentage 34.6% of the patients with SARS-COV2 fall in the age group (50–69 years).

Again, Patients' group included 38 (54.3 %) males and 32 (45.7 %) females as shown in table (3.1), whereas, control group included 33 (33.0 %) males and 67 (67.0 %) females. These results are agreed with the published studies; in Italy Meini *et al*., (2021) revealed that the highest percentage (59.2%) of the patients with SARS-COV2 were males, while Hernández-Garduño, (2020) reported that 58.7% of the patients with SARS-COV2 was for males opposite 41.3% of them was for females. Also, The results of this study agreed with Scully *et al*., (2020) who revealed that most of the patients with SARS-COV2 were males.

Table 3.2: Univariate Logistic regression analysis to identify variables independently associated with hospitalization for SARS-COV2

Age groups	B	P. value	OR	95% C.I. for OR	
				Lower	Upper
<30 year	**Reference**				
30-39 year	2.827	**<0.001***	16.891	3.720	76.698
40-49 year	3.291	**<0.001***	26.857	5.496	131.239
≥ 50 year	4.578	**<0.001***	97.357	18.919	500.991
Gender					
Female	**Reference**				
Male	0.880	**0.006***	2.411	1.286	4.520

CI: Confidence interval, B:Beta

Table 3.2 revealed Univariate Logistic regression analysis to identify variables independently associated with hospitalization for SARS-COV2. The results found that those in who age group (≥ 50 year) were significantly more likely to be hospitalized for SARS-COV2 compared with those in who age group less than <30 year (OR 97.357; 95% CI, 18.919-500.991,

p<0.001). While males were significantly more likely to be hospitalized for SARS-COV2compared with females (OR 2.411; 95% CI, 1.286 -4.520, *p=0.006*). These results agreed with several studies; Boddington *et al*., (2020) revealed that the age group (50–69 years) was likely to be at higher risk than age group (18-35 years) (OR 0.86 (95% CI 0.57–1.30), Meini *et al.*, (2021) showed that males were likely to be at higher risk than females (OR =0.54, CI=0.37-0.79, *P=0.001*). While Klein *et al.*, (2020) reported Females are less affected by SARS-COV2 than males.

Many studies have shown that elder age groups are more susceptible to SARS-CoV2 (WHO, 2020-1; Robert Koch Institut, 2020; Kassem, 2020). Leng and Goldstein, (2010) reported there is a correlation between age and natural immunity as reviewed elsewhere and concluded that older people are particularly prone to develop more infections as natural immunity declines gradually at older ages.

Regarding gender, these results agreed with studies explaining that male patients may have higher expression of angiotensin-converting enzyme 2 (ACE2), which may be regulated by male sex hormones rendering them to more risk for SARS-CoV-2 infection and poor clinical outcomes as well (La Vignera *et al*., 2020). In addition, this may be partly because ACE2 expression encoded by the ACE2 gene lays on the X-chromosome, thus allowing females to be potentially heterozygous whereas men who are definitely homozygous allowing males to be potentially high ACE2 expressor . Moreover, it is hypothesized that females may counteract the progression of the SARS-CoV-2 infection and severe clinical outcomes due to carrying X-linked heterozygous alleles called sex dimorphism by activating a mosaic advantage (Gemmati *et al*.,2020).

3.2. Frequency distribution of patients with SARS-COV2 according to body mass index and smoking.

Table 3.3: The distribution of patients with SARS-COV2 and control subjects according to BMI categories, and smoking status

BMI Categories		Control (No.=100)	Patients (No.=70)
Non- Obesity	No	73	34
	%	73.0%	48.6%
Obesity ≥30kg/m2	No	27	36
	%	27.0%	51.4%
Smoking			
No	No	87	42
	%	87.0%	60.0%
Yes	No	13	28
	%	13.0%	40.0%

Table 3.3 results found that the patients group included 36 (51.4%) patients with SARS-COV2 were obese (BMI ≥ 30 kg/m2), whereas 34 (48.6%) of them were non-obesity. The control group included 73 (73.0%) of the participants who had no SARS-COV2 were non- obesity, and only 27 (27.0%) of them were obese. These results agreed with the study findings done in Mexico (Hernández-Garduño, 2020) which found that 38.8% of patients have obesity opposite 33.3% of control, while 66.7% of control (negative SARS-CoV-2) have no obesity opposite 61.2% patients (positive SARS-CoV-2).

Regarding smoking status, the current study shows that the Patients group included 28 (40.0%) patients with SARS-COV2 were smokers, whereas 42 (60.0%) of them were non-smokers. The control group included

87 (87.0%) of the participants who had no SARS-CoV2 were non-smokers, and only 13 (13.0%) of them were smoker. These findings agreed with Berumen *et al*., (2020), which found that 69% of non-confirm cases with SARS-COV2 were non-smokers.

Table 3.4: The distribution of patients with SARS-COV2 and control subjects according to BMI categories and gender

Gender	BMI		Control	Patients
Male	**Non- Obesity**	No	26	17
		%	78.8%	44.7%
	Obesity	No	7	21
		%	21.2%	55.3%
Female	**Non- Obesity**	No	47	17
		%	70.1%	53.1%
	Obesity	No	20	15
		%	29.9%	46.9%

Table 3.4 reveals that obese males were more at risk of SARS-COV2 infection than females. This agreed with the study findings conducted in Shenzhen, China (Cai *et al*., 2020), which found that a high percentage of 78.1% of patients (males) have obesity compared to females. Another study done in Italy (Busetto *et al*., 2020) who revealed that 72.4% of patients with SARS-COV2 (males) have obesity. The possible explanation for these results due to that different patterns of fat distribution in men versus women could account for the increased death in men. In other words, overweight men carry most of their fat in their abdominal region, [and that] has a detrimental effect on lung function, which is more prominent when fighting serious lung infections like SARS-COV2 pneumonia (Mundell, 2021).

Table 3.5: Univariate Logistic regression analysis to identify variables independently associated with hospitalization for SARS-COV2

BMI Categories	B	P. value	OR	95% C.I. for OR	
				Lower	Upper
Non- obesity	**Reference**				
Obesity	1.052	0.001*	2.863	1.504	5.450
Smoking					
No	**Reference**				
Yes	1.495	<0.001*	4.462	2.099	9.482

CI: Confidence interval, B:Beta

Table 3.5 reveals a Univariate Logistic regression analysis to identify variables independently associated with hospitalization for SARS-COV2. The results found that those who were obese were significantly more likely to be hospitalized for SARS-COV2 compared with those who were non-obese (OR 2.863; 95% CI, 1.504-5.450, *p=0.001*). Whereas those smokers were significantly more likely to be hospitalized for SARS-Cov2 compared with those in who non-smokers (OR 4.462; 95% CI, 2.099-9.482, *p=<0.001*). These results are consistent with the study findings conducted in South Korea (Jung *et al*., 2021), reported compared to normal-weight individuals, the adjusted odds ratios in the overweight and obese individuals were 1.13 (95% confidence interval [CI], 1.03-1.25) and 1.26 (95% CI, 1.15-1.39). another study done by Hernández-Garduño, (2020) which revealed that the obesity were likely at higher risk to be infected for SARS-Cov2 than those who were non-obese (OR 1.31; 95% CI, 1.25–1.37, *p=0.0001*), and those smokers were likely at higher risk to be infected for SARS-Cov2 compared with those in who non-smokers (OR 0.8; 95% CI, 0.74–0.86, *p=0.0001*). Also, the current results agreed with Ji *et al*., (2021), who found that Overweight (body mass index [BMI] 23 to 24.9 kg/m2; adjusted odds ratio [aOR], 1.16; 95% confidence interval [CI], 1.1.03 to 1.30) and class 1

obesity (BMI 25 to 29.9 kg/m2; aOR, 1.27; 95% CI, 1.14 to 1.42) had significantly increased SARS-Cov2 risk, while classes 2 and 3 obesity (BMI ≥30 kg/m2) showed similar but non-significant trend. (Ji *et al*., 2021).

A recent meta-analysis Patanavanich and Glantz, (2020) including a total of 11,590 SARS-COV2 patients showed that smokers have 1.91 times the odds of progression towards severe forms of SARS-COV2 than never smokers; so this is directly consistent this explanation of the current findings.

From another perspective to discuss the results of the current study, many studies have proven that obesity, as defined by a body mass index (BMI) greater than 30 kg/m^2, is a risk factor for bacterial and viral pneumonia, ARDS acute respiratory distress syndrome, and acute respiratory failure after lung transplantation (Lederer *et al*.,2011 ;Mertz *et al*.,2013), and this observed by (Peng *et al*.,2020; Wu *et al*.,2020; Docherty *et al*.,2020; Petrilli *et al*.,2020) reported that during the SARS-COV2 pandemic, obesity was implicated as a clinically significant risk factor for severe disease.

There are multiple mechanisms that may underlie the observed association of obesity with acute respiratory failure and death from SARS-CoV2 infection. First, adipose tissue expansion in obesity leads to immune activation, resulting in increased circulating concentrations of inflammatory molecules, including interleukin-6, tumor necrosis factor-α, and monocyte chemoattractant protein-1 (Nishimura *et al*.,2009).

In this study, demonstrated what other studies have shown since SARS-Cov2 has a severe stage of pneumonia or respiratory failure, smokers are much more susceptible to severe disease because of their immunocompromised lungs (Pötschke-Langer *et al*.,2015). In an article from the area of pulmonology, Brake *et al*.,(2020). explained the biological process of coronavirus attacking the human body, where they stated that

smoking is one factor that accelerates the production of the angiotensin-converting enzyme-2 (ACE2) receptor, which is known to be the receptor of coronavirus, and therefore smokers are more vulnerable to this disease than non-smokers .Reviewing seven study reports, Zhao *et al*.,(2020). concluded that although the association between smoking and SARS-Cov2 is non-significant, active smoking increases the risk of having severe SARS-Cov2 and smokers have a 1.98 times greater risk of having the severe stage. In an evidence-based study conducted in Bangladesh from 2009 to 2012, found that 23% of adults above 15 years of age are smokers, while in another study, the prevalence of second-hand smokers who are exposed to smoking at home was found to be 53.5%(Fischer *et al*.,2015; Nargis *et al*.,2015).

3.3 Results of the parameters between case and control groups

Table 3.6 : Comparison of the mean values of the parameters between case and control groups

Parameters		Median (IQR)	Mean Rank	Sum of Ranks	Mann-Whitney U	P. value
Age	**Control**	31.0 (13.0)	61.34	6133.50	1083.500	<0.001*
	Patients	47.0(19.25)	120.02	8401.50		
WBCs(*10^3) cells/mcL	**Control**	8.20 (2.58)	82.18	8218.00	3168.000	0.293
	Patients	8.30(6.65)	90.24	6317.00		
Ferritin ng/ml	**Control**	50.0 (40.0)	50.97	5096.50	46.500	<0.001*
	Patients	395.25(955.75)	134.84	9438.50		
Dimer mg/L	**Control**	115.0(49.75)	52.47	5247.00	197.000	<0.001*
	Patients	460.0(196.25)	132.69	9288.00		
CRP mg/L	**Control**	6.50 (5.30)	53.70	5370.00	320.000	<0.001*
	Patients	15.68(18.92)	130.93	9165.00		
Lymphocytes (%)	**Control**	37.50(13.58)	102.45	10244.50	1805.500	<0.001*
	Patients	21.39 (19.52)	61.29	4290.50		

Neutrophil (%)	**Control**	55.10 (16.44)	68.68	6868.00	1818.000	<0.001*
	Patients	71.28 (27.12)	109.53	7667.00		

Table 3.6 reveals that all parameters for the case (with SARS-COV2) group was significantly higher than that for the control group, except WBCs were no significant difference between patients and control groups (*P. value*=0.293). These results are consistent with Marouf *et al*., (2021) revealed that CRP (mg/L) for the case group was significantly higher than that for the control group (*P<0.001*; control (median 5.4) ; case (median 24.3), D-Dimer (mg/l) for the case group was significantly higher than that for the control group (P. value 0.003; control (median 5.4) ; case (median 24.3), and whereas Lymphocyte (103/µl) was for control (median 2.0) , and for case (median 9.9). Also, these results agreed with Kamel *et al*., (2021), which found that Neutrophils (K/uL) for the case (positive SARS-COV2) group was significantly higher than that for the control (negative SARS-COV2) group, which *P. value=0.001*.

Table 3.7: The distribution of patients with SARS-COV2 and control subjects according to laboratory tests

			Control (No.100)	**Patient (No.70)**
Lymphocyte(%) levels	**Low (<20%)**	No	3	32
		%	3.0%	45.7%
	Normal (20-45%)	No	97	35
		%	97.0%	50.0%
	High (>45%)	No	0	3
		%	0.0%	4.3%
	Mean± SD (Range)		33.14±7.29 (8.22-39.67)	23.91±11.18 (8.22-48.90)
Neutrophil(	**Low**	No	-	-

%) Level	**(<40.0 %)**	%	-	-
	Normal (40.0-74.0%)	No	92	38
		%	92.0%	54.3%
	High (>74.0 %)	No	8	32
		%	8.0%	45.7%
	Mean± SD (Range)		55.74±9.37 (44.50-55.74)	67.99±14.61 (41.43-88.90)
CRP mg/L level	**Normal (0-10 mg/L)**	No	81	0
		%	81.0%	0.0%
	High (>10 mg/ L)	No	19	70
		%	19.0%	100.0%
	Mean± SD (Range)		7.41±4.22 (3.10-22.30)	29.44±29.71 (11.0-144.0)
D-Dimer mg/L	**Normal (<500mg/L)**	No	100	39
		%	100.0%	55.7%
	High (≥500 mg/L)	No	0	31
		%	0.0%	44.3%
	Mean± SD (Range)		147.08±60.05 (101.00-333.00)	542.05±335.27 (100.00-2345.00)
Ferritin ng/ml	**Negative**	No	99	20
		%	99.0%	28.6%
	Positive	No	1	50
		%	1.0%	71.4%
	Mean± SD (Range)		55.75±43.63 (17.00-320.00)	926.86±1318.72 (89.00-7156.00)

In table 3.7 the current study shows that very high percentages of laboratory tests for the control group fell within normal values. While there are patients with SARS-COV2 who have a marked increase from the normal values, and some of them decrease for some patients in laboratory tests, and this indicates the interpretations of much previous research that clarified significant changes in various laboratory parameters including lymphopenia, high C reactive protein levels, ferritin, and D-dimer among SARS-COV2 cases (Wang *et al*., 2020; Li *et al*., 2020). Another study reported significant leukopenia present in around 30% of 24 asymptomatic SARS-COV2cases (Hu *et al*., 2020).

The comparison of some markers between patients and control groups has been carried out and the results were demonstrated in table (3-7). Mean levels of CRP were 29.44±29.71 and 7.41±4.22, in patients with SARS-COV2 and healthy control group respectively; the mean levels was higher in patients group in comparison with control group. Also, the Mean levels of D-Dimer were 542.05±335.27 and 147.08±60.05, in patients with SARS-COV2 and healthy control group respectively; the mean levels was higher in patients group in comparison with control group. These results is in agreement with the study findings done by Petrushevska *et al*., (2021), which found that mean levels of CRP (mg/L) were 144.7±21.38 and 2.1±0.05, in patients with SARS-COV2 and healthy control group respectively, while the mean of D-dimer (mg/L)for cases was 2688±499.1, for control was 225±22.75

According to S-Ferritin, the present study show the mean levels of S-Ferritin highly significant increase in patients group in compared to healthy controls groups, 926.86±1318.72 and 55.75±43.63 respectively.

The results of this study indicate that 82.0% control, and 58.6% case fell within normal range for WBCs, and 45.7% of patients have low level for

Lymphocyte compare to control, which agreed with Liu *et al*.,(2020) had observed that nearly 80% of the patients had normal or decreased white blood cell counts, and 72.3% (99/137) had lymphocytopenia .The results of the previous studies indicated that the number of lymphocytes in the peripheral blood of SARS-COV2 infected individuals was significantly reduced, which is consistent with the results of previous studies (Huang *et al*.,2020; Wang *et al*.,2020; Xu *et al*.,2020).

In this study, increased level of neutrophils 32(45.7%) along with a decrease in lymphocyte numbers 32 (45.7%) in patients with SARS-COV2. These findings agreed with several studies have addressed an increased level of neutrophils along with a decrease in lymphocyte numbers in patients with SARS-COV2 (Xu *et al*.,2020; Tatum *et al*.,2020). Qin *et al*.,(2020)found that severe cases were likely to have a higher neutrophil count but a lower lymphocyte count compared with non-severe patients; the neutrophil-lymphocyte ratio (NLR) thus tended to be higher in the severe group (Tatum *et al*.,2020). In combination with the concomitant lymphopenia, an elevated neutrophil-to-lymphocyte ratio has emerged as a hallmark of severe SARS-COV2 **(**Guan *et al*.,2020; Qin *et al*.,2020).

The explanation of the reasons for the change of values in patients with SARS-COV2 in this study is attributed to; D-dimers are products of fibrin degradation and have been useful in a clinical decision for the diagnosis of pulmonary embolism, and deep vein thrombosis (DVT) (Page, 2006). The group attributes lymphopenia to the destruction of lymphocytes by the virus and subsequent cell death. Notably, lymphocytopenia is also common in patients with Middle East Respiratory Syndromes (MERS) because of lymphocyte apoptosis (Chu *et al*., 2016). Therefore, previous research corroborates the results in this study, indicating that lymphocyte count could be an indicator of disease severity.

Table 3.8: Univariate Logistic regression analysis to identify variables independently associated with hospitalization for SARS-COV2

parameters		B	P. value	OR	95% C.I. for OR	
					Lower	Upper
WBCs*10^3 cells/mcL Categories	**Low (<4)**	**Reference**				
	Normal (4-11)	-21.896-	0.999	0.000	0.000	.
	High (>11)	-20.835-	0.999	0.000	0.000	-
Lymphocyte Level	**Low (<20%)**	**Reference**				
	Normal (20-45%)	-3.386-	<0.001	0.034	0.010	0.117
	High (>45%)	18.836	0.999	151450766.500	0.000	-
Neutrophil (%)Level	**Normal (40.0-74.0%)**	**Reference**				
	High (>74.0%)	2.270	<0.001	9.684	4.090	22.932
CRP mg/L level	**Normal (0-10 mg/L)**	**Reference**				
	High (>10 mg/L)	22.507	0.996	5951749136.000	0.000	.
D-Dimer mg/L	**Normal (<500mg/L)**	**Reference**	-	-	-	-
	High (≥500 mg/L)	22.145	0.998	4142243187.000	0.000	.
Ferritin ng/ml	**Negative**	**Reference**				
	Positive	22.145	0.998	4142243187.000	0.000	.

Table 3.8 reveals a Univariate Logistic regression analysis to identify variables independently associated with hospitalization for SARS-COV2.

The results found that those who have normal levels of lymphocytes were significantly less likely to be hospitalized for SARS-COV2 compared with those who have low levels of Lymphocyte (B= -3.386, OR 0.034; 95% CI, 0.010-0.117, *p<0.001*).

Whereas those who have a high level of neutrophils were significantly more likely to be hospitalized for SARS-COV2 compared with those who have a normal level of neutrophils (B= 2.270, OR 9.684; 95% CI, 4.090-22.932, *p<0.001*).

These results are consistent with *Yang et al*., (2020), and Ruan *et al*., (2020), which found that Lymphocytopenia was reported to be associated with a more severe form of SARS-COV2.

Fan et al., (2021) reported that normal levels of lymphocytes were significantly less likely to be at higher risk for SARS-COV2 than those who have low levels of Lymphocyte (B= −0.238, OR 1.269; 95% CI, 1.023-1.573, *p=0.030*), and those who have a high level of Neutrophil were significantly more likely to be at higher risk for SARS-COV2 than those who have a normal level of Neutrophil (B= 0.289, OR 1.335; 95% CI, 1.105-1.612, *p=0.003*).

In this study, only two factors were significantly more likely to be hospitalized for SARS-COV2 were shown, and this may be explained according to several studies; Sun *et al*., (2020) reported that the main risk factor for lymphopenia and eosinopenia is a highly severe clinical diagnosis. The state of inflammatory cell depletion is strengthened in SARS-COV2 patients' recoveryphase, but this continues or worsens in SARS-COV2 patients' exacerbated process. They explained the reduction of peripheral inflammatory cells by the migration of the neutrophils, eosinophils, and lymphocytes from peripheral blood to the lungs, leading to neutropenia, lymphopenia, and eosinopenia which led to aggravate respiratory distress. Although the mechanism of significant lymphocyte reduction in severe

SARS-COV2 remains unclear, there are hypothesis other than lymphocyte infiltration and sequestration in the lungs, gastrointestinal tracts, and or lymphoid tissues: (1) lymphocytes express the ACE2 receptor and may be a direct target of SARS-CoV-2 infection (Xu *et al*.,2020)., and (2) an increase of pro-inflammatory cytokines in SARS-COV2, especially IL-6, could induce further lymphocyte reduction (Lin *et al.*,2020).

3.4 Subjects Immunological Analysis Results

Table 3.9 reveals that all parameters (TNF-a, IL33, GM-CSF) for the case (with SARS-COV2) group was significantly higher than that for the control group.

Table 3.9: Comparison of the mean values of the parameters between case and control groups

Immunological parameters		Median (IQR)	Mean Rank	Sum of Ranks	Mann-Whitney U	P. value
TNF-a	Control	3.84 (3.89)	58.10	5810.00	760.000	<0.001*
	Patients	9.81 (7.68)	124.64	8725.00		
IL33	Control	3.09 (2.47)	50.89	5089.00	39.000	<0.001*
	Patients	67.04 (158.40)	134.94	9446.00		
GM-CSF	Control	2.52 (0.42)	70.55	7054.50	2004.500	<0.001*
	Patients	3.02 (3.45)	106.86	7480.50		

The results of this study agreed with Bonaventura *et al*., (2020) reported that increased levels of GM-CSF have been recently described in patients with SARS-COV2 compared to healthy controls.

While this study is very consistent with other studies that support an increase in the level of immunological examination results during infection with SARS-COV2 disease, various interpretations; the increased IL-33 levels in severe infection could result from epithelial damage caused by

strong interactions between the airway epithelium and activated immune cells. SARS-CoV2-derived papain-like protease (PLpro), a powerful inducer of IL- 33 in epithelial cells (Shin *et al*., 2020), may also trigger epithelium-derived IL-33 to initiate inflammatory responses in the lungs. To test whether SARS-CoV2 infection induces IL-33 expression in epithelial cells (Liang *et al*., 2021).

Notably, IL-33 promotes rapid neutrophil migration via macrophage-derived CXCL1 and CXCL2, whereas neutrophil elastase and cathepsin G further contribute to IL-33 processing and maturation to exacerbate inflammatory responses. It is plausible that pathogenic γδ17 T cells may also accelerate neutrophil recruitment to the lungs via IL-17 production. Furthermore, immature neutrophils have been reported in severe SARS-COV2 cases (Schulte-Schrepping *et al*., 2020). Neutrophil dysregulation may be attributed to increased IL-33/ILC2 responses, since IL-33 can educate neutrophils towards a unique immunosuppressive phenotype via ILC2s and dampen the appropriate antiviral T cell immune response (Liang *et al*., 2019), which is potentially involved in the control of SARS-CoV2 infection. Importantly, elevated IL-33 levels and the associated type 2 immunity in chronic viral infection are considered potential inducers of pulmonary fibrosis, which is a recognized sequelae of acute respiratory distress syndrome (ARDS) observed in approximately 40% of SARS-COV2 patients (Spagnolo *et al*., 2020).

Regarding GM-CSF, Increased levels of GM-CSF have been found in the bronchoalveolar fluid of patients with ARDS compared with healthy controls . Higher levels were observed in the early phases (1–3 days) with a progressive decrease in late stages (day 14). GM-CSF may indirectly contribute to ARDS by the suppression of neutrophil apoptosis (Aggarwal *et al*., 2000; Matute-Bello *et al*., 2000) as activated neutrophils play a major role in the microvascular damage contributing to lung damage (Rebetz *et*

al., 2018; Bonaventura *et al*., 2019). In the early phases of viral infections, GM-CSF's role may be protective as it helps limit virus-related injury. For this reason, an inhaled formulation of sargramostim is being tested in patients with SARS-COV2 related acute hypoxic respiratory failure (Bonaventura *et al*., 2020).

TNF-a is important in nearly all acute inflammatory reactions, acting as an amplifier of inflammation, and its blockade has been used to treat more than ten different autoimmune inflammatory diseases, suggesting that TNF-a blockade may be an interesting therapeutic approach to reduce organ damage in SARS-COV2 patients (Feldmann *et al*.,2020)

3.4.1. TNF-a levels in patients and control groups.

Table 3.10 : Sensitivity and specificity of TNF-α level in SARS-COV2

TNF-α level (fold)	Patients NO. = 70	Control NO. = 100
Predictive-Patients > 4.96	56(80.0%)	10 (10.0%%)
Predictive-Control ≤ 4.96	14 (20.0%%)	90 (90.0%)
Sensitivity %	80.0 %	
Specificity %	90.0 %	
PPV %	84.8 %	
NPV %	86.5 %	
AUC (95% CI)	0.891 (0.840- 0.943) **"Good"**	
P. value	<0.001*	

CI: Confidence interval, AUC: Area under curve, NPV: Negative predictive value, PPV: Positive predictive value.

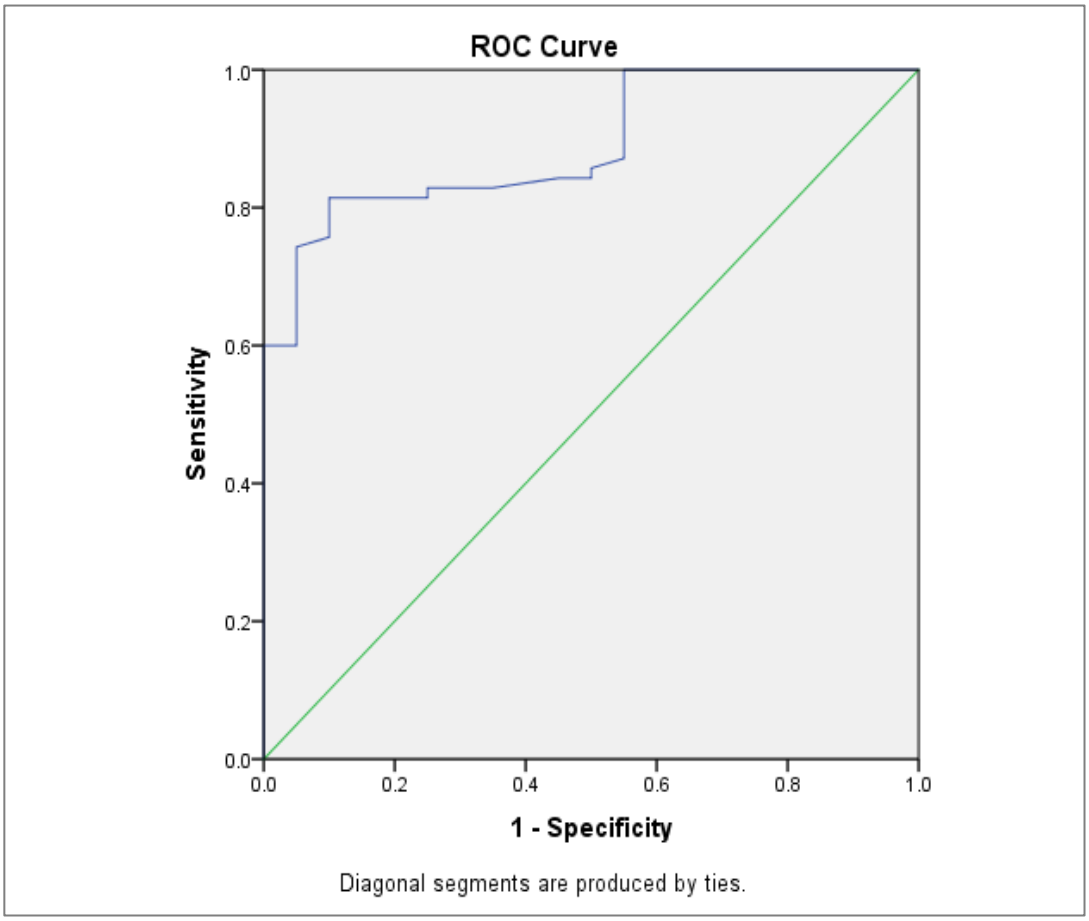


Figure (3.1) Receiver operator characteristic curve analysis for the calculation of TNF-α possible diagnostic cutoff value.

To evaluate the TNF-α cutoff value as well as to predict the SARS-COV2 as diagnostic tests or adjuvant diagnostic tests, receiver operator characteristic (ROC) curve analysis was carried out and the results are shown in table (3.10), and figure (3.1). The TNF-α cutoff value was > 4.96 with sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), Area under curve, and *P. value* of 80.0%, 90.0 %, 84.8 %, 86.5% and 0.891 (0.840- 0.943), and <*0.001*, respectively.

3.4.2. IL33 levels in patients and control groups.

Table 3.11: Sensitivity and specificity of IL33 level in SARS-COV2

IL33 level (fold)	**Patients NO.= 70**	**Control NO.= 100**
Predictive-Patients > 6.92	68(97.1%)	6 (6.0%)
Predictive-Control ≤ 6.92	2 (2.9%)	94 (94.0%)
Sensitivity %	97.1 %	
Specificity %	94.0 %	
PPV %	91.9 %	
NPV %	97.9 %	
AUC (95% CI)	0.994 (0.986- 1.000) **"Excellent"**	
P. value	<0.001*	

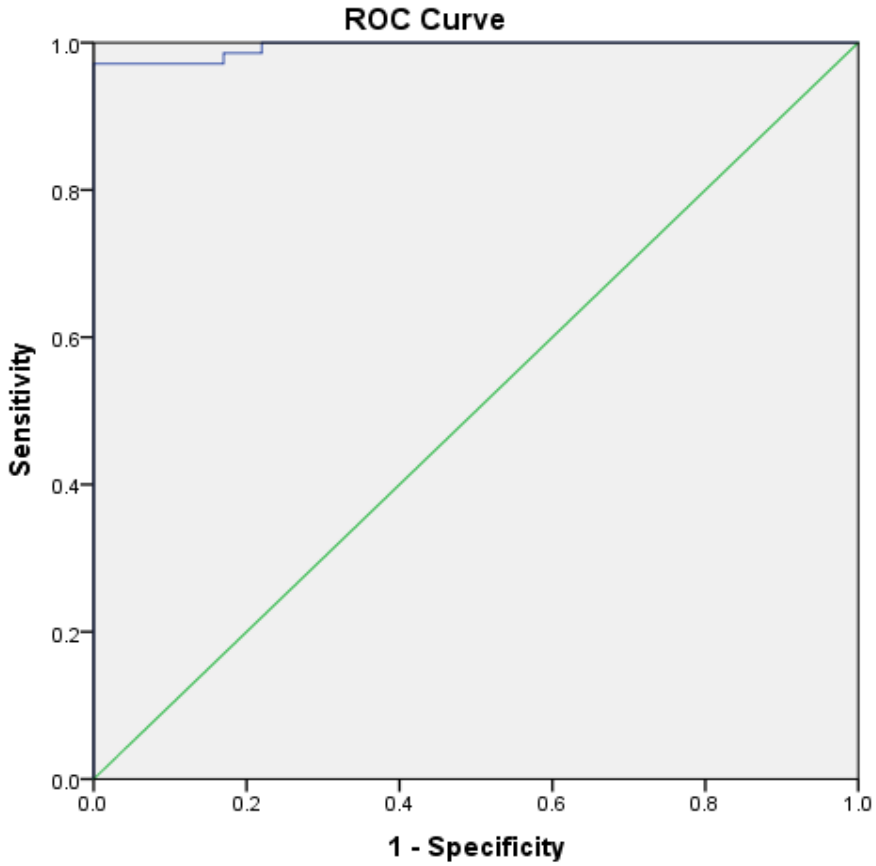


Figure (3.2) Receiver operator characteristic curve analysis for the calculation of IL33 possible diagnostic cutoff value.

To evaluate the IL33 cutoff value as well as to predict the SARS-COV2 as diagnostic tests or adjuvant diagnostic tests, receiver operator characteristic (ROC) curve analysis was carried out and the results are shown in table (3.11), and figure (3.2). The IL33 cutoff value was > 6.92 with sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), Area under curve, and *P. value* of 97.1 %, 94.0 %, 91.9%, 97.9% , 0.994 (0.986- 1.000), *<0.001*, respectively. These results are consistent with Moustafa, (2022), which found that the AUC of the ROC curve was 0.889 for IL33.

3.4.3. GM-CSF levels in patients and control groups.

Table 3.12: Sensitivity and specificity of GM-CSF level in SARS-COV2

GM-CSF level (fold)	Patients NO. = 70	Control NO. = 100
Predictive-Patients > 2.31	55(78.6%)	71 (71.0%)
Predictive-Control ≤ 2.31	15 (21.4%)	29 (29.0%)
Sensitivity %	78.6 %	
Specificity %	29.0 %	
PPV %	43.6 %	
NPV %	65.9 %	
AUC (95% CI)	0.714 (0.628- 0.800) **"Fair"**	
P. value	<0.001*	

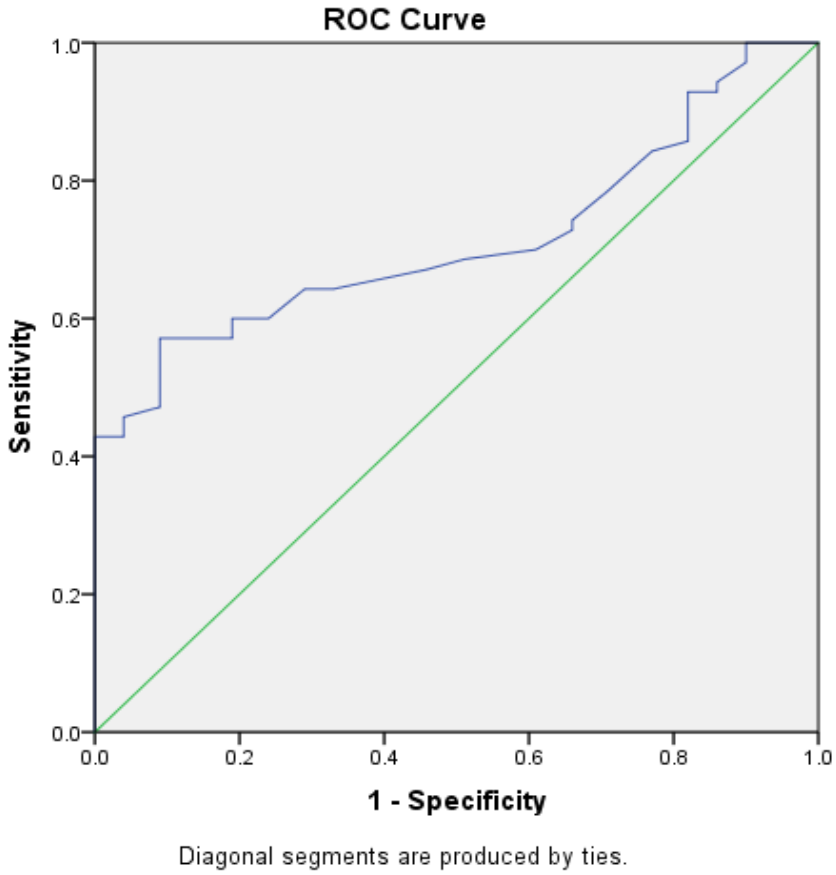


Figure (3.3) Receiver operator characteristic curve analysis for the calculation of GM-CSF possible diagnostic cutoff value.

To evaluate the GM-CSF cutoff value as well as to predict the SARS-COV2 as diagnostic tests or adjuvant diagnostic tests, receiver operator characteristic (ROC) curve analysis was carried out and the results are shown in table (3-12), and figure (3.3). The GM-CSF cutoff value was > 2.31 with sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), Area under curve and P. value of 78.6 %%, 29.0 %, 43.6 %, 65.9% , 0.714 (0.628- 0.800), and <0.001, respectively.

Table 3-13: Correlations of serum immunological markers level to Demographic characteristics and some parameters in patients with SARS-COV2

Parameters		WBCs	Ferritin	Dimer	CRP	Lymphocytes	Neutrophil	IL33	TNF-a	GM-CSF	Age
WBCs *10^3	R	1	0.328**	0.337**	0.345**	-0.480-**	.482**	0.366**	0.113	0.194*	0.261**
	P		0.000	0.000	0.000	0.000	0.000	0.000	0.142	0.011	0.001
Ferritin ng/ml	R	.328**	1	.554**	.377**	-0.510-**	.491**	.323**	.422**	.342**	.439**
	P	.000		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
D-Dimer mg/L	R	.337**	.554**	1	.468**	-0.543-**	.529**	.474**	.557**	.296**	.517**
	P	.000	0.000		0.000	0.000	0.000	0.000	0.000	0.000	0.000
CRP mglL	R	.345**	.377**	.468**	1	-0.506-**	.544**	.438**	.485**	.336**	.321**
	P	.000	0.000	0.000		0.000	0.000	0.000	0.000	0.000	0.000
Lymphocytes(%)	R	-.480-**	-.510-**	-.543-**	-.506-**	1	-.692-**	-.430-**	-.423-**	-.308-**	-.448-**
	P	0.000	0.000	0.000	0.000		.000	.000	.000	.000	.000
Neutrophil(%)	R	.482**	.491**	.529**	.544**	-0.692-**	1	.457**	.437**	.312**	.452**
	P	0.000	0.000	0.000	0.000	0.000		0.000	0.000	0.000	0.000
IL33	R	.366**	.323**	.474**	.438**	-0.430-**	.457**	1	.481**	.395**	.462**
	P	.000	0.000	0.000	0.000	0.000	0.000		0.000	0.000	0.000
TNF-a	R	.113	.422**	.557**	.485**	-0.423-**	.437**	.481**	1	.545**	.478**
	P	.142	0.000	0.000	0.000	0.000	0.000	0.000		0.0	0.0

										00	00
GM-CSF	R	.194**	.342**	.296**	.336**	-0.308-**	.312**	.395**	.545**	1	.339**
	P	.011	.000	.000	.000	.000	.000	.000	.000		.000
Age	R	.261**	.439**	.517**	.321**	-0.448-**	.452**	.462**	.478**	.339**	1
	P	.001	.000	.000	.000	.000	.000	.000	.000	.000	

P ; p vale at $P \leq 0.05$, R: correlation

The correlations between some serum Immunological markers levels and demographic characteristics and some markers in patients with SARS-COV2 are shown in table (3-13). All parameters had a positive significant relationship with each other except for lymphocytes, which had a negative relationship for all parameters (WBCs/ r= -0.480, *P <0.001*), (Ferritin/ r= -0.510, *P <0.001*), (D-Dimer/ r= -0.543, *P <0.001*), (CRP/ r= -0.506, *P <0.001*), (Neutrophil / r= -0.692, P <0.001), (IL33 / r= -430, P <0.001), (TNF-a / r= -423, *P <0.001*), (GM-CSF / r= -0.308, *P <0.001*), and (Age / r= -0.448, *P <0.001*). While there was no significant association between WBCs and TNF-a (*P. value*=0.142).

3.5. Molecular analysis

3.5.1 DNA Extraction

Genomic DNA from blood samples were extracted by using G-spin™ Total DNA Extraction Mini Kit and checked by using Nano drop spectrophotometer at (260/280 nm), it was ranging between 1.8- 2.2, with purity average equal 2, and DNA concentration mean was 20 ng/µl.

3.5.2. Detection of Genes Polymorphisms

3.5.2.1. Detection of *TNFa (rs1800629)* gene Polymorphism

The distribution of *TNFa (rs1800629)* gene Polymorphism was detected by ARMS-PCR technique. At this locus there are three genotypes; GG, GA and AA. The wild type homozygote genotype were showed only G allele amplification. The mutant type homozygote genotype were showed only A allele amplification. Whereas, the heterozygote genotype were showed G and A alleles amplification at 290 bp product size, figure (3-4). The genotype distribution had no deviation from Hardy-Weinberg equilibrium.

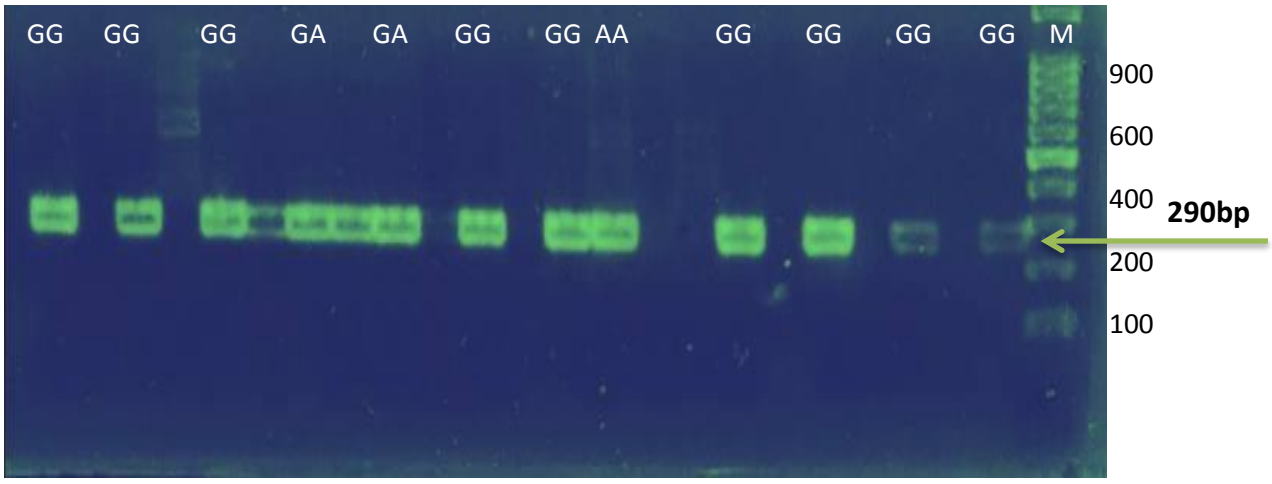


Figure (3-4): Agarose gel electrophoresis image that show the ARMS -PCR product analysis of TNF-a (rs1800629) gene polymorphism in 2% agarose. Where M: marker (1500-100bp), lane (GG) wild type homozygote, lane (AA) mutant type homozygote, and lane (GA) heterozygote, that show at 290bp bands.

Hardy Weinberg equation was applied to *TNFα (rs1800629)* genotypes, GG, GA and AA, distribution within the control group and results are shown in table 3.14.The homozygous wild genotype GG was encountered in 10 out of 20 control subjects; the heterozygous GA genotype was seen in 7 out of 20 control subjects and the homozygous mutant AA genotype was seen in 3 out of 20 control subjects. The observed distribution of control subjects according to *TNF-a (rs1800629)* genotypes was non-significantly different from the expected one (P = 0.668), as shown in table 3.14.The Genotype frequency distribution in patients and control group according to *TNF-a (rs1800629)* genotypes was non-significantly*(P=0.606)* as shown in table 3.15,The allele frequency distribution in patients and control group according to *TNF-a (rs1800629)* genotypes was non-significantly*(p= 0.347)*as shown in table 3.16.

Table 3.14 : Hardy Weinberg equation among control group

Genotypes	Observed	Expected	χ^2	*P*
Homozygote reference GG	10	9.12	0.818	0.668 ¥ NS
Heterozygote GA	7	8.78		
Homozygote variant AA	3	2.11		

¥: Chi-square test; NS: Non-significant at *P >0.05*

Table 3.15: *TNFα (rs1800629)* Genotype frequency distribution in patients and control group

Genotype	Patients *No.* = 70	Control *No.*= 20	χ^2	*P* ¥
GG	31 (44.3%)	10 (50.0%)	1.002	0.606 NS
GA	21(30 %)	7 (35.0%)		
AA	18 (25.7%)	3 (15.0%)		

No. number of cases; ¥: Chi-square test; NS: Non-significant at $P > 0.05$

Table 3.16: *TNFα (rs1800629)* allele frequency distribution in patients and control group.

Allele	Patients *NO.*= 140	Control *NO.*= 40	*P*	OR	95 % CI		EF	PF
					Lower	Upper		
A	57	13	0.347¥ NS	1.426	0.678	2.99	0.289	
G	83	27		0.701	0.333	1.473		0.289

***NO.*number of alleles; ¥: Chi-square test; OR: odds ratio; CI: confidence interval; EF: etiologic fraction; PF: preventive fraction; HS: highly significant at $P \leq 0.01$**

Table 3.17: Association between *TNFa (rs1800629)* genotype and some biochemical and immunological biomarkers

Biomarkers	*TNFa (rs1800629) genotype*			
	GG *NO.*= 31	GA NO.= 21	AA *NO.*= 18	*P*
White blood cells*10^3				
Mean± SD	**10.27** ± 3.65	**9.85**± 3.38	**8.86** ± 4.04	**0.533 ¥ NS**
Range	5.45 – 15.70	3.40 – 14.70	3.40 – 19.50	
Neutrophils				
Mean± SD	**69.76** ± 13.31	**68.09** ± 14.81	**66.62** ± 15.59	**0.780¥ NS**
Range	45.79 – 85.60	43.60 – 88.90	41.43 – 88.13	
Lymphocyte				
Mean± SD	**24.79** ± 13.26	**23.07** ± 10.27	**23.24** ± 8.61	**0.837 ¥ NS**
Range	8.88 – 49.90	8.22 – 39.67	11.47 – 38.60	
C-reactive protein (CRP) mg/L				
Mean± SD	**28.34** ± 13.74	**28.09** ± 11.81	**31.50** ± 11.94	**0.928 ¥ NS**
Range	11.10 – 144.00	11.00 – 77.00	16.70 – 122.00	
D-Dimer ng/L				
Mean± SD	**612.15** ± 163.05	**454.95** ± 162.9	**504.11** ± 151.2	**0.229 ¥ NS**
Range	123.00 – 2345.0	100.0 – 700.0	312.0 – 843.0	
S-Ferritin ng/ml				

Mean± SD	**1042.11** ± 168.7	**862.33** ± 235.4	**808.58** ± 142.7	**0.816 ¥ NS**
Range	289.39– 4033.61	89.00 – 3404.0	230.0 – 4543.0	
IL33 level				
Mean± SD	**153.83** ± 138.2	**138.2** ± 136.80	**237.16** ± 162.1	**0.553 ¥ NS**
Range	7.27 – 1033.62	7.21– 966.78	4.82– 1379.91	
TNF-α				
Mean± SD	**10.63** ± 4.96	**10.34** ± 7.76	**9.27** ± 9.27	**0775 ¥ NS**
Range	4.93– 28.95	1.85 – 32.21	2.01– 128.95	
GM-CSF				
Mean± SD	**4.29** ± 3.03	**6.85** ± 5.98	**8.03** ± 5.75	**0.293 ¥ NS**
Range	1.80– 30.47	1.63 – 28.91	1.89– 50.37	

n: number of cases; ¥: Chi-square test; **NS**: not significant at $P > 0.05$**; S**: significant at $P \leq 0.05$

The association between *TNFa (rs1800629)* genotype and some serum Immunological markers levels and some blood parameters in patients with SARS-cov2 are shown in table (3-17), All parameters had a non –significant association with *TNFa(rs1800629)* genotype.

The current case-control study is aimed to evaluate the associations of genetic polymorphisms in (TNFs) with SARS-COV2 and its mortality rate.ARMS technique was recruited to detect (−308G>A, rs1800629 G>A)TNFα polymorphisms among the Iraqi SARS-COV2 patients and controls.

Results established non-significant genotypes and alleles frequency distribution between the two groups($P = 0.668$).previous studies and scientific evidences were exploring molecular mechanisms of SARS-COV2 in other ethnicities.

Heidari *et al*.,(2022), findings provided insights into the relationship between TNFα polymorphisms and severe acute respiratory syndrome coronavirus among iranian population.

Nikpouraghdam *et al*., (2020) suggested that older age, male gender, and having comorbid conditions were significantly correlated with death rates among Iranian SARS-COV2 patients(Shan *et al*.,2020; Lukassen *et al*.,2020).

Besides genetics, psychological distress, smoking, poor sleep quality, and body mass index are among the risk factors associated with SARS-COV2 susceptibility and incubation time By performing a case-control study,present results were inline with Chong *et al*.,(2006) who reported that the (TNFα)-308G/A polymorphism, is associated with the onset progress of SARS-CoV2 infection but not the progress of SARS-CoV (Saleh *et al*.,2022).

A recent study established that when the cytokine storm syndrome happened in SARS-COV2, it caused increasing levels of TNF-α, interleukin 1 (IL-1), IL-6, IL-8, IL-12, and IFN-γ; therefore, increasing some cytokines, for example, IL-6 and TNF-α cause poor prognosis in patients with SARS-COV2 (Rokni *et al*., 2020).

More recently, Kirtipal and Bharadwaj, (2021) reported that IL6 SNPs could be considered an indicator of SARS-COV2severity in humans. This indicates that SNPs in genes encoding inflammatory cytokines and other innate immune genes might also impact the susceptibility to acute respiratory disorders, including SARS-COV2.

The host genetics plays a fundamental role in the immune response to the SARS-CoV-2 virus and influences the risk of SARS-COV2, severity, and outcome in affected patients. statistical analysis of the current study revealed that GG and GA genotypes were more frequent in the control group compared to SARS-COV2 subjects .these genotypes may decrease the risk of SARS-COV2by (OR =0.674 , 95% CI = 0.291–1.55, $p = 0.354$ ¥).

Moreover, these genotypes showed a protective role that decresed the risk of SARS-COV2 moderately among healthy subjects in the study. On the

other hand, the correlation between disease severity, prognosis, and signs/symptoms of SARS-COV2 subjects, laboratory and immunological parameters in concered by this study and different genotypes of studied variations assessed by statistical analysis showed no significant association concerning the evaluated parameters between different genotypes(Saleh *et al*.,2022).

Several case-control studies (Rokni *et al*.,2019;Ovsyannikova *et al*.,2020) of assorted designs have emerged to elucidate the association of specific host genetic variants with clinical disease severity or susceptibility and duration of hospitalization times to SARS-CoV2 infection beside correlation to some selected biochemical, hematological, and immunological parameters (Anastassopoulou *et al*., 2020).

Present study findings revealed non significant correlations between TNFα-311A/G, rs1800629 G>A polymorphisms and SARS-CoV-2 infection and mortality rate. However, GG and AG genotypes seemed to decreased the risk of SARS-CoV2 disease, as they conferred protection against SARS-CoV2 susceptibility and hospitalization.

The rs1800629 polymorphism is the most studied TNFα variation, which is a G/A substitution and is located in the promoter region at position −308(Roszak *et al*.,2015). It has been established that the presence of the GG genotype for this SNP confers strong in vivo and in vitro transcriptional activity(Muñoz-Valle *et al*.,2014;Agliardi *et al*.,2018).

Previous analyses of this polymorphism in different populations gave inconclusive results.

Zhang *et al*.,(2017) discovered that TNFα rs1800629 is associated with enhanced risk of sepsis, a systemic inflammatory response to infection, under allelic A versus G, GA versus GG, and GA + AA versus GG inheritance models.

Tharwat *et al*., (2019) reported that the AA genotype of this variant confers susceptibility to hepatitis C virus infection in Egyptian patients. In connection with respiratory diseases, Yang *et al*.,(2014) proposed that TNFα rs1800629 is a risk factor for asthma. This can be explained by the role of TNFα in the pathophysiology of respiratory diseases(Rutigliano and Graham, 2004).

Ding *et al*.,(2019) showed that allele A of the TNFα rs1800629 polymorphism is associated with risk of ARDS in a Chinese population, whereas the GG genotype was linked to lower mortality. It has also been shown that the G allele of this variation was overrepresented in patients with influenza A/H1N1 and correlated with disease severity in a Mexican population(Martinez-Ocaña *et al*.,2013) confirming to current study findings, Wang *et al*.,(2008) study showed no difference between the genotype distribution of TNFα SNPs, including -308G/A, between cases with the SARS and healthy subjects. In present study, found that the G allele and GA + GG genotypes decrease the risk of SARS-CoV-2 infection among healthy controls. This conflicting results can be explained by the role of TNF in the pathophysiology of ARDS of SARS-CoV2 among different ethinicities.

Results reached through this work illustrate the significant increament of TNF alpha serum level among SARS-COV2 patients . As an inflammatory mediator, TNF-α affects the production of other cytokines. However, the interaction between cytokines might result in antagonistic (TNF-α and TNF-β, for instance) or synergistic (e.g., TNF-α with IL-1 interactions) effects. TNFs also regulate receptor expression of other cytokines or stabilize cytokine messages by another, and, therefore, play pivotal roles in signal transduction(Neta *et al*.,1992) confirmed current study findings and hypothesized that serum concentration of TNF-α is elevated in

subjects with SARS-CoV2; thus, these patients have a greater probability of developing ARDS and death by initiating the cytokine storm.

Accumulating evidence shows that genetic background may influence the outcome and severity of SARS-COV2. Herein, for the first time, this study reported a possible although nonsignificant association between TNFα polymorphisms and SARS-COV2risk and outcome. However, there are some shortcomings to the current study. First, small sample size was relatively small.Second, depending on their interactions with other risk factors and SARS-COV2 candidate SNPs, allelic variants of TNFα gene might exhibit different impacts in other races. So, further studies on a larger population and different ethnicities are needed to confirm present study results.

3.5.2.2 Detection of *IL-13 (rs20541)* gene Polymorphism

The distribution of *IL-13 (rs20541)* gene Polymorphism was detected by ARMS-PCR technique. At this locus there are three genotypes; GG, AG and AA. The 418bp as a control wild type homozygote genotype were showed only G allele amplification at 251 bp product size. The mutant type homozygote genotype were showed only A allele amplification at 214 bp product size. Whereas, the heterozygote genotype were showed A and G alleles amplification at 418bp and 251 bp product size respectively, figure (3-5). The genotype distribution had no deviation from Hardy-Weinberg equilibrium.

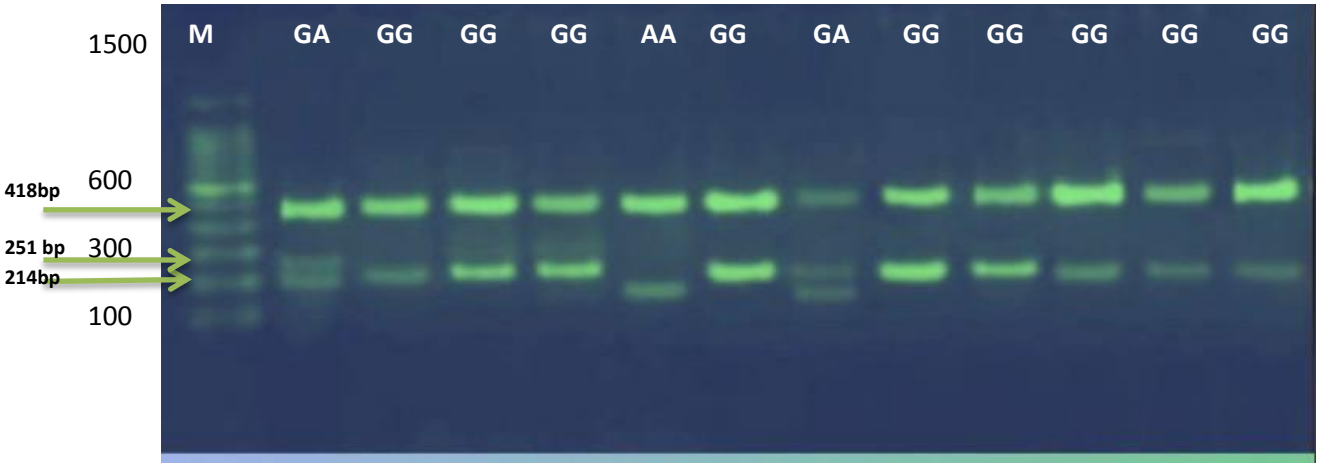


Figure (3-5): Agarose gel electrophoresis image that show the ARMS -PCR product analysis of IL-13 (rs20541) gene polymorphism in 2% agarose. Where M: marker (1500-100bp),418 as a control, lane (GG) wild type homozygote that show at 251bp band, lane (AA) mutant type homozygote, that show at 214bp band, and lane (GA) heterozygote, that show at 418bp .

Hardy Weinberg equation was applied to *IL-13 (rs20541)* genotypes, GG, AG and AA, distribution within the control group and results are shown in table 3.18. The homozygous wild genotype GG was encountered in 12 out of 20 control subjects; the heterozygous GA genotype was seen in 6 out of 20 control subjects and the homozygous mutant AA genotype was seen in 2 out of 20 control subjects.The observed distribution of control subjects according to *IL-13 (rs20541)* genotypes was non-significantly different from the expected one (P = 0.670), as shown in table 3.18. The Genotype frequency distribution in patients and control group according to *IL-13 (rs20541)* genotypes was non-significantly*(P=0.490)* as shown in table 3.19,The allele frequency distribution in patients and control group according to *IL-13 (rs20541)* genotypes was non-significantly*(p= 0.418)*as shown in table 3.20.

Table 3.18: Hardy Weinberg equation among control group

Genotypes	Observed	Expected	χ^2	*P*
Homozygote reference GG	12	11.25	0.80	0.670 ¥ NS
Heterozygote GA	6	7.5		
Homozygote variant AA	2	1.25		

¥: Chi-square test; NS: Non-significant at $P > 0.05$

Table 3.19: *IL-13 (rs20541)* Genotype frequency distribution in patients and control group

Genotype	Patients *NO.*= 70	Control *NO.* = 20	χ^2	*P* ¥
GG	33 (47.1 %)	12 (60.0%)	1.429	0.490 NS
AG	23 (32.9 %)	6 (30.0%)		
AA	14 (20.0%)	2 (10.0%)		

NO: number of cases; ¥: Chi-square test; NS: Non-significant at $P > 0.05$

Table 3.20: *IL-13 (rs20541)* allele frequency distribution in patients and control group.

Allele	Patients *NO.*= 140	Control *NO.* = 40	*P*	OR	95 % CI		EF	PF
					Lower	Upper		
A	51	10	0.178 ¥ NS	1.719	0.777	4.219	0.418	
G	89	30		0.581	0.262	1.287		0.418

***NO*: number of alleles; ¥: Chi-square test; OR: odds ratio; CI: confidence interval; EF: etiologic fraction; PF: preventive fraction; HS: highly significant at $P \leq 0.01$**

Table 3.21: Association between *IL-13 (rs20541)* Genotype and some biochemical and immunological biomarkers

Biomarkers	*IL-13 (rs20541) Genotype*			*P*
	GG *NO.* = 33	AG NO.= 23	AA *NO.*= 14	
White blood cells *10^3				
Mean± SD	**9.82** ± 4.05	**9.07** ± 3.33	**9.6285** ± 4.01	**0.764 ¥ NS**
Range	4.33 – 19.50	3.40 – 14.70	3.40 – 15.70	
Neutrophils				
Mean± SD	**71.54** ± 12.81	**64.19** ± 16.15	**66.78** ± 14.68	**0.177 ¥ NS**

Range	42.73 – 88.90	41.43 – 88.13	45.79 – 85.60	
Lymphocyte				
Mean± SD	**20.52** ± 9.88	**26.67** ± 12.43	**25.74** ± 10.72	**0.103 ¥ NS**
Range	8.88 – 41.97	8.22– 48.90	10.88 – 43.80	
C-reactive protein (CRP) mg/L				
Mean± SD	**32.22** ± 15.24	**26.13** ± 16.60	**27.75**± 13.67	**0.747 ¥ NS**
Range	11.10 – 144.00	11.00 – 124.00	11.00 – 80.00	
D-Dimer ng/L				
Mean± SD	**607.40** ± 242.05	**435.32**± 140.9	**573.53**± 203.1	**0.154 ¥ NS**
Range	100.00 – 2345.0	123.0 – 700.0	312.0 – 1508.0	
S-Ferritin ng/ml				
Mean± SD	**1031.19** ± 954.2	**610.72** ± 209.7	**1265.8**± 969.8	**0.280 ¥ NS**
Range	129.0- 6876.00	89.00 – 3404.0	230.0 – 7156.0	
IL33 level				
Mean± SD	**235.19** ± 381.28	**158.94**± 251.2	**150.31** ± 218.8	**0.603 ¥ NS**
Range	11.50 – 1379.91	4.82– 1033.62	12.10– 966.7	
TNF-α				
Mean± SD	**10.21** ± 6.19	**9.10** ± 4.46	**12.07** ± 8.72	**0.368 ¥ NS**
Range	2.33– 28.95	1.85 – 18.83	2.01– 32.21	
GM-CSF				
Mean± SD	**4.98** ± 6.71	**5.87**± 6.50	**8.15** ± 13.17	**0.500 ¥ NS**
Range	2.07– 30.47	1.63 – 28.91	1.89– 50.37	

NO.: number of cases; ¥: Chi-square test; **NS**: not significant at $P > 0.05$**; S**: significant at $P \leq 0.05$

The association between *IL-13 (rs20541)* genotype and some serum Immunological markers levels and some blood parameters in patients with

SARS-cov2 are shown in table (3-21), All parameters had a non –significant association with *IL-13 (rs20541)*genotype.

The distribution of *IL-13* (*rs20541*) gene Polymorphism was detected by ARMS-PCR technique. The genotype distribution had no deviation from Hardy-Weinberg equilibrium. During the time of this study it was clear that GG genotype was more prevalent among healthy control(OR:0.575), while AA genotype was more detected among SARS-COV2 patients(OR:1.737).

Previously, lots of studies researched the association of Interleukin *IL 13* gene polymorphisms and the risk of some respiratory disease, however to our knowledge no studies have attempted to link this gene polymorphism to SARS-COV2 (Omraninava *et al*., 2020).

The coronavirus disease (Covid-19) pandemic is the most serious event of the year 2020, causing considerable global morbidity and mortality. This thesis seeks to present a thorough analysis of potential relationships between interindividual immunogenic variations and illness susceptibility or symptoms brought on by the coronavirus strains. Coronavirus associated with severe acute respiratory syndrome (Darbeheshti *et al*.,2021).

Present study results provides promising clues related to the potential benefits of using immunotherapy and immune modulation for respiratory infectious disease treatment in a personalized manner(Alvarez *et al*.,2018).

Association studies concerning the earlier strain of coronavirus, namely SARS-CoV, could provide some clues, comming in line with present study data, previous recent studies concluded that the differences in the vulnerability of individuals to the coronavirus infections have been proposed to be linked to the key role cytokines early host defense mechanisms, especially before producing specific antibodies(Omraninava *et al*.,2020).
Polymorphisms in the IL13 researched through this work were deeply based by another study in Korea, investigation of *IL13* genes in Korean children revealed various SNPs in various regions of this gene, which were

significantly associated with severe RSV infection. However, after statistical correction, the results related to this SNP were marginal(Choi *et al* .,2002).

It is noteworthy that association studies demonstrated different results in different populations; that is, a specific SNP might be significantly associated with a specific disease in one population, but not in another. These finding highly confirmed current study data about the partial association between IL13 polymorphism and SARS-COV2 (Darbeheshti and Rezaei.,2020).

The infammatory cytokine storm described in SARS-COV2 appears to be closely related to the development and progression of ARDS.Concerning the infammatory biomarkers studied in this work, the SARS-COV2 group corroborates the T2 response predominance (Feng *et al*.,2015).

IL-13 actively participates in the T2 pathway, Infammatory cytokines have been the key mediators in the innate immune response and infammatory reaction in both Middle East Syndrome Coronavirus (MERS-CoV) and SARS-CoV-2. Interestingly, patients with MERS-CoV have presented an immune response lacking on T1 cells and directed towards a T2 immune(Rogo *et al* .,2016). response, and studies have related the exacerbated T2 response to more aggressive forms of its disease(Alvarez *et al*.,2018).

IL-13 is an immunoregulatory protein produced mainly by activated Th2 cells(Chomarat and Banchereau,1998). and is involved in the maturation and differentiation of B cells(Briere *et al*.,1993). This cytokine is considered part of the Th2 immune response and a reasonable hypothesis is that the imbalance in the Th1/Th2 balance could be involved in the pathogenesis of chronic diseases associated with age and are the result of a dysfunction in immune system regulation (Álvarez-Rodríguez *et al*.,2012).

Results reached by present study were parallel with other studies (Passalacqua *et al*., 2017;Sarzi-Puttini *et al*., 2020; Diao *et al*., 2020) .They established that a genetic predisposition in the expression of genes coding for Th2 cytokines like *IL13* gene polymirphism could influence the severity and/or susceptibility of SARS-COV disease.

This study analyzed for the first time the influence of the *rs20541* polymorphism in the gene encoding the *IL-13* in a large series of Iraqi patients with SARS-COV2 disease. Current study results about a possible association were marginal showing a protective role for GG genotype and risk assiciation with AA genotype.

However, statistical power for comparisons in allele frequencies and genotype of patients with SARS-COV2 is low, (*P= 0.548*) so the results should be taken with caution and confirmed in studies containing a higher number of patients. In fact, the polymorphism studied in this work is more clearly associated with SARS-COV2 disease, especially in elderly male patients. In contrast, the association of other polymorphisms in the gene for *IL-13*, such as *rs1800925 (IL13-1055),* and rs2243204 have been described in other inflammatory rheumatic disease, which did not evaluate(Bowes *et al*., 2011; Prompetchara *et al*., 2020).

In summary, the *rs20541* (R130Q) polymorphism of the *IL13* gene is associated with susceptibility to SARS-COV. The utility of this gene to predict prognosis in SARS-COV disease should be confirmed in studies with more patients. Further studies are needed regarding Th2 cytokine genes that allow us to clarify this issue(Vaz de Paula *et al*., 2020).

3.5.2.3. Detection of *TLR6 (rs 5743789)* gene Polymorphism

The distribution of *TLR6 (rs 5743789)*gene Polymorphism was detected by ARMS-PCR technique. At this locus there are three genotypes; TT, TA and AA. The 306 bp as a controlwild type homozygote genotype were showed only T allele amplification at 153 bp product size. The mutant type

homozygote genotype were showed only A allele amplification at 202 bp product size. Whereas, the heterozygote genotype were showed T and A alleles amplification at 153 and 202 bp product size respectively, figure (3-6). The genotype distribution had no deviation from Hardy-Weinberg equilibrium.

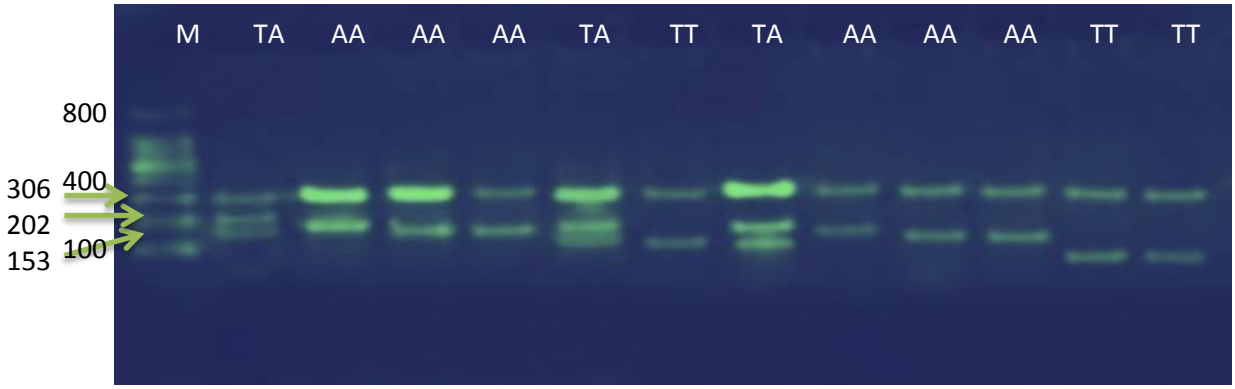


Figure (3-6): Agarose gel electrophoresis image that show the ARMS -PCR product analysis of *TLR6 (rs5743789)* gene polymorphism in 2% agarose. Where M: marker (1500-100bp),306 as a control, lane (TT) wild type homozygote that show at 153bp band, lane (AA) mutant type homozygote, that show at 202bp band, and lane (TA) heterozygote, that show at 306bp .

Hardy Weinberg equation was applied to *TLR6 (rs 5743789)* genotypes, TT, TA and AA, distribution within the control group and results are shown in table 3.22. The homozygous wild genotype TT was encountered in 16 out of 20 control subjects; the heterozygous TA genotype was seen in 3 out of 20 control subjects and the homozygous mutant AA genotype was seen in 1 out of 20 control subjects. The observed distribution of control subjects according to *TLR6 (rs 5743789)* genotypes was non-significantly different from the expected one (P = 0.372), as shown in table 3.22. The Genotype frequency distribution in patients and control group according to *TLR6 (rs 5743789)* genotypes was non-significantly*(P=0.059)* as shown in table 3.23,The allele frequency distribution in patients and control group

according to *TLR6 (rs 5743789)* genotypes was significantly*(p= 0.026)*as shown in table 3.24.

Table 3.22: Hardy Weinberg equation a mong control group.

Genotypes	Observed	Expected	χ^2	*P*
Homozygote reference TT	16	15.3	1.975	0.372 ¥ NS
Heterozygote TA	3	4.37		
Homozygote variant AA	1	0.312		

¥: Chi-square test; NS: Non-significant at $P > 0.05$

Table 3.23: *TLR6 (rs 5743789)* Genotype frequency distribution in patients and control group

Genotype	Patients *NO.*= 70	Control *NO.* = 20	χ^2	*P* ¥
TT	42 (60.0 %)	16 (80.0%)	7.441	0.059 NS
TA	14 (20.0 %)	3 (15.0%)		
AA	14 (20.0%)	1 (5.0%)		

NO.: number of cases; ¥: Chi-square test; NS: Non-significant at $P > 0.05$

Table 3.24: *TLR6 (rs 5743789)* allele frequency distribution in patients and control group.

Allele	Patients *NO.* = 140	Control *NO.*= 40	*P*	OR	95 % CI		EF	PF
					Lower	Upper		
A	42	5	0.026 ¥ S	3.0	1.011	8.90	0.666	
T	98	35		0.333	0.112	0.989		0.666

***NO.*: number of alleles; ¥: Chi-square test; OR: odds ratio; CI: confidence interval; EF: etiologic fraction; PF: preventive fraction; HS: highly significant at $P \leq 0.01$**

Table 3.25: Association between *TLR6 (rs 5743789)* Genotype and some biochemical and immunological biomarkers

Biomarkers	*TLR6 (rs 5743789) Genotype*			*P*
	TT *NO.* = 42	TA NO. = 14	AA *NO.*= 14	
White blood cells *10^3				
Mean± SD	**9.83** ±3.49	**9.70** ± 3.18	**8.39** ± 5.11	**0.468 ¥ NS**
Range	4.33 – 15.70	5.36 – 14.89	3.40 – 19.50	
Neutrophils				

Mean± SD	**69.31** ± 13.38	**68.56** ± 15.83	**63.03**± 16.42	**0.388 ¥ NS**
Range	42.73 – 88.13	41.43 – 88.90	43.60– 83.70	
Lymphocyte				
Mean± SD	**22.99** ± 10.41	**22.75**± 11.53	**27.70** ± 13.00	**0.393 ¥ NS**
Range	8.88 – 41.97	9.10– 48.90	10.88– 46.90	
C-reactive protein (CRP) mg/L				
Mean± SD	**31.89** ± 15.37	**28.06** ± 13.73	**22.78** ± 16.76	**0.620 ¥ NS**
Range	11.10 – 144.00	11.00 – 88.00	11.00 – 60.00	
D-Dimer ng/L				
Mean± SD	**540.58** ± 272.89	**471.00**± 148.1	**615.8** ± 584.9	**0.486 ¥ NS**
Range	100.00 – 1788.0	123.0 – 698.0	300.0 – 2345.0	
S-Ferritin ng/ml				
Mean± SD	**1005.31**± 397.8	**735.3**± 569.35	**980.58**± 821.3	**0.774 ¥ NS**
Range	281.0- 6876.00	99.00 – 1689.0	214.0 – 7156.0	
IL33 level				
Mean± SD	**187.3** ± 303.38	**228.17**± 291.9	**61.0** ± 45.5	**0.202 ¥ NS**
Range	4.82 – 1379.9	14.54– 966.7	12.10– 182.4	
TNF-α				
Mean± SD	**9.90**± 6.82	**9.74**± 5.33	**11.64** ± 6.61	**0.646 ¥ NS**
Range	2.33– 20.46	1.85 – 32.21	2.01– 32.21	
GM-CSF				
Mean± SD	**5.93** ± 9.29	**7.12** ± 9.01	**4.68** ± 4.55	**0.723 ¥ NS**
Range	1.89– 50.37	1.63 – 30.47	1.89– 18.87	

No.: number of cases; ¥: Chi-square test; **NS**: not significant at $P > 0.05$**; S**: significant at $P \leq 0.05$

The association between *TLR6 (rs 5743789)* genotype and some serum Immunological markers levels and some blood parameters in patients with

SARS-cov2 are shown in table (3-25), All parameters had a non –significant association with *TLR6 (rs 5743789)* genotype.

Toll-like receptor genes are involved in the host immune response against viral infections including SARS-COV-2. Results reached by this study showed that the observed distribution of control subjects according to *TLR6 (rs 5743789)* genotypes was non-significantly different from the expected one (*P = 0.372*), although TT genotypes was marginally associated with control subjects than SARS-COV2 patients with odd ratio(OR= 3.0, C.I.=1.011- 8.90).

Results of the present study established that The mutant ‘A/A' genotypes and the ‘A' alleles of *TLR6(rs 5743789)* polymorphism were significantly associated with increased risk of SARS-COV2 patients.

The innate immune system provides the first-line protection against invading organisms and it is potentially important in SARS-CoV-2 infection(Shereen *et al*., 2020) . The innate immunity includes a family of receptor proteins, named pattern recognition receptors (PRRs); of which, toll-like receptors (TLRs) are important members. TLRs can identify pathogen-associated molecular patterns (PAMP) and respond by eliciting inflammatory responses to eliminate the invading organisms (Sadik *et al*.,2015).Data reached by present study were confirmed by previous studies (Alseoudy *et al*., 2022).

TLR signaling has a pivotal role in the regulation of cytokine expression; hence TLR signaling could be crucially implicated in cytokine storm of SARS-CoV-2 infection (Mehta *et al*.,2020; Florindo *et al.*,2020). de Rivero Vaccari *et al*., (2020);found that TLRs have a dual role in in confronting SARS-COV2 infection.

TLRs play an important role in recognition of viral particles and initiation of the innate immune system with secretion of pro-inflammatory cytokines,

although it can also harm the host due to persistent inflammation and tissue destruction via activation of inflammasome and production of IL-1β, which induces IL-6 leading to hyperactivation of the immune system which can contribute to acute lung injury (Wang *et al* .,2020).

Different TLRs, like TLR2, TLR3, TLR4, TLR6, TLR7, TLR8, and TLR9 are potentially important in eliminating SARS-COV-2 infection (Barton *et al*.,2020), results about TLR6 established by our study were deep seeted by recent research (Motta Junior *et al*.,2020) , this study like current study illustrated that the mutant ‘A/A' genotype and the ‘A' allele of TLR6(rs 5743789)were significantly associated with an increased risk of SARS-COV2 pneumonia.

TLR genes display genetic variations and allelic polymorphisms resulting in numerous immunopathological consequences in viral infections (Yuki *et al*.,2020). Up to the time of this work that hypothesized the study of TLRs immunopolymorphisms may provide important clues on the susceptibility and clinical outcomes of SARS-COV-2 infection among Iraqi patients.

Furthermore, present study illustrated that the elderly males harboring the ‘A/A' genotype of TLR6 (rs 5743789) polymorphism could be more susceptible to SARS-COV2 pneumonia than females having the same genotype. This sex-dependent difference may be owing to gender-specific behaviors, genetic and hormonal factors, and sex differences related to SARS-COV-2 infection (Prompetchara *et al*.,2020).

However, current study results were dissimilar to those of Mosaad *et al*.,(2019) which showed no significant association between *TLR6 (rs 5743789*) polymorphism and chronic HCV infection. In the genetic association studies of infectious diseases, these conflicting findings are frequent. These discrepancies could be due to microbe genetic heterogeneity,

differences in the exposure rate to infectious agents, and various pathogen-induced immune responses, in addition to the interaction with environmental factors(Alseoudy *et al*., 2020).

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Conclusions & Recommendations

Conclusions:

1. The highest percentage of 29 (41.4%) of patients with SARS-COV2 fall in the age group (≥ 50 years),Most of the patients with SARS-COV2 fall in male-gender.Those in who age group (≥ 50 year) were significantly more likely to be hospitalized for SARS-COV2 compared with those in who age group less than <30 year while males are significantly more likely to be hospitalized for SARS-COV2 compared with females.
2. Mostly patients with SARS-COV2 were obese having BMI ≥ 30 kg/m2. Likewise, Patients group included 28 (40.0%) patients with SARS-COV2 were smokers. Obese males were more at risk of SARS-CO2 infection than females and significantly more likely to be hospitalized for SARS-COV2 compared with those who were non-obese and non-smokers.
3. CRP, D-Dimer, serum ferritin, Lymphocyte, and Neutrophils counts were significantly high among SARS-COV2 patients compared to healthy controls.
4. For SARS-COV2 those who have normal levels of CRP, D-Dimer, serum ferritin lymphocytes, and neutrophils were significantly less likely to be hospitalized compared with those who have low levels of Lymphocyte.
5. All the immunological parameters (TNF-a, IL33, GM-CSF) serum levels for the case (with SARS-COV2) group was significantly higher than that for the control group.
6. All the parameters evaluated in this study had a positive significant relationship with each other except for lymphocytes, which had a negative relationship for all parameters.
7. Results of genotype and allele frequency distribution of *TNFa (rs1800629*) Polymorphism, *IL-13 (rs20541*) Polymorphism, and *TLR6 (rs 5743789*) Polymorphism showed non-significant differences between patients with SARS-COV2 and healthy controls.

Recommendations:

1. The most effective strategy to avoid SARS-CoV-2 infection is to get vaccinated against SARS-COV2 . The SARS-COV2 Treatment Guidelines Panel (the Panel) recommends SARS-COV2 vaccination for transplant and cellular immunotherapy candidates and recipients, based on the effectiveness of SARS-COV2 vaccines in the general population and the increased risk of SARS-COV2 causing worse clinical outcomes in these patients.
2. Wear a mask, stay in a different room, and use a separate bathroom if possible to keep others safe in your home.
3. Because their use would decrease budgetary demands on health institutions and help diagnose patients more precisely, the Ministry of Health should implement the assessment of the number of SARS-COV2 Antigen-specific test diagnoses and permit them to be utilized to identify the virus.
4. The confusing acquired response associated with SARS-COV2 infections should be thoroughly investigated, and more research into the mechanisms that cause variation in response to most viruses should be conducted.