وزارة التعليم العالي والبحث العلمي

جامعة بابل

كلية العلوم

قسم علوم الحياة

دراسة تعدد الاشكال في جينات HLA-C و HLA-DRB1 بين مرضى COVID-19 وارتباطها بالالتهاب الرئوي البكتيري

اطروحة مقدمة لمجلس كلية العلوم جامعة بابل وهي جزء من متطلبات نيل درجة الدكتوراة فلسفة في العلوم / علوم الحياة

مقدمة من قبل

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بكالوريوس علوم حياة ٢٠٠٩

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بأشراف

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Ministry of Higher Education and Scientific Research

University of Babylon

College of Science

Department of Biology

Study of HLA-C and HLA-DRB1 Genes Polymorphism among Patients with COVID-19 and Association with Bacterial Pneumonia

Thesis Submitted

to the Council of the College of Science University of Babylon in a Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Biology

By

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2023 A.D **1445 H**

بِسْمِ اللَّـهِ الرَّحْمَـٰنِ الرَّحِيمِ

((أَمَّنْ هُوَ قَانِتٌ آنَاءَ اللَّيْلِ سَاجِدًا وَقَائِمًا يَحْذَرُ الْآخِرَةَ وَيَرْجُو رَحْمَةَ رَبِّهِ ۗ قُلْ هَلْ يَسْتَوِي الَّذِينَ يَعْلَمُونَ وَالَّذِينَ لَا يَعْلَمُونَ ۗ إِنَّمَا يَتَذَكَّرُ أُولُو الْأَلْبَابِ))

صدق اللَّهُ العظيم

[سورة الزمر:9]

Dedication

First and foremost, I thank God (Allah), the Almighty for endowing his immense blessing that helped me in each step of my progress toward the successful completion of my research work.

To my father… who has become under the dust and whom God has commanded me to be righteous and benevolent

To my brother… May God have mercy on him

I am dedicating this thesis to my family and many friends.

A special feeling of gratitude to my loving mother…

Whose her words of encouragement and push for tenacity ring in my ears, which provide me inspiration for success and keenness throughout my life.

To my lovely family……

My beloved, steadfast husband…

Who encourages me with passion and endless support. I am very lucky to have a man who loves me so much and stands beside me.

My children…

Who have been affected in every way possible by this quest.

To my sisters...

They never left my side and are very special They have always helped me and believed that I could do it.

To My supervisor…

I appreciate how you have been constantly encouraging and guiding me over the past two years. It was a lot of fun working under your guidance and I knew I genuinely appreciated all the support and guidance you gave me throughout my studies. It was a pleasure working with you, and I learned a lot from you.

Ruqia 2023

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Finally, great thanks go to my friends and family members for encouragement and moral support, and to everyone who supported me and stood by my side.

Ruqia 2023

الخلاصة

الخلاصة

الالتهاب الرئوي هو نوع من أنواع عدوى الجهاز التنفسي السفلي الحاد وهي شائعة وحادة ، والمعروف باعتباره مشكلة صحية عالمية رائدة وسببًا مباشرًا للمرض والوفيات. أجريت الدراسة الحالية للتحقيق في الأسباب البكتيرية والفيروسية للالتهاب الرئوي وكذلك التحقيق في تأثير خلايا الدم البيضاء ، وعدد الصفائح الدموية ، والخلايا الليمفاوية ، ومستضد كريات الدم البيضاء البشرية- C ، ومستضد كريات الدم البيضاء البشرية- DRB1.

تضمنت الدراسة الحالية ٢٠٠ عينة دم من مرضى (كوفيد-١٩ والالتهاب الرئوي البكتيري) الذين تتراوح أعمارهم (١٣ إلى ٨٠) سنة وزعت حسب شدة المرض على النحو التالي: (٤٠ مجموعة أ ، ٤٠ مجموعة ب ، ٤٠ مجموعة ج ، و ٤٠ من المجموعة د) تم نقلهم إلى المستشفى في أجنحة كوفيد-١٩ في مدينة مرجان الطبية ومستشفى الإمام صادق في محافظة بابل لمدة شهرين (ديسمبر ٢٠٢١ ويناير ٢٠٢٢) و ٤٠ مجموعة هـ . والنتيجة توضح أن مستوى خلايا الدم البيضاء كان مرتفعا معنويا ($P \leq$ ٠،٠٥) في المرضى مقارنة بمجموعات السيطرة السليمة ظاهريا. بينما انخفضت نسبة عدد الصفائح الدموية والخلايا الليمفاوية بشكل ملحوظ بين المرضى مقارنة بمجموعات الضبط السليمة. تم التعرف على البكتيريا عن طريق الزراعة ، ونظام فايتك ٢ المضغوط ، في الدراسة الحالية ، كانت البكتيريا الأكثر شيوعًا هي العقدية الرئوية و الكلبسيلة الرئوية.

أظهرت تقنية الفحص المناعي المرتبط بالإنزيم أن تركيز المصل بين فيروس كورونا المتلازمة التنفسية الحادة ، المجموعة (أ) بمستوى ٤٠،٠٣ نانوغرام / مل ، المجموعة (ب) بمستوى ٤٧،٩٣ نانوغرام / مل ، المجموعة (ج) ذات المستوى ٤٦،٨٣ نانوغرام / مل ، المجموعة (د) بمستوى ٦١،١٥ نانوغرام / مل والمجموعة (هـ) بمستوى ١٧،٦٥ نانوغرام / مل ،($P \leq$ ٠،٠٠١). بينما أظهرت مجموعة المريض ترددات أقل لمستضد كريات الدم البيضاء البشرية DRB1 مع متلازمة تنفسية حادة يسببها فيروس كورونا ، مع المجموعة (أ) التي تحتوي على مستويات ٣٨،٨٨ نانوغرام / لتر ، المجموعة (ب) لديها مستويات ٣٤،٣٥ نانوغرام / لتر ، المجموعة (ج) لها مستويات ٤٦،٢٠ نانوغرام / لتر ، المجموعة (د) بمستويات ٥١،١٣ نانوغرام / لتر ، والمجموعة (هـ) بمستويات ٥٩،٩٥ نانوغرام / لتر ($P \leq$ ٠،٠٢).

الخلاصة

أظهرت نتيجة تفاعل البلمرة المتسلسل (PCR-المتسلسل) لجين مستضد كريات الدم البيضاء البشرية ، كما يلي في النوع الطبيعي rs1050445 CC تواترًا منخفضًا بشكل ملحوظ في جميع الحالات مقارنة بالتحكم الصحي الظاهر. في المتحولة متغايرة الزيجوت CA و الطفرة متماثلة الزيجوت AA كانت مرتفعة بشكل ملحوظ مقارنة بالتحكم الصحي الظاهر. وفقًا لهذه النتائج ، كان تواتر الأليل C أقل بشكل ملحوظ في كوفيد-١٩ والالتهاب الرئوي الجرثومي مقارنةً بالضوابط التي تبدو صحية (قيمة P ≥٠،٠٠٢). في rs1050446 من الأنماط الجينية لوحظ وجود فروق ذات دلالة إحصائية بين النيوكليوتيدات SNPs بين الأليلات G و T مع زيادة الأليل G في المرضى وأيضًا زيادة في الأليلات T في مرضى كوفيد-١٩ ولكن انخفاض في مرضى الالتهاب الرئوي البكتيري مقارنةً بالضوابط السليمة على ما يبدو . تمت زيادة تواتر rs1050446 SNPs GG النوع الطبيعي و GT الطفرة متغايرة الزيجوت بشكل كبير في المرضى من الضوابط التي تبدو صحية (قيمة P =٠،٠٠٨) (قيمة P ≥٠،٠٠٤) على التوالي. لم يتم زيادة تواتر rs1050446 SNPs الطفرة متماثل اللواقح بشكل ملحوظ في كوفيد-١٩ وانخفض في الالتهاب الرئوي البكتيري مقارنة بالضوابط الصحية على ما يبدو (قيمة P ≥٠،٠٣). في rs1071650 ، كان تواتر T أليل أقل بكثير في الحالات مقارنة بالضوابط التي تبدو صحية (قيمة P ≥٠،٠٠٢). في النوع البري (TT) أظهر النمط الجيني زيادة ملحوظة في التردد في الحالات مقارنةً بالضوابط الصحية على ما يبدو. في المتحولة متغايرة الزيجوت ، كان تواتر الحالات مع الأنماط الجينية TG و GG مرتفعًا بشكل ملحوظ مقارنةً بالضوابط التي تبدو صحية. في rs9264668 الأنماط الجينية SNPs ، كان تواتر الأليل C أعلى بكثير في الحالات من الضوابط التي تبدو صحية. في النوع البري (TT) في جميع المرضى ، أظهر مرضى متماثل الزيجوت (AA) في مرضى كوفيد-١٩ تواترًا منخفضًا بشكل ملحوظ مقارنةً بالضوابط الصحية على ما يبدو. في الطفرة متغايرة الزيجوت ، لم يكن تواتر الحالات مع الأنماط الجينية CA و AA في الالتهاب الرئوي الجرثومي مرتفعًا بشكل ملحوظ مقارنةً بالضوابط التي تبدو صحية.

فيما يتعلق بتعدد الأشكال لمستضد كريات الدم البيضاء البشرية DRB1 ، كانت النتيجة لأربعة SNPs ، كما يلي rs13192471 SNPs ، أظهر (TT) انه لا يوجد فرق معنوي بشكل ملحوظ في جميع الحالات مقارنةً بالضوابط الصحية الظاهرة ، في (TC) كان تردد جميع الحالات غير مرتفع بشكل ملحوظ في الالتهاب الرئوي الجرثومي وليس له تأثير في كوفيد-١٩ مقارنةً بالضوابط السليمة ظاهريًا ، في (CC) لم يظهر تواترًا منخفضًا بشكل

الخلاصة

ملحوظ في مرضى كوفيد-١٩ ولم يظهر أي تأثير لمرضى الالتهاب الرئوي الجرثومي مقارنةً بالضوابط السليمة. في rs9275431 ، أظهر (GG) عدم وجود انخفاض ملحوظ في التردد في جميع الحالات ولكنه زاد في مرضى المجموعة (أ) مقارنةً بالضوابط السليمة ظاهريًا ، في (GA) لم ينخفض تكرار جميع الحالات بشكل ملحوظ مقارنةً بالضوابط الصحية الظاهرة ، في (AA) أظهر انخفاضًا ملحوظًا في التردد في المرضى مقارنةً بالضوابط الصحية. في rs9275432 ، أظهر (GG) انخفاضًا ملحوظًا في التردد في الحالات مقارنة بعناصر التحكم الصحية الظاهرة ، في تواتر الحالات مع (GT) كان مرتفعًا بشكل ملحوظ مقارنة بالضوابط الصحية الظاهرة ، في تواتر الحالات مع (TT) كان مرتفعًا بشكل ملحوظ مقارنةً بعناصر التحكم الصحية الظاهرة ، في rs9275433 ، في النمط الجيني GG في جميع المرضى لم يظهر أي زيادة ملحوظة في التردد مقارنةً بالضوابط الصحية الظاهرية ، في تواتر الحالات مع GC انخفض بشكل ملحوظ مقارنةً بالضوابط الصحية الظاهرة ، في تواتر الحالات مع CC لم يكن مرتفعًا بشكل ملحوظ مقارنة بالضوابط الصحية الظاهرة. يتأثر التسبب في العوامل المسببة للالتهاب الرئوي بواسطة مستضد كريات الدم البيضاء البشرية C ومستضد كريات الدم البيضاء البشرية DRB1. يشارك مستضد كريات الدم البيضاء البشرية C في التسبب في عدوى فيروس كورونا المتلازمة التنفسية الحادة ويساهم في الشدة والوفاة في كوفيد-١٩ ومرضى الالتهاب الرئوي الجرثومي. لكن مستضد كريات الدم البيضاء البشرية DRB1 له تأثير وقائي ضد سارس كوفيد-٢ والعدوى البكتيرية.

Summary

Summary

Pneumonia is a type of acute lower respiratory tract infection that is common and severe, it is recognized as a leading global health problem and as a direct cause of morbidity and mortality. The current study is conducted to investigate the bacterial and viral causes of pneumonia and also investigate the impact of white blood cells, platelet count, lymphocytes, human leukocyte antigen-C, and human leukocyte antigen-DRB1.

This study included 200 blood specimens from (COVID-19 and bacterial pneumonia) patients ages (13-to 80) years were distributed according to the severity of disease as the following: (40 group A, 40 group B, 40 group C, and 40 group D) who have been hospitalized at the COVID-19 Wards in Merjan Medical City and Imam Sadiq Hospital in Babylon Province for 2 months (December 2021 and January 2022) and 40 group E. The result clarifies that the level of white blood cells was elevated significantly ($P \leq 0.05$) in patients compared to the apperently healthy control groups. While the percentage of platelet count and lymphocytes was decreased significantly among patients compared to the apperently healthy control groups.

Identification of bacteria has been done by culture, and Vitek 2 compact system ,In the present study the most common bacteria was *Streptococcus pneumoniae* and *Klebsiella pneumoniae*.

By Enzyme Linked Immunosorbent Assay technique showed that the concentration serum among acute respiratory syndrome coronavirus, group (A) having a level of 40.03 ng/ml, group (B) having a level of 47.93 ng/ml, group (C) having a level of 46.83 ng/ml, group (D) having a level of 61.15 ng/ml and group (E) having a level of 17.65 ng/ml, (P

≤0.001). While the patient's group showed lower frequencies of human leukocyte antigen-DRB1 with a coronavirus-induced acute respiratory syndrome, with group (A) having levels of 38.88 ng/L, group (B) having levels of 34.35 ng/L, group (C) having levels of 46.20 ng/L, group (D) having levels of 51.13 ng/L, and group (E) having levels of 59.95 ng/L (P ≤0.02).

The result of polymerase chain reaction (PCR-Sequence) for human leukocyte antigen-C gene, as the following in rs1050445 Wild type (CC) showed a significantly decreased frequency in all cases compared to apparently healthy control. In the Mutant heterozygous (CA) and Mutant homozygous (AA) were significantly elevated compared to apperently healthy control. According to these findings, the frequency of the C allele was significantly lower in COVID-19 and bacterial pneumonia compared to apparently healthy controls (P-Value ≤0.002). In rs1050446 genotypes SNPs were noticed statistically significant differences between the G, and T alleles were with an increase of the G allele in patients and also an increase of T alleles in COVID-19 patients but a decrease in bacterial pneumonia patients compared with apparently healthy controls. The frequency of rs1050446 SNPs GG wild type and GT Mutant heterozygous was significantly increased in patients than apparently healthy controls (p-value=0.008) (P-Value ≤0.04) respectively. The frequency of rs1050446 SNPs TT Mutant homozygous was not significantly increased in COVID-19 and decreased in bacterial pneumonia than apparently healthy controls (P-Value ≤0.3). In rs1071650, The frequency of the T allele was significantly lower in cases than in apparently healthy controls (P-Value ≤0.02). In wild type (TT) genotype showed a significantly increased frequency in cases compared

to apparently healthy controls. In the Mutant heterozygous, the frequency of cases with the genotypes TG and GG was significantly elevated compared to apparently healthy controls. In rs9264668 genotypes SNPs the frequency of the C allele was significantly higher in cases than apparently healthy controls. In the wild type (TT) in all patients and Mutant homozygous (AA) in COVID-19 patients showed a significantly decreased frequency compared to apparently healthy controls. In the Mutant heterozygous, the frequency of cases with the genotypes CA and AA in bacterial pneumonia was not significantly elevated compared to apparently healthy controls.

Regarding human leukocyte antigen-DRB1 polymorphism the result for four SNPs, as the following rs13192471 SNPs, the (TT) showed a no significantly decreased frequency in all cases compared to apperently healthy controls, in the (TC) the frequency of all cases was no significantly elevated in bacterial pneumonia and no effect in COVID-19 compared to apperently healthy controls, in the (CC) showed a no significantly decreased frequency in COVID-19 patients and no effect bacterial Pneumonia patients compared to apperently healthy controls. In the rs9275431, the (GG) showed a no significantly decreased frequency in all cases but increased in group (A) patients compared to apperently healthy controls, in the (GA)the frequency of all cases was no significantly decreased compared to apperently healthy controls, in the (AA) showed a significantly decreased frequency in patients compared to apperently healthy controls. In the rs9275432, In the (GG) showed a significantly decreased frequency in cases compared to apperently healthy controls, in the frequency of cases with the (GT) was significantly elevated compared to apperently healthy controls, in the frequency of

cases with the (TT) was significantly elevated compared to apperently healthy controls, in the rs9275433, In the GG genotype in all patients showed no a significantly increased frequency compared to apperently healthy controls, in the frequency of cases with the GC was significantly decreased compared to apperently healthy controls, in the frequency of cases with the CC was no significantly elevated compared to apperently healthy controls. Pathogenesis of pneumonia causative agents is impacted by human leukocyte antigen-C and human leukocyte antigen-DRB1. human leukocyte antigen-C is involved in the pathogenesis of acute respiratory syndrome coronavirus infection and contributes to severity and death in COVID-19 and bacterial pneumonia patients. but human leukocyte antigen-DRB1 has a protective effect against SARS-CoV-2 and bacterial infection.

Appendices

Sample number		
Patient's name:		**Age:**
Weight	**Height**	**BMI**
Chronic Disease ○ **Hypertension** ○ **Cardiovascular diseases** ○ **Diabetes** ○ **Other**		
Diagnostic method: ○ **RT-PCR** ○ **CT scan** ○ **Rapid COVID-19Ab**		
Date of admission	**Case severity** ○ **Severe** ○ **Sever with Pneumonia** ○ **Non Severe** ○ **Non severe with Pneumonia**	
Date of sample collection		

Laboratory tests

HEMALTOLOGY

WBC	
Hb	
Platelet count	
Lym	
ESR	

RANDOM BLOOD SUGAR

LIVER FUNCTIONS TESTS

GPT	
GPT	
Alkaline Phosphatase	

RENAL FUNCTIONS TESTS

S. creatinine	
Urea	
Uric acid	

CRP

○ **Positive** ○ **Negative**

LIPID PROFILE

Cholesterol	
Triglycerides	

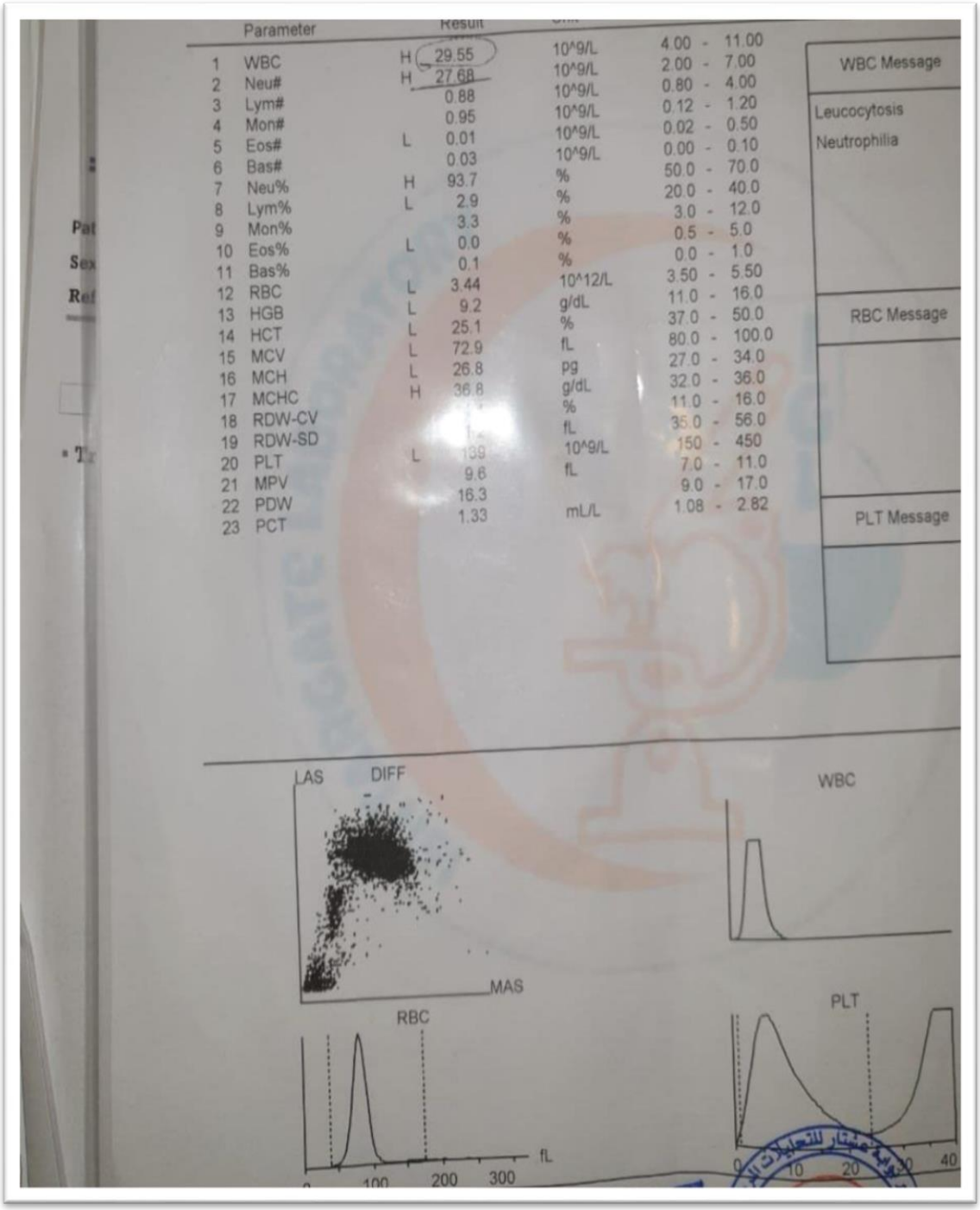
ELECTROLYTES LEVELS

Ca	
Na	
K	
Cl	

Patient's consent

Vaccination ○ **Yes** ○ **No**

Notes:



	Parameter		Result	Unit	Reference Range
1	WBC	H	29.55	10^9/L	4.00 - 11.00
2	Neu#	H	27.68	10^9/L	2.00 - 7.00
3	Lym#		0.88	10^9/L	0.80 - 4.00
4	Mon#		0.95	10^9/L	0.12 - 1.20
5	Eos#	L	0.01	10^9/L	0.02 - 0.50
6	Bas#		0.03	10^9/L	0.00 - 0.10
7	Neu%	H	93.7	%	50.0 - 70.0
8	Lym%	L	2.9	%	20.0 - 40.0
9	Mon%		3.3	%	3.0 - 12.0
10	Eos%	L	0.0	%	0.5 - 5.0
11	Bas%		0.1	%	0.0 - 1.0
12	RBC	L	3.44	10^12/L	3.50 - 5.50
13	HGB	L	9.2	g/dL	11.0 - 16.0
14	HCT	L	25.1	%	37.0 - 50.0
15	MCV	L	72.9	fL	80.0 - 100.0
16	MCH	L	26.8	pg	27.0 - 34.0
17	MCHC	H	36.8	g/dL	32.0 - 36.0
18	RDW-CV			%	11.0 - 16.0
19	RDW-SD			fL	35.0 - 56.0
20	PLT	L	139	10^9/L	150 - 450
21	MPV		9.6	fL	7.0 - 11.0
22	PDW		16.3		9.0 - 17.0
23	PCT		1.33	mL/L	1.08 - 2.82

WBC Message

Leucocytosis
Neutrophilia

RBC Message

PLT Message

Appendices

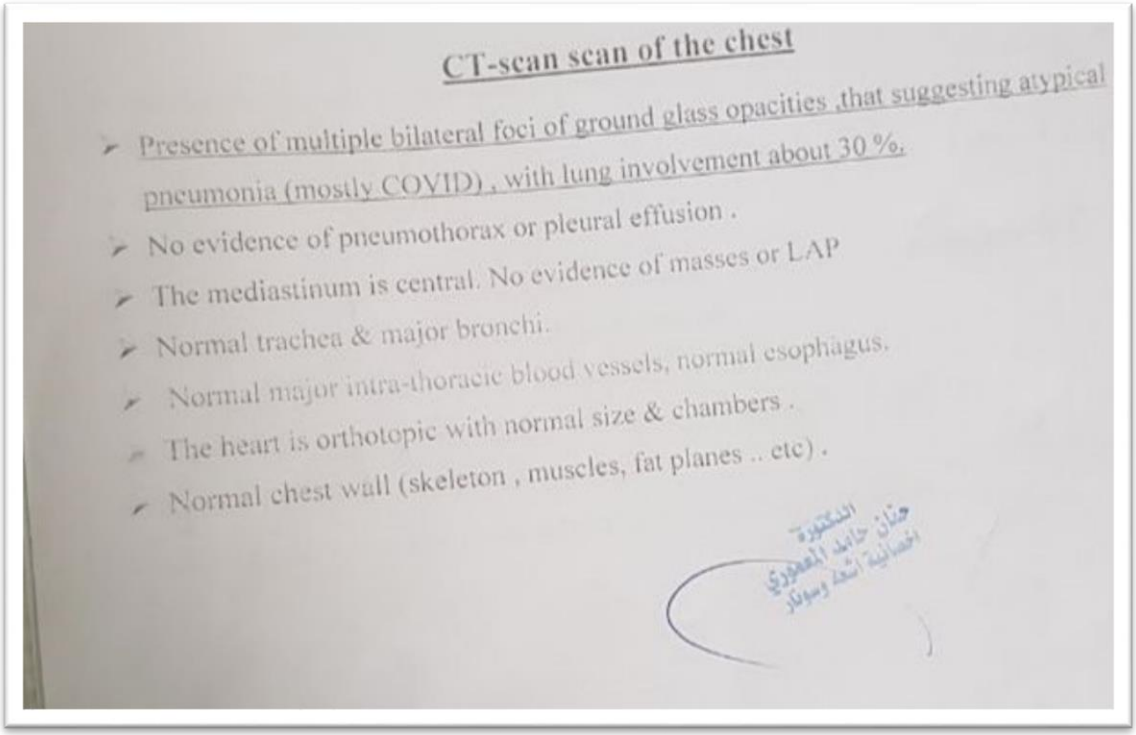
الجنس :

Seq	Test	Result	Unit	Normal Value
1	CRP	38.9	mg/L	10 -0
2	SA -Ag	20.76		Negative: <1. Positive: > 1.

34701

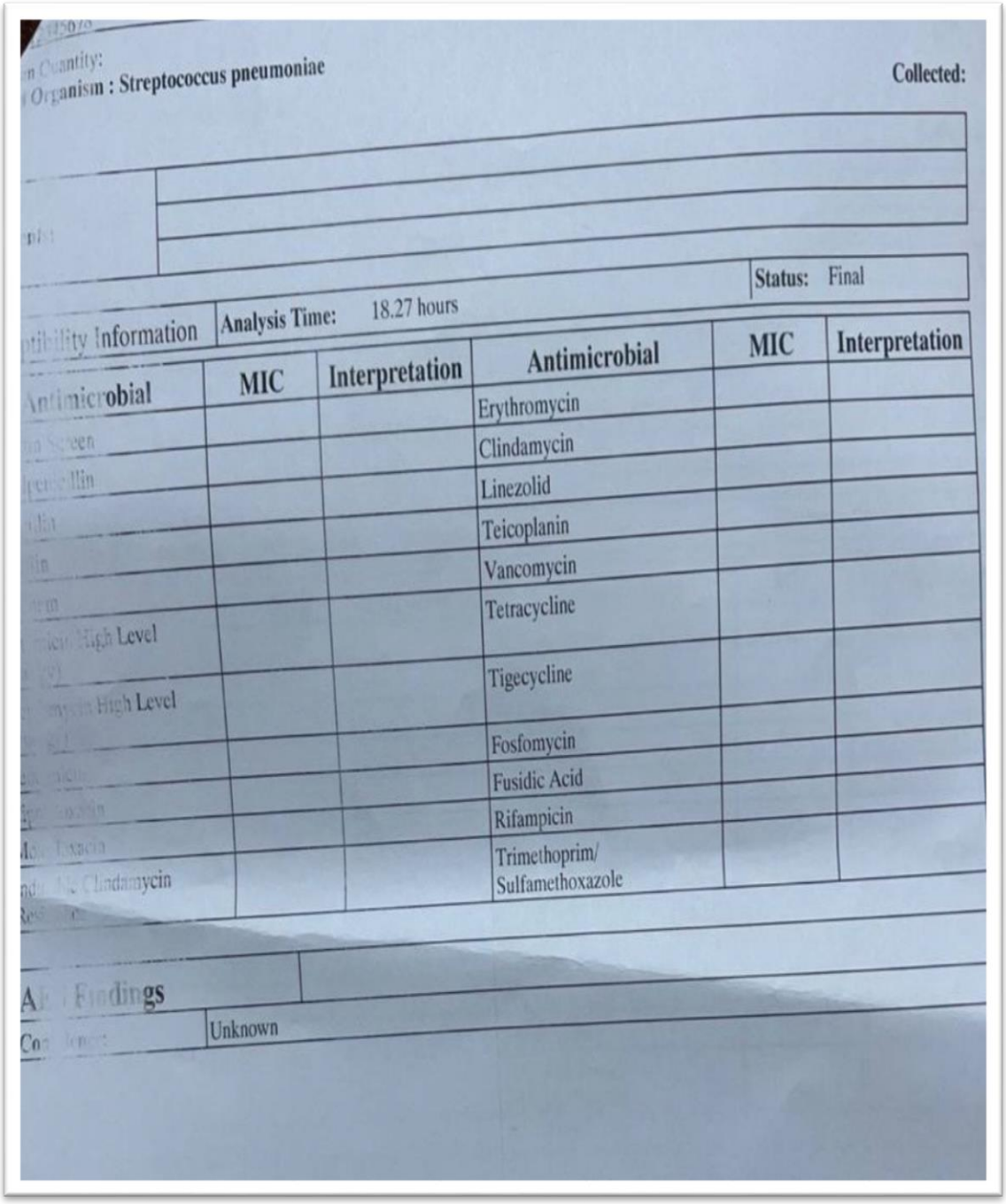
رقم المراجعة :

احمد كاظم



CT-scan scan of the chest

- Presence of multiple bilateral foci of ground glass opacities ,that suggesting atypical pneumonia (mostly COVID) , with lung involvement about 30 %.
- No evidence of pneumothorax or pleural effusion .
- The mediastinum is central. No evidence of masses or LAP
- Normal trachea & major bronchi.
- Normal major intra-thoracic blood vessels, normal esophagus.
- The heart is orthotopic with normal size & chambers .
- Normal chest wall (skeleton , muscles, fat planes .. etc) .



Quantity:
Organism : Streptococcus pneumoniae
Collected:

Status: Final

tibility Information | Analysis Time: 18.27 hours

Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation
n Screen			Erythromycin		
cillin			Clindamycin		
in			Linezolid		
			Teicoplanin		
m			Vancomycin		
High Level			Tetracycline		
			Tigecycline		
High Level					
			Fosfomycin		
			Fusidic Acid		
			Rifampicin		
Clindamycin			Trimethoprim/ Sulfamethoxazole		

Findings

Unknown

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List of Abbreviations

Abbreviation	Definition
ACE-2	Angiotensin-Converting Enzyme 2
AMPs	Anti-Microbial Peptides
ARDS	Acute Respiratory Distress Syndrome
BALT	Bronchus-Associated Lymphoid Tissue
BMI	Body Mass Index
CBC	Complete Blood Count
CD4	Cluster of Differentiation 4
CLTs	Cytotoxic CD8+ T cells

COPD	Chronic Obstructive Pulmonary Disease
COVID-19	Coronavirus Disease-19
CoVs	Coronaviruses
CRP	C-Reactive Protein
CT	Chest Computerized Tomography
CVD	Cardio Vascular Disease
DC	Dendritic Cells
DM	Diabetes Mellitus
DNA	Deoxyribonucleic Acid
EDTA	Ethylenediamine Tetraacetic Acid
ELISA	Enzyme-Linked Immunosorbent Assays
ESR	Erythrocyte Sedimentation Rate
Hb	Hemoglobin
HEV	High Endothelial Venule
HLA	Human Leukocyte Antigen
HWE	Hardy Weibery Equilibrium
HC	Healthy Controls
ICTV	International Committee on Taxonomy of Viruses
IL-6	Interleukin-6
INF-I	Type I Interferon
IQR	Interquartile Range
LDH	Lactate Dehydrogenase
MERS	Middle- East-Respiratory-Syndrome
NAD	Nicotinamide Adenine Dinucleotide
NK	Natural Killer
NLR	Neutrophil to Lymphocyte
OD	Optical Density
OR	Odds Ratio
ORFs	Open Reading Frames
P	Probability
PC	Plasma Cells
PCR	Polymerase Chain Reaction
PH	Potential of Hydrogen
PLT	Platelet Count

PP	Polyproteins
PT	Prothrombin Time
PTT	Partial Thromboplastin Time
RCU	Respiratory Care Unit
RNA	Ribonucleic Acid
ROC	Receiver Operating Characteristic
ROS	Reactive Oxygen Species
RT	Reverse Transcription
RT-PCR	Reverse-Transcriptase Polymerase Chain Reaction
S	Spike
SARS	Severe Acute Respiratory Syndrome
SARS COV-2	Severe Acute Respiratory Syndrome Coronavirus 2
SD	Standard Deviation
STAT	Signal Transducers and Activators of Transcription
SNP	Single Nucleotide Polymorphism
TBE	Tris-Borate EDTA
TLR7	Toll-Like Receptor 7
TNF	Tumor Necrosis Factor
TNF-α	Tumor Necrosis Factor-alpha
TRSs	Transcriptional Regulatory Sequences
UTR	Untranslated Region
Vit-D	Vitamin D
WBC	White Blood Cell
WHO	World Health Organization

Introduction

Infection by bacteria or viruses that interact with lung tissue to create inflammation is the most frequent cause of pneumonia. A frequent acute lower respiratory tract infection known as pneumonia is thought to be a major cause of morbidity and mortality on a global scale (Gajewska *et al*., 2020).

The outbreak of pneumonia caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), named coronavirus disease-2019 (COVID-19) that started in Wuhan- China, has become a global pandemic. SARS-CoV-2 enter host cells via the angiotensin-converting enzyme 2 (ACE2) receptor, which is expressed in various human organs after binding to spike a surface glycoprotein on the virus, this respiratory infection has spread globally to become a pandemic affecting millions of people with a mortality rate reaching 2% (Synowiec *et al*., 2021). The clinical features of COVID-19 pneumonia range from a mild illness to a very severe illness with acute hypoxemic respiratory failure requiring ventilation and Intensive Care Unit admission (Thüsen, and Eerden, 2020). Bacterial pneumonia is the major cause of morbidity and mortality globally. *S.pneumoniae* is a common leading bacterial cause of pneumonia (Katsurada *et al*.,2017).

Several genetic variables, particularly those expressed by genes of the human leukocyte antigen (HLA) system, that are linked to susceptibility or resistance to bacterial, viral, fungal, and parasitic illnesses also influence the immune response against pathogens (Crux and Elahi, 2017). The HLA genes are divided into three classes (I, II, and III), and the HLA gene products are essential for both innate and adaptive immune responses. Antigen presentation of intracellular and extracellular peptides is mediated, respectively, by the traditional class I and class II HLA molecules (Wieczorek *et al.,* 2017). In this context, individual differences in the immune response to SARS-COV-2 and bacterial infection are influenced by the HLA genes'

extraordinary variety among various cultures (Debnath *et al.*, 2020). HLA genes, the most polymorphic genetic system, may serve as susceptibility loci or perhaps may confer protection against viral infections given the crucial role that HLA molecules play in binding to viral antigenic peptides and presenting them to virus-specific cytotoxic T lymphocytes (Debnath *et al*., 2020; Nguyen *et al*.,2020). Genetic sequence changes in the exons that code for this portion of the HLA molecules lead to amino acid variations inside the peptide-binding groove, and these variations determine whether the HLA genes are positively or negatively associated with any infectious disorders (Nguyen, *et al*., 2020; Poulton, *et al.*, 2020).

An efficient presentation of peptides by HLA molecules to the cells of the immune system is essential to initiate a potent and effective response against pathogens such as SARS-CoV-2 to swiftly clear the infection. Class I HLA-C antigen presents peptides from inside the cell on the cell surface. If the cell is infected by a virus, peptides of viral origin will be presented so that the cell can be lysed by CD8+ cytotoxic lymphocytes (Nie *et al.*, 2001). Regarding the specific association of HLA alleles with COVID-19, healthy individuals carrying HLA-C may be more susceptible to being infected with SARS-CoV-2 and spreading the infection within the population (Correale *et al.*, 2020). Similar to CD8+ cells, the counts of CD4+ cells and their function in controlling the replication of SARS- CoV-2 have been indicated to be diminished (Wen *et al.*, 2020).

In addition to CD4+ and CD8+ cells, B cell populations were also observed to have lower counts in COVID-19 patients with severe disease than in healthy individuals (Zhang *et al.*, 2020). However, despite this numerical decline, the function of these cells is preserved as they continue to produce IgG and IgM antibodies against the virus and this was correlated with the recovery or persistence of the virus (Sette and Crotty, 2021). Another study

showed a link between the presence of HLA-DRB1, and severe symptoms of COVID-19 (Novelli *et al.*,2020). The alleles HLA-C, as well as HLA-DQB1were, were found to be linked with higher COVID-19 mortality (Lorente *et al.*, 2021).

Several studies have shown various changes in selected immune parameters during the pneumonia infection by comparing infected (Severe without Pneumonia, Severe with Pneumonia, Non-Severe without Pneumonia, and Non-Severe with Pneumonia) patients and healthy controls.

This study was aimed to look at the relationships between HLA-C, HLA-DRB1 polymorphism, and severity of COVID-19 patients with and without Bacterial pneumonia infections, to achieve these the following objectives:

1. Blood specimens were collected from severe COVID-19 patients and apparently healthy controls.
2. Isolation and identification of bacterial pneumonia specimens from sputum.
3. Estimation of some hematological markers WBC, Lymphocytes, and Platlete count in the study population.
4. Detection of serum levels of HLA-C and HLA-DRB1 by ELISA in the study population.
5. Human DNA extraction to study HLA-C and HLA-DRB1 polymorphism in the study population.

Literatures Review

2. Pneumonia

2.1.Definition of Pneumonia

Pneumonia is an infection of the lungs that causes inflammation of the alveolar ducts and alveolar sacs, which makes breathing difficult and reduces oxygen intake (Regunath, and Oba,2021). Its symptoms, which include coughing, fever, sweats, sputum, dyspnea, and chest pain, are known as winter fever. Although the illness can strike healthy, young individuals, it is more prevalent and dangerous in youngsters, the elderly, immunocompromised individuals, and those who have co-morbidities (Cilloniz *et al*., 2020). Along with other lower respiratory tract infections (LRTI), pneumonia is the fourth greatest cause of death globally, accounting for 2.38 million fatalities in 2016 (Abajobir *et al*., 2017).

Infectious pneumonia is the result of the acute invasion of the lung parenchyma by one or more viruses, bacteria, or fungi. It can affect one or both lungs and can either affect only a portion of the affected lung or the entire lung, causing an infection that can range in severity from a mild illness to a serious, life-threatening condition (Egelund *et al*.,2017). Various types of pneumonia can develop after using a breathing support device like a ventilator, including community-acquired pneumonia, pneumonia linked to hospital settings, and ventilator-associated pneumonia (Shi *et al*., 2019).

2.2.Historical Background of Pneumonia

The word Pneumonia came from the Greek πνεύμων (pneúmōn) meaning " lung". Symptoms of pneumonia are first described by the Greek physician Hippocrates, Peripneumonia, and pleuritic affections, are observed If the fever is acute if there be pains in one side or both and if expiration if cough is present, and the sputa expectorated (Zubieta-Calleja and Zubieta-

DeUrioste, 2021).

Pneumonia was the third most common cause of death overall and the primary infectious illness killer in the late 1800s and early 1900s. Pneumonia patient care has grown into a significant public health program. Several infectious agents were being discovered at this time, and great consideration was being given to the best ways to combat the pathogens and treat patients (Mani, 2018).

The history of pneumonia also referred to as "Winter Fever," is well documented. Scholars weren't able to recognize pneumonia as its infection and not only a symptom of other diseases until the 19th century, even though it went by numerous names and was frequently classified as a disease. The discovery of pneumonia bacteria under a microscope by German pathologist Edwin Klebs in 1875 marked a significant advance in the battle against respiratory illness. Subsequently, in the 1880s, two of the most prevalent bacterial causes of pneumonia were discovered by Carl Friedlander and Albert Frankel. By the 1930s, numerous treatments for pneumonia had been created. Even though pneumonia mortality decreased globally during this time, infection rates were still quite high. Its drop was significantly influenced by the invention of the antibiotic penicillin (ul Haque *et al*., 2022).

Most infections that cause pneumonia are bacterial or viral. Most frequently, it is brought on by the bacteria *Streptococcus pneumoniae*. Some strains of the illness have been reported to produce walking pneumonia, a mild form of pneumonia. For those who have impaired immune systems or compromised immune systems, such as the old, ill, or immunosuppressed individuals, the lung infection can be very deadly (Heathorn and Slagle,2020). Viral pneumonia is first described by Hobart Reimann in 1938. had established the practice of routinely typing the pneumococcal organism

and the distinction between viral and bacterial strains (Donati *et al*.,2013). The SARS epidemic began quietly at the turn of the 21st century. Middle East respiratory syndrome (MERS) is first reported in September 2012 in Saudi Arabia, following the isolation of MERS- CoV from a male patient who died from severe pneumonia and multiple organ failure. On December 2019 Atypical unknown pneumonia is first recorded in Wuhan City, Hubei province. Patients have shown high fever (more than 38°C), dry cough, malaise, and breathing difficulties (Schwartz and Graham 2020).

2.3.Epidemiology

Pneumonia is a common respiratory infection, affecting approximately 450 million people a year and occurring in all parts of the world. It is a primary cause of death for people of all ages, accounting for 1.4 million fatalities in 2010 (7% of all deaths worldwide that year), and 3.0 million in 2016, the 4th leading cause of death in the world (Ruuskanen *et al.*,2011; Rana *et al.*, 2021). Pneumonia can be brought on by a variety of bacteria, viruses, and fungi (Brima *et al.*, 2021).

Pneumonia can be caught in a variety of places, including hospitals, the general public, and ventilator use (Joseph *et al*., 2020). As of 2016, pneumonia, a form of lower respiratory tract infection, it was the most fatal communicable disease (Özer and Aykar 2022). Children under the age of five and people over the age of 75 have the highest rates. Compared to the industrialized world, it happens around five times more frequently in the developing world (Ruuskanen *et al.,* 2011). The highest rates of pneumonia are found in Sub-Saharan Africa and South Asia (Dwinantoaji, and Sumarni 2020). Vaccines, appropriate therapies, and healthy lifestyle choices can help prevent and manage pneumonia (Chen *et al.*, 2020).

Each year, there are around 200 million instances of viral community-acquired pneumonia, with 100 million cases affecting children and 100 million cases affecting adults. The influenza virus, respiratory syncytial virus, parainfluenza virus, adenoviruses, and coronavirus are the most common viral culprits (Jain *et al.*,2015).

SARS-CoVs, coronavirus infections that cause high fevers, dyspnea, and pneumonia, first appeared in Guangdong, south China, in 2002. They then spread quickly to many other parts of the world. In 26 nations, the illness has spread, resulting in around 8096 cases and 774 fatalities. whereas MERS-CoV was initially discovered in 2012 in Saudi Arabia. A severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-related pneumonia outbreak that originated in Wuhan, China, has spread to more than 25 other countries, causing 2494 cases of illness and 858 fatalities. More than 150 nations and territories were afflicted by the disease in March 2020 (Helmy *et al.,* 2020).

On February 24, 2020, an Iranian student from that country was reported to have the first confirmed case of COVID-19 in Iraq. On February 25, four other cases from one family in Kirkuk province were also revealed, and both families had traveled to Iran previously. On February 27 in Baghdad, another case involving a patient who had recently been to Iran is noted. As of March 12, 2020, there have been 74 confirmed cases and 8 fatalities across Iraq. On April 16, 2020, there were 1415 confirmed cases. By May 24, 2020, there were 4469 confirmed cases of COVID-19, with 160 deaths reported, while 2738 individuals had recovered from the virus (Sarhan *et al.*,2020).

2.4.Pathophysiology

The pathogenesis of pneumococcal infection is a complex interplay between pneumo- coccal virulence determinants and the host immune response. Molecular studies have considerably advanced knowledge and understanding of the precise structures and functions of the different determinants and their pathogenic roles. Aspiration of fluid, inhalation of droplets, or hematogenous dissemination are all ways that pathogens might travel to the lungs. When host defense mechanisms fail to keep the respiratory network clear of pathogenic pathogens, pneumonia develops. Several consequences appear shortly after, such as immune deficiency and cytokine storm which is marked by elevated levels of pro-inflammatory cytokines in addition to altered redox balance in infected cells due to changes in nicotinamide adenine dinucleotide (NAD) biosynthesis. As a result, excessive tissue damage occurs (Nasi *et al.*, 2020).

In viral Pneumonia, Five steps make up the "SARS-CoV-2" replication cycle: attachment, penetration, biosynthesis, maturity, and release. The main host receptor for viral entry into cells is ACE-2, which is seen to be highly expressed in adult nasal epithelial cells. The virus undergoes local replication and propagation, along with the infection of ciliated cells in the conducting airways. This stage lasts a couple of days and the immune response generated during this phase is a limited one. Despite having a low viral load at this time, the individuals are highly infectious, and the virus can be detected via nasal swab testing (Wang, X *et al*., 2020; Hoffmann *et al*., 2020).

However, there are three coronaviruses (severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory syndrome coronavirus (MERS- CoV), and (SARS-CoV-2) that can replicate in the lower respiratory

tract and cause pneumonia, which can be fatal. SARS-CoV-2 belongs to the beta coronavirus genus. Its closest relative among human coronaviruses is SARS- CoV, with 79% genetic similarity, however, among all known coronavirus sequences, SARS-CoV-2 is most similar to bat coronavirus RaTG13, with 98% similarity (Fehr and Perlman, 2015; Zhou, D *et al*., 2020) and coronavirus sequences in the pangolin (a scaly anteater) also share high similarity (Andersen *et al*., 2020). Therefore, disease severity in patients is due to not only the viral infection but also the host response. The pattern of increasing severity with age is also broadly consistent with the epidemiology of SARS- CoV and MERS- CoV (Guan *et al*., 2020; Huang, A *et al*., 2020).

Bacterial Pneumonia, The two types of pneumonia are lobar and bronchopneumonia. In lobar pneumonia, the germs have spread to affect the majority of one lung's lobe. The bacteria that cause bronchopneumonia have propagated via the microscopic air passages to affect areas of more than one lung lobe. (Garg *et al*.,2019). When bacteria enter the lungs and alveoli from the nose or mouth, bacterial pneumonia develops.

Additionally, through the blood, bacteria can move from other areas of the body to the lungs. The immune system of the body will try to eliminate the bacteria from the body once it detects them in the alveoli, where they will continue to develop in the air space. Acute inflammation results from the opening of small capillaries surrounding the alveoli, which increases blood flow to the lungs and allows inflammatory cells, such as neutrophils, to enter the lung tissue (Katherine *et al*.,2020). Pneumonia is the most common of the *S.pneumoniae* diseases which includes symptoms such as fever and chills, cough, rapid breathing, difficulty breathing, and chest pain. For the elderly, they may include confusion, low alertness, and the formerly listed symptoms

to a lesser degree (Baucells *et al.*,2015). Individuals with *Klebsiella pneumoniae* tend to cough up a characteristic sputum, as well as have a fever, nausea, tachycardia, and vomiting. *Klebsiella pneumoniae* tends to affect people with underlying conditions, such as alcoholism (Hirsch and Tam 2010).

To get a *K.pneumoniae* infection, a person must be exposed to the bacteria. In other words, *K.pneumoniae* must enter the respiratory tract to cause pneumonia, or the blood to cause a bloodstream infection. In healthcare settings, *K.pneumoniae* bacteria can be spread through person-to-person contact (for example, contaminated hands of healthcare personnel, or other people via patient-to-patient) or, less commonly, by contamination of the environment; the role of transmission directly from the environment to patients is controversial and requires further investigation. However, the bacteria are not spread through the air. Patients in healthcare settings also may be exposed to *K.pneumoniae* when they are on ventilators or have intravenous catheters or wounds. These medical tools and conditions may allow *K.pneumoniae* to enter the body and cause infection (Xu *et al.*,2021).

Although neutrophils are required to kill and remove the bacteria in the lungs, they can also damage the pneumocytes lining the alveoli (Katherine *et al*.,2020).

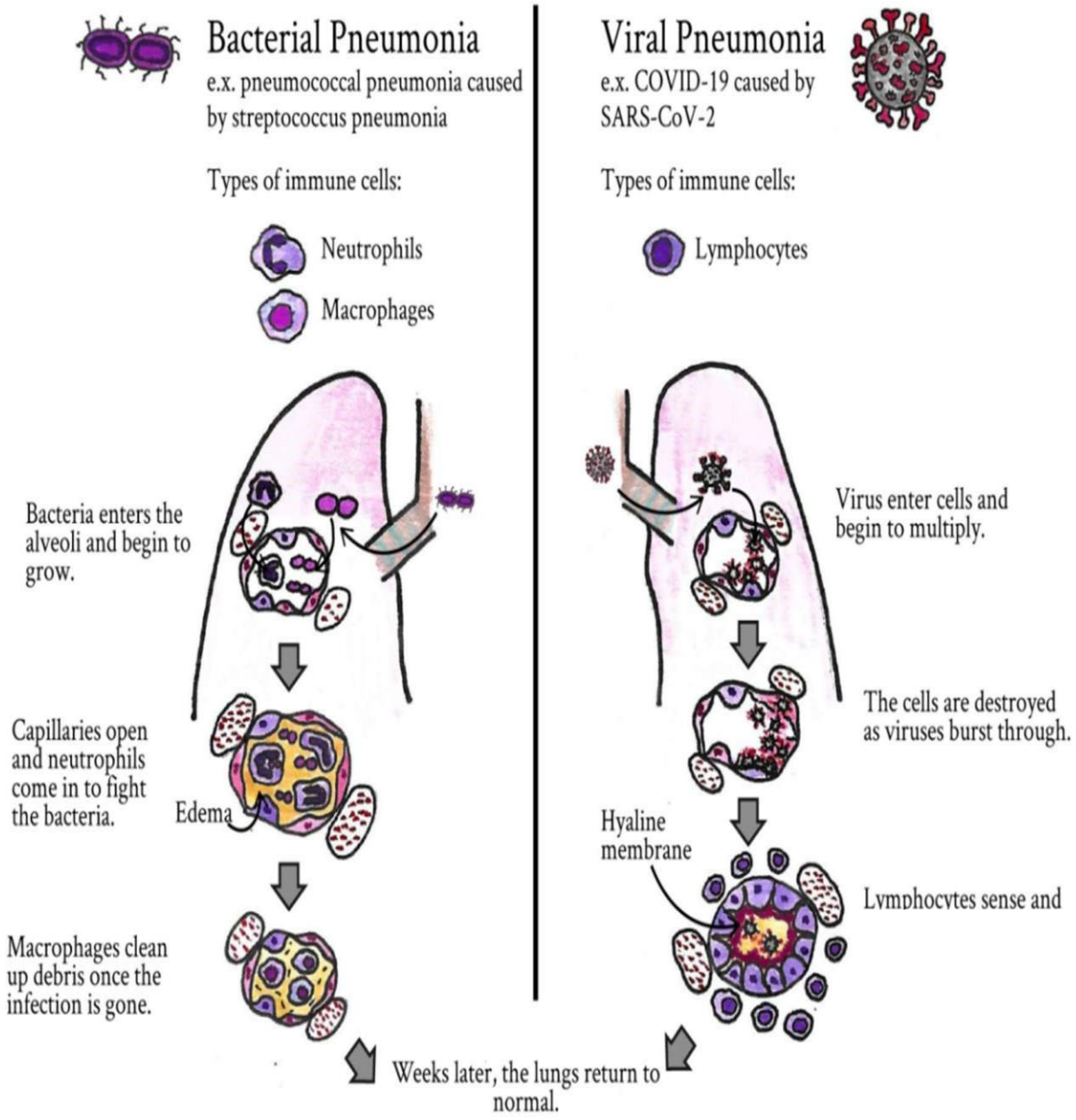


Fig (2-1): The Pathophysiology of Pneumonia in Viral or Bacterial Causes (Katherine *et al*.,2020).

2.4.1.Factors Associated with Pathogenesis of Viral and Bacterial Pneumonia

Pneumonia is an infection of the lungs that can cause mild to severe illness in people of all ages. However, some people are at increased risk of getting pneumonia. Being a certain age, having certain medical conditions, and smoking can increase a person's risk for pneumonia. The first of these factors is the patient's age, as surveys showed that ages ≥ 65 years showed higher rates of mortalities. These patients also showed low lymphocyte count or had a high ratio of neutrophil to lymphocyte (NLR) (Qin *et al.*, 2020; Wu *et al.*, 2020).

Bacterial pneumonia can affect all ages, but you are at greater risk if you abuse alcohol, smoke cigarettes, are debilitated, have a respiratory disease or viral infection, or have a weakened immune system. Other serious conditions, such as malnutrition, diabetes, heart failure, sickle cell disease, or liver or kidney disease, are additional risk factors (Martin-Loeches *et al.*,2015).

As the frequency in males was shown to be higher than in females, gender is also a significant determinant. This can be ascribed to variations in steroid hormones between men and women, which have an impact on a variety of immunity-related factors. Additionally, several immune-regulatory genes on the X chromosome, such as toll-like receptor 7 (TLR7), can reduce viral loads and inflammation in females (Conti and Younes, 2020).

Besides, patients with diabetes mellitus (DM) undergo severe complications compared to non-diabetics and this can be attributed to an imbalance in ACE2 activation pathways, resulting in an inflammatory response that can cause pancreatic beta-cell malfunction (Wang *et al.*,2020). Obesity is another risk factor, and people who have a high body mass index (BMI) have a poor prognosis because they have a chronic inflammatory condition that intensifies a

cytokine storm (Dutta *et al.*,2020). Since the patient's lung epithelium is already compromised, chronic respiratory illnesses have been recognized as risk factors from the beginning. ACE2 receptor levels are typically higher in those with chronic obstructive pulmonary disease (COPD). In the end, this facilitates the transmission of SARS CoV-2 and the invasion of the respiratory epithelium (Hasanagic and Serdarevic, 2020).

2.5.Etiology of Pneumonia

Pneumonia can be brought on by many things, including bacteria, viruses, and fungi. Children under the age of five, persons over the age of 65, and people with underlying medical conditions are the groups most at risk for pneumonia (Zhang *et al*.,2022). Respiratory infection with fever and dry cough is most prominent in COVID-19 patients. Some other distinct features like nausea, vomiting, diarrhea, and gastrointestinal infections appeared in a significant proportion of patients (Galanopoulos *et al*.,2020).

Unexpectedly, respiratory viruses were found in patients' samples more frequently than bacteria, making up 62% of the total patients. In particular, 23% of patients had one or more viruses, 11% of patients had one or more bacteria, 3% of patients had both bacterial and viral pathogens, and 1% of patients had a fungal or mycobacterial infection. *Streptococcus pneumoniae* (in 5% of patients), influenza virus (in 6% of patients), and human rhinovirus were the most prevalent pathogens (Jain *et al.*, 2015).

2.5.1.Etiology of Coronaviruses

Since it was first reported, the novel coronavirus disease has surprisingly received a great deal of attention worldwide. Early in December 2019, the WHO claimed that China (Hubei region), had reported that several individuals had been hospitalized for pneumonia with an unknown etiology. These patients were seafood market workers, but fairly shortly human-to-

human transmission has been confirmed, posing a public health concern (Chung *et al*., 2020; Haddad *et al*., 2011; Jiang *et al*., 2020).

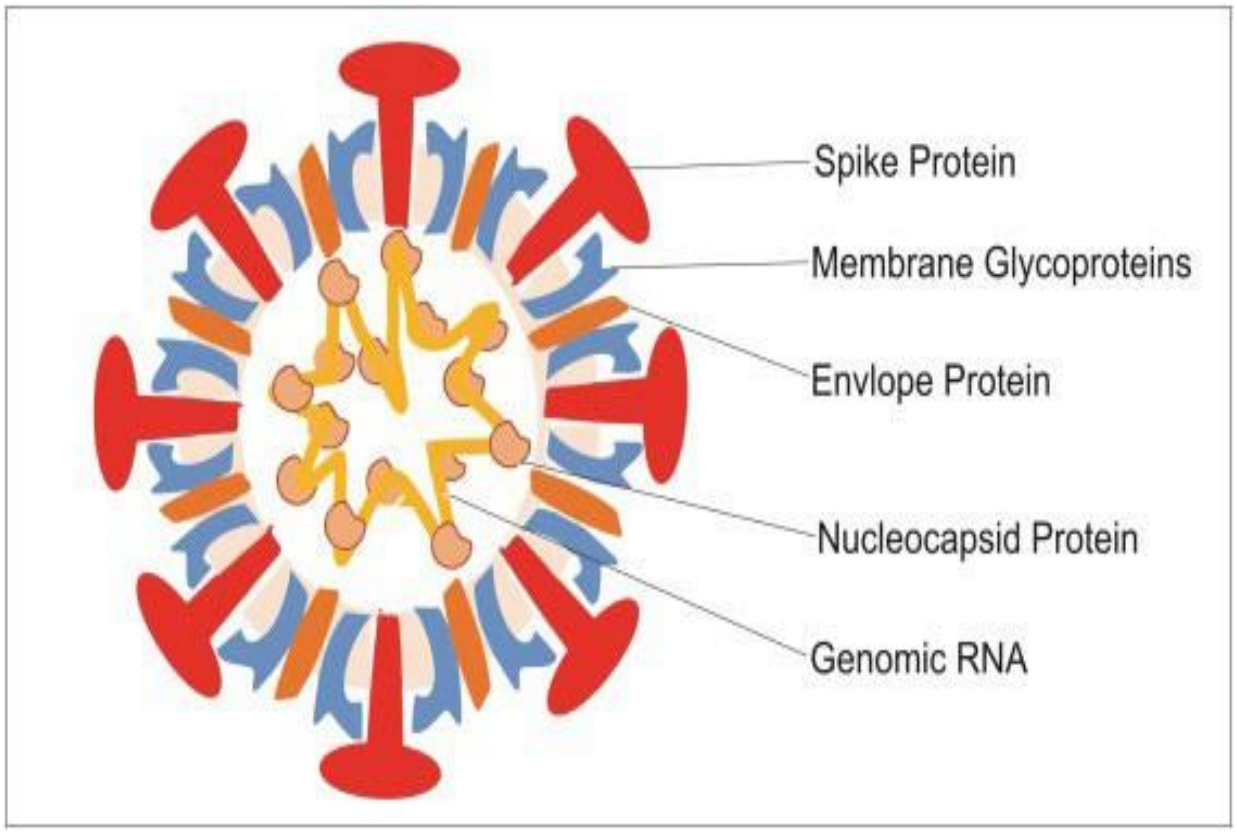


Fig (2-2): Structure of Human Acute Respiratory Syndrome Coronaviruses (Zinzula *et al*., 2021).

Soon after the patients started displaying symptoms similar to those of the SARS CoV-2 epidemic, the cause was identified as a new strain of the coronavirus family, which was given the name 2019 nCoV. On February 11, 2020, The ICTV changed the designation to "SARS-CoV2" (Din and Boppana, 2020). This is the third outbreak, following SARS in 2003 and MERS in 2012 (Chowell *et al*., 2015). In contrast to SARS and MERS, the outbreak has aggressively spread to virtually all territories, resulting in a global healthcare crisis (Peery *et al*., 2020), and is considered the seventh virus of the family that can cause severe illnesses in humans (Hedman *et al*.,

2021).

The illness quickly spread over the world, leading to a pandemic that put strains on all countries as they announced a lockdown, social distance laws due to asymptomatic carriers, a lack of targeted therapeutics, and a variety of risk factor categories (Yuki *et al*., 2020). Due to their high incidence and wide distribution, new coronaviruses occasionally appear in humans. The spread of coronaviruses, their genetic diversity, and their sporadic genome recombination, as well as an increase in human-animal interaction activities, have all been noted (Cui *et al*., 2019).

The virus's outer structure, including the newly discovered SARS-CoV-2, are spherical positive single-stranded RNA viruses that are characterized by spike proteins projecting from the virion surface. The virus has three membrane-bound proteins denoted S (spike), M(membrane), and E (envelop), as well as a fourth nucleocapsid (N) protein (Das, 2020). SARS-CoV-2 is an RNA virus with positive sense RNA with a size ranging from 26 to 31 kilobases having a single-stranded genome encased in a lipid bilayer adorned with proteins. SARS-CoV is a human virus that causes severe acute respiratory syndrome and shares 82 percent of homology with SARS-CoV-2 (SARS) (Chen *et al*.,2019).

The genome contains a 5′ cap structure along with a 3′ poly (A) tail, allowing it to act as an mRNA for translation of the replicates polyproteins. The replicas gene encoding the non-structural proteins occupies two-thirds of the genome, about 20 kb, as opposed to the structural and accessory proteins, which make up about 10 kb of the viral genome. The 5′ end of the genome contains a leader sequence and untranslated region (UTR) that contains multiple stem-loop structures required for RNA replication and transcription. Additionally, at the beginning of each structural or accessory gene are transcriptional regulatory sequences (TRSs) that are required for the

expression of each of these genes. The 3′UTR also contains RNA structures required for the replication and synthesis of viral RNA. The organization of the CoVs genome is 5′-leader-UTR-replicase S-(Spike) E- (Envelope) M-(Membrane) N-(Nucleocapsid) -3′UTR-poly (A) tail with accessory genes interspersed within the structural genes at the 3′ ends of the genome (Yuhang *et al.*, 2020).

Droplets that contaminate the air, surfaces, or hands can spread the infection. Human-to-human transmission is the route to manifest especially in pre-symptomatic and asymptomatic patients, fecal-oral transmission is also another way of transmission as studies proved fecal shedding of the virus (Fuk- Woo *et al.*, 2020; Rossi *et al.*, 2020).

The body is first exposed to virus particles, commonly through the mouth or nose. The virus enters the lungs where it infects the pneumocytes or other cells in the alveoli and airways. An interstitial inflammation results from the virus's infiltration of the alveoli, which damages the surfaces of the ciliated epithelium Congestion, hemorrhage, and intracellular viral inclusions may develop in the lungs. Some of the virus particles can be eliminated by local host defenses including mucociliary clearance or the release of certain secretory IgA antibodies. However, the infection persists if mucociliary clearance is compromised if secretory IgA antibodies are not present. Viral replication takes place after the invasion of respiratory epithelial cells. Newer viruses then infect larger numbers of epithelial cells, shut off the synthesis of critical proteins, and ultimately lead to host cell death (Lee *et al*., 2016).

Viruses use specialized proteins found on their surface to attach to other proteins on the surface of cells in the lungs. For example, SARS-CoV2 uses a Spike protein on its surface to attach to the protein angiotensin-converting enzyme 2 (ACE2) in cells in the lung. After attaches it uses the machinery of

the cell to make new copies of the virus. Pneumocytes infected by a virus can become damaged and die. The body responds to this injury by replacing the thin type 1 pneumocyte with the thicker, stronger type 2 pneumocytes. Injured pneumocytes also release signals that attract lymphocytes to the lungs.

As in bacterial pneumonia, fluid fills the air spaces which makes it difficult to breathe hyaline membranes (Brittona, 2019). These hyaline membranes are frequently observed alongside type 2 pneumocytes, fluid, and fluid-filled alveolar walls. breathing difficulties since it is more difficult for oxygen to transfer from the lungs to the blood due to the thicker alveolar walls. Because the alveoli have less air than normal in these areas, the alterations associated with viral pneumonia on an X-ray or CT scan will also appear more grey or white (Wan *et al*., 2020).

2.5.2.Etiology of Bacterial Pneumonia

Pneumonia is due to infections caused primarily by bacteria or viruses and less commonly by fungi and parasites. Although more than 100 strains of infectious agents have been identified, only a few are responsible for the majority of cases. Mixed infections with both viruses and bacteria may occur in roughly 45% of infections in children and 15% of infections in adults (Ruuskanen *et al.*, 2011).

People with infectious pneumonia often have a productive cough, fever accompanied by shaking chills, shortness of breath, sharp or stabbing chest pain during deep breaths, and an increased rate of breathing. In elderly people, confusion may be the most prominent sign (Zhang, R *et al*., 2020). The typical signs and symptoms in children under five are fever, cough, and fast or difficult breathing. Fever is not very specific, as it occurs in many other common illnesses and may be absent in those with severe disease,

malnutrition, or in the elderly.

In addition, a cough is frequently absent in children less than 2 months old. More severe signs and symptoms in children may include blue-tinged skin, unwillingness to drink, convulsions, ongoing vomiting, extremes of temperature, or a decreased level of consciousness (Rambaud-Althaus *et al.*, 2015). The green or yellow sputum produced when a person with pneumonia coughs is made up of millions of neutrophils and debris from damaged tissue and dead bacteria. It is a sign that active inflammation is taking place in the lungs.

Types of bacteria that commonly cause pneumonia in this study include:

2.5.2.1.Streptococcus pneumoniae

Streptococcus pneumoniae, or pneumococcus, is a gram-positive α-hemolytic, (under aerobic conditions) or beta-hemolytic (under anaerobic conditions), aerotolerant anaerobic member of the genus Streptococcus. They are usually found in pairs (diplococci) and do not form spores and are non-motile. As a significant human pathogenic bacterium, *S.pneumoniae* was recognized as a major cause of pneumonia in the late 19th century and is the subject of many humoral immunity studies (Mahato *et al.*, 2019).

Pneumococcus is a highly invasive, bacterium, responsible for high rates of morbidity and mortality worldwide, pneumococcal diseases range from mild respiratory tract mucosal infections such as otitis media and sinusitis to more severe diseases such as pneumonia, septicemia, and meningitis (Oligbu *et al*.,2019).

The nasopharynx is where *S.pneumoniae* colonizes, but over time it can transfer to the lungs where it causes pneumonia. Pneumococcal pneumonia is a global health concern and vastly affects children under the age of five as well as the elderly and individuals with pre-existing health conditions,

Pneumonia accounts for 15% of all deaths of children <5 years of age and is the single largest infectious cause of death in children worldwide (Mathur *et al*., 2018). WHO reported that pneumonia accounts for 16% of all deaths of children under 5 years old, killing 920,136 children in 2015, with the most common cause of bacterial pneumonia being *S.pneumoniae* (Brooks and Mias, 2018).

The genome of *S.pneumoniae* is a closed, circular DNA structure that contains between 2.0 and 2.1 million base pairs depending on the strain. It has a core set of 1553 genes, plus 154 genes in its virulome, which contribute to virulence, and 176 genes that maintain a noninvasive phenotype. Genetic information can vary up to 10% between strains. The pneumococcal genome is known to contain a large and diverse repertoire of antimicrobial peptides, including 11 different antibiotics (Rezaei Javan *et al*., 2018).

Natural bacterial transformation involves the transfer of DNA from one bacterium to another through the surrounding medium. Transformation is a complex developmental process requiring energy and is dependent on the expression of numerous genes. In *S.pneumoniae*, at least 23 genes are required for transformation. For a bacterium to bind, take up, and recombine exogenous DNA into its chromosome, it must enter a special physiological state called competence (Engelmoer *et al*.,2011).

2.5.2.2. *Klebsiella Pneumoniae*

Klebsiella pneumoniae (*K.pneumoniae*) is an opportunistic, Gram-negative, facultatively anaerobic, rod-shaped 0.3-1 μm in diameter and 0.6- 6 μm in length, singly arranged, in pairs, or short chains. *K. pneumoniae* is lactose-fermenting, non-motile, non-spore-forming, oxidase negative, with a prominent polysaccharide capsule of extensive thickness which gives a

mucoid appearance for colonies on agar plates. *K.pneumoniae* grows rapidly on ordinary media and their colonies look large pink, round, and mucoid on MacConkey agar indicating fermentation of lactose and acid production (Amraei *et al.,* 2022).

Gram-negative bacteria related to the Enterobacteriaceae family cause a wide range of diseases including pneumonia, UTIs, bloodstream infections, and sepsis, all these infections are characterized by their morbidity and mortality (Bengoechea *et al*.,2019). *K.pneumoniae* isolates gaining attention due to the rise in the number of infections and the increasing number of strains resistant to antibiotics. Pathogenic features of Klebsiella-induced pneumonia include cell death associated with bacterial replication, avoidance of phagocytosis by phagocytes as a result of heavily encapsulated strains, and the attenuation of host defense responses, chiefly the production of antimicrobial factors, also utilizes a variety of virulence factors, especially capsule polysaccharide, lipopolysaccharide, fimbriae, which act as adhesins to epithelial cells, outer membrane proteins, and determinants for iron acquisition and nitrogen source utilization, for survival and immune evasion during infection (Ming *et al*., 2021).

Klebsiella pneumoniae is an important cause of community-acquired pneumonia in individuals with impaired pulmonary defenses and is a major pathogen for nosocomial pneumonia the clinical manifestations of pneumonia caused by *K.pneumoniae* are cough, fever, pleuritic chest pain, and shortness of breath with necrotic destruction of alveolar spaces, the formation of cavities, and production of blood-tinged sputum, Currant jelly. Finally, Pneumonia caused by *K.pneumoniae* can be complicated by bacteremia, lung abscesses, and the formation of an empyema (Efah *et al*.,2020).

2.6. Immunopathogenesis

It has been illustrated that the immune system is a major player in the

pathogenesis of COVID-19 because the causative virus (SARS-CoV-2) can induce dysregulated innate and adaptive immune responses that are ultimately associated with widespread damage to tissues and organs (Thierry and Roch, 2020).

2.6.1.Innate Immune Response

Lungs are the vital organs designed not only for the gaseous exchange but also serve as a major immune organ to protect the host from diseases caused by the pathogen inhalation during respiration along with allergens and xenobiotics (allergic asthma, pneumonia, sepsis-associated) (Qian *et al*.,2013). The innate immune system serves as the first line of defense against foreign pathogens by recognizing their pathogen-associated molecular patterns (PAMPs) or microbe-associated molecular patterns (MAMPs). Also, innate immune cells recognize the damage or danger-associated molecular patterns (DAMPs) generated during the pro-inflammatory conditions disturbing immune homeostasis (Schaefer, 2014).

Pneumonia is a serious life-threatening infection among the children and older population. Pneumonia pathogenesis is a very complex process involving microbial invasion of the lower respiratory tract through community or hospital spread. It may occur through inhalation of the causal pathogen. For example, *S.pneumoniae* is the most common pathogen respons ible for community-acquired pneumonia (CAP). In addition to the *S.pneumoniae*, *Legionella pneumophila*, *Mycoplasma pneumoniae*, *Chlamydophila pneumoniae*, *Chlamydophila psittaci*, and *Coxiella burnetii* are several other common pathogens responsible for CAP (Arnold *et al*.,2016; Cillóniz *et al*.,2016).

Most hospital-acquired pneumonia is caused by Gram-negative pathogens (*Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, etc.). The pulmonary innate immune response during pneumonia initiates with the activation of residential innate immune cells (AECs, AMs, etc.) inducing neutrophil infiltration into the lungs. Toll-like receptor 4 (TLR4) activation-induced immune response protects the experimental animals infected with Gram-positive (*Streptococcus pneumoniae*) or Gram-negative bacteria (*Klebsiella pneumoniae*) induced pneumonia (Kumar, 2020).

Three major cell types line the airway: the ciliated cell, the mucous-secreting goblet cell, and the secretory Clara cell. In addition, in the upper airways, there are submucosal glands that contribute to airway secretions. It remains controversial at present if the lung below the glottis is sterile or if there is a lung microbiome. Many recent studies have focused on the ability of respiratory epithelium to respond to pathogens through PRRs such as Toll-like receptors (TLRs). Most TLRs (TLR1–6, 9) are found on the respiratory epithelium, and the function of TLRs in response to several pathogens resulting in lower respiratory infection has been well characterized.

A compartment of lymphocytes residing in the respiratory tract epithelium over the epithelial membrane and between the epithelial cells known as bronchus-associated lymphoid tissue (BALT) that is comprised of B cells a major immune cell population to generate IgA, T cells, and DCs. Balts also have high endothelial venule (HEV), which serves to transport lymphocytes and antigens to and from circulation. The IgA produced may bind to the lymphocytes to increase their Ab-dependent cytotoxic action. The secreted IgA also protects against viral and bacterial infections along with the allergy. These pulmonary innate immune cells serve as antigen-presenting cells (APCs) and secrete several cytokines and chemokines to regulate both the

pulmonary innate and adaptive immunity. The pulmonary microbiota helps in the pulmonary immune system development, tolerance induction, and homeostasis. The pulmonary innate immune response during pneumonia initiates with the activation of residential innate immune cells (AECs, AMs, etc.) inducing neutrophil infiltration into the lungs (Invernizzi, 2020).

Antimicrobial peptides (AMPs) are further important innate immune factors suggested to have a role in the resistance to SARS-CoV-2 infection (Ghosh and Weinberg, 2021). They are low molecular weight proteins with a broad range of antimicrobial actions against bacteria, viruses, and fungi (Dijksteel *et al.*, 2021). The AMPs are positively charged with a hydrophobic and a hydrophilic side, allowing them to be soluble in aqueous conditions, and can also enter lipid-rich membranes (Annunziato and Costantino, 2020). These peptides can kill target cells through a variety of processes once they enter a target microbial membrane. The major families of AMPs in mammals are cathelicidins and defensins (Bahar and Ren, 2013).

2.6.2.Adaptive Immune Response

Adaptive immunity plays a critical role in pulmonary immunity to many pathogens and is the increasing focus of vaccine-induced immunity. In the case of adaptive immunity, it has been shown that both types of T-lymphocytes (CD4+ and CD8+) are markedly involved in COVID-19 pathogenesis (Huang *et al.*, 2020).

The available evidence indicates that both cell populations show declined counts and impaired functions during the progression of SARS-CoV-2 infection, particularly in severe cases (Jacques and Apedaile, 2020). Further, it has been reported that virus-specific memory CD8+ T cells were effective

in protecting the patients from the lethality of disease due to their role in controlling viral replication (Schulien *et al.*, 2021).

Besides these cellular defects in immunity against SARS-CoV-2, the immunopathogenesis of COVID-19 is probably mediated by pro-inflammatory cytokines (Khosroshahi *et al.*, 2021). It has been depicted that the acute respiratory distress in COVID-19 and the development of severe illness were related to up-regulated levels of pro-inflammatory cytokines, and accordingly, this condition was termed cytokine release syndrome or cytokine storm (Pum *et al.*, 2021). The most important cytokines in this context are IL-1β, IL-6, IL-18, TNF-α, and IFN-γ, as shown in Figure (2-3) (Olbei *et al.*, 2021). However, IL-6 is the most encountered cytokine, and it has been found that IL-6 is a key cytokine linked with a higher risk of severity and death in COVID-19 patients (Santa Cruz *et al.*, 2021). It is produced by dendritic cells and macrophages in the course of recognizing the pathogens via toll-like receptors at infection sites (Ascierto *et al.*, 2021).

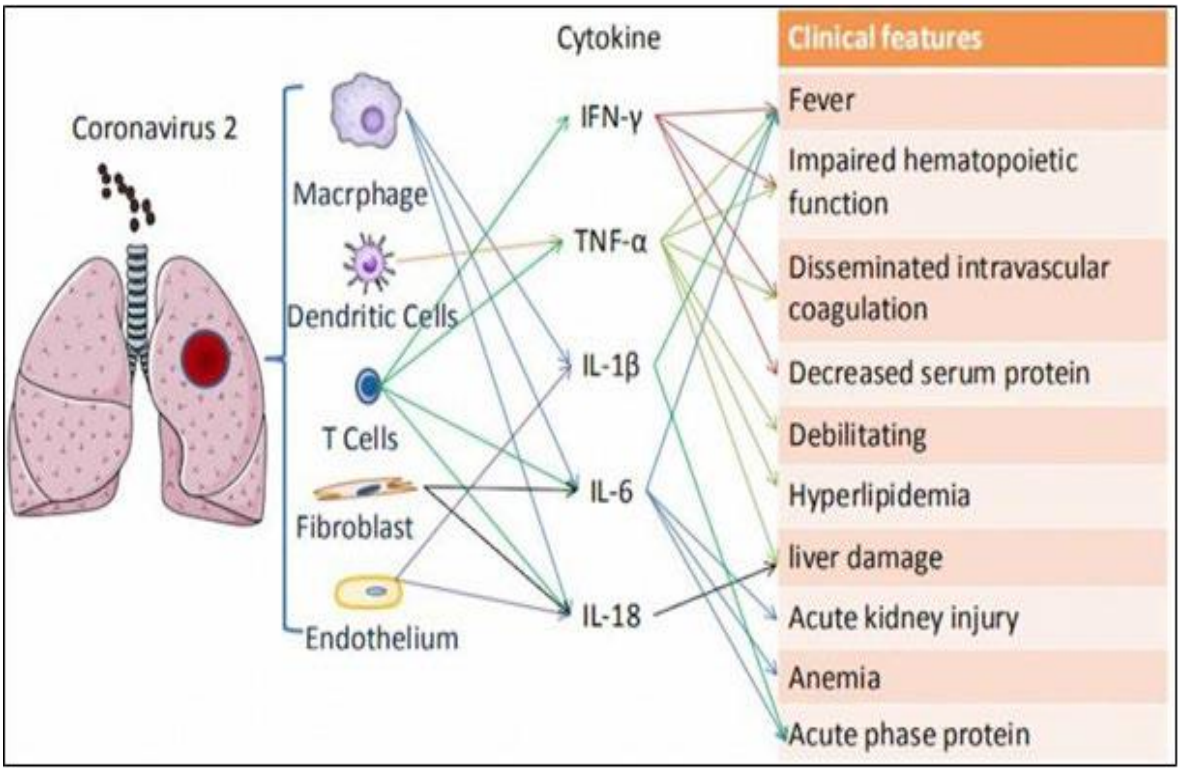


Fig (2-3): Cytokines involved in the pathogenesis of SARS-CoV-2 infection and their cellular source and associated clinical feature (Olbei *et al.*, 2021).

2.7.Human Leukocyte Antigen (HLA) system

The human leukocyte antigen (HLA) gene complex is a region of chromosome 6 genes that produce the major histocompatibility complexes (MHC) protein that contains (Kulski *et al*.,2019). The immune response against pathogens is also controlled by some genetic factors associated with susceptibility or resistance to viral, bacterial, fungal, and parasitic infections, in particular, those encoded by genes of the human leukocyte antigen (HLA) system, which are mapped to the short arm of human chromosome 6 (Crux and Elahi, 2017). The HLA genes are organized into three classes (I, II, and III) and a more detailed region has been addressed, as in Figure (2-4) The classical class I (A, B, and C) and class II (DR, DQ, and DP) HLA molecules are involved in mediating antigen presentation of intracellular and extracellular peptides, respectively, while HLA-class III are serum proteins (Wieczorek *et*

al., 2017). Non-classical class I HLA molecules (E, F, and G) are other important products encoded by genes in the HLA region, and constitute the core molecules involved in controlling the immune response to infectious agents, as well as inflammatory reactions (Helenius *et al*., 2015).

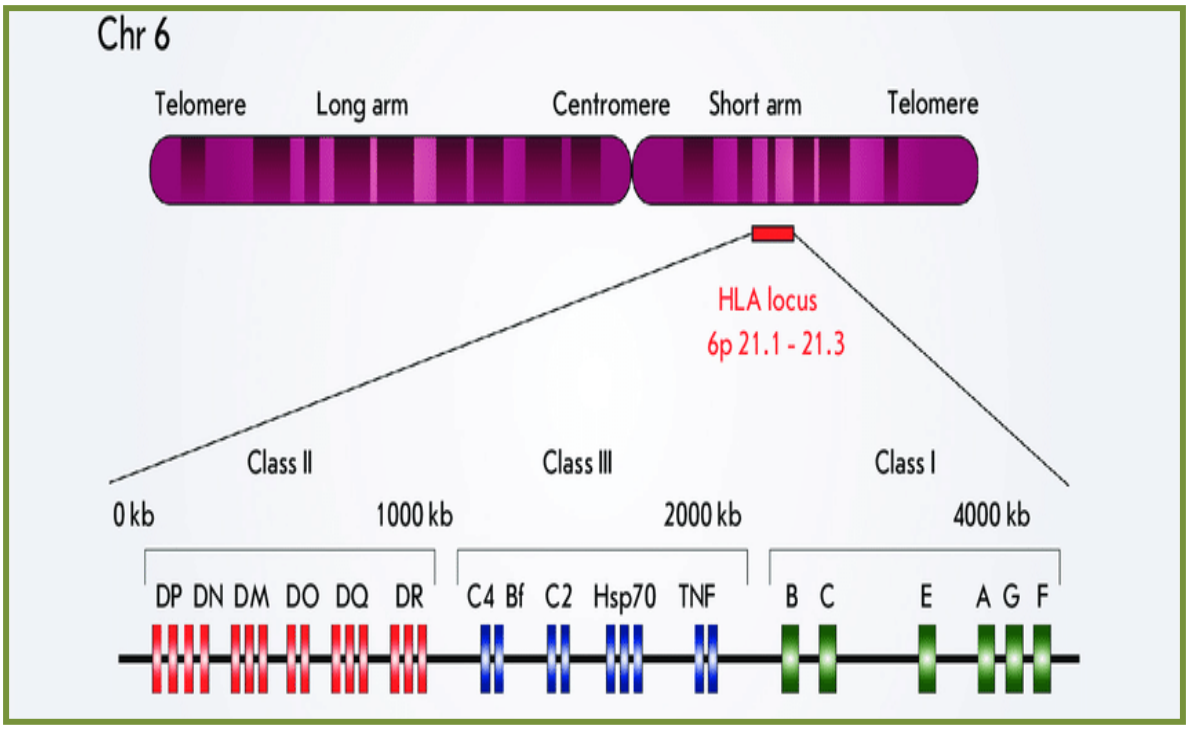


Fig.(2-4): Gene map of the human leukocyte antigen (HLA) region (Zakharova *et al*.,2019).

The antigen-presenting cells (APCs) engulf the pathogen through a process called phagocytosis. Proteins from the pathogen are digested into small pieces (peptides) and loaded onto human leukocyte antigens (specifically, MHC II), and then presented by antigen-presenting cells to helper T cells, (Taylor *et al*., 2011). In the process, proteins (both local and foreign) produced within most cells are presented to human leukocyte antigens (specifically, MHC class I) at the cell surface. Infected cells can be recognized and destroyed by T cells (Agarwal *et al*., 2017). Any cell expressing another type of HLA is non-self and is viewed by the body's immune system as an invader (Jokiniemi *et al*.,

2020).

2.7.1.Structure and Expression of HLA Molecules

2.7.1.1.HLA-C molecules

The HLA-C gene is one of the HLA-class II molecules that belongs to the MHC (human = HLA) class I heavy chain receptors. The HLA-C receptor is a heterodimer consisting of an HLA-C mature gene product and β2-microglobulin. The mature C chain is anchored in the membrane. MHC Class I molecules, like HLA-C, are expressed in nearly all cells, and present small peptides to the immune system which surveys for non-self peptides. HLA-C is a locus on chromosome 6, which encodes for many HLA-C alleles that are Class-I MHC receptors. HLA-C, localized proximal to the HLA-B locus, is located on the distal end of the HLA region. Most HLA-C: B haplotypes are in strong linkage disequilibrium and many are as ancient as the human species itself (Siegel *et al*.,2019).

HLA class I genes are located on the short arm of chromosome 6. The transcription of HLA-C is regulated by core promoter elements but also by distal regulators. The core promoter consists of the EnhancerA, ISRE, and an SXY box. Compared to HLA-A and -B, the EnhancerA of HLA-C has no functional binding site for NFκB. ISRE activation is mediated through IFNγ stimulation which recruits the transcription factor IRF. The SXY box is composed of the W/S, X1, X2, and Y and is important for the binding of NLRC5 and the formation of the enhanceosome. Transcription factors for W/S are still unknown, but X1 has binding sites for RFX and ETS, X2 has binding sites for CREB and ATF1, and Y for NFY. Moreover, the non-coding region of HLA-C contains an OCT1 binding site ~800 bp upstream of the core promoter region. HLA-C has 8 exons. Exon 1 encodes the signal peptide. Exons 2 and 3 encode the α1 and α2 domains which build the peptide binding

grove. The α3 domain (exon 4) is connected to the transmembrane domain (TM) and cytoplasmic tail (CYT) (exon 5-7), anchoring the molecule to the cell membrane (Vollmers *et al.,* 2021). Exon 1 encodes the signal peptide. Exons 2 and 3 encode the α1 and α2 domains which build the peptide binding grove. The α3 domain (exon 4) is connected to the transmembrane domain (TM) and cytoplasmic tail (CYT) (exon 5-7), anchoring the molecule to the cell membrane (Vollmers *et al*., 2021).

2.7.1.2.HLA-DRB1 molecules

HLA-DR is a class II human leukocyte antigen (HLA) expressed on the cell surface of antigen-presenting cells, including monocytes, differentiated macrophages, and dendritic cells, as well as B cells. Since the first description of the role of HLA-DR in immunosuppression, HLA-DR expression on monocytes has been subsequently proven to be a reliable marker for evaluating immune dysfunction and risk of secondary bacterial infections in sepsis and trauma patients (Zhuang *et al.,*2020). Thus, reduced amounts of HLA-DRB1 can also place COVID-19 patients at high risk of secondary and severe bacterial nosocomial infections. This observation is consistent with a clinical report of secondary bacterial infections and end-organ injury among COVID-19 patients requiring ICU care (Zhou *et al.,* 2020). HLA class II histocompatibility antigen, DRB1 beta chain is a protein that in humans is encoded by the *HLA-DRB1* gene. DRB1 encodes the most prevalent beta subunit of HLA-DR. HLA class II is expressed on the surface of antigen-presenting cells (including macrophages, B cells, and dendritic cells) and is essential to display peptides to T-helper CD4+ cells, inducing their activation. HLA class II antigens are encoded by DR, DQ, and DP (classical) and DM, DO (nonclassical) loci. Individual amino acid position variance in HLA class II molecules, especially within HLA-DR molecules forming antigen-binding

grooves, within the HLA-DRB1 subregion there is an outstandingly polymorphic HLA-DRB1 gene (Wysocki *et al.*,2020).

2.7.2.Function of HLA-Classes

Within the host factors that may influence the susceptibility to develop the most severe forms of SARS-CoV-2, the human leukocyte antigen (HLA) complex has been appointed as a potential candidate. HLA classes have different functions that encode glycoproteins that regulate the immune response by presenting exogenous and endogenous peptides to T lymphocytes and Natural Killer (NK) cells (Migliorini *et al*., 2021).

The primary function of Class II (HLA-DR) is to present peptide antigens, potentially foreign in origin, to the immune system to elicit or suppress T-(helper)-cell responses that eventually lead to the production of antibodies against the same peptide antigen. Antigen-presenting cells (macrophages, B-cells, and dendritic cells) are the cells in which DR is typically found. Increased abundance of DR 'antigen' on the cell surface is often in response to stimulation, and, therefore, DR is also a marker for immune stimulation (Benlyamani *et al*., 2020).

The first reported function of the HLA-DRB1 gene provides instructions for making a protein that plays a critical role in the immune system. The HLA-DRB1 gene is part of a family of genes called the human leukocyte antigen (HLA) complex. The HLA complex is the human version of the major histocompatibility complex (MHC), a gene family that occurs in many species.

The HLA-DRB1 gene belongs to a group of MHC genes called MHC class II. MHC class II genes provide instructions for making proteins that are present on the surface of certain immune system cells. These proteins attach to protein fragments (peptides) outside the cell. MHC class II proteins display these peptides to the immune system. If the immune system recognizes the peptides as foreign (such as viral or bacterial peptides), it triggers a response to attack the invading viruses or bacteria. The protein produced from the HLA-DRB1 gene, called the beta chain, attaches (binds) to another protein called the alpha chain, which is produced from the HLA-DRA gene (Novelli *et al.*, 2020).

Together, they form a functional protein complex called the HLA-DR antigen-binding heterodimer. This complex displays foreign peptides to the immune system to trigger the body's immune response. Each MHC class II gene has many possible variations, allowing the immune system to react to a wide range of foreign invaders. Researchers have identified hundreds of different versions (alleles) of the HLA-DRB1 gene, each of which is given a particular number (such as HLA- DRB1*04:01) (Degenhard *et al*., 2021).

2.8.Genetic polymorphism of HLA genes

It is well known that host genetic polymorphisms play a key role in the susceptibility or resistance to different viral infections (Debnath *et al.,* 2020; Ramos-Lopez *et al.,* 2020). Given the primary function of host genes in SARS-CoV-2 entrance, replication in cells, and mounting of the immune response, it appears that a combination of several genes may be implicated in COVID-19 pathogenesis (Debnath *et al.*,2020).

Accordingly, to date, numerous studies have been conducted on the association between genetic polymorphisms and COVID-19 (Anastassopoulou *et al.*,2020; Öztürk *et al.,* 2020). In some studies, COVID-19 development and/or severity may be correlated with polymorphisms in genes involved in the innate and

adaptive immune response (toll-like receptors (TLRs), human leukocyte antigen (HLA) class I and II, and cytokines/chemokines), as well as genes involved in viral binding and entry into host cells (angiotensin converting enzyme-2 (ACE2) and transmembrane serine protease-TMPR (Grolmusz *et al*., 2021; SeyedAlinaghi *et al*., 2021).

However, it is still unclear which and to what degree specific polymorphisms contribute to the susceptibility to this disease (Anastassopoulou *et al.*,2020).

The human Leukocyte Antigen (HLA), located in the short arm of human chromosome 6, represents one of the most highly polymorphic systems in the human genome and plays a central role in the regulation of immune response. The HLA system includes near to 27,000 alleles in three distinct classes of genes (Class I, II, and III). Of the three classes of genes, HLA class I-A, B, C, and class II-DR, DP, and DQ play a crucial role in various immunological functions in humans including antigen presentation to T lymphocytes and recognition of self and non-self proteins. HLA class I and II gene polymorphisms provide the strongest and most consistent alleles for autoimmune disease susceptibility (Zakharova *et al.*,2019; Muñiz-Castrillo *et al.*, 2020).

Since the beginning of the COVID-19 worldwide pandemia, the amino acid sequences of SARS-CoV-2 and HLA antigen interactions have been studied through in silico prediction models of spatial interactions between them (Nguyen *et al.*, 2020; Barquera *et al.*, 2020). While this allows the prediction of the host's immune response to viral antigens. For example, HLA-C positively correlated with the expansion of COVID-19 (Correale *et al.*, 2020).

3. Materials and Method

3.1.Materials

3.1.1.Laboratory Equipment and Instruments

The laboratory types of equipment and instruments used in the present study were listed in Table (3-1).

Table (3-1): Laboratory Equipment and Instruments

Instruments and Equipment	Origin	Company
Autoclave	Hirayama	(Japan)
Centrifuge	Germany	Eppendorf
Complete blood count	China	
Distillation	GFL	(Germany)
Electric sensitive balance	Denver	(USA)
ELISA reader, ELISA washer	BioTeck	(USA)
Gel documentation system	Vilber	(France)
Gel electrophoresis system	Cleaver Scientific	(UK)
Incubator	Germany	Memmert
Microcentrifuge	Germany	BECKMAN COULTER
Micropipette 100-1000 µL	Japan	DRAGON LAB

Micropipette 20-200 µL	Japan	GHADIR. LAB
Nanodrop 2000c	Fisher Scientific	(Canada)
PCR Thermal cycler	Techne	(UK)
Refrigerator	Concord	(Lebanon)
Vitek-2 system	France	Biomerieux
Vortex	Holland	LABINCO

3.1.2.Kits, Biological and Chemical Materials

The kits, biological and chemical materials used in this study are listed in Table (3-2).

Table (3-2): Kits, biological and chemical materials

Type of Kit	Origin	Company
Agarose, TBE buffer	Condalab	Spain
DNA extraction Kit	Favorgen	Taiwan
DNA ladder	Bioner	Korea
Green master mix	Bioner	Korea
HLA-C ELISA Kit	Biotech	China
HLA-DRB1 ELISA Kit	Biotech	China
Nuclease Free water	Bioner	Korea
Primers	Macrogen	Korea
Simply Safe	Eurx	Poland

3.1.3.Primer Pairs

The Primer pair (Macrogen/Korea) Table (3-3)

Table (3-3): Primer Sequencing

Primer	Sequences 5 to 3	product
HLA- C Forward	**TCTCAGACGGGGAGACTCTG**	**624bp**
HLA- C Reverse	**CGCAGTCCCGGTTCTAAAGT**	
HLA-DRB1 Forward	**TCTCCCTCCTAATGGTAACTGAG**	**409bp**
HLA-DRB1 Reverse	**GAGTTTCCCCTGAGAGTGCT**	

3.1.4.Culture Media

All culture media are prepared according to the manufacturer's instructions and sterilized by autoclave at 121°C of 15 lb/inch2 for 15min after adjusted pH for 7.2.

Table (3-4): Culture Media

Medium	Company	Origin
Blood agar	NEOGEN	UK
Mac-Conkey agar	NEOGEN	UK

3.2.The Study Subjects

3.2.1.Patients and Control Groups

This case-controls study includes 200 blood specimens from acute respiratory syndrome coronavirus patients aged (13 to 80) years distributed according to the severity of disease as the following: (40) severe without pneumonia and 40 Severe with pneumonia, who needed ventilators and lying in the respiratory care unit (RCU) with severe respiratory distress, respiratory rate ≥30 breaths/minute and pulse oxygen saturátion (SpO2) ≤ 93% on resting state, the case wás considered to be in severe illness with PCR positive, 40 non-severe without pneumonia and 40 non-severe with pneumonia with positive PCR result those who did not need respirators with an oxygen rate higher than 93% (SpO2) ≥93% on resting state) who have been hospitalized at the acute respiratory syndrome coronavirus Wards in Merjan Medical City and Al-Imam Al-Sadiq Hospital in Babylon Province during 2 months (December 2021 and January 2022) and 40 healthy controls.

All patients were diagnosed based on previous clinical reports, clinical examinations, and PCR tests, and the result of the plate late, WBC, and lymphocyte count was taken from the CBC medical reports. These cases were compared with each other, all of them were asked to fill out a questionnaire and all had no family history of any disease. All patients suffering from acute respiratory syndrome coronavirus were included and excluded from other types of respiratory diseases.

3.2.2.Collection of Specimens

3.2.2.1.Blood Specimens

All subjects had venous blood specimens of five milliliters obtained. The blood specimens are then separated into two groups. For DNA extraction, the initial part (2.5 ml) is transferred to the anticoagulant tube (EDTA) tube. The remaining fraction (2.5ml) is transferred to a Gel tube for serum separation, the blood is allowed to coagulate for about 30 minutes at room temperature and then centrifuged for 5 minutes at 3000 rpm. The serum was then collected in four repeaters in a sterile Eppendorf tube and kept frozen at -20 ºC.

3.2.2.2.Sputum Specimens

Specimens of morning sputum are obtained from patients before antimicrobial agents are given and placed in sterile containers as follows: The patient was instructed to wash his mouth with normal saline to reduce the number of the oral flora, then directed to breathe deeply and cough deeply to bring up sputum into a container and transported to the laboratory (Orikiriza, 2019).

3.2.2.3. Laboratory Methods

Level of lymphocyte, platelet count, and total WBC count were determined using Complete blood count (CBC) a fully automated hematology analyzer.

3.2.3.Ethical Approval

1-The study was done and the cases were collected after getting the agreement of the patients (verbal acceptance).

2-Approval of Babylon Science Collage Ethical Committee.

3- Before starting the study, permission was taken from Babylon's health presidency

3.2.4. Study Design

The specimens proceeded according to the study design that showed in Figure (3-1).

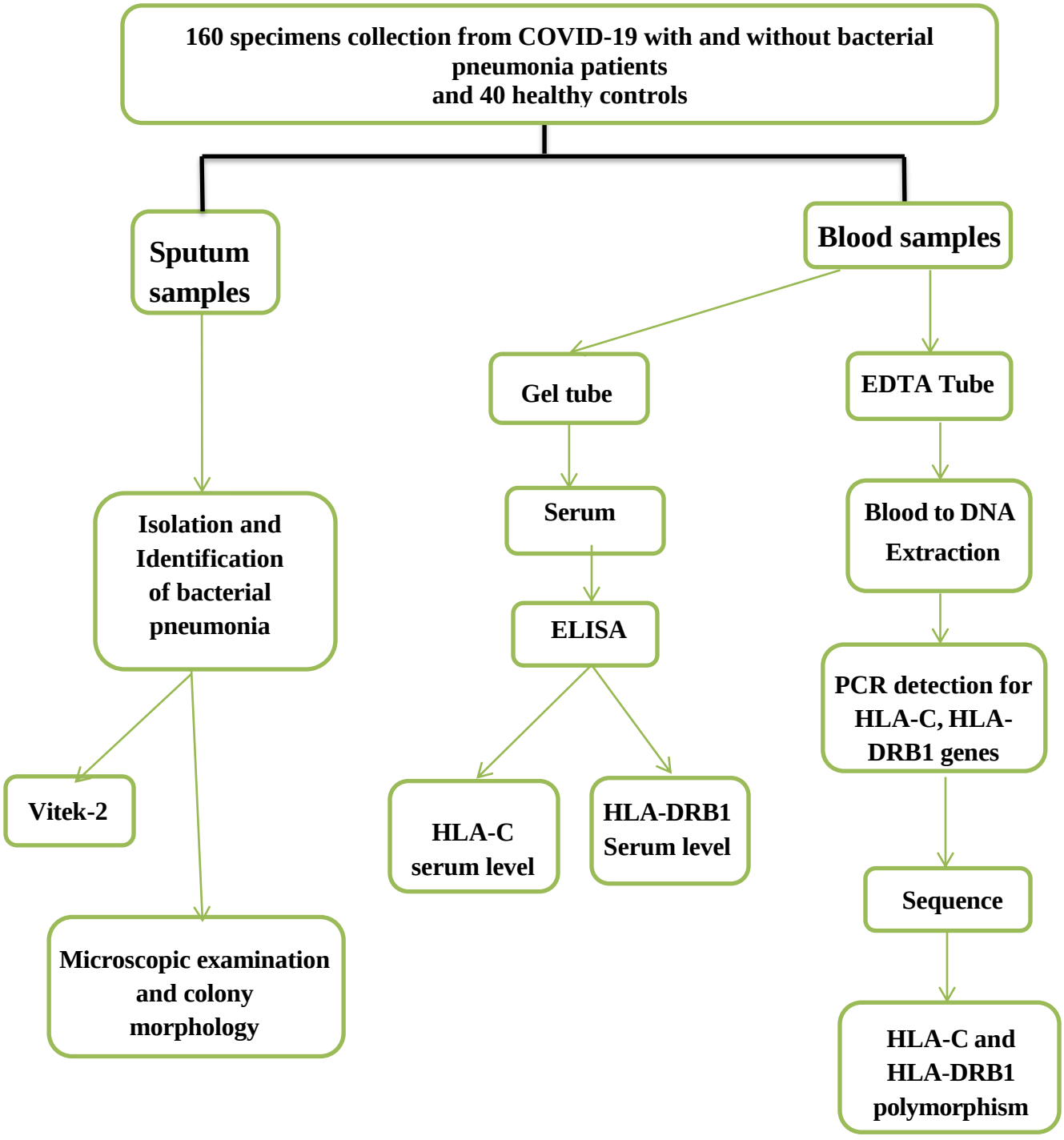


Fig (3-1): Study Design

3.2.5.Preparation of Cultural Media

The culture media were prepared according to the instruction of the manufacturing company and sterilized by autoclave at 121 ºC and 15 pounds (Macfadden, 2000).

3.2.5.1.Blood Agar Medium

Blood agar medium was prepared according to manufacturer instructions by dissolving 40 g of blood agar base in 1000 ml D.W. The medium was autoclaved at 121 ºC for 15 min and pressure 15 pounds per square (psi), left cooled to 50 ºC, and 5% of fresh human blood was added and mixed well inside a sterilized hood. This medium was used as an enrichment medium for the cultivation of the bacterial isolates and to determine their ability to do blood hemolysis (Forbes *et al.*, 2007).

3.2.5.2.MacConkey Agar Medium

It was made by dissolving 51.5 gm into 1000 ml of DW. it was used for the primary isolation of most Gram-negative bacteria and to differentiate lactose fermenters from non-lactose fermenters (Winn, and Shotton 2006).

3.2.6.Isolation and Identification of Bacterial Isolates

1-Bacterial Isolation Assays

The specimens were inoculated on blood agar, and MacConkey agar as predominant enrich media, selective and differential media for the isolation, purification, and identification of bacteria. The plates were incubated at 37˚C for 24 hours.

2- Bacterial Identification Assays

Colony Morphology and Microscopic Examination

A single colony was taken from each primary positive culture media and repeated growth to gain pure culture and then it was identified depending on its morphology and culture characteristics (colony shape, size, color and nature of pigments, translucency, edge, elevation, texture, blood hemolysis, lactose fermentation, mannitol fermentation,) and then it was examined under the microscope after making smear from the pure colony on clean slid and stain with Gram's stain for observation arrangement and shape, and reaction bacteria with stain (MacFaddin,2000).

3.2.7. Vitek-2 System

The Vitek-2 system was used to confirm the biochemical test according to the manufacturer's instructions. This system consists of the personal computer, a reader incubator that is prepared up of many inner constituents including a card filler mechanism, card cassette, bar code reader, cassette loading processing mechanism, card sealer cassette carousel, and incubator. In addition to transmittance optics, instruments control electronics, waste processing, and firmware. The system was equipped with an extended identification database for all routine identification tests that provide improved efficiency in microbial diagnosis which reduces the need to perform any additional tests, so that will increase safety for both the test and the users.

All the following steps were prepared according to the manufacturer's instructions. Three ml of normal saline were placed in a plane test tube and inoculated with a loop full of a single colony. The colony must be aged 24 hr. The test tube was inserted into a dens check machine for standardization of the colony to McFarland's standard solution (1.5×108 cells/ml). The standardized

inoculums are placed into the cassette and a sample identification number was entered into the computer software via a barcode. Thus the VITEK 2 card is connected to the sample ID number. Then, the cassette was placed in the filler module, and when the cards are filled, transferred the cassette to the reader incubator module All the following steps were handled by the instrument, the instrument controls the incubation temperature, the optical reading of the cards and continually monitors and transfers test data to the computer for analysis.

A.Standardization

After primary isolation, handling was minimized in a simple inoculum preparation, standardization, and dilution step. The standardized inoculum was placed into the cassette and a sample identification number was entered into the computer software via barcode.

B.Traceability

The VITEK-2 card type was then read from the barcode placed on the card during manufacturing and the card is thus connected to the sample ID. Manufacturer barcodes link the card to patient information in this one easy barcode reading step.

C.Load and Go

Place the cassette in the filler module. When the cards were filled, transfer the cassette to the reader/incubator module. All subsequent steps are handled by the instrument.

3.2.8.Immunological Study

3.2.8.1.Estimation of Serum Human HLA-C and HLA-DRB1

ELISA kit was applied to the in vitro quantitative determination of Human HLA-C and HLA-DRB1.

1. Test Principle

In the ELISA kit, the Sandwich-ELISA method is used to detect HLA-C and HLA-DRB1, respectively. A Human HLA-DRB1 antibody is pre-coated on a 96-well microtiter plate included in this kit. Samples or standards were mixed with the appropriate antibody in the appropriate wells. A biotinylated detection antibody for HLA-C was then added. To each well, we added an avidin-horseradish peroxidase conjugate (HRP). Washing was performed after the chromogenic step to remove any remaining free components. Each well was incubated with a different substrate. Only the wells containing HLA-C and HLA-DRB1 contain biotinylated detection antibodies and Avidin HRP conjugates. The enzyme-substrate reaction turns yellow after the stop solution is added. The optical density (OD) at an excitation wavelength was measured using a spectrophotometric technique (450 nm 2 nm). The OD value and Perforin concentration were found to be highly correlated. The concentration of HLA-C and HLA-DRB1 was determined by comparing the OD of the samples to the standard curve.

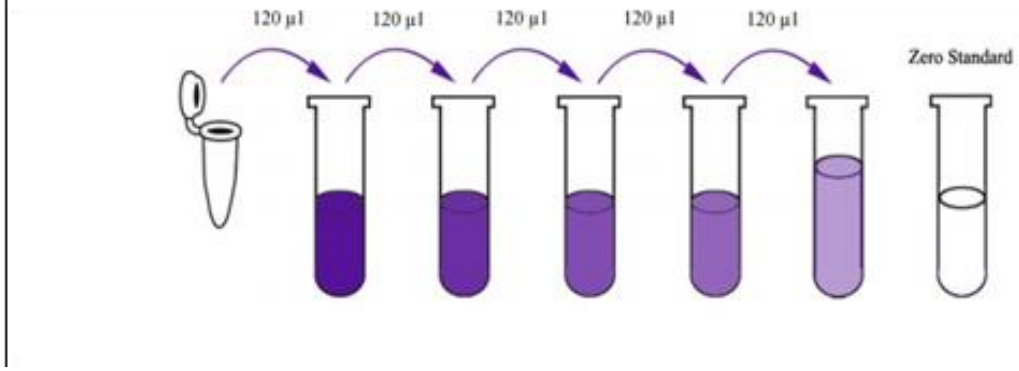
2. Reagent Preparation

I-HLA-C

1- All reagents was brought to room temperature before use.

2- Standard solution preparation: Reconstitute the 120µl of the standard (160ng/ml) with 120µl of standard diluent to generate an 80ng/ml standard stock solution. Was allowed the standard to sit for 15 mins with gentle agitation before making dilutions. Prepared duplicate standard points by serially diluting the standard stock solution (80ng/ml) 1:2 with standard diluent to produce 40ng/ml, 20ng/ml, 10ng/ml, and 5ng/ml solutions. Standard

diluent serves as the zero standards (0 ng/ml). Any remaining solution should be frozen at -20°C and used within one month. The dilution of standard solutions



Standard Concentration	Standard No.5	Standard No.4	Standard No.3	Standard No.2	Standard No.1
160ng/ml	80ng/ml	40ng/ml	20ng/ml	10ng/ml	5ng/ml

suggested is as follows:

Fig (3-2): Schematic presentation of a standard preparation of HLA-C

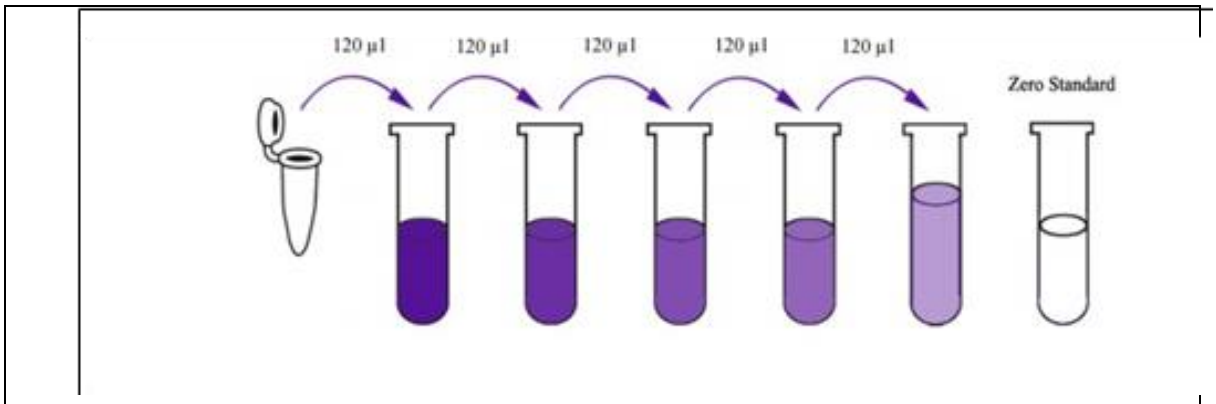
Wash Buffer: Diluted 20ml of washed buffer concentrate 25x into deionized or distilled water to yield 500 ml of 1x Wash Buffer. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.

2-HLA-DRB1

1- All reagents was brought to room temperature before use.

2- Standard solution preparation: Reconstitute the 120µl of the standard (9600ng/L) with 120µl of standard diluent to generate a 4800ng/L standard stock solution. Was allowed the standard to sit for 15 mins with gentle agitation before making dilutions. Prepared duplicate standard points by serially diluting the standard stock solution (4800ng/L) 1:2 with standard diluent to produce 2400ng/L, 1200ng/L, 600ng/L, and 300ng/L solutions. Standard diluent serves

as the zero standards (0 ng/L). Any remaining solution should be frozen at -20°C and used within one month. The dilution of standard solutions suggested is as follows**:**



Standard Concentration	Standard No.5	Standard No.4	Standard No.3	Standard No.2	Standard No.1
9600ng/L	4800ng/L	2400ng/L	1200ng/L	600ng/L	300ng/L

Fig (3-3): Schematic presentation of a standard preparation of HLA-DRB1

Wash Buffer: Diluted 20ml of washed buffer concentrate 25x into deionized or distilled water to yield 500 ml of 1x Wash Buffer. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.

3- ELISA Assay Procedure

1. Prepared all reagents, standard solutions, and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature.

2. Determined the number of strips required for the assay. Insert the strips in the frames for use. The unused strips should be stored at 2-8°C.

3. Adde d 50µl standard to the standard well. Note: Don't adde d a

biotinylated antibody to a standard well because the standard solution contains a biotinylated antibody.

4. Added 40µl sample to sample wells and then add 10µl anti-HLA antibody to sample wells, then add 50µl streptavidin-HRP to sample wells and standard wells (Not blank control well). Mix well. Cover the plate with a sealer. Incubate for 60 minutes at 37°C.

5. Removed the sealer and wash the plate 5 times with wash buffer. Soak wells with 300ul wash buffer for 30 seconds to 1 minute for each wash. For automated washing, aspirate or decant each well and wash 5 times with wash buffer. Blot the plate onto paper towels or other absorbent material.

6. Added 50µl substrate solution A to each well and then add 50µl substrate solution B to each well. Incubate plate covered with a new sealer for 10 minutes at 37°C in the dark.

7. Added 50µl Stop Solution to each well, the blue color will change into yellow immediately.

8. Determined the optical density (OD value) of each well immediately using a microplate reader set to 450 nm within 10 minutes after adding the stop solution**.**

3.2.8.2. Calculating of Results of ELISA Test

The standard curve was created by plotting the mean OD value for each standard on the y-axis against the concentration on the x-axis and drawing a best-fit curve through the points on the graph, when specimens have been diluted, the concentration calculated from the standard curve was multiplied by the dilution factor. the OD of the specimen that surpassed the upper limit of

the standard curve was tested after appropriate dilution as shown in the figure (3-4) and (3-5).

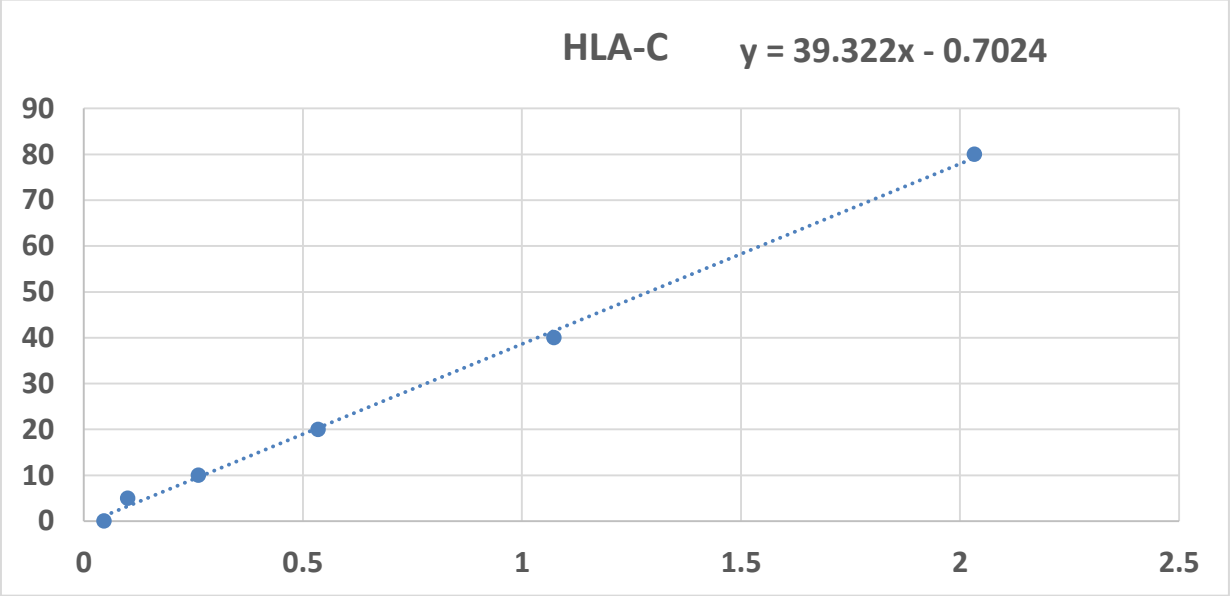


Fig (3-4): Standard Curve of HLA-C

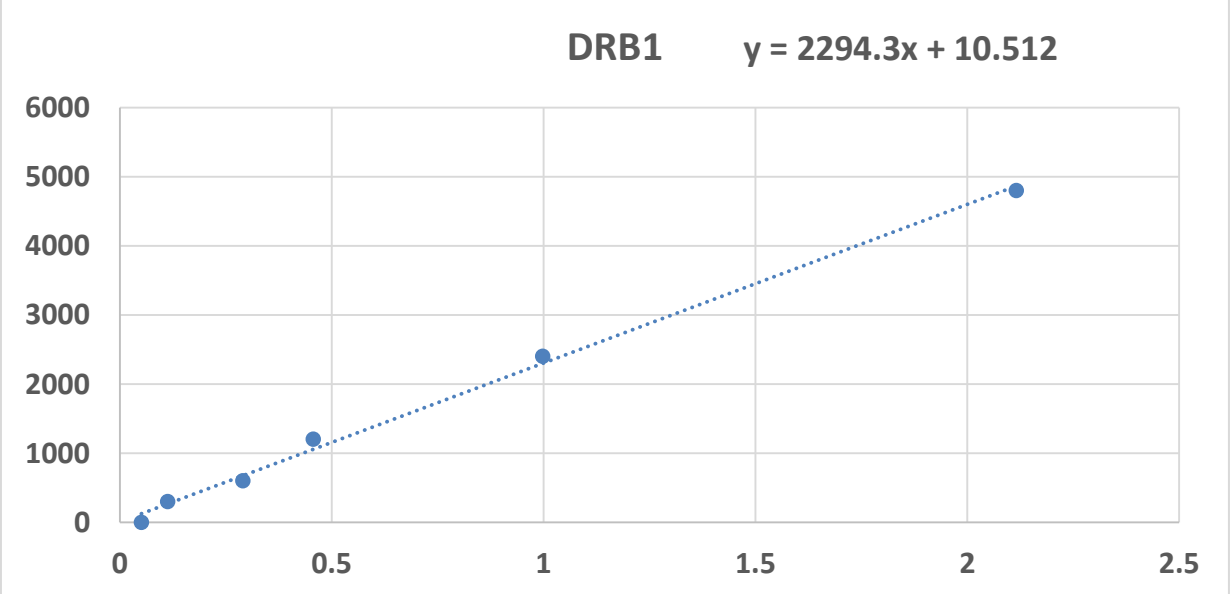


Fig (3-5): Standard Curve of HLA-DRB1

3.2.9. Genomic Study

3.2.9.1. Genomic DNA Extraction from Frozen Human Blood

Favor the Prep TM Genomic Mini Kit obtained genomic DNA from frozen human blood following the manufacturer's protocol.

1-A 1.5 mL microcentrifuge tube was used to transfer approximately 200 µL of thawed frozen blood from an EDTA tube. Following this step, the sample was incubated in the incubator for 15 minutes at 60 ° C with 30 µL of proteinase K (10mg/ml, not provided).

2-As soon as the blood turned greenish-black from the addition of 200 µL of FABG Buffer for cell lysis, the sample was incubated for 15 minutes at 70 ° C. and vortexed or shaken to ensure that the cells were fully lysed before being placed back into the assay. Every three minutes, the sample was inverted to create a new one. For DNA elution, the Elution Buffer was placed in an incubator set to 70°C.

3-To ensure that no precipitation formed during DNA binding was added 200 µL of ethanol (96-100%) was to the sample and vortexed for 10 seconds. This was followed by one minute of 14000-rpm centrifugation on the FABG column after it was carefully transferred to the FABG column. Discarded the Flow- Through Two milliliters of new collection tubes were used to hold the FABG column in place.

4-After adding 400 µL of W1 Buffer to the FABG tube, the tube was centrifuged at 1400 rpm for 30 seconds. After discarding the flow-through, the FABG column was reinserted into the collection tube. well and toward the positive (red) electrodes. Add power by turning on the switch.

5-To dry the column, 600 µL of wash Buffer was added to the FABG tube and centrifuged at 1400 rpm for 30 seconds. The flow-through was then discarded and the FABG tube was placed in the collection tube and centrifuged for an additional 3 minutes.

6-After that, the dry FABG column was transferred to a new 1.5 ml microcentrifuge tube, and 100 µL of preheated elution buffer or TE was added directly to the FABG column membrane. After 10 minutes of incubation at 37°C, the DNA was extracted by centrifugation at 1400 rpm for 1 minute. DNA was kept at 4°C until it was needed.

3.2.9.2.Primer Preparation

TB Buffer (8.0) was used to dissolve the primer pairs used in this study, which contained Tris-HCL (10 Mm) and EDTA-Na2. Initially, the primer stock tube is prepared, and then the working solution is prepared from the primer stock tube.

The nuclease-free water was added following the manufacturer's instructions (micro gen/Korea) to obtain a (300 picomole/microliter) concentration of the primer stock solution. By diluting the stock solution with nuclease-free water, the working solution was obtained at a concentration of 10 picomole/microliter (Green and Sambrook,2012).

3.2.9.3. Reaction Mixture

1-Amplification of DNA was carried out in a final volume of 50 µL reaction mixture as mentioned in Table (3-4).

Table (3-5): Contents of the Reaction Mixture

No.	Contents of the reaction mixture	Volume
1.	Green master mix	25 µL
2.	Upstream primer (10pmol/ µL)	3 µL
3.	Downstream primer (10pmol/ µL)	3 µL
4.	Nuclease free water	14 µL
5.	DNA from sample	5 µL
Total volume		50µL

3.2.9.4. Polymerase Chain Reaction (PCR)

The target DNA was amplified using specific primer pairs in a conventional PCR as previously described (Table 3-6). The PCR product (amplicon) is obtained by repeating three consecutive steps for a specific number of cycles, which can then be visualized after agarose gel electrophoresis, as shown in Table (3-6) which contains information on the thermal cycling conditions.

Table (3-6): **PCR thermal cycling conditions.**

Steps	Temperature (°C)	Conditions time	Cycle number	Reference
Initial denaturation	95	2 minutes	1	This Study
Denaturation	95	30 seconds	35	
Annealing	62.2	30 seconds		
Extension	72	1 minute		
Final extension	72	5 minutes	1	
Initial denaturation	95	5 minutes	1	This Study
Denaturation	95	30 seconds	30	
Annealing	63	30 seconds		
Extension	72	1 minute		
Final extension	72	5 minutes	1	

3.2.9.5. TBE Buffer (Tris-Borate-EDTA)

DNA gel electrophoresis was commonly performed using a TBE running buffer. The stock solution of TBE was prepared and stored at a concentration of 10x working solution. Dissolving 108 grams of Tris, 55 grams of boric acid, and 40 ml of 0.5 M EDTA in 1000 ml of D.W. yielded the 1x solution. When preparing gel electrophoresis gel, the final concentration of the TBE solution was prepared by adding 100ml of 10x TBE buffer to the final volume

of D.W of 900 ml and using a concentration of 1x TBE solution prepared by adding 100ml of 10x TBE buffer to the final volume of 950ml (Sambrook and Russel,2001).

3.2.9.6. Agarose Gel Electrophoresis

Agarose gel electrophoresis is a good way to separate DNA fragments. The amount of agarose in a gel depends on the size of the DNA fragments that need to be separated. It ranges from 0.5% to 2%. (Lee *et al*.,2012). A 1.5 gel was used to get a good picture of small parts of the PCR product (100-700 bp). However, the specific weight of agarose was added to 100 ml of 1x TBE buffer and then melted in the microwave until the solution was clear. This is how the solution was made. Afterward, the agarose gel cooled down to 50-55°C. 5 ml of a dye called "simply safe" (10 mg/ml) was added to 100 ml of melting agarose gel to get a concentration of 0.5%. (Sambrook and Russel,2006). The gel tray was filled with agarose, the comb was put in the right place, and then it was left to dry. They were put in a separate well of the gel, and markers were put in another well. Using the gel's percentage and size, a run was done in the correct way for the electrodes to connect (The time of agarose gel electrophoresis is 70 minutes for PCR product).

1- When using a plastic tray, made sure that the comb's teeth are at least 0.5mm higher than the tray's bottom.

2- 1mL TBE (10X) stock solution + 1000 mL deionized water = 1 mL TBE (10X) solution (1X).

3- Mixed the buffer and 1.5g agarose in a 500 ml flask. Microwave the solution for 90 seconds on high power to dissolve the agarose. Made sure the agarose

the solution is completely dissolved and has no visible agarose particles.

4- Used 50°C agarose solution with 5 µL Simple safe stock solution. Poured the agarose into the gel-casting tray in a steady stream. Remove any air bubbles with a yellow tip.

5- When applying the gel, kept the comb 1.5cm away from the gel's edge. Waited 20– 30 minutes before using the agarose solution. Taking the comb out of the agarose gel after it has solidified requires extreme caution to avoid tearing it. Set the gel-casting tray on the gel box's central platform.

6- After removing the gel tray from the gel box, place it on the central platform.

7- The electrophoresis buffer should be raised 0.5–1cm above the gel surface by adding a buffer to the buffer chamber.

8- Place the samples in the yellow-tipped wells. Made sure that the syringe tip is well above the electrophoresis buffer if injecting. Wait a few moments before slowly ejecting the sample. Avoid contaminating nearby water sources. Sequentially fill each well with a sample. One gel well should be sufficient if you have enough samples to fill the other gel wells.

9- First load 5µl of ladder molecular weight marker to each side of the gel (flanking the sample line) and 5 µl of DNA specimen in the other well.

10- The electrodes can be connected to the gel box lid. The positive electrode can be used to draw DNA from a well (red). The machine needs the power supply turned on to work properly.

11- No electrophoresis should be done until the tracking dye has traveled at least 10 cm across the gel.

3.2.9.7.Sequencing of PCR Product

Forty microliters of HLA-C and HLA-DRB1 products were sent to Macrogene/ Korea for Sanger sequencing. After trimming each sequence, the result of the trimmed sequence was blasted in NCBI to check the similarities and differences with the database. Finch TV version 1.4.0 (Geospiza, Inc.; Seattle, WA, USA; (http: //www.geospiza.com) was used to check the polymorphism of the genes above.

2.3.Biosafety and Hazard Material Disposing

Biosafety aspects were followed during the work including wearing all personal safety equipment such as masks, paws, gloves, and glasses when taking samples from patients sleeping in the epidemiological corridor. The samples were stored after sterilization with ethanol in freezers designated for storing COVID-19 samples. Then all contaminated syringes and supplies were disposed of supplies by autoclaving and then incineration. All benches were cleaned with alcohol (70%) before and after work. Simply Safe used red safe instead of ethidium bromide to reduce biohazard. (Bergen, and Shelhamer, 1996).

2.4. Statistical analysis

Number and percentage were used to express categorical variables. Parametric variables were given as mean ± standard deviation (SD) and significant differences were assessed using the least significant difference (LSD) test. Nonparametric variables were expressed as the median and interquartile range (IQR), and the Mann-Whitney U test (to compare two groups) and the Kruskal-walls test (to compare three or more groups) were used to assess significant differences between medians. Multinomial logistic

regression analysis was applied to determine the odds ratio (OR) and 95% CI. The association between the polymorphism and susceptibility to acute respiratory syndrome coronavirus was expressed as OR and 96% CI. A probability (p) value ≤ 0.05 was considered significant. The statistical analysis was performed using IBM SPSS Statistics 25.0 (Armonk, NY: IBM Corp.) and GraphPad Prism version 9.5.0 (San Diego, California USA).

4. Result and Discussion

4.1. Clinical Characteristics of SARS-CoV-2 and Bacterial Pneumonia Patients

Clinical characteristics for (COVID-19 and Bacterial Pneumonia) patients who have been hospitalized at the COVID-19 Wards in Merjan Medical City and Al-Imam Al-Sadiq Hospital in Babylon Province and healthy control groups for two months (December 2021 and January 2022). This case-control study aims to reveal the following results. The study population was divided into five groups (A-severe corona patients without bacterial pneumonia, B-severe corona patients with bacterial pneumonia, C- non-severe corona patients without bacterial pneumonia, D- non-severe corona patients with bacterial pneumonia, and E- apparently healthy controls).

4.1.1.The Demographic Distribution of the Study Subjects

During this study, HLA-C and HLA-DRB1 were studied from an immunological study, which was done for (180) specimens including (40) group (A), (40) group (B), (40) group (C), (40) group (D), and (20) group (E). The second section of this study examines HLA-C and HLA-DRB1 genes polymorphism in (200) specimens, including (160) acute respiratory syndrome coronavirus patients, ((40) group (A), (40) group (B), (40) group (C) and (40) group (D),) and (40) group (E). The study revealed that all groups of COVID-19 patients have positive results for PCR tests and C-reactive protein (CRP) tests and some groups of bacterial pneumonia patients have positive results for *Streptococcus pneumoniae* or *Klebsiella pneumoniae* in bacterial infection.

Age of Participants

The mean age of acute respiratory syndrome coronavirus patients cases was significantly increased as compared to healthy controls (HC) (53.95 ± 17.67 *vs*. 35.43 ± 16.03 years; $P \leq 0.0001$) (Figure 4-1). The age median range in

group (A) is (13-87), in the group (B) is (15-78), in the group (C) is (10-83), in the group (D) is (19-80), and in the group (E) is (12-72). The study showed that there are significant differences in p-value (0.05) among acute respiratory syndrome coronavirus patients compared with healthy controls.

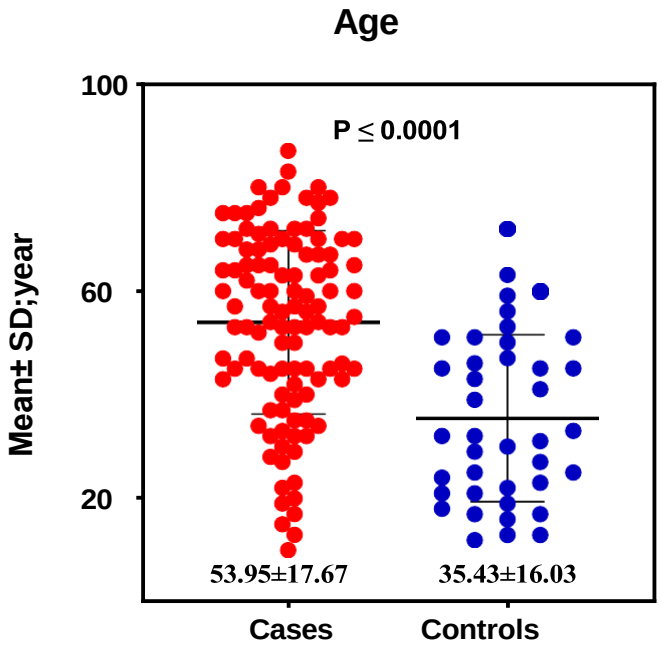


Fig (4-1): Study population distributed according to mean age.

Chronic diseases associated with age progressions such as diabetes mellitus, heart conditions, and hypertension increase with age. A decline in the number and size of ciliated cells in the lung as well as a decrease in nasal resistance can be used to explain the idea that older people are more susceptible to SARS CoV-2. Lung involvement in the elderly, lower oxygen saturation levels, and a larger need for oxygen supplementation were all strongly correlated. These patients had more severe COVID-19 than younger patients, necessitating frequent mechanical breathing and ICU admission (Lian *et al.*,2020).

As previously reported (Mueller *et al.*,2020; Speretta and Leite, 2020; Starke *et al*.,2020), adults in their sixth decade of life account for the bulk of hospitalizations and have a higher risk of death than younger adults. This is because of the severity and result of COVID-19. This may be connected to immunosenescence and cellular senescence, two immune aging processes that can degrade immune responses to SARS-CoV-2 and increase systemic inflammation as a result of irreversible cell cycle arrest (Zhou *et al*.,2021).

The age impact on acute respiratory syndrome coronavirus patient's outcome has been further attributed to immune- and inflammatory-mediated mechanisms. In this regard, a pronounced pro-inflammatory condition has been observed in the lungs of elderly patients with chronic diseases, and this may dysregulate the immune responses in patients having COVID-19 (Costagliola *et al.*,2021).

Yang *et al*.,(2020) reported that elderly patients were more susceptible to COVID-19 than younger adults. Also, (Wu *et al*.,2020) revealed that the median age of patients was 51 years (43-60 years), and 41.8% developed Acute Respiratory Disease, especially in older age. Further, the severity and outcome of the disease largely depend on the age of the patient. Also agreed with Jaaffar *et al*.,(2019) who reported that most bacterial pneumonia patients most of their ages were between 10 and 30 years 49% followed by 31–45 years recorded 37% and older than 45 years. (Mohammed *et al.*,2019) in Thi-Qar found that bacteria pneumonia patients' ages range between (15-80 years) and the median age is 22 years.

4.1.2. Estimation of Blood Parameters in COVID-19, Bacterial Pneumonia Patients, and Healthy Controls

The result clarifies that the mean level of W.B.C was elevated significantly (P≤ 0.05) in bacterial pneumonia patients (severe COVID-19 with pneumonia (17.16±5.74), and non-severe COVID-19 with pneumonia (9.42±4.23)), then in COVID-19 patients (severe COVID-19 without pneumonia (12.41±5.65), and non-severe COVID-19 without pneumonia (9.46±4.06)), compared to the healthy control groups (8.43±2.86), Table (4-1). While the percentage of PLT also decreased significantly among both patients compared to the control group, Likewise, the percentage of LYM%, was decreased significantly among COVID-19 patients compared to the control groups Figure (4-2).

Table (4-1): Blood parameters in COVID-19 with and without Bacterial Pneumonia patients and healthy controls

Parameters	COVID-19 with Bacterial Pneumonia		COVID-19 without Bacterial Pneumonia		Healthy Controls	P-Value
	Severe	Non-Sever	Severe Pneumonia	Non-Severe Pneumonia		
WBC	17.41±5.65	19.46±4.06	12.16±5.74	9.42±4.23	8.43±2.86	0.000*
Lym	5.54±7.16	12.57±14.18	5.26±6.24	9.39±7.81	6.32±1.36	0.000*
PLT	198.8±57.19	218.4±78.76	209.5±61.34	251.2±53.95	296.6±61.22	0.002*

*Significant (P≤0.05).

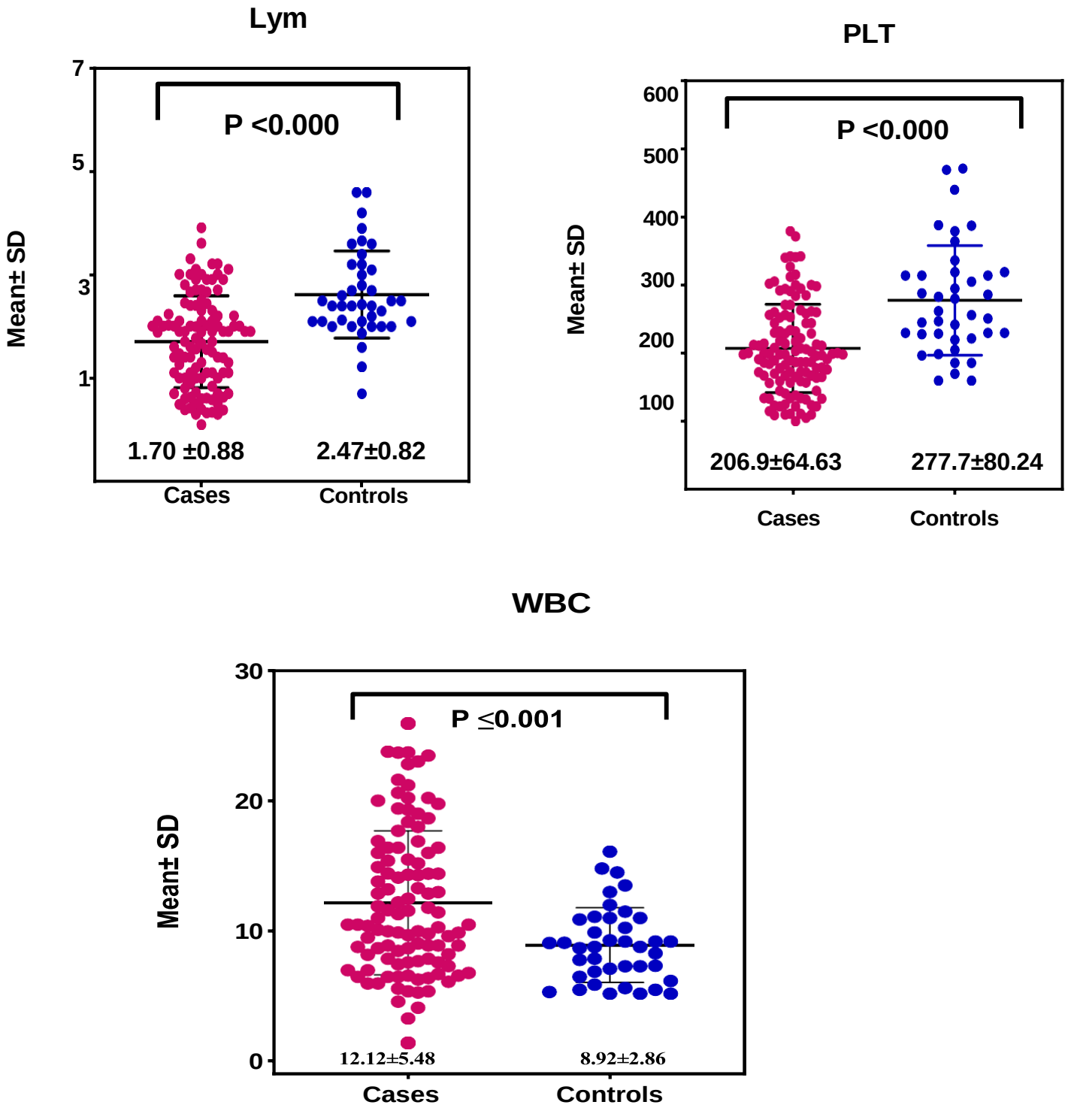


Fig (4-2): Comparsion between Cases and Cotrols in blood parameters (WBC, LYM, and PLT).

According to Kawamata *et al.*,(2015) explanation of pneumonia severity classifications-mild, moderate, severe, and no high severity pneumonia patients and found that WBC counts increased in patients compared to healthy subjects but no significant difference according to the severity of the disease.

Zhou *et al*.,(2020) suggested that lymphopenia can be considered a surrogate biomarker of ineffective immune response to the SARS-CoV-2 infection. According to Mousavi, (2020), at the height of the SARS-CoV-2 outbreak in Tehran, Iran, 60% of participants had lymphopenia, with 92.8 percent of ICU patients also having lymphopenia. The mean neutrophil count was also significantly higher in non-survived patients. So according to their findings, the majority of patients have a high percentage of neutrophils and few lymphocytes.

Identical line. Attiyah, *et al*.,(2021) demonstrated noticeably greater neutrophil and WBC levels as well as reduced lymphocyte and monocyte levels. It has been suggested that lymphopenia in severe/critically infected patients may result from the virus's direct involvement or the redistribution of WBC through chemotaxis or apoptosis. While lymphocyte albumin and low-density lipoprotein levels in the severe pneumonia infection group are lower than in the normal infection group, Thomas *et al*.,(2020) found that WBC, neutrophil, monocyte, CRP, serum creatinine, and urea nitrogen levels are higher.

4.2. Isolation Bacteria in Patients with Bacterial Pneumonia Specimens

In this study, 80 sputum specimens were collected from patients suffering from pneumonia of both sexes with age (13-87) years, carried out from Merjan Medical City and Imam Sadiq Hospital in Babylon province for 2,

months (December 2021 and January 2022). Pneumonia is caused by bacteria of which 80 (40%) showed positive bacterial growth and 120 (60%) showed no growth. Out of 80 positive bacterial growths, 60 (or 75%) are *Streptococcus pneumoniae*, followed by 20 (or 25%) *Klebsiella pneumoniae.* Gram-positive bacteria are pneumonia's most frequent cause. The illness is common worldwide and affects people of all ages (Najem *et al.*,2015). According to Ibrahim *et al.,(*2017) out of the 90 specimens, 50 specimens (55.5%) included Streptococcus spp. while 40 specimens revealed 44.4% non-Streptococcus spp. species. Among the isolates, *Streptococcus pneumoniae* made up eleven (22%).

According to Jameel *et al*., (2020), out of the 20597 sputum specimens they tested, 1155 (5.6%) were positive and 19442 (94.4%) were negative. 169 samples from the 17268 that were analyzed for TB tested positive at a rate of (1.1%), 898 samples from the 3241 that were studied tested positive for bacterial growth at a rate of (27.7%), and only 88 samples (0.4%) tested positive for fungal growth. *Pseudomonas aeruginosa* and *Moraxella catarrhalis* were the most prevalent Gram-negative bacteria, followed by *Klebsiella pneumoniae* and *Escherichia coli* (15.86%) and (13.31%), respectively. 53%29%18% *S.aureus*, and *K.pneumoniae*, respectively. At the top of the Gram-positive bacteria list is *Streptococcus pneumoniae* (15.0%).

Table (4-2): Bacterial Pneumpnia that associated with COVID-19

Bacterial species	Severe	Non-Severe	N(%)
Streptococcus pneumoniae	28	32	75%
Klebsiella pneumoniae	12	8	25%
Total	40	40	100%

4.2.1.The Diagnostic Characteristics of *Streptococcus Pneumoniae*

The current study shows that 60 isolates of *Streptococcus pneumoniae* were found in the patient's sputum. Based on phenotypic traits such as colony morphology, and Gram's stain) alpha-hemolytic (under aerobic conditions) or beta-hemolytic (under anaerobic conditions) facultative anaerobic.

All specimens were cultured on blood agar after incubation at 37 C for 24 hours, and colonies appear forming highly mucoid and glistening. The most frequent cause of pneumonia is *Streptococcus pneumoniae*. The illness affects people of all ages and is widespread around the world. The gram-positive bacteria *Streptococcus pneumoniae* are short pairs of diplococci, facultatively anaerobic, and alpha-hemolytic. They also test negative for oxidase and catalase, and their colonies are 1-2 mm in diameter and immobile (Ibrahim *et al.*, 2019).

Table (4-3): Diagnostic feature of *Streptococcus pneumoniae*

Test	Results
Growth on blood agar	*S.pneumoniae* appears as small, grey, moist mucoidal), colonies, and characteristically produces a zone of alpha-hemolysis (green)
Hemolysis	alpha- hemolytic
Gram stain	Gram-positive
Morphology	Pairs of cocci (diplococci), but they may also occur singly and in short chains
Catalase	Negative
Optochin	Positive

4.2.2.The Identification Characteristics of *Klebsiella Pneumoniae*

In total, 20 of 80 patients, had a positive microbiological result for *Klebsiella pneumoniae*. The phenotypic characteristics of *K. pneumoniae*, such as the colony morphology of the bacteria on blood agar, which is mucoid and 3 to 4 mm in diameter, are used to make a diagnosis of the organism in a lab. setting. *K.pneumoniae* colonies on MAC are typically pink, mucoid, and 3 to 4 mm in diameter.

According to (Effah *et al*., 2020) observations, *K. pneumoniae* is a naturally occurring bacillus that is categorized as Gram-negative, non-motile, and capsulated. It can be found in the environment as well as on human mucous membranes in the gastrointestinal tract and oropharynx microbiome. The bacteria can spread from these colonized areas to distinct tissues causing acute infections, including pneumonia, urinary tract infections, wound infections, bacteremia, and liver abscesses.

Table (4-4): Diagnostic features of *Klebsiella Pneumoniae*

Test	Results
Growth on blood and MaCconkey agar	Mucoid colonies on blood agar, and lactose fermenting colonies on MaCconkey agar appear red to pink in color.
Gram stain	Gram-negative bacilli
Morphology	Large shiny and dark pink. Gram-negative, the short rod-shaped bacterium, plump, straight rods, non-motile, encapsulated, facultatively anaerobic, large, dome-shaped.

4.3.Immunological Study

4.3.1. Estimation of Human Leukocyte Antigen-C (HLA-C) Serum Level in the Study Population

Getting rid of COVID-19 patients includes a strong immune response capable of controlling the infection and appropriate treatment. The current study demonstrated that serum HLA-C levels were significantly elevated among acute respiratory syndrome coronavirus patients regardless of disease severity.

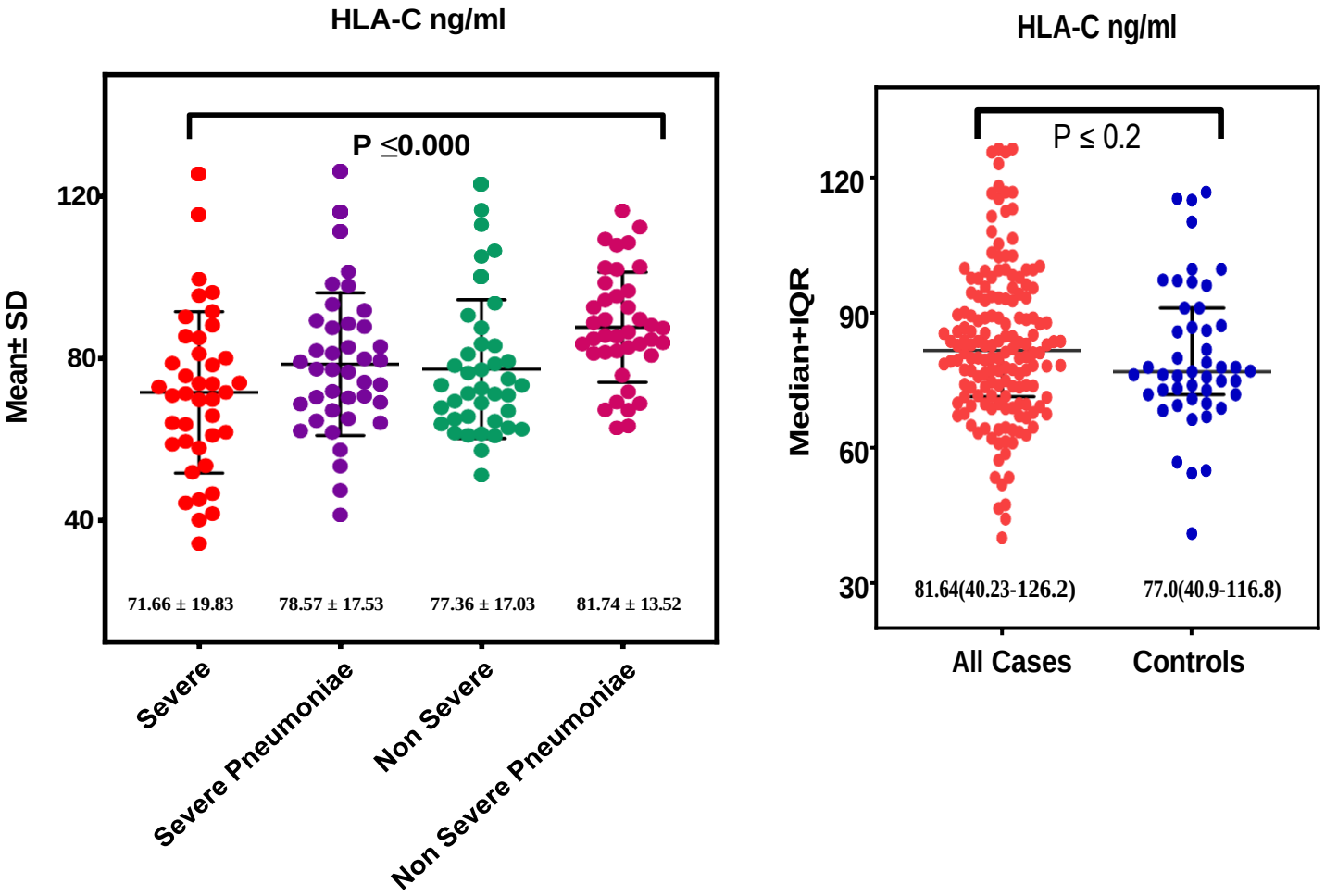
The present results revealed a significant difference (P ≤0.05) in the serum levels of HLA-C between patients and healthy controls. By graph pad prism In the case of disease severity, HLA-C levels show significant differences between patients with, group (A) having a level of 71.66±19.83 ng/ml, group (B) having a level of 78.57±17.53 ng/ml, group (C) having a level of 77.36±17.03 ng/ml, group (D) having a level of 81.74±13.52 ng/ml, (P ≤0.000). The HLA-C serum levels were no significantly increased in (COVID-19 and Bacterial pneumonia) patients having a level 81.64 (40.23-126.2) ng/ml, and apparently healthy controls having a level of 77.0 (40.9-116.8) ng/ml, (P ≤0. 2). Shown in figure (4-3).

In the case of disease severity, HLA-C levels show significant differences between patients with Severe, Severe Pneumonia, Non-Severe, or Non-Severe Pneumonia ($P \leq 0.000$), As shown in Figure (4-3).

The main function of HLA-C is to inhibit innate and adaptive immune responses by interacting with its ligands on target cells to transmit inhibitory signals. Natural killer cells, CD4+ and CD8+ T cells, B cells, macrophages, dendritic cells, and neutrophils are among the target cells functionally influenced by HLA-C. These findings were consistent with previous observations that HLA-C serum levels were up-regulated in the serum of patients with severe disease (Al-Bayatee, and Ad'hiah,2021). These data confirm the recently addressed hypothetical views implicating HLA-C in the immunopathogenesis of COVID-19 and disease severity (Saulle *et al*., 2021).

Under normal physiological conditions, HLA-C can function as an inhibitor of T and NK cell cytotoxicity and an enhancer of T regulatory cells (Bian, and Fu, 2022). Interestingly, in vitro, evidence showed that a large number of genes related to HLA-class I (including HLA-C), HLA-class II, or antigen presentation were found to be up-regulated 36 h post-infection of a human lung epithelial cell line with SARS-CoV (Josset *et al*., 2013).

The results of the current study were confirmed by Nelde, A *et al.*, (2022), who also recommended more investigation into serum HLA class I levels and their connection to various symptoms. Convalescent people who had COVID-19 symptoms such as a cough, headache, and weariness reported having elevated serum HLA class I levels.



Horizontal lines indicate medians, while vertical lines indicate interquartile range (IQR). Significant differences were assessed with the Mann-Whitney U test (to compare two groups), and the Kruskal-Wallis test (to compare three groups) by Graph Pad Presim.

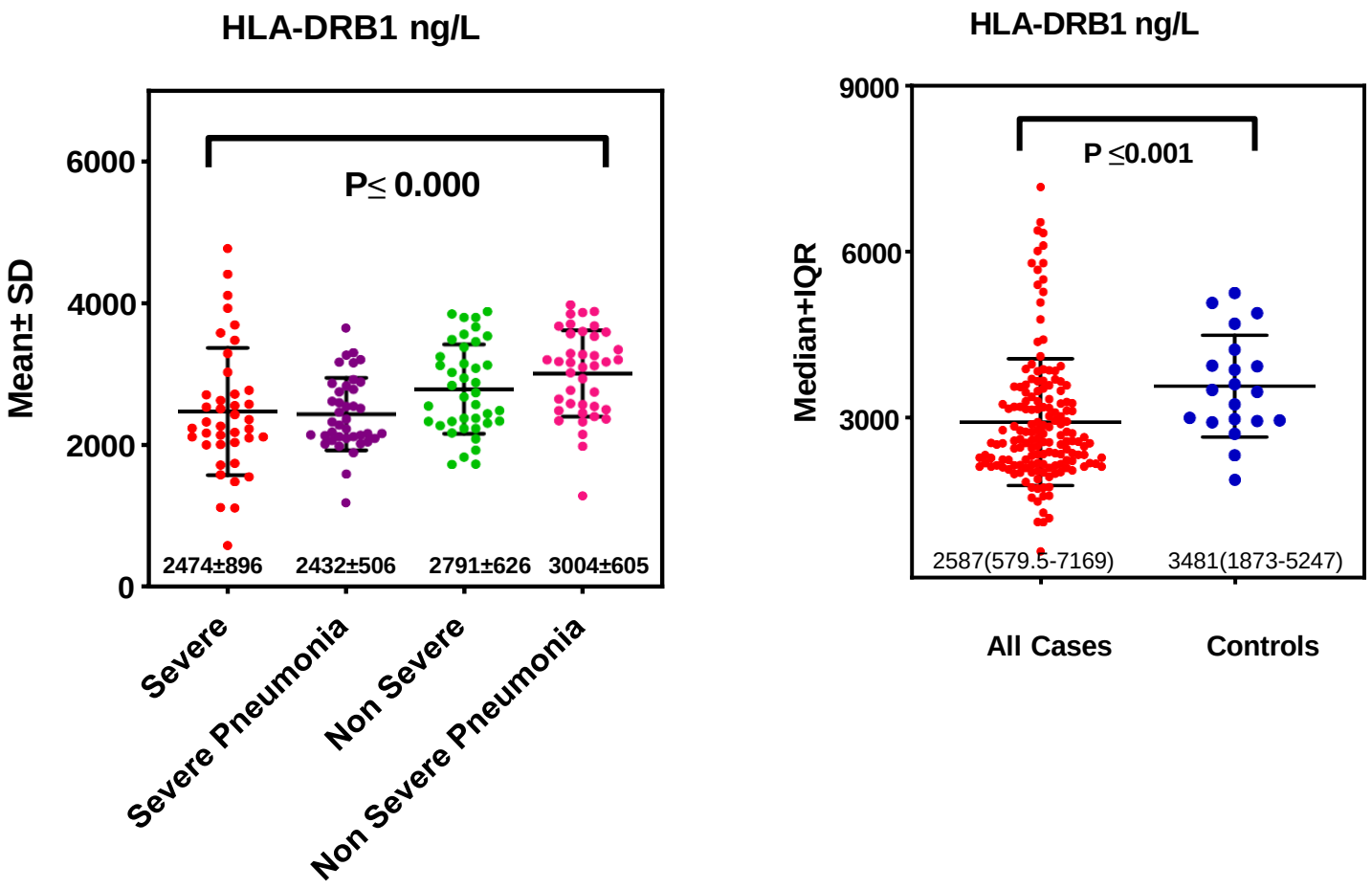
Fig (4-3): Scatter dot plots of concentration HLA-C in COVID-19 with and without pneumonia, and cases distributed according to the severity of the disease.

4.3.2. Estimation of Human Leukocyte Antigen-DRB1 (HLA- DRB1) Serum Level in the Study Population

In the present study, you examined the severity of the illness among 160 people with acute respiratory syndrome and the prevalence of HLA-DRB1 in comparison to 20 healthy controls. The patient's group showed lower frequencies of HLA-DRB1 with a coronavirus-induced acute respiratory syndrome.

By graph pad prism In the case of disease severity, HLA-DRB1 levels show significant differences between patients with, group (A) having a level of 2474 ± 896 ng/L, group (B) having a level of 2432 ± 506 ng/L, group (C) having a level of 2791 ± 626 ng/L, group (D) having a level of 3004 ± 605 ng/L, ($P \leq 0.000$). The HLA-C serum levels were significantly decreased in (COVID-19 and Bacterial pneumonia) patients having a level 2587 (579.5-7169) ng/L, and apparently healthy controls having a level of 3481 (1873-5247) ng/L, ($P \leq 0.001$). Shown in figure (4-4).

In the case of disease severity, HLA-DRB1 levels show significant differences between patients with Severe, Severe Pneumonia, Non-Severe, or Non-Severe Pneumonia ($P \leq 0.001$), As shown in Figure (4-4).



Horizontal lines indicate medians, while vertical lines indicate interquartile range (IQR). Significant differences were assessed with the Mann-Whitney U test (to compare two groups), and the Kruskal-Wallis test (to compare three groups) by Graph Pad Presim.

Fig (4-4): Scatter dot plots of concentration HLA-DRB1 in COVID-19 with and without pneumonia, and cases distributed according to the severity of the disease.

Given the central role of HLA molecules for the stimulation and induction of T cell-mediated anti-viral immunity, it is speculative that HLA_DRB1 may determine the genetic susceptibility to or possibly protection against SARS COV-2 and they can be related to severity, prognosis, and outcome of diseases

(Littera, *et al*., 2020)., (Nguyen, *et al*.,2020).

Valtierra-Alvarado, M *et al*.,(2022) supported the findings of the current study, A lower frequency of HLA-DR is observed in type 2 diabetes (T2DM) patients, HLA-DR-/low cells have been associated with an MDSC immunosuppressive phenotype, therefore an increased frequency of these cells could be associated with impairment of immune protective functions. The frequency of HLA-DR-/low (negative or low expression of HLA-DR) cells in HC and T2DM patients was evaluated by flow cytometry. To this end, the HLA-DR-/low population was defined by HLA-DR downregulation and was delimited by fluorescence minus one (FMO) control.

Restrepo, B. I *et al*.,(2021) support this finding showing lower HLA-DR expression in all the monocyte subsets from diabetes patients, regardless of their dyslipidemic state. Lipids may further affect HLA-DR expression, with triglycerides promoting depression, while cholesterols, particularly LDL, counterbalance the reductive effect.

Domon, H.,& Terao, Y.(2021) support this study and found that expression of the human leukocyte antigen (HLA) class II molecule was decreased in THP-1-derived macrophages treated with supernatants from dead neutrophils. Neutrophil elastase also cleaved HLA-DRB1 on extracellular vesicles isolated from macrophages without triggering morphological changes. Thus, leakage of neutrophil elastase may disrupt innate immune responses, antigen presentation, and T-cell activation. Additionally, inhibition of neutrophil elastase is a potential therapeutic option for treating bacterial and viral pneumonia.

4.3.3. Correlation Among the Immunological Parameter

This study correlated HLA-C and HLA-DRB1 serum levels in (COVID-19 and bacterial pneumonia) patients, and correlation analysis revealed the

following results: Positive correlation is significant at the 0.000 level (2-tailed).

As shown in table (4-7). This correlation was significant, These data may underline some causal relationships between parameters in (COVID-19 and bacterial pneumonia) patients and their synergistic effects in predisposing the individual to the disease or its severity.

Table (4-7): Correlations between HLA-C and HLA-DRB1 serum levels in COVID-19 and bacterial pneumonia patients.

Parameter	HLA C	HLA DRB1
HLA C **Sig**	**1**	**0.410**** **0.000**
HLA DRB1 **Sig**	**0.410**** **0.000**	**1**

** Correlation is significant at the 0.01 level (2-tailed).

Individual HLA genotypes might play a role in determining susceptibility to COVID-19 infection and infection outcome. Although HLA class I is well known as a molecule that presents intracellular molecules, HLA class II can have a role in viral peptide presentation. A recent study showed that peptides from SARS-COV2 can bind HLA-DR molecules present on monocytes (Parker *et al.,* 2021).in which an association between HLA- DRB1 and the incidence of COVID-19 infection was suggested (Novelli *et al*., 2020).

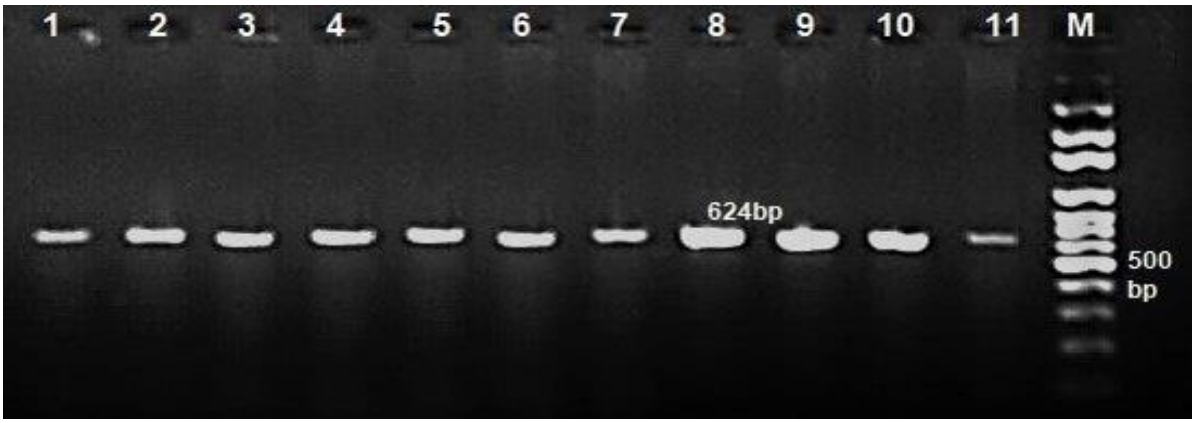
In contrast, a study found a positive significant correlation between the HLA-C and HLA-DRB1) haplotype and the incidence and death from COVID-19 (haplotype of susceptibility) (Pisanti *et al.*,2020).

Sakuraba, *et al*.(2020) who support this study clarified that antigen presentation stimulates the body's humoral and cellular immunity, mediated by virus-specific immune cells. Studied the association between HLA class I, and the risk of death due to COVID-19 and have identified HLA-C as

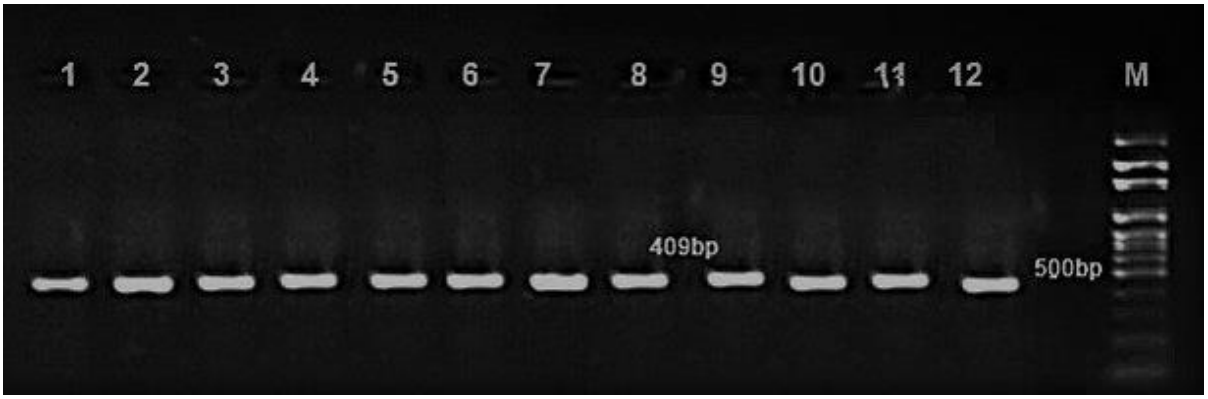
a potential candidate that may influence mortality. The results of study demonstrated a correlation, so additional research on the functional properties of HLA-C and a subsequent study to assess the causal relationships are warranted.

4.4. Genetic study

Detecting and recognizing particular PCR-amplified fragments via human DNA extraction (HLA-C and HLA-DRB1-1) are depicted in Figures (4-5), and (4-6).



Fig(4-5): (1.5 %) Agarose gel electrophoresis at 72 volts for 60 minutes of PCR product of HLA-C amplicon (624bp), 1-11 represented sample, M (DNA marker size (100bp).



Fig(4-6): (1.5 %) Agarose gel electrophoresis at 72 volts for 60 minutes of PCR product of HLA-DRB1A amplicon (409bp), 1-12 represented samples, M (DNA marker size (100bp).

4.4.1.Hardy-Weinberg Equilibrium Analysis of HLA-C

Analysis of Hardy-Weinberg equilibrium (HWE) demonstrated that the three genotypes of HLA-C GG (wild type), GT (Mutant heterozygous), and TT (Mutant homozygous) polymorphism deviated significantly from HWE in patients and healthy controls (HC), as significant variations were found between observed and expected frequencies (Table 4-8).

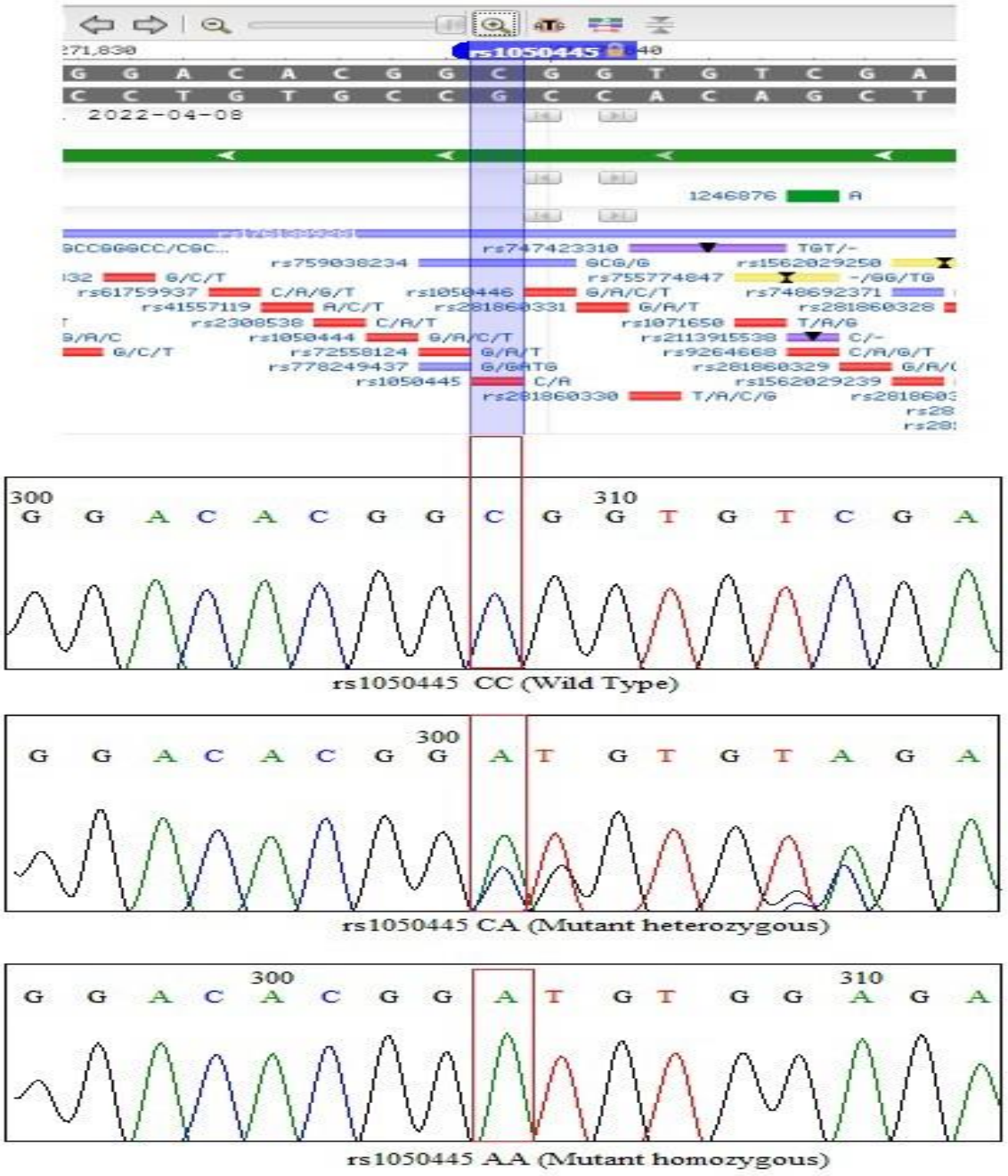
Table (4-8): Hardy-Weinberg equilibrium analysis of HLA-C (GG, GT, and TT) polymorphism in the study population

Genotype	Cases (N = 134)				HC (N = 40)			
	Observed		Expected		Observed		Expected	
	N	%	N	%	N	%	N	%
GG (wild type)	58	43.28	37.6	28.05	27	67.5	19.6	49.0
GT (Mutant heterozygous)	26	19.40	66.8	49.85	2	5.0	16.8	42.0
TT (Mutant homozygous)	50	37.32	29.6	22.10	11	27.5	3.6	9.0
HWE P-value	0.000				0.09			

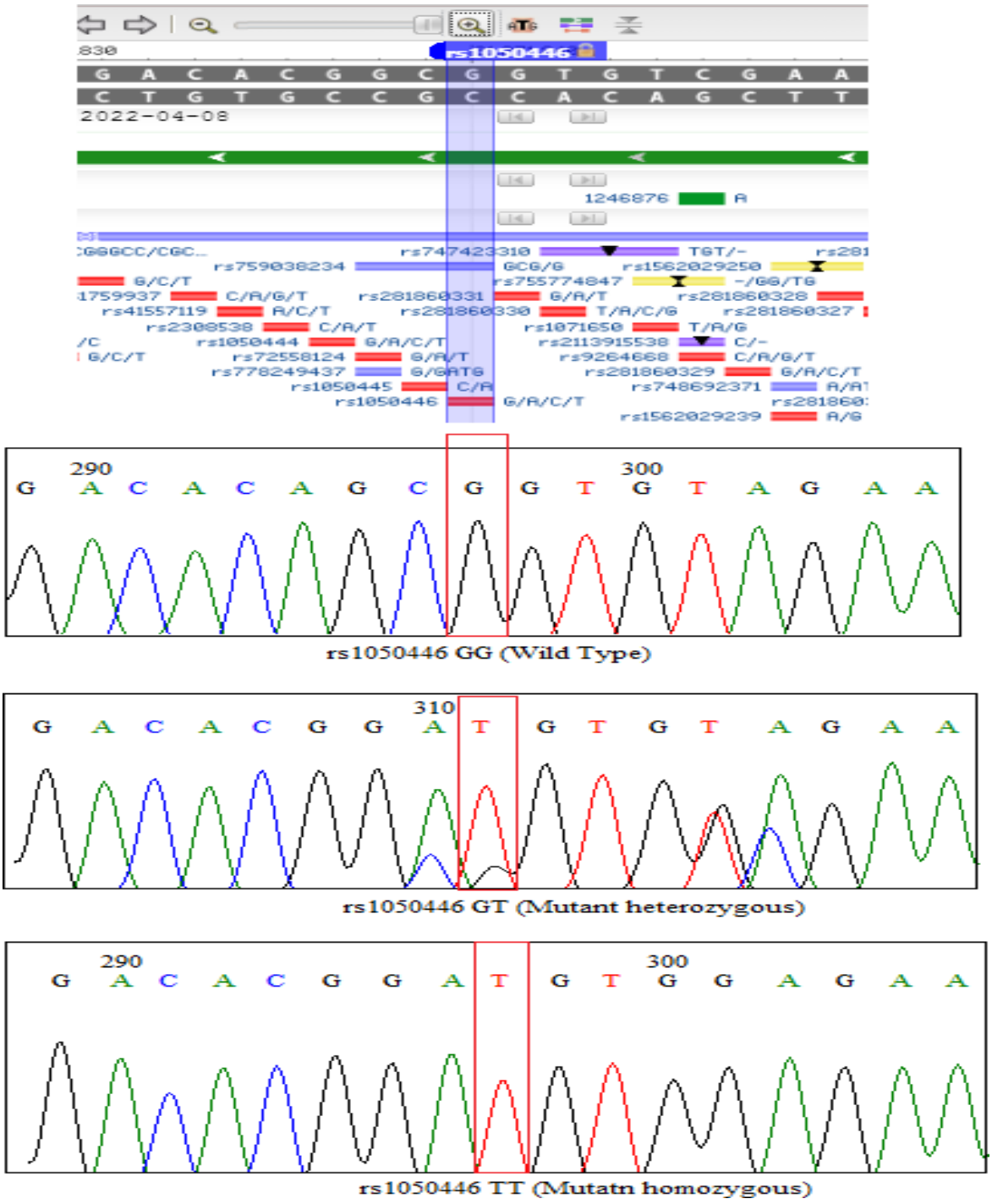
4.4.2.Association of HLA-C A Single Nucleotide Polymorphisms (SNPs) and Risk of Patients and Healthy Controls

Trimming of the HLA-C PCR product's Sanger sequencing data. To determine the genotype variations between (COVID-19 and Bacterial Pneumonia) patients and healthy controls, multiple alignments for each were created using Finch TV version 1.4.0 (Geospiza, Inc.; Seattle, WA, USA; (http://www.geospiza.com)) and compared with the NCBI database. Figures

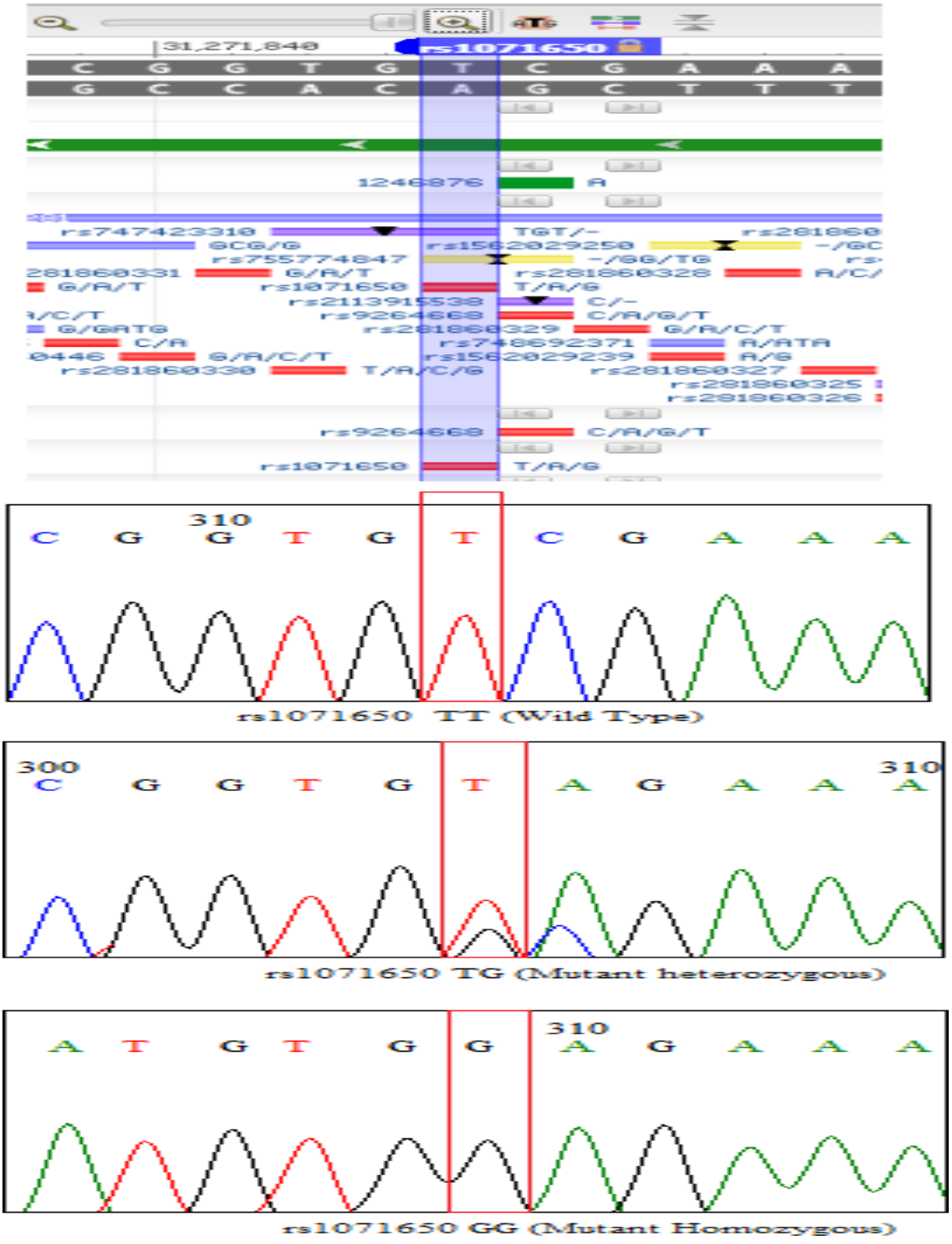
(4-7), (4-8), (4-9), and Figure (4-10) show SNPs distributions on the HLA-C gene.



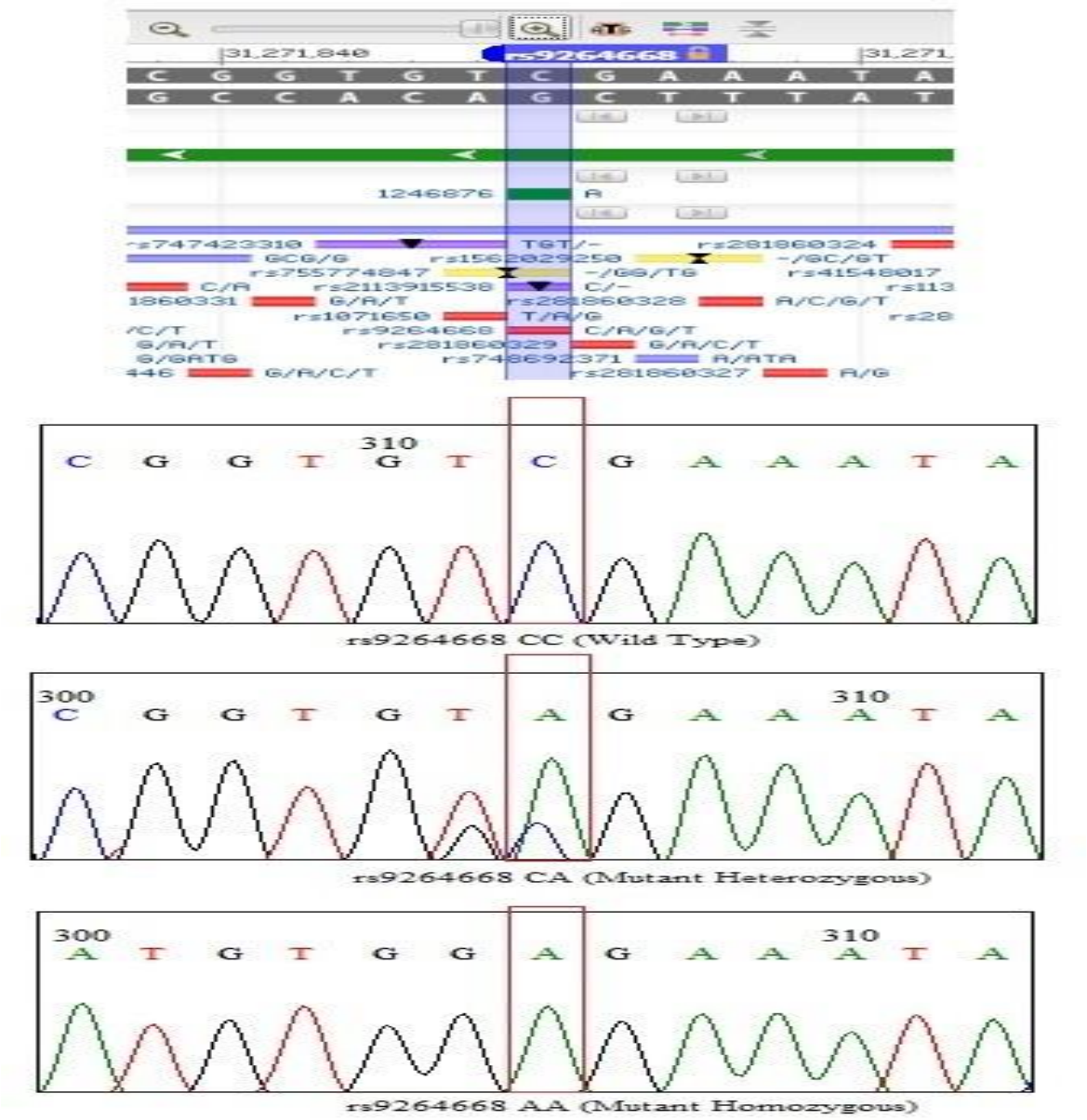
Fig(4-7): Distributions of rs1050445 genotypes SNPs on HLA-C gene in study population



Fig(4-8): Distributions of rs1050446 genotypes SNPs on HLA-C gene in study population



Fig(4-9): Distributions of rs1071650 genotypes SNPs on HLA-C gene in study population



Fig(4-10): Distributions of rs9264668 genotypes SNPs on HLA-C gene in study population

A-HLA-C rs1050445 SNPs

HLA-C rs1050445 SNPs, By using this study's first SNP, it was discovered (19.00% CC, 35.05% CA, and 45.95% AA) genotype carries in the group (A) patients, with an allele frequency of (0.36) for C-allele and (0.64) for A-allele. The genotype distribution in the group (B) patients is as the following (48.57% CC, 22.85% CA, and 28.58% AA), The C-allele and

A-allele frequencies are (0.61) and (0.39) in the group (B) cases respectively. The genotype distribution in group (C) patients is as the following (17.14% CC, 28.57% CA, and 54.29% AA). The C-allele and A-allele frequencies are (0.31) and (0.69) in the group (C) cases respectively, with (81.00% CC,0% CA, and 19.00% AA genotype carried in group (D) patients, with an allele frequency of (0.8) for C- allele and (0.2) for A-allele and The genotype distribution in the group (E) are as the following (67.5% CC, 5.00% CA, 27.5% AA. The C-allele and A-allele frequencies are (0.7) and (0.3) in the group (E) healthy controls respectively.

According to these findings, the frequency of the C allele was significantly lower in COVID-19 and Bacterial Pneumonia compared to healthy controls (HC) ($P \leq 0.002$). Such deviation scored an OR value of (0.44) (95% CI: 0.74 - 3.52). In the HLA-C rs1050445 Wild type, the CC genotype showed a significantly decreased frequency in all cases compared to healthy control (HC) (P-Value $\leq$0.001; OR = 0.30; 95% CI =0.14 to 0.64). In the Mutant heterozygous (CA), the frequency of all cases with the genotypes CA was significantly elevated compared to healthy control (HC) (P-Value $\leq$0.02; OR = 4.71; 95% CI =1.30 to 25.05). In the HLA-C rs1050445 Mutant homozygous, the AA genotype showed a no significantly increased frequency in COVID-19 patients and decreased in COVID-19 with bacterial Pneumonia patients compared to healthy control (HC) (P-Value=0.2; OR = 1.61; 95% CI =0.74 to 3.52). These results indicate that there is a relationship between HLA-C rs1050445 SNPs and infection severity in the study population as shown in (Table 4-9).

Table (4-9): Distributions of genotypes and allele frequencies in HLA-C rs1050445 SNP in the Study population.

rs1050445 genotypes		Severe COVID-19 n=37 (%)	Severe COVID-19 + Pneumonia n=35 (%)	Non-Severe COVID-19 n=35 (%)	Non-Severe COVID-19 + Pneumonia n=27(%)	Healthy Control n=40 (%)	P-Value	OR	CL=95%
CC (wild type)		7(19.00)	17(48.57)	6(17.14)	22(81.00)	27(67.5)	0.001	0.30	0.14 to 0.64
CA (Mutant heterozygous)		13(35.05)	8(22.85)	10(28.57)	0	2(5.00)	0.02	4.71	1.30 to 25.05
AA (Mutant homozygous)		17(45.95)	10(28.58)	19(54.29)	5(19.00)	11(27.5)	0.2	1.61	0.74 to 3.52
Alleles (%)	C	27(0.36)	42(0.61)	22(0.31)	44(0.8)	56(0.7)	0.002	0.44	0.25 to 0.74
	A	47(0.64)	28(0.39)	48(0.69)	10(0.2)	24(0.3)			

OR=Odd ratio, Cl (95%)=confidence interval, P-value ≤0.05 calculated for estimation of significant difference of the patient's genotypes and alleles.

B- HLA-C rs1050446 genotypes SNPs

HLA-C rs1050446 genotypes SNPs is the second SNP conducted by the current study found (32.43% GG, 21.42% GT, and 46.15% TT) genotype carries in the group (A) patients, with an allele frequency of (0.43) for G-allele and (0.57) for T-allele. The genotype distribution in the group (B) patients is as the following (51.42% GG, 20.00% GT, and 28.58% TT), The G-allele and T- allele frequencies are (0.61) and (0.39) in the group (B) cases respectively, The genotype distribution in a group (C) patients is as the following (17.14% GG, 31.42% GT, and 51.44% TT), The G-allele and T-allele frequencies are (0.32) and (0.68) in the group (C) cases respectively, The genotype distribution in the group (D) patients is as the following (81.00% GG, 0% GT, and 19.00% TT), with an allele frequency of (0.81) for G-allele and (0.19) for T-allele. And found (67.5% GG, 5.00% GT, and

27.5% TT) genotype carried in the group (E) healthy controls, The G-allele and T-allele frequencies are (0.7) and (0.3) in the group (E) control respectively. All demographic characteristics of the patients and healthy control and associated risk factors for COVID-19 and bacterial Pneumonia were demonstrated in Table (4-10).

In this study were noticed statistically significant differences between the G, and T alleles with an increase of the G allele in patients and also an increase of T alleles in COVID-19 patients but a decrease in bacterial pneumonia patients compared with healthy controls. The frequency of HLA-C rs1050446 genotypes SNPs GG wild type and GT Mutant heterozygous was significantly increased in patients than healthy controls (OR = 0.37; 95% CI =0.17 to 0.77 and p-value ≤0.008) (OR = 4.57; 95% CI =1.03 to 20.19 and p-value=0.04) respectively. The frequency of HLA-C rs1050446 genotypes SNPs TT Mutant homozygous was not significantly increased in COVID-19 and decreased in bacterial Pneumonia than healthy controls (OR = 1.56; 95% CI =0.72 to 3.41 and p-value ≤0.3).

This study verified that the HLA-C rs1050446 genotype SNPs have a significant effect on the risk factors for COVID-19 and bacterial pneumonia where the GT-genotype conferred an increased risk for COVID-19 and bacterial pneumonia but the TT genotype formed an intermediate risk group in COVID- 19 and protective in bacterial Pneumonia.

Table (4-10): Distributions of genotypes and allele frequencies in HLA-C rs1050446 genotype SNPs in the study population.

rs1050446 genotypes		Severe COVID-19 n=37 (%)	Severe COVID-19 + Pneumonia n=35 (%)	Non-Severe COVID-19 n=35 (%)	Non- Severe COVID-19 + Pneumonia n=27(%)	Healthy Control n=40 (%)	P-Value	OR	CL=95%
GG (wild type)		12(32.43)	18(51.42)	6(17.14)	22(81.00)	27(67.5)	0.008	0.37	0.17 to 0.77
GT (Mutant heterozygous)		8(21.42)	7(20.00)	11(31.42)	0	2(5.00)	0.04	4.57	1.03 to 20.19
TT (Mutant homozygous)		17(46.15)	10(28.58)	18(51.44)	5(19.00)	11(27.5)	0.3	1.56	0.72 to 3.41
Alleles (%)	G	32(0.43)	43(0.61)	23(0.32)	44(0.81)	56(0.7)	0.007	0.48	0.28 to 0.82
	T	42(0.57)	27(0.39)	47(0.68)	10(0.19)	24(0.3)			

OR=Odd ratio, Cl (95%)=confidence interval, p-value ≤0.05 calculated for estimation of significant difference of the patient's genotypes and alleles.

C- HLA-C rs1071650 genotypes SNPs

The third HLA-C rs1071650 genotypes SNPs, and the results were (27.00% TT, 29.37% TG, and 43.27% GG) genotype carries in the group (A) patients, with an allele frequency of (0.42) for T-allele and (0.58) for G-allele. The genotype distribution in the group (B) patients is as the following (42.85% TT, 31.43% TG, and 25.72% GG), The T-allele and G-allele frequencies are (0.59) and (0.41) in the group (B) cases respectively, The genotype distribution in a group (C) patients is as the following (31.32% TT, 34.34% TG, and 34.34% GG), The T-allele and G-allele frequencies are (0.49) and (0.51) in the group (C) cases respectively, The genotype distribution in the group (D) patients is as the following (81.00% TT, 0% TG, and 19.00% GG), with an allele frequency of (0.81) for G-allele and (0.19) for

T-allele. And found (67.5% TT, 5.00% TG, and 27.5% GG) genotypes carried in the group (E) controls, The T-allele and G-allele frequencies are (0.7) and (0.3) in the group (E) control respectively. All demographic characteristics of the patients and healthy control and associated risk factors for COVID-19 and bacterial Pneumonia were demonstrated in Table (4-11).

The association of HLA-C rs1071650 polymorphism with COVID-19 and bacterial pneumonia was evaluated in cases with disease versus healthy controls (HC) using allele, wild type, Mutant heterozygous, and Mutant homozygous. The frequency of the T allele was significantly lower in cases than in apparently healthy controls (OR=0.54; 95% CI =0.31 to 0.93 and $P \leq 0.02$). In the wild type, the TT genotype showed a significantly decreased frequency in cases compared to apparently healthy controls (OR = 0.37; 95% CI =0.17 to 0.77; and $P \leq 0.008$). In the Mutant heterozygous, the frequency of cases with the genotypes TG and GG was significantly elevated compared to HC (OR = 6.46; 95% CI = 1.47 to 28.21 and $P \leq 0.01$). These results indicate that the carriers of alleles associated with a higher background level of the T and G transcript are at a higher risk for pneumonia development.

Table (4-11): Distributions of genotypes and allele frequencies in HLA-C rs1071650 genotype SNPs in the study population.

rs1071650 genotypes		Severe COVID-19 n=37 (%)	Severe COVID-19 + Pneumonia n=35 (%)	Non-Severe COVID-19 n=35 (%)	Non-Severe COVID-19 + Pneumonia n=27(%)	Healthy Control n=40 (%)	P-Value	OR	CL=95%
TT (wild type)		10(27.00)	15(42.85)	11(31.32)	22(81.00)	27(67.5)	0.008	0.37	0.17 to 0.77
TG (Mutant heterozygous)		11(29.73)	11(31.43)	12(34.34)	0	2(5.00)	0.01	6.46	1.47 to 28.21
GG (Mutant homozygous)		16(43.27)	9(25.72)	12(34.34)	5(19.00)	11(27.5)	0.02	1.2	1.57to 2.66
Alleles (%)	T	31(0.42)	41(0.59)	34(0.49)	44(0.81)	56(0.7)	0.02	0.54	0.31 to 0.93
	G	43(0.58)	29(0.41)	36(0.51)	10(0.19)	24(0.3)			

OR=Odd ratio, Cl (95%)=confidence interval, p-value ≤0.05 calculated for estimation of significant difference of the patient's genotypes and alleles.

D-HLA-C rs9264668 genotypes SNPs

The fourth HLA-C rs9264668 genotypes SNPs and the results of the following (18.9% CC, 32.4% CA, and 48.7% AA) as the genotype distribution in the group (A) patients, with C-allele frequency (0.35) and A-allele frequency (0.65). The genotype distribution in group (B) patients was (42.85% CC, 25.71% CA, and 31.44% AA), with C-allele frequency (0.56), and A-allele frequency (0.44). the results of the following (22.85% CC, 31.42% CA, and 45.73% AA) as the genotype distribution in group (C) patients, with C-allele frequency (0.38) and A-allele frequency (0.62). The genotype distribution in group (D) patients was (70.00% CC, 0% CA, and 30.00% AA), with C-allele frequency (0.7), and A-allele frequency (0.3) and the results of the following (60.00% CC, 12.5% CA, and 27.5% AA) as the genotype distribution in group (E) controls, with a C-allele frequency (0.66)

and an A-allele frequency (0.34). These results indicate that there is a relationship between HLA-C rs9264668 genotypes SNPs and infection severity in the study population as shown in Table (4-12).

The frequency of the C allele was significantly lower in cases than in HC (OR=0.48; 95% CI =0.28 to 0.80 and P ≤0.005). In the wild type, the CC genotype in all patients and Mutant homozygous, the AA genotype in COVID-19 patients showed a significantly decreased frequency compared to HC (OR = 0.38; 95% CI =0.18 to 0.79 and P ≤ 0.009). In the Mutant heterozygous, the frequency of cases with the genotypes CA and AA in bacterial pneumonia was not significantly elevated compared to HC (OR = 2.19; 95% CI = 0.79 to 6.07 and P ≤ 0.1) these results indicate that the carriers of alleles associated with a higher background level of the C and A transcript are at a higher risk for pneumonia development.

Table (4-12): Distributions of genotypes and allele frequencies in HLA-C rs9264668 genotypes SNPs in the Study population.

rs9264668 genotypes		Severe COVID-19 n=37 (%)	Severe COVID-19 + Pneumonia n=35 (%)	Non-Severe COVID-19 n=35 (%)	Non-Severe COVID-19 + Pneumonia n=27(%)	Healthy Control n=40 (%)	P-Value	OR	CL=95%
CC (wild type)		7(18.9)	15(42.85)	8(22.85)	19(70.00)	24(60.00)	0.009	0.38	0.18 to 0.79
CA (Mutant heterozygous)		12(32.4)	9(25.71)	11(31.42)	0	5(12.5)	0.1	2.19	0.79 to 6.07
AA (Mutant homozygous)		18(48.7)	11(31.44)	16(45.73)	8(30.00)	11(27.5)	0.1	1.7	0.79 to 3.74
Alleles (%)	C	26(0.35)	39(0.56)	27(0.38)	38(0.7)	53(0.66)	0.005	0.48	0.28 to 0.80
	A	48(0.65)	31(0.44)	43(0.62)	16(0.3)	27(0.34)			

OR=Odd ratio, Cl (95%)=confidence interval, p-value ≤0.05 calculated for estimation of significant difference of the patient's genotypes and alleles.

HLA class I is a critical component of the viral antigen presentation pathway and confer differential viral susceptibility and severity of the disease (Rosendahl Huber *et al*.,2014). Recent studies have shown that SARSCoV- 2-specific CD8+ T cells can be detected in peripheral blood mononuclear cells of COVID- 19 patients,16–19 suggesting that some SARS- CoV- 2- derived peptides can be naturally processed in human cells, presented on HLA class I molecules and induce SARS- CoV- 2- specific cytotoxic CD8+ T cells in vivo. Accumulating evidence highlights the importance of investigating associations between HLA variation and the development and/or severity of COVID- 19 (Nguyen *et al.*,2020).

A higher frequency of HLA-C*01 has been found in patients with acute viral encephalitis than in control subjects (Tuttolomondo et al.,2018). Numerous studies have reported alleles of protection or susceptibility to COVID-19. A study conducted with patients found that the HLA-C) alleles were more frequently detected in the COVID-19 group than in the control population (Wang *et al.*,2020).

Interestingly, found certain HLA genetic polymorphisms that could increase the risk of death in patients with COVID-19. Previously, regarding prognosis, the presence of HLA-C*16 increases the risk of rapid progression in patients with Acquired Immune Deficiency Syndrome. In addition, the HLA allele associated with mortality (HLA-C*01), previously was associated with poor evolution in other infectious diseases, or with the risk of other infectious diseases (Dutta *et al*.,2018).

The possibility of using large datasets for which SNP genotypes are available to infer the HLA alleles is attractive on several grounds. First, it allows existing genetic data, often generated without the goal of characterizing HLA variation, to be used to gain a better understanding of these genes. second

it implicitly allows the imputation of genetic variation at a high molecular level of resolution. For example, the inference of a second-field level HLA allele implies that amino acid residues at specific positions can be inferred and have their effects tested upon phenotypes of interest. Because there is a priori evidence that the variation at specific amino acids can contribute in a statistically significant way to disease phenotypes, obtaining data at this level of resolution is an important methodological resource as will be discussed in the next section (Sanchez-Mazas and Meyer 2014).

At the beginning of the outbreak of the COVID-19 pandemic in Europe in the spring of 2020, an international collaboration of European centers was established to address the question of whether there were potential genetic host factors associated with the severe clinical course of SARS-CoV-2 infection.

HLA-C is a potential risk allele, which was associated with increased risk factors when present in the form of at least one allele. This is the first significant description of this HLA allele as a relevant allele for the severe clinical course of SARS-CoV in Asia. Specifically, it has been shown that in patients infected with HIV, HLA-C in combination with polymorphisms is associated with high viral load and severe clinical course (Iturrieta-Zuazo *et al.*,2020). Moreover, HLA-C has been reported to occur at a higher frequency in COVID-19 patients than in the healthy population (Littera *et al.*,2020).

Chandran *et al.*,(2014) showed that genotypes positive for the activating KIR2DS2 in the absence of HLA-C 1 ligands for homologous inhibitory KIR2DL2/2DL3 conferred a significantly higher risk of prostate-specific antigen (PSA). The study findings support a possible role for NK cells and innate immunity in the pathogenesis of PSA. Notably, these investigators found an increased risk for PSA with high-expression HLA-C alleles. The

contribution of the genetic components, including the HLA system, to COVID-19 susceptibility, has been shown in many genetic studies (Pojero *et al.*,2021).

Results obtained from an ethnically homogenous group suggest that patients who were homozygous for the HLA-C allele were at higher risk of severe COVID-19. Similar results have also been shown in multicentre studies in Europe (Littera *et al*.,2020 and Weiner *et al.*,2021).

4.4.3.Hardy-Weinberg equilibrium analysis

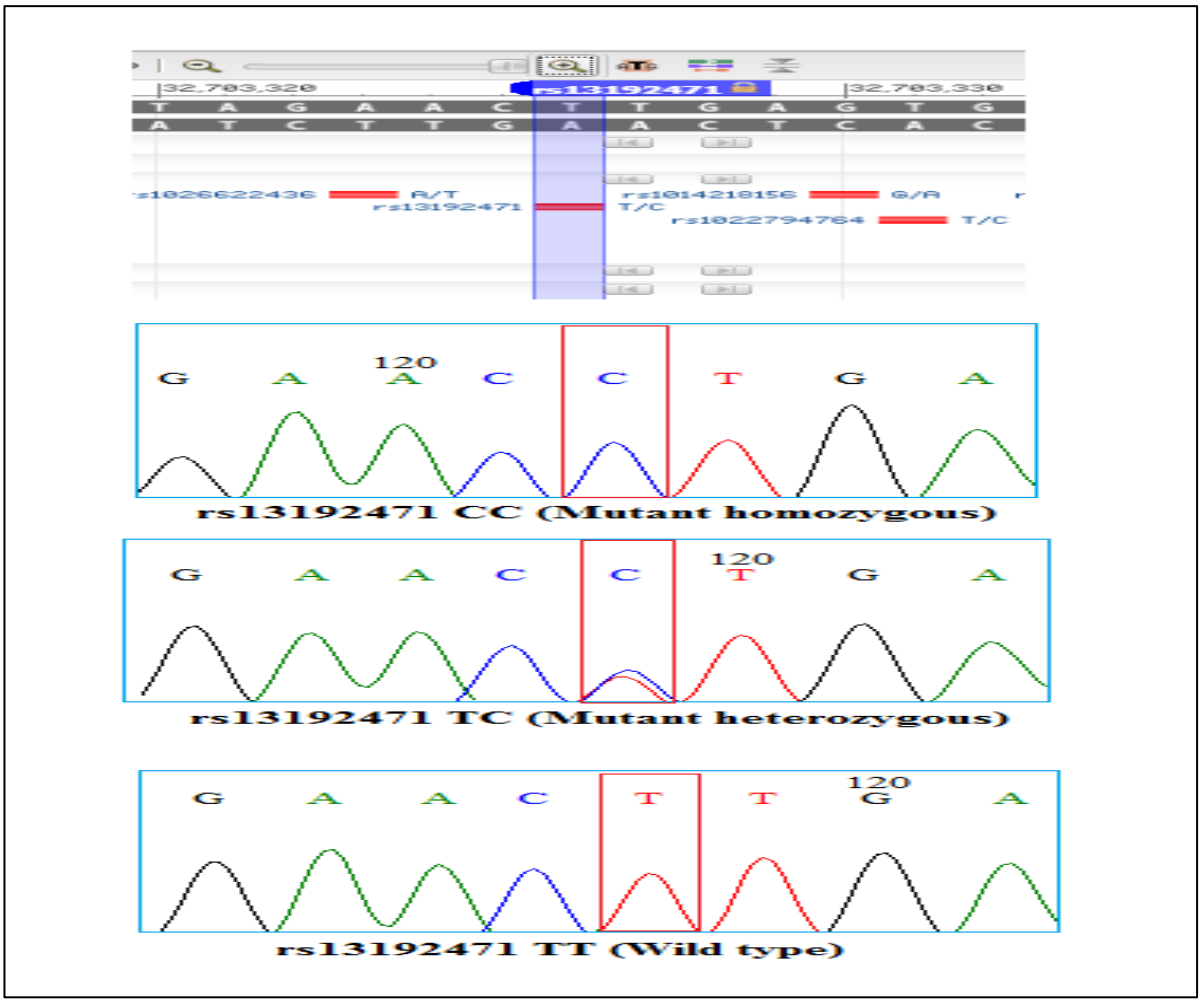
Analysis of Hardy-Weinberg equilibrium (HWE) demonstrated that the three genotypes of HLA-DRB1 GG (wild type), GA (Mutant heterozygous), and AA (Mutant homozygous) polymorphism deviated significantly from HWE in patients and healthy controls (HC), as significant variations were found between observed and expected frequencies (Table 4-13).

Table (4-13): Hardy-Weinberg equilibrium analysis of HLA-DRB1 GG, GA, and AA polymorphism in the study population

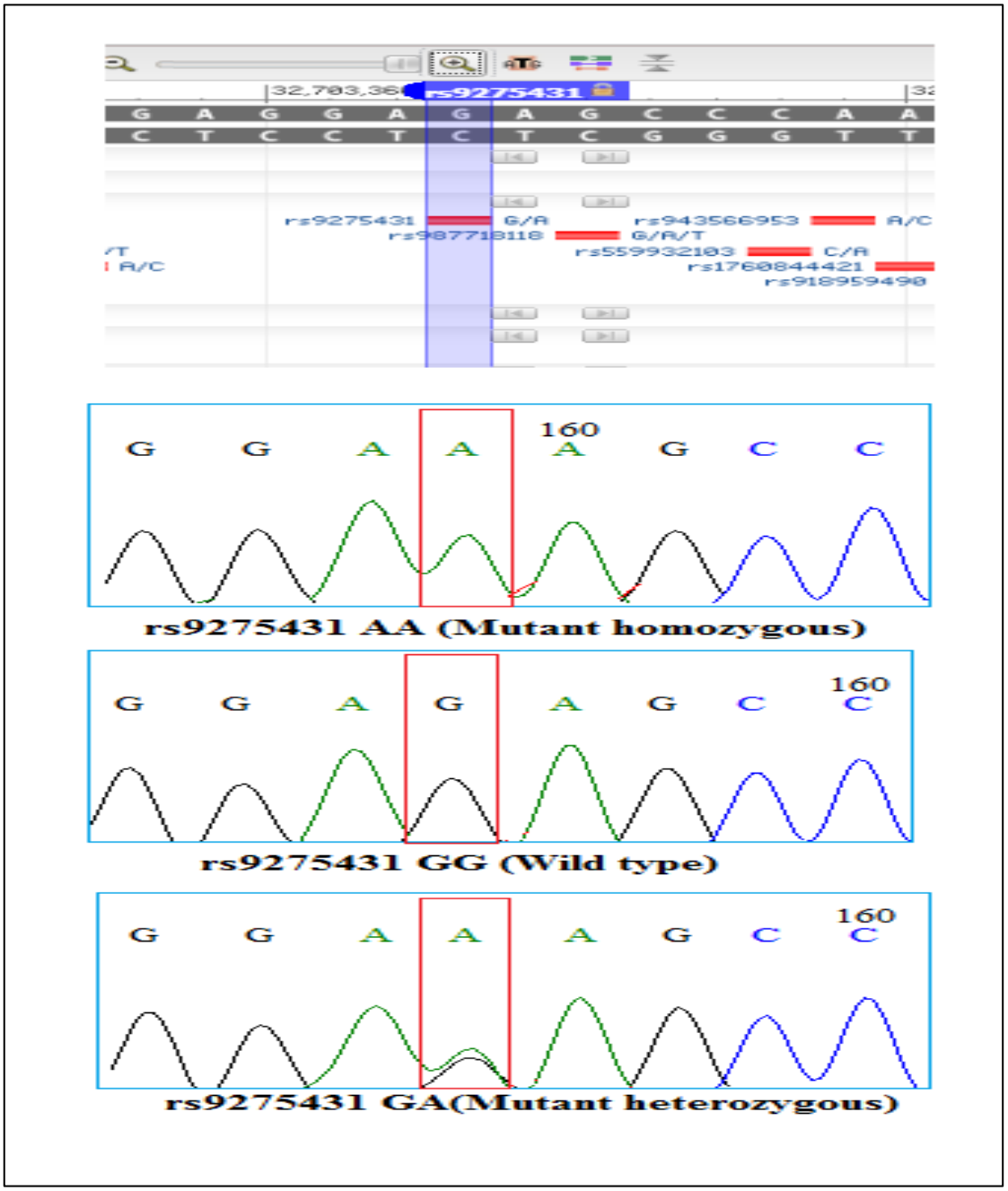
Genotype	Cases (N = 66)				HC (N = 27)			
	Observed		Expected		Observed		Expected	
	N	%	N	%	N	%	N	%
GG (wild type)	44	66.66	39.4	59.68	16	59.25	14.1	52.22
GA (Mutant heterozygous)	14	21.21	23.2	35.11	7	25.52	10.8	40.0
AA (Mutant homozygous)	8	12.13	3.4	5.21	4	15.23	2.1	7.78
HWE p-value	0.001				0.06			

4.4.4.Association of HLA-DRB1 a Single Nucleotide Polymorphisms (SNPs) and Risk of Patients and Healthy Controls

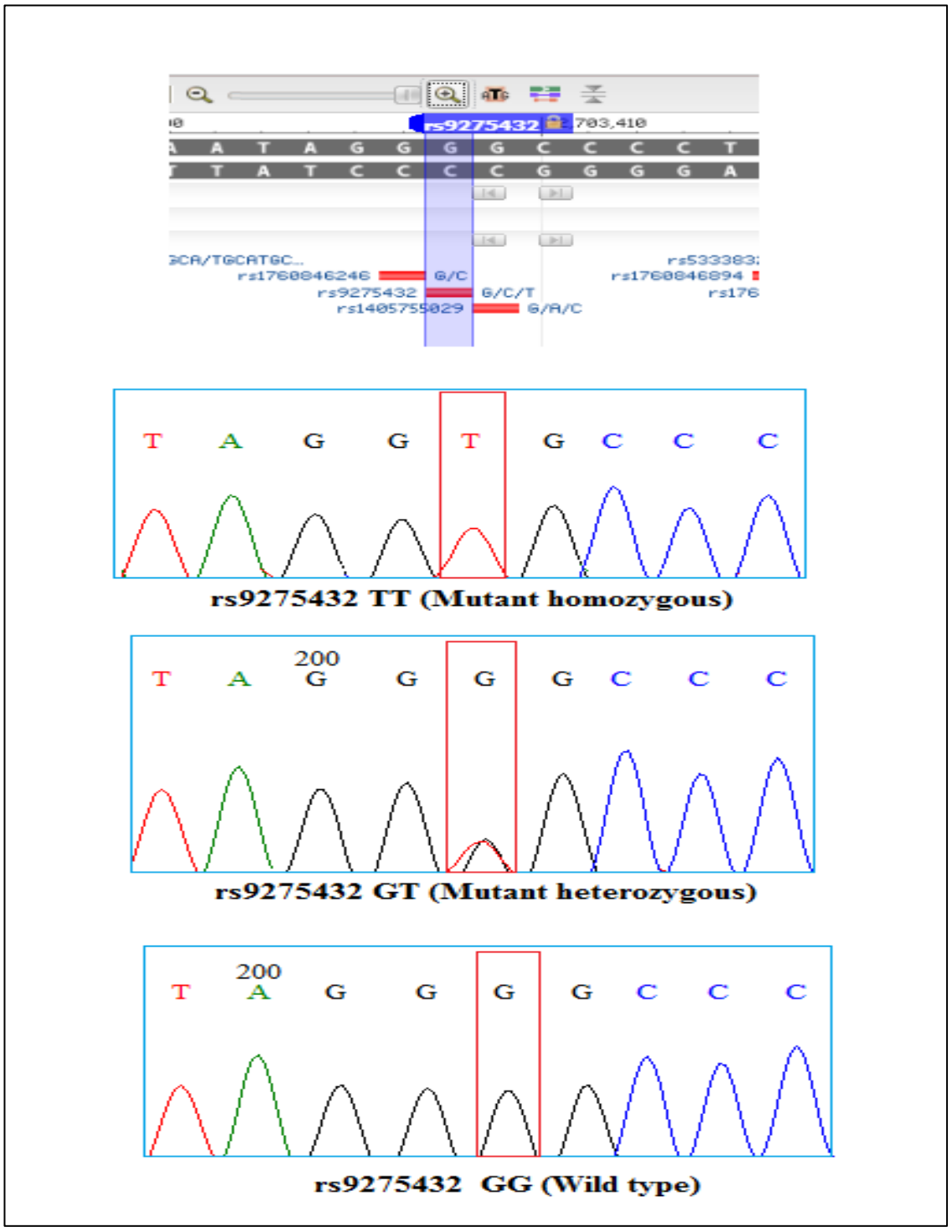
Trimming of the HLA-DRB1 PCR product's Sanger sequencing data. To determine the genotype variations between (COVID-19 and Bacterial Pneumonia) patients and healthy controls, multiple alignments for each were created using Finch TV version 1.4.0 (Geospiza, Inc.; Seattle, WA, USA; (http://www.geospiza.com)) and compared with the NCBI database. Figure (4-11), (4-12), (4-13), and figure (4-14) show SNP distributions on the HLA-DRB1 genes.



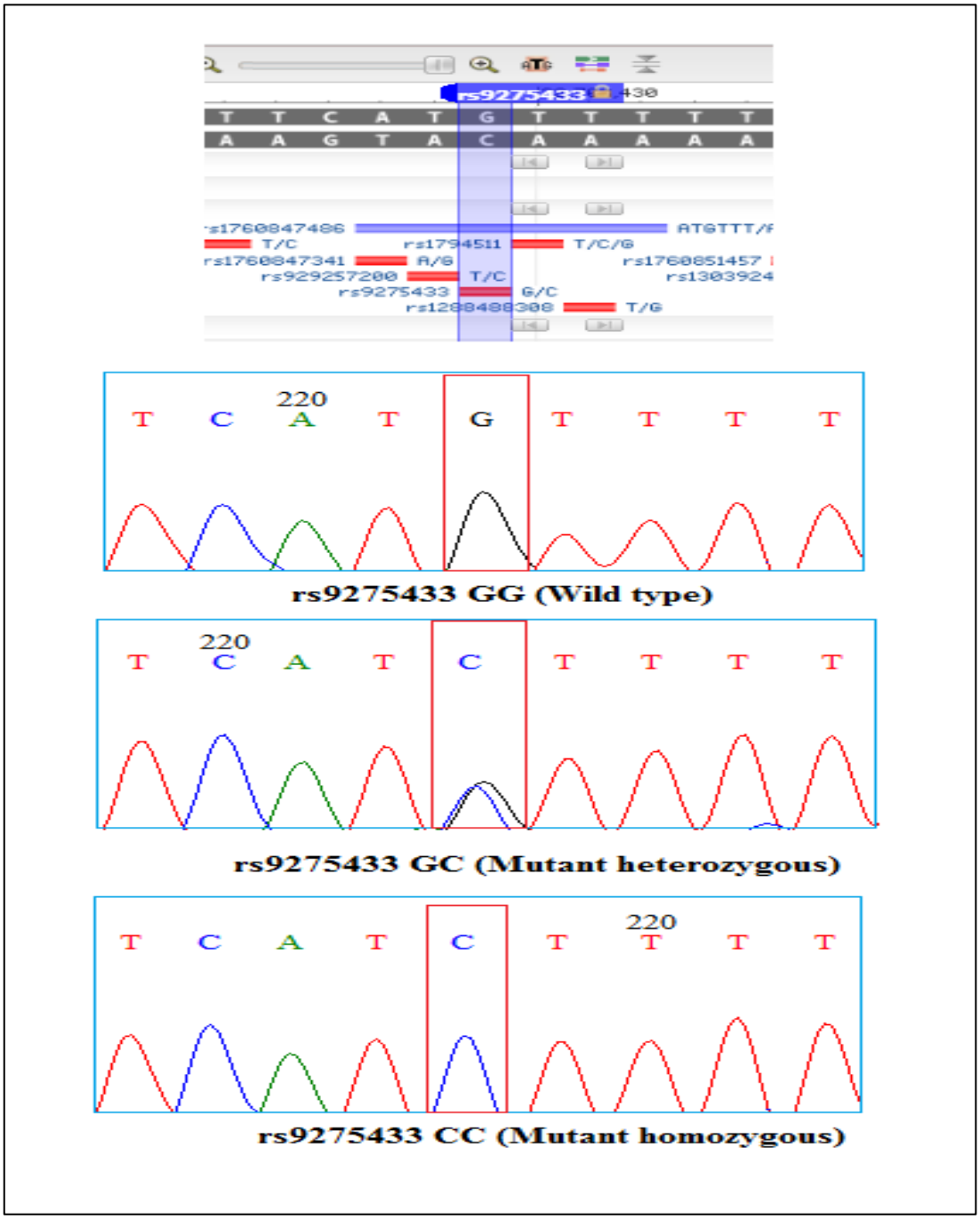
Fig(4-11): Distributions of rs13192471 genotypes SNPs on HLA-DRB1 gene in study population



Fig(4-12): Distributions of rs9275431 genotypes SNPs on HLA-DRB1 gene in study population



Fig(4-13): Distributions of rs9275432 genotypes SNPs on HLA-DRB1 gene in study population



Fig(4-14): Distributions of rs9275433 genotypes SNPs on HLA-DRB1 gene in study population

A-HLA-DRB1 rs13192471 SNPs

HLA-DRB1 rs13192471 genotypes SNPs, by using this study's first SNP, it was discovered (86.36% TT, 0% TC, and 13.64% CC) genotype carries in the group (A) patients, with an allele frequency of (0.86) for T-allele and (0.14) for C-allele. The genotype distribution in group (B) patients is as the following (85.35% TT, 14.29% TC, and 0% CC), The T-allele and C-allele frequencies are (0.93) and (0.07) respectively. The genotype distribution in group (C) patients is as the following (82.35% TT, 0% TC, and 17.65% CC), The T-allele and C-allele frequencies are (0.82) and (0.18) respectively. With (76.92% TT, 23.08% TC, and 0% CC genotypes carried in group (D) patients, with an allele frequency of (0.88) for T- allele and (0.12) for C-allele, and The genotype distribution in the group (E) are as the following (100% TT, 0% TC, and 0% CC, the T-allele and C-allele frequencies are (1) and (0) in the healthy controls respectively.

According to these findings, the frequency of the T allele was no significantly lower in COVID-19 and Bacterial Pneumonia compared to healthy controls (HC) ($P \leq 0.06$). Such deviation scored an OR value of (0.0.09) (95% CI: 0.003 to 1.02). In the HLA-DRB1 rs13192471Wild type, the TT genotype showed no significantly decreased frequency in all cases compared to healthy control (HC) (P-Value ≤0.09; OR = 0.0.08; 95% CI =0.005 to 1.54). In the Mutant heterozygous (TC), the frequency of all cases with the genotypes TC was no significantly elevated in bacterial pneumonia and had no effect in COVID-19 compared to healthy control (HC) (P-Value ≤0.2; OR = 4.91; 95% CI =0.26 to 92.09). In the HLA-DRB1 rs13192471 Mutant homozygous, the CC genotype showed no significantly decreased frequency in COVID-19 patients and no effect bacterial Pneumonia patients compared to healthy control (HC) (P-Value=0.2; OR = 5.90; 95% CI =0.32 to 108.64). These results indicate that there is a relationship between HLA-DRB1

rs13192471 SNPs and infection severity in the study population as shown in (Table 4-14).

Table (4-14): Distributions of genotypes and allele frequencies in HLA-DRB1 rs13192471 genotypes SNPs in the Study population.

rs13192471 genotypes		Severe COVID-19 n=22 (%)	Severe COVID-19 + Pneumonia n=14 (%)	Non-Severe COVID-19 n=17 (%)	Non-Severe COVID-19 + Pneumonia n=13(%)	Healthy Control n=27 (%)	P-Value	OR	CL=95%
TT (wild type)		19(86.36)	12(85.71)	14(82.35)	10(76.92)	27(100)	0.09	0.08	0.005 to 1.54
TC (Mutant heterozygous)		0	2(14.29)	0	3(23.08)	0	0.2	4.91	0.26 to 92.09
CC (Mutant homozygous)		3(13.64)	0	3(17.65)	0	0	0.2	5.90	0.32 to 108.64
Alleles (%)	T	38(0.86)	26(0.93)	28(0.82)	23(0.88)	54(1)	0.06	0.09	0.003 to 1.02
	C	6(0.14)	2 (0.07)	6(0.18)	3(0.12)	0			

OR=Odd ratio, Cl (95%)=confidence interval, p-value ≤0.05 calculated for estimation of significant difference of the patient's genotypes and alleles.

B-HLA-DRB1 rs9275431 SNPs

HLA-DRB1 rs9275431genotypes SNPs, By using this study's second SNP, it was discovered (90.91% GG, 0% GA, and 9.09% AA) genotype carries in the group (A) patients, with an allele frequency of (0.9) for G-allele and (0.1) for A-allele. The genotype distribution in group (B) patients is as the following (57.14% GG, 42.86% GA, and 0% AA), The G-allele and A-allele frequencies are (0.76) and (0.24) respectively. The genotype distribution in group (C) patients is as the following (41.17% GG, 35.29% GA, and 23.54% AA), The G-allele and A-allele frequencies are (0.61) and (0.39) respectively. With (69.23% GG, 15.32% GA, and 15.45% AA genotype carried in group (D) patients, with an allele frequency of (0.77) for G- allele and (0.23) for A-allele,

and the genotype distribution in group (E) was as the following (59.25% GG, 25.92% GA, and 14.83% AA, the G-allele and A-allele frequencies are (0.66) and (0.34) in the healthy controls respectively.

According to these findings, the frequency of the G allele was not significant in COVID-19 and Bacterial Pneumonia compared to healthy controls (HC) (P ≤ 0.1). Such deviation scored an OR value of (1.79) (95% CI: 0.85 to 3.77). In the HLA-DRB1 rs9275431 Wild type, the GG genotype showed no significantly decreased frequency in all cases but increased in group (A) patients compared to healthy control (HC) (P-Value ≤0.4; OR = 1.37; 95% CI =0.54 to 3.45). In the Mutant heterozygous (GA), the frequency of all cases with the genotypes GA was not significantly decreased in all cases compared to healthy control (HC) (P-Value ≤0.6; OR = 0.76; 95% CI =0.27 to 2.18). In the HLA-DRB1 rs9275431 Mutant homozygous, the AA genotype showed a significantly decreased frequency in COVID-19 patients and bacterial Pneumonia patients compared to healthy control (HC) (P-Value=0.000; OR = 41.68; 95% CI =11.43 to 152.01). These results indicate that there is a relationship between HLA-DRB1 rs13192471 SNPs and infection severity in the study population as shown in (Table 4-15).

Table (4-15): Distributions of genotypes and allele frequencies in HLA-DRB1 rs9275431 genotypes SNPs in the Study population.

rs9275431 genotypes		Severe COVID-19 n=22 (%)	Severe COVID-19 + Pneumonia n=14 (%)	Non-Severe COVID-19 n=17 (%)	Non-Severe COVID-19 + Pneumonia n=13(%)	Healthy Control n=27 (%)	P-Value	OR	CL=95%
GG (wild type)		20(90.91)	8(57.14)	7(41.17)	9(69.23)	16(59.25)	0.4	1.37	0.54 to 3.45
GA (Mutant heterozygous)		0	6(42.86)	6(35.29)	2(15.32)	7(25.92)	0.6	0.76	0.27 to 2.18
AA (Mutant homozygous)		2(9.09)	0	4(23.54)	2(15.45)	4(14.83)	0.000	41.68	11.43 to 152.01
Alleles (%)	G	40(0.9)	22(0.76)	22(0.61)	20(0.77)	29(0.66)	0.1	1.79	0.85 to 3.77
	A	4(0.1)	6(0.24)	14(0.39)	6(0.23)	15(0.34)			

OR=Odd ratio, Cl (95%)=confidence interval, p-value ≤0.05 calculated for estimation of significant difference of the patient's genotypes and alleles.

C-HLA-DRB1 rs9275432 SNPs

The third HLA-DRB1 rs9275432 genotypes SNPs, and the results were (72.72% GG, 27.28% GT, and 0% TT) genotype carries in the group (A) patients, with an allele frequency of (0.86) for G-allele and (0.14) for T-allele. The genotype distribution in the group (B) patients is as the following (85.71% GG, 14.29% GT, and 0% TT), The G-allele and T-allele frequencies are (0.93) and (0.07) in the group (B) cases respectively. The genotype distribution in the group (C) patients is as the following (47.05% GG, 23.52% GT, and 29.43% TT), the G-allele and T-allele frequencies are (0.59) and (0.41) in the group (C) cases respectively. The genotype distribution in the group (D) patients is as the following (76.92% GG, 0% GT, and 23.08% TT), with an allele frequency of (0.77) for G-allele and (0.23) for T-allele. And found (100% GG, 0% GT, and

0% TT) genotype carried in the group (E) controls, The G-allele and T-allele frequencies are (0.1) and (0) in the group (E) control respectively. All demographic characteristics of the patients and healthy control and associated risk factors for COVID- 19 and bacterial pneumonia were demonstrated in Table (4-16).

The association of HLA-DRB1 rs9275432 polymorphism with COVID-19 and bacterial pneumonia was evaluated in cases with disease versus healthy controls (HC) using allele, Wild type, Mutant heterozygous, and Mutant homozygous. The frequency of the G allele was significantly lower in cases than in HC (OR=0.03; 95% CI =0.002 to 0.56 and $P \leq 0.01$). In the Wild type, the GG genotype showed a significantly decreased frequency in cases compared to HC (OR = 0.04; 95% CI =0.002 to 0.70; and $P \leq 0.02$). In the Mutant heterozygous, the frequency of cases with the genotype GT was significantly elevated compared to HC (OR = 239.8; 95% CI = 13.68 to 4202.74 and $P \leq 0.000$), In the Mutant homozygous, the frequency of cases with the genotypes TT was significantly elevated compared to HC (OR = 378.5; 95% CI = 21.07 to 6797.35 and $P \leq 0.000$). these results indicate that the Mutant heterozygous and the Mutant homozygous are a higher risk factors for COVID-19 and bacterial pneumonia development.

Table (4-16): Distributions of genotypes and allele frequencies in HLA-DRB1 rs9275432 genotypes SNPs in the Study population.

rs9275432 genotypes		Severe COVID-19 n=22 (%)	Severe COVID-19 + Pneumonia n=14 (%)	Non-Severe COVID-19 n=17 (%)	Non-Severe COVID-19 + Pneumonia n=13(%)	Healthy Control n=27 (%)	P-Value	OR	CL=95%
GG (wild type)		16(72.72)	12(85.71)	8(47.05)	10(76.92)	27(100)	0.02	0.04	0.002 to 0.70
GT (Mutant heterozygous)		6(27.28)	2(14.29)	4(23.52)	0	0	0.000	239.8	13.68 to 4202.74
TT (Mutant homozygous)		0	0	5(29.43)	3(23.08)	0	0.000	378.5	21.07 to 6797.35
Alleles (%)	G	38(0.86)	26(0.93)	20(0.59)	20(0.77)	54(1)	0.01	0.03	0.002 to 0.56
	T	6(0.14)	2 (0.07)	14(0.41)	6(0.23)	0			

OR=Odd ratio, Cl (95%)=confidence interval, p-value ≤0.05 calculated for estimation of significant difference of the patient's genotypes and alleles.

D-HLA-DRB1 rs9275433 SNPs

The fourth HLA-DRB1rs9275433 genotypes SNPs and the results of the following (90.91% GG, 0% GC, and 9.09% CC) as the genotype distribution in the group (A) patients, with G-allele frequency (0.9) and C-allele frequency (0.1). The genotype distribution in group (B) patients was (64.28% GG, 35.72% GC, and 0% CC), with G-allele frequency (0.82), and C-allele frequency (0.18). The results of the following (47.06% GG, 35.31% GC, and 17.63% CC) as the genotype distribution in group (C) patients, with G-allele frequency (0.65) and C-allele frequency (0.35). The genotype distribution in the group (D) patients was (69.23% GG, 15.37% GC, and 15.40% CC), with G-allele frequency (0.77), and C-allele frequency (0.23) and the results of the following (81.48% GG, 0% GC, and 18.52% CC) as the genotype distribution

in group (E) healthy controls, with a G-allele frequency (0.8) and a C-allele frequency (0.2). These results indicate that there is a relationship between HLA-DRB1 rs9275433 genotypes SNPs and infection severity in the study population as shown in Table (4-17).

The frequency of the G allele was significantly lower in cases than in HC (OR=0.40; 95% CI =0.18 to 0.86 and P ≤0.01). In the Wild type, the GG genotype in all patients showed no significantly decreased frequency compared to HC (OR = 0.52; 95% CI =0.17 to 1.57 and P ≤ 0.2). In the Mutant heterozygous, the frequency of cases with the genotypes GC was no significantly decreased compared to HC (OR = 13.8; 95% CI =0.79 to 242.32 and P ≤ 0.07), In the Mutant homozygous, the frequency of cases with the genotypes CC was no significantly decreased compared to HC (OR = 0.52; 95% CI =0.14 to 1.81, and P ≤ 0.3). these results indicate that the Mutant heterozygous was a higher risk factor for COVID-19 and bacterial pneumonia development.

Table (4-17): Distributions of genotypes and allele frequencies in HLA-DRB1 rs9275433 genotypes SNPs in the Study population.

rs9275433 genotypes		Severe COVID-19 n=22 (%)	Severe COVID-19 + Pneumonia n=14 (%)	Non-Severe COVID-19 n=17 (%)	Non-Severe COVID-19 + Pneumonia n=13(%)	Healthy Control n=27 (%)	P-Value	OR	CL=95%
GG (wild type)		20(90.91)	9(64.28)	8(47.06)	9(69.23)	22(81.48)	0. 2	0.52	0.17 to 1.57
GC (Mutant heterozygous)		0	5(35.72)	6(35.31)	2(15.37)	0	0.07	13.8	0.79 to 242.32
CC (Mutant homozygous)		2(9.09)	0	3(17.63)	2(15.40)	5(18.52)	0.3	0.52	0.14 to 1.81
Alleles (%)	G	40(0.9)	23(0.82)	22(0.65)	20(0.77)	44(0.8)	0.01	0.40	0.18 to 0.86
	C	4 (0.1)	5(0.18)	12(0.35)	6(0.23)	10(0.2)			

OR=Odd ratio, Cl (95%)=confidence interval, p-value ≤0.05 calculated for

COVID-19 is an acute respiratory disease caused by SARSCoV2 infection and has a wide range of clinical manifestations and severity; ranging from asymptomatic carriers and mild flulike symptoms to severe respiratory distress and death (Castelli *et al*.,2021; Adli *et al*.,2022 and Kim, and Jeong 2020). HLA class I and II genetic variants are implicated in the modulation of immune response to diverse viral antigens (Hammer *et al*.,2015 and Tian *et al.,2017).*

Kachuri, L *et al*., (2020) have stated that HLA-II controls the landscape of antiviral humoral immune response, They considered HLA-DRB1 as a crucial genetic factor governing host vulnerability to viral infections. The scientific literature on the association of HLA allelic repertoire with COVID- 19 clinical severity has rapidly expanded in the past two years. Many studies showed accordant or discordant results about HLA alleles that were protective or resistant to SARS- CoV- 2 infection. Furthermore, these studies often described the allelic repository within a unique cultural cohort to minimize genotypic heterogeneity. Previous genome-wide association studies have identified in the human leukocyte antigen (HLA) class II region multiple independent SNPs that are significantly associated with COVID-19 and bacterial pneumonia risk.

Poulton *et al*.,(2020) Reported a significant positive association between HLA-DRB1*15:01 and COVID-19 disease in the UK population. The study showed an impaired presentation of viral epitopes in those COVID-19 patients carrying HLA-DRB1*15:01 and HLA-DRB1*10 alleles. This, in turn, may prompt an inefficient T cell response and consequently inadequate antibody response, which is required for viral neutralization and elimination in synergy with T cell-mediated immunity. These findings are consistent with those previously published by (Kachuri *et al.*,2020) and other studies,

who identified DRB1 as a significant genetic determinant influencing several diseases and especially viral infections susceptibilities such as Epstein Barr virus (EBV), Varicella zoster virus (VZV), human herpesvirus 7, (HHV7), and Merkel cell polyomavirus (MCV) (Wang *et al*.,2020; Alves *et al.*,2006, and Kachuri *et al*.,2020).

The association between COVID-19 disease and the HLA-DRB1*15 allele was also observed in the Iranian population and indeed the allele frequency of HLA-DRB1*15 was found higher in patients with lymphopenia compared to those cases without lymphopenia. It has been reported that this clinical outcome was likely linked to the development of severe and critical COVID-19 disease, suggesting the HLA-DRB1*15 allele is a genetic marker for severe forms of the disease (Ebrahimi *et al.*,2021).

I.Conclusions

The study reached the following conclusions:

1- Pathogenesis of pneumonia causative agents is impacted by HLA-C and HLA-DRB1.

2- HLA-C is involved in the pathogenesis of acute respiratory syndrome coronavirus infection and contributes to severity and death in COVID-19 and bacterial pneumonia patients. but HLA-DRB1 has a protective effect against SARS-CoV-2 and bacterial infection. It is one of the elements in the prevention of coronavirus in people with acute respiratory syndrome.

4-The severity and the outcome of COVID-19 and bacterial pneumonia course of infection depend on the age of patients; moreover, Susceptibility to COVID -19 and bacterial pneumonia may be increased with the presence of chronic diseases,

5- rs13192471 genotypes SNPs, rs9275431genotypes SNPs, rs9275432 genotypes SNPs, and rs9275433 genotypes SNPs on HLA-DRB1 gene was protective factor for COVID-19 patients and bacterial pneumonia.

6-Polymorphism in HLA-C and HLA-DRB1 was significantly associated with the disease risk.

II.Recommendations

The results of this study encouraged the investigator to propose the following recommendations:

1- Detection of other HLA genes such as HLA-A and determining their specificity and their polymorphism.

2- Further studies are required to detect HLA-DRB1 and determine its specificity and its polymorphism to detect if it contributes to the severity of COVID-19 with and without bacterial pneumonia.

References

Abajobir, A. A., Abbafati, C., & Abbas, K. M. (2017). Global, regional, and national age-sex specific mortality for 264 causes of death, 1980–2016: A systematic analysis for the global burden of disease study 2016. Lancet, 390(10100), 1151–1210.

Adli, A., Rahimi, M., Khodaie, R., Hashemzaei, N., & Hosseini, S. M. (2022). Role of genetic variants and host polymorphisms on COVID- 19: From viral entrance mechanisms to immunological reactions. Journal of Medical Virology, 94(5), 1846-1865.

Agarwal, R. K., Kumari, A., Sedai, A., Parmar, L., Dhanya, R., & Faulkner, L. (2017). The case for high-resolution extended 6-loci HLA typing for identifying related donors in the Indian subcontinent. Biology of Blood and Marrow Transplantation, 23(9), 1592–1596.

Al-Bayatee, N. T., & Ad'hiah, A. H. (2021). Soluble HLA-G is upregulated in serum of patients with severe COVID-19. Human Immunology, 82(10), 726-732.

Alves, C., Souza, T., Meyer, I., Toralles, M. B. P., & Brites, C. (2006). Immunogenetics and infectious diseases: special reference to the mayor histocompatibility complex. Brazilian Journal of Infectious Diseases, 10, 122-131.

Ammad Ud Din, M. A. U., & Boppana, L. K. T. (2020). An update on the 2019-nCoV outbreak. American Journal of Infection Control, 48(6), 713.

Amraei, S., Eslami, G., Taherpour, A., & Hashemi, A. (2022). The role of ACT and FOX genes in Klebsiella pneumoniae strains isolated from hospitalized patients. Micro Nano Bio Aspects, 1(2), 18–25.

Anastassopoulou, C., Gkizarioti, Z., Patrinos, G. P., & Tsakris, A. (2020). Human

genetic factors associated with susceptibility to SARS-CoV-2 infection and COVID-19 disease severity. Human Genomics, 14(1), 40.

Andersen, K. G., Rambaut, A., Lipkin, W. I., Holmes, E. C., & Garry, R. F. (2020). The proximal origin of SARS- CoV-2. Nature Medicine, 26(4), 450–452.

Annunziato, G., & Costantino, G. (2020). Antimicrobial peptides (AMPs): A patent review (2015–2020). Expert Opinion on Therapeutic Patents, 30(12), 931–947.

Ascierto, P. A., Fu, B., & Wei, H. (2021). IL-6 modulation for COVID-19: The right patients at the right time? Journal for ImmunoTherapy of Cancer, 9(4).

Astuti, I. (2020). Severe acute respiratory syndrome coronavirus 2 (SARS- CoV-2): An overview of viral structure and host response. Diabetes and Metabolic Syndrome: Clinical Research and Reviews, 14(4), 407–412.

Attiyah, S. M., Elsayed, H. M., Al-Mughales, J. A., Moharram, A. B., & Abdel-Fattah, M. (2021). Critical cases of COVID-19 patients can be predicted by the biomarkers of complete blood count. Indian Journal of Science and Technology, 13(48), 4739-4745.

Azkur, A. K., Akdis, M., Azkur, D., Sokolowska, M., van de Veen, W., Brüggen, M. C., O'Mahony, L., Gao, Y., Nadeau, K., & Akdis, C. A. (2020). Immune response to SARS-CoV-2 and mechanisms of immunopathological changes in COVID-19. Allergy, 75(7), 1564–1581.

Babapoor-Farrokhran, S., Gill, D., Walker, J., Rasekhi, R. T., Bozorgnia, B., & Amanullah, A. (2020). Myocardial injury and COVID-19: Possible mechanisms. Life Sciences, 253, 117723.

Bahar, A. A., & Ren, D. (2013). Antimicrobial peptides.

Pharmaceuticals, 6(12), 1543–1575.

Barquera, R., Collen, E., Di, D., Buhler, S., Teixeira, J., Llamas, B., Nunes, J. M., & Sanchez-Mazas, A. (2020). Binding affinities of 438 HLA proteins to complete proteomes of seven pandemic viruses and distributions of strongest and weakest HLA peptide binders in populations worldwide. HLA, 96(3), 277–298.

Baucells, B. J., Mercadal Hally, M., Álvarez Sánchez, A. T., & Figueras Aloy,J. (2015). Asociaciones de probióticos para la prevención de la enterocolitis necrosante y la reducción de la sepsis tardía y la mortalidad neonatal en recién nacidos pretérmino de menos de 1.500g: Una revisión sistemática. Anales de Pediatría, 85(5), 247–255.

Bengoechea, J. A., & Sa Pessoa, J. (2019). Klebsiella pneumoniae infection biology: Living to counteract host defences. FEMS Microbiology Reviews, 43(2), 123–144.

Benlyamani, I., Venet, F., Coudereau, R., Gossez, M., & Monneret, G. (2020). Monocyte HLA-DR measurement by flow cytometry in COVID-19 patients: An interim review. Cytometry. Part A, 97(12), 1217–1221.

Bian, Q & Fu, B. (2022). Immunological microenvironment at the maternal-fetal interface. Journal of Reproductive Immunology, 151, 103632.

Brima, Y., Atemkeng, M., Tankio Djiokap, S., Ebiele, J., & Tchakounté, F. (2021). Transfer learning for the detection and diagnosis of types of pneumonia including pneumonia induced by COVID-19 from chest X-ray images. Diagnostics, 11(8), 1480.

Brittona, L. (2019). Bovine respiratory disease. All-island animal disease surveillance report, 36.

References

Brooks, L. R. K., & Mias, G. I. (2018). Streptococcus pneumoniae's virulence and host immunity: Aging, diagnostics, and prevention. Frontiers in Immunology, 9, 1366.

Castelli, E. C., De Castro, M. V., Naslavsky, M. S., Scliar, M. O., Silva, N. S., Andrade, H. S., ... & Zatz, M. (2021). MHC variants associated with symptomatic versus asymptomatic SARS-CoV-2 infection in highly exposed individuals. Frontiers in Immunology, 12, 742881.

Chavez, S., Long, B., Koyfman, A., & Liang, S. Y. (2021). Coronavirus Disease (COVID-19): A primer for emergency physicians. American Journal of Emergency Medicine, 44, 220–229.

Chen, G., Wu, D. I., Guo, W., Cao, Y., Huang, D., Wang, H., ... & Ning, Q. (2020). Clinical and immunological features of severe and moderate coronavirus disease 2019. The Journal of clinical investigation, 130(5), 2620-2629.

Chen, N., Zhou, M., Dong, X., Qu, J., Gong, F., Han, Y., Qiu, Y., Wang, J., Liu, Y., Wei, Y., Xia, J., Yu, T., Zhang, X., & Zhang, L. (2020). Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: A descriptive study. Lancet, 395(10223), 507–513. .

Chen, W., Wang, Q., Li, Y. Q., Yu, H. L., Xia, Y. Y., Zhang, M. L., ... & Feng, Z. J. (2020). Early containment strategies and core measures for prevention and control of novel coronavirus pneumonia in China. Zhonghua yu fang yi xue za zhi [Chinese journal of preventive medicine], 54(3), 239-244.

Chung, E., Subramaniam, G., & Dass, L. C. (2020). Online learning readiness among university students in Malaysia amidst COVID-19. Asian Journal of University Education, 16(2), 45-58.

References

Chandran, V., Bull, S. B., Pellett, F. J., Ayearst, R., Pollock, R. A., & Gladman, D. D. (2014). Killer-cell immunoglobulin-like receptor gene polymorphisms and susceptibility to psoriatic arthritis. Rheumatology, 53(2), 233-239.

Chowell, G., Viboud, C., Hyman, J. M., & Simonsen, L. (2015). The Western Africa ebola virus disease epidemic exhibits both global exponential and local polynomial growth rates. PLoS currents, 7.

Cillóniz, C., Dominedò, C., Pericàs, J. M., Rodriguez-Hurtado, D., & Torres, A. (2020). Community-acquired pneumonia in critically ill very old patients: a growing problem. European Respiratory Review, 29(155).

Conti, P., & Younes, A. (2020). Coronavirus COV-19/SARS-CoV-2 affects women less than men: Clinical response to viral infection. Journal of Biological Regulators and Homeostatic Agents, 34(2), 339–343.

Correale, P., Mutti, L., Pentimalli, F., Baglio, G., Saladino, R. E., Sileri, P., & Giordano, A. (2020). HLA-B* 44 and C* 01 prevalence correlates with Covid19 spreading across Italy. International Journal of Molecular Sciences, 21(15), 5205.

Correale, P., Mutti, L., Pentimalli, F., Baglio, G., Saladino, R. E., Sileri, P., & Giordano, A. (2020). HLA-B*44 and C*01 Prevalence Correlates with Covid19 Spreading across Italy. International Journal of Molecular Sciences, 21(15), 1–12.

Costagliola, G., Spada, E., & Consolini, R. (2021). Age-related differences in the immune response could contribute to determine the spectrum of severity of COVID-19. Immunity, Inflammation and Disease, 9(2), 331–339.

Crux, N. B., & Elahi, S. (2017). Human leukocyte antigen (HLA) and immune regulation: How do classical and non-classical HLA alleles modulate immune

response to human immunodeficiency virus and hepatitis C virus infections? Frontiers in Immunology, 8, 832.

Cui, Y., Yao, H., Zhang, J., Zhang, T., Wang, Y., Hong, L., ... & Hou, J. (2019). Over 16% efficiency organic photovoltaic cells enabled by a chlorinated acceptor with increased open-circuit voltages. Nature communications, 10(1), 2515.

Das, S. K. (2020). The pathophysiology, diagnosis and treatment of corona virus disease 2019 (COVID-19). Indian Journal of Clinical Biochemistry, 35(4), 385–396.

Debnath, M., Banerjee, M., & Berk, M. (2020). Genetic gateways to COVID-19 infection: Implications for risk, severity, and outcomes. FASEB Journal, 34(7), 8787–8795.

Degenhardt, F., Mayr, G., Wendorff, M., Boucher, G., Ellinghaus, E., Ellinghaus, D., ... & International IBD Genetics Consortium. (2021). Transethnic analysis of the human leukocyte antigen region for ulcerative colitis reveals not only shared but also ethnicity-specific disease associations. Human molecular genetics, 30(5), 356-369.

Dijksteel, G. S., Ulrich, M. M. W., Middelkoop, E., & Boekema, B. K. H. L. (2021). Review: Lessons learned from clinical trials using antimicrobial peptides (AMPs). Frontiers in Microbiology, 12, 616979.

Din, M. A. U., & Boppana, L. K. T. (2020). An update on the 2019-nCoV outbreak. American Journal of Infection Control, 48(6), 713.

Domon, H., & Terao, Y. (2021). The role of neutrophils and neutrophil elastase in pneumococcal pneumonia. Frontiers in Cellular and Infection Microbiology, 11, 615959.

References

Donati, A., Damiani, E., Domizi, R., Romano, R., Adrario, E., Pelaia, P., Ince, C., & Singer, M. (2013). Alteration of the sublingual microvascular glycocalyx in critically ill patients. Microvascular Research, 90, 86–89.

Dutta, A., Das, D., Jana, R. K., & Vo, X. V. (2020). COVID-19 and oil market crash: Revisiting the safe haven property of gold and Bitcoin. Resources Policy, 69, 101816.

Dutta, M., Dutta, P., Medhi, S., Borkakoty, B., & Biswas, D. (2018). Polymorphism of HLA class I and class II alleles in influenza A (H1N1) pdm09 virus infected population of Assam, Northeast India. Journal of medical virology, 90(5), 854-860.

Dwinantoaji, H., & Sumarni, D. W. (2020). Human security, social stigma, and global health: The COVID-19 pandemic in Indonesia. Journal of Medical Sciences (Berkala Ilmu Kedokteran), 52(3).

Ebrahimi, S., Ghasemi-Basir, H. R., Majzoobi, M. M., Rasouli-Saravani, A., Hajilooi, M., & Solgi, G. (2021). HLA-DRB1* 04 may predict the severity of disease in a group of Iranian COVID-19 patients. Human Immunology, 82(10), 719-725.

Effah, C. Y., Sun, T., Liu, S., & Wu, Y. (2020). Klebsiella pneumoniae: An increasing threat to public health. Annals of Clinical Microbiology and Antimicrobials, 19(1), 1.

Egelund, G. B., Jensen, A. V., Andersen, S. B., Petersen, P. T., Lindhardt, B. Ø., von Plessen, C., Rohde, G., & Ravn, P. (2017). Penicillin treatment for patients with Community-Acquired Pneumonia in Denmark: A retrospective cohort study. BMC Pulmonary Medicine, 17(1), 66.

Engelmoer, D. J., & Rozen, D. E. (December 2011). Competence increases

survival during stress in Streptococcus pneumoniae. Evolution; International Journal of Organic Evolution, 65(12), 3475–3485.

Fehr, A. R., Perlman, S., Maier, H. J., Bickerton, E., & Britton, P. (2015). An overview of their replication and pathogenesis; section 2 genomic organization. Methods in Molecular Biology, 1282, 1–23.

Forbes, J. M. (Ed.). (2007). Voluntary food intake and diet selection in farm animals. Cabi.

Fuk-Woo, C. J., Shuofeng, Y., & Kin-Hang, K. (2020). To Kelvin Kai-Wang, Chu Hin, Yang Jin. A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. Lancet, 395(10223), 514-23.

Gajewska, M., Goryński, P., Paradowska-Stankiewicz, I., Lewtak, K., Piotrowicz, M., Urban, E., ... & Izurieta, P. (2020). Monitoring of community-acquired pneumonia hospitalisations before the introduction of pneumococcal conjugate vaccine into Polish National Immunisation Programme (2009–2016): A nationwide retrospective database analysis. Vaccine, 38(2), 194-201.

Galanopoulos, M., Gkeros, F., Doukatas, A., Karianakis, G., Pontas, C., Tsoukalas, N., Viazis, N., Liatsos, C., & Mantzaris, G. J. (2020). COVID-19 pandemic: Pathophysiology and manifestations from the gastrointestinal tract. World Journal of Gastroenterology, 26(31), 4579– 4588.

Garg, M., Prabhakar, N., Gulati, A., Agarwal, R., & Dhooria, S. (2019). Spectrum of imaging findings in pulmonary infections. Part 1: Bacterial and viral. Polish Journal of Radiology, 84, e205–e213.

Ghosh, S. K., & Weinberg, A. (2021). Ramping up AMPs against SARS- CoV-2. Frontiers in Molecular Biosciences, 8, 547.

References

Green, M. R., & Sambrook, J. (2012). Molecular cloning. A Laboratory Manual 4th.

Grolmusz, V. K., Bozsik, A., Papp, J., & Patócs, A. (2021). Germline genetic variants of viral entry and innate immunity may influence susceptibility to SARS-CoV-2 infection: Toward a polygenic risk score for risk stratification. Frontiers in Immunology, 12, 653489.

Guan, W. J., Ni, Z. Y., Hu, Y., Liang, W. H., Ou, C. Q., He, J. X., ... & Zhong, N. (2020). otros (2020),"Clinical characteristics of coronavirus disease 2019 in China". N Engl J Med, 382(18), 1708-1720.

Haddad, N. M., Crutsinger, G. M., Gross, K., Haarstad, J., & Tilman, D. (2011). Plant diversity and the stability of foodwebs. *Ecology letters*, *14*(1), 42-46.

Helenius, A., Gerke, C., & Hengel, H. (2015). Classical and non-classical MHC I molecule manipulation by human cytomegalovirus: So many targets—But how many arrows in the quiver? Cellular and Molecular Immunology, 12(2), 139–153.

Hammer, C., Begemann, M., McLaren, P. J., Bartha, I., Michel, A., Klose, B., ... & Fellay, J. (2015). Amino acid variation in HLA class II proteins is a major determinant of humoral response to common viruses. The American Journal of Human Genetics, 97(5), 738-743.

Hansen, T. H., & Bouvier, M. (2009). MHC class I antigen presentation: Learning from viral evasion strategies. Nature Reviews. Immunology, 9(7), 503–513.

Hasanagic, S., & Serdarevic, F. (2020). Potential role of memantine in the prevention and treatment of COVID-19: Its antagonism of nicotinic acetylcholine receptors and beyond. European Respiratory Journal, 56(2).

References

Heathorn, A., & Slagle, S. (2020). Penicillin & Pneumonia. Science, Technology, & Society: A Student-Led Exploration.

Hedman, P., Srinivasan, P. P., Mildenhall, B., Barron, J. T., & Debevec, P. (2021). Baking neural radiance fields for real-time view synthesis. In Proceedings of the IEEE/CVF International Conference on Computer Vision (pp. 5875-5884).

Helmy, Y. A., Fawzy, M., Elaswad, A., Sobieh, A., Kenney, S. P., & Shehata, A. A. (2020). The COVID-19 pandemic: a comprehensive review of taxonomy, genetics, epidemiology, diagnosis, treatment, and control. Journal of clinical medicine, 9(4), 1225.

Hemalatha, S. (2011). A Study on Aerobic Bacterial and Fungal Isolates in Bronchoalveolar Lavage in Patients with Lower Respiratory Tract Infection in a Tertiary Care Hospital (Doctoral dissertation, Madras Medical College, Chennai).

Hirsch, E. B., & Tam, V. H. (2010). Detection and treatment options for Klebsiella pneumoniae carbapenemases (KPCs): an emerging cause of multidrug-resistant infection. Journal of Antimicrobial Chemotherapy, 65(6), 1119-1125.

Hoffmann, M., Kleine-Weber, H., Schroeder, S., Krüger, N., Herrler, T., Erichsen, S., Schiergens, T. S., Herrler, G., Wu, N. H., Nitsche, A., Müller, M. A., Drosten, C., & Pöhlmann, S. (2020). SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. Cell, 181(2), 271–280.e8.

Hosseini, A., Hashemi, V., Shomali, N., Asghari, F., Gharibi, T., Akbari, M., Gholizadeh, S., & Jafari, A. (2020). Innate and adaptive immune responses

against coronavirus. Biomedicine and Pharmacotherapy, 132, 110859.

Huang, A. T., Garcia-Carreras, B., Hitchings, M. D., Yang, B., Katzelnick, L. C., Rattigan, S. M., ... & Cummings, D. A. (2020). A systematic review of antibody mediated immunity to coronaviruses: kinetics, correlates of protection, and association with severity. *Nature communications*, *11*(1), 4704.

Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y., ... & Cao, B. (2020). Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. The lancet, 395(10223), 497-506.

Ibrahim, H. J., Al-Taai, H. R. R., Al-Dolaymi, A. A. F., & Aboud, A. A. (2019). Molecular detection of TETM gene in and TN916 tetracycline resistant Streptococcus pneumoniae in Baqubah City, Iraq. Biochemical and Cellular Archives, 19(1), 999–1004.

Invernizzi, R., Lloyd, C. M., & Molyneaux, P. L. (2020). Respiratory microbiome and epithelial interactions shape immunity in the lungs. Immunology, 160(2), 171–182.

Iturrieta-Zuazo, I., Rita, C. G., García-Soidán, A., de Malet Pintos-Fonseca, A., Alonso-Alarcón, N., Pariente-Rodríguez, R., Tejeda-Velarde, A., Serrano-Villar, S., Castañer-Alabau, J. L., & Nieto-Gañán, I. (2020). Possible role of HLA class-I genotype in SARS-CoV-2 infection and progression: A pilot study in a cohort of Covid-19 Spanish patients. Clinical Immunology, 219, 108572.

Izurieta, P. (2020). Monitoring of community-acquired pneumonia hospitalisations before the introduction of Pneumococcal Conjugate Vaccine into Polish National Immunisation Programme (2009–2016): A nationwide

retrospective database analysis. Vaccine, 38(2), 194–201.

Jaaffar, A. I., Al-Mahmood, S., Maeh, R. K., & Alyasiry, M. (2019). Microbiological profile with antibiotic resistance pattern in patients of pneumonia in Iraq. Drug Invention Today, 11(11).

Jacques, F. H., & Apedaile, E. (2020). Immunopathogenesis of COVID-19: Summary and possible interventions. Frontiers in Immunology, 11, 564925.

Jain, S., Self, W. H., Wunderink, R. G., Fakhran, S., Balk, R., Bramley, A. M., ... & Finelli, L. (2015). Community-acquired pneumonia requiring hospitalization among US adults. New England Journal of Medicine, 373(5), 415-427.

Jameel, S. K., Abbas, A. H., Mohammed, N. A., Hayyawi, A. H., Al Ismee, A. M., Ibrahim, E. M., ... & Assi, M. S. (2020). Microbial examination outcome of sputum at tertiary hospital in Baghdad-Iraq. EurAsian Journal of BioSciences, 14(2).

Jokiniemi, A., Magris, M., Ritari, J., Kuusipalo, L., Lundgren, T., Partanen, J., & Kekäläinen, J. (2020). Post-copulatory genetic matchmaking: HLA-dependent effects of cervical mucus on human sperm function. Proceedings. Biological Sciences, 287(1933), 20201682.

Joseph, I. K., Nweze, N. O., Sulaiman, A. S., & Loko, A. Z. (2020). Comparative model profiles of COVID-19 occurrence in Nigeria. International Journal of Mathematics Trends and Technology, 66(6), 297–310.

Josset, L., Menachery, V. D., Gralinski, L. E., Agnihothram, S., Sova, P., Carter, V. S., ... & Katze, M. G. (2013). Cell host response to infection with novel human coronavirus EMC predicts potential antivirals and important differences with SARS coronavirus. MBio, 4(3), 10-1128.

References

Kachuri, L., Francis, S. S., Morrison, M. L., Wendt, G. A., Bossé, Y., Cavazos, T. B., ... & Witte, J. S. (2020). The landscape of host genetic factors involved in immune response to common viral infections. *Genome medicine, 12*, 1-18.

Katsurada, N., Suzuki, M., Aoshima, M., Yaegashi, M., Ishifuji, T., Asoh, N., Hamashige, N., Abe, M., Ariyoshi, K., & Morimoto, K. (2017). The impact of virus infections on pneumonia mortality is complex in adults: A prospective multicentre observational study. BMC Infectious Diseases, 17(1), 1–11.

Kawamata, R., Ishigaki, M., Shimasaku, K., Oguri, M., & Ouchi, M. (2015). The sizes of z∼ 6–8 lensed galaxies from the Hubble Frontier Fields Abell 2744 data. The Astrophysical Journal, 804(2), 103.

Khosroshahi, L. M., Rokni, M., Mokhtari, T., & Noorbakhsh, F. (2021). Immunology, immunopathogenesis and immunotherapeutics of COVID-19; an overview. International immunopharmacology, 93, 107364.

Kim, Y. C., & Jeong, B. H. (2020). Strong correlation between the case fatality rate of COVID-19 and the rs6598045 single nucleotide polymorphism (SNP) of the interferon-induced transmembrane protein 3 (IFITM3) gene at the population-level. Genes, 12(1), 42.

Kubysheva, N., Soodaeva, S., Novikov, V., Eliseeva, T., Li, T., Klimanov, I., Kuzmina, E., Baez-Medina, H., Solovyev, V., Ovsyannikov, D. Y., & Batyrshin, I. (2018). Soluble HLA-I and HLA-II molecules are potential prognostic markers of progression of systemic and local inflammation in patients with COPD. Disease Markers, 2018, 3614341.

Kulski, J. K., Shiina, T., & Dijkstra, J. M. (2019). Genomic diversity of the major histocompatibility complex in health and disease. Cells, 8(10), 1270.

Kumar, V. (2020). Pulmonary innate immune response determines the outcome

of inflammation during pneumonia and sepsis-associated acute lung injury. Frontiers in Immunology, 11, 1722.

Lechien, J. R., Chiesa-Estomba, C. M., De Siati, D. R., Horoi, M., Le Bon, S. D., Rodriguez, A., Dequanter, D., Blecic, S., El Afia, F., Distinguin, L., Chekkoury-Idrissi, Y., Hans, S., Delgado, I. L., Calvo-Henriquez, C., Lavigne, P., Falanga, C., Barillari, M. R., Cammaroto, G., Khalife, M., & Saussez, S. (2020). Olfactory and gustatory dysfunctions as a clinical presentation of mild-to-moderate forms of the coronavirus disease (COVID-19): A multicenter European study. European Archives of Oto- Rhino-Laryngology, 277(8), 2251–2261

Lee, J. S., Giesler, D. L., Gellad, W. F., & Fine, M. J. (2016). Antibiotic therapy for adults hospitalized with community-acquired pneumonia: A systematic review. JAMA, 315(6), 593–602.

Lian, J., Jin, C., Hao, S., Zhang, X., Yang, M., Jin, X., Lu, Y., Hu, J., Zhang,
S., Zheng, L., Jia, H., Cai, H., Zhang, Y., Yu, G., Wang, X., Gu, J., Ye, C., Yu, X., . . . Sheng, J. (2020). High neutrophil-to-lymphocyte ratio associated with progression to critical illness in older patients with COVID-19: A multicenter retrospective study. Aging, 12(14), 13849– 13859.

Littera, R., Campagna, M., Deidda, S., Angioni, G., Cipri, S., Melis, M., Firinu, D., Santus, S., Lai, A., Porcella, R., Lai, S., Rassu, S., Scioscia, R., Meloni, F., Schirru, D., Cordeddu, W., Kowalik, M. A., Serra, M., Ragatzu, P., . . . & Chessa, L. (2020). Human leukocyte antigen complex and other immunogenetic and clinical factors influence susceptibility or protection to SARS-CoV-2 infection and severity of the disease course. The Sardinian experience. Frontiers in Immunology, 11, 605688.

Lorente, L., Martín, M. M., Franco, A., Barrios, Y., Cáceres, J. J., Solé-Violán, J.,

Perez, A., Marcos Y Ramos, J. A., Ramos-Gómez, L., Ojeda, N., Jiménez, A., Working Group on COVID-19 Canary ICU, & Annex. Members of the BIOMEPOC group. HLA genetic polymorphisms and prognosis of patients with COVID-19. (2021). Medicina Intensiva (English Edition), 45(2), 96–103-103.

Lowery, S. A., Sariol, A., & Perlman, S. (2021). Innate immune and inflammatory responses to SARS-CoV-2: Implications for COVID-19. Cell Host and Microbe, 29(7), 1052–1062.

Lupia, T., Scabini, S., Mornese Pinna, S. M., Di Perri, G., De Rosa, F. G., & Corcione, S. (2020). 2019 novel coronavirus (2019-nCoV) outbreak: A new challenge. Journal of Global Antimicrobial Resistance, 21, 22–27.

MacFadden, B. J. (2000). Cenozoic mammalian herbivores from the Americas: reconstructing ancient diets and terrestrial communities. Annual Review of Ecology and Systematics, 31(1), 33-59.

Mahato, S., Sah, H. K., & Yadav, S. (2019). Isolation of Streptococcus pneumoniae from the sputum samples and their antimicrobial resistance in Biratnagar, Nepal. Journal of Microbiology and Experimentation, 7(6), 299–304.

Malik, Y. A. (2020). Properties of coronavirus and SARS-CoV-2. Malaysian Journal of Pathology, 42(1), 3–11.

Mani, C. S. (2018). Acute pneumonia and its complications. Principles and Practice of Pediatric Infectious Diseases, 238.

Mantlo, E., Bukreyeva, N., Maruyama, J., Paessler, S., & Huang, C. (2020). Antiviral activities of type I interferons to SARS-CoV-2 infection. Antiviral Research, 179, 104811., 107364.

References

Martin-Loeches, I., Torres, A., Rinaudo, M., Terraneo, S., de Rosa, F., Ramirez, P., Diaz, E., Fernández-Barat, L., Li Bassi, G. L., & Ferrer, M. (2015). Resistance patterns and outcomes in intensive care unit (ICU)- acquired pneumonia. Validation of European Centre for Disease Prevention and Control (ECDC) and the Centers for Disease Control and Prevention (CDC) classification of multidrug resistant organisms. Journal of Infection, 70(3), 213–222.

Mathur, S., Fuchs, A., Bielicki, J., Van Den Anker, J., & Sharland, M. (2018). Antibiotic use for community-acquired pneumonia in neonates and children: WHO evidence review. Paediatrics and International Child Health, 38(sup1), S66–S75.

Mendes-Frias, A., Oliveira, A. I., Dias, L., Matos, A. R., Carvalho, A., . . . & Silvestre, R. (2021). Interleukin-6 is a biomarker for the development of fatal severe acute respiratory syndrome coronavirus 2 pneumonia. Santa Cruz, A. Frontiers in Immunology, 12, 613422.

Migliorini, F., Torsiello, E., Spiezia, F., Oliva, F., Tingart, M., & Maffulli, N. (2021). Association between HLA genotypes and COVID-19 susceptibility, severity, and progression: A comprehensive review of the literature. European Journal of Medical Research, 26(1), 1.

Ming, L., Sun, T., Ding, H., He, J., Wu, W., Zhang, M., . . . & Lin, Y. (2021). Lung disease. In (pp. 595–640). Clinical Molecular Diagnostics. Springer.

Mohamed Khosroshahi, L. M., Rokni, M., Mokhtari, T., & Noorbakhsh, F. (2021). Immunology, immunopathogenesis and immunotherapeutics of COVID-19; an overview. International Immunopharmacology, 93, 107364.

Mohammad, Z. A. A., & Hbaiel, M. K. (2019). Morphological and molecular

study of contracaecum larvae with a new record of contracaecum septentrionale in al-Sanaf marsh southern Thi-Qar Province, Iraq. Indian Journal of Public Health Research and Development, 10(10).

Mousavi, S. F. (2020). Psychological well-being, marital satisfaction, and parental burnout in Iranian parents: The effect of home quarantine during COVID-19 outbreaks. Frontiers in Psychology, 11, 3305.

Mueller, A. L., McNamara, M. S., & Sinclair, D. A. (2020). Why does COVID-19 disproportionately affect older people? Aging (albany NY), 12(10), 9959–9981.

Muñiz-Castrillo, S., Vogrig, A., & Honnorat, J. (2020). Associations between HLA and autoimmune neurological diseases with autoantibodies. Auto Immun Highlights, 11(1), 2.

Najem, R. D., Abdulabbas, A. M., Abd-Al-Azeez, E. A. A., & Mohammed, A. K. (2015). Lobar pneumonia in hospitalized children and response to empirical antibiotic treatment. Iraqi Postgraduate Medical Journal, 14(3).

Nasi, A., McArdle, S., Gaudernack, G., Westman, G., Melief, C., Rockberg, J., Arens, R., Kouretas, D., Sjölin, J., & Mangsbo, S. (2020). Reactive oxygen species as an initiator of toxic innate immune responses in retort to SARS-CoV-2 in an ageing population, consider N-acetylcysteine as early therapeutic intervention. Toxicology Reports, 7, 768–771.

Nelde, A., Flötotto, L., Jürgens, L., Szymik, L., Hubert, E., Bauer, J., ... & Wethmar, K. (2022). Upstream open reading frames regulate translation of cancer-associated transcripts and encode HLA-presented immunogenic tumor antigens. Cellular and Molecular Life Sciences, 79(3), 171.

References

Nguyen, A., David, J. K., Maden, S. K., Wood, M. A., Weeder, B. R., Nellore, A., & Thompson, R. F. Journal of Virology. (2020). Human Leukocyte Antigen Susceptibility Map for Severe Acute Respiratory Syndrome Coronavirus 2, 94(13), 1–12.

Ni, L., Ye, F., Cheng, M. L., Feng, Y., Deng, Y. Q., Zhao, H., Wei, P., Ge, J.,Gou, M., Li, X., Sun, L., Cao, T., Wang, P., Zhou, C., Zhang, R., Liang, P., Guo, H., Wang, X., Qin, C. F., . . . & Dong, C. (2020). Detection of SARS-CoV-2-specific humoral and cellular immunity in COVID-19 convalescent individuals. Immunity, 52(6), 971–977.e3.

Nie, Y., Yang, G. Y., Song, Y., Zhao, X., So, C., Liao, J., Wang, L. D., & Yang, C. S. (2001). DNA hypermethylation is a mechanism for loss of expression of the HLA class I genes in human esophageal squamous cell carcinomas. Carcinogenesis, 22(10), 1615–1623.

Novelli, A., Andreani, M., Biancolella, M., Liberatoscioli, L., Passarelli, C., Colona, V. L., Rogliani, P., Leonardis, F., Campana, A., Carsetti, R., Andreoni, M., Bernardini, S., Novelli, G., & Locatelli, F. (2020). HLA allele frequencies and susceptibility to COVID-19 in a group of 99 Italian patients. HLA, 96(5), 610–614.

Olbei, M., Hautefort, I., Modos, D., Treveil, A., Poletti, M., Gul, L., Shannon-Lowe, C. D., & Korcsmaros, T. (2021). SARS-CoV-2 causes a different cytokine response compared to other cytokine storm-causing respiratory viruses in severely ill patients. Frontiers in Immunology, 12, 629193.

Oligbu, G., Fry, N. K., & Ladhani, S. N. (2019). The pneumococcus and its critical role in public health. In Streptococcus pneumoniae (pp. 205–213). Humana Press.

References

Orikiriza, P. (2019). Improving diagnosis of childhood tuberculosis in a high TB-HIV prevalent setting (Doctoral dissertation, Université Montpellier).

ÖZER, Z. C., & AYKAR, F. Ş. (Eds.). (2022). Kardiyovasküler Hemşirelik. Akademisyen Kitabevi.

Öztürk, R., Taşova, Y., & Ayaz, A. (2020). COVID-19: Pathogenesis, genetic polymorphism, clinical features and laboratory findings. Turkish Journal of Medical Sciences, 50(SI–1), 638–657.

Parker, R., Partridge, T., Wormald, C., Kawahara, R., Stalls, V., Aggelakopoulou, M., ... & Ternette, N. (2021). Mapping the SARS-CoV-2 spike glycoprotein-derived peptidome presented by HLA class II on dendritic cells. Cell reports, 35(8), 109179.

Pisanti, S., Deelen, J., Gallina, A. M., Caputo, M., Citro, M., Abate, M., ... & Martinelli, R. (2020). Correlation of the two most frequent HLA haplotypes in the Italian population to the differential regional incidence of Covid-19. Journal of translational medicine, 18(1), 1-16.

Pojero, F., Candore, G., Caruso, C., Di Bona, D., Groneberg, D. A., Ligotti, M. E., ... & Aiello, A. (2021). The role of immunogenetics in COVID-19. International Journal of Molecular Sciences, 22(5), 2636.

Poulton, K., Wright, P., Hughes, P., Savic, S., Welberry Smith, M., Guiver, M., Morton, M., van Dellen, D., Tholouli, E., Wynn, R., & Clark, B. (2020). A role for human leucocyte antigens in the susceptibility to SARS-Cov-2 infection observed in transplant patients. International Journal of Immunogenetics, 47(4), 324–328.

Pourbagheri-Sigaroodi, A., Bashash, D., Fateh, F., & Abolghasemi, H. (2020). Laboratory findings in COVID-19 diagnosis and prognosis. Clinica Chimica Acta; International Journal of Clinical Chemistry, 510, 475–482.

References

Pum, A., Ennemoser, M., Adage, T., & Kungl, A. J. (2021). Cytokines and chemokines in SARS-CoV-2 infections—Therapeutic strategies targeting cytokine storm. Biomolecules, 11(1), 91.

Qian, Z., Travanty, E. A., Oko, L., Edeen, K., Berglund, A., Wang, J., Ito, Y., Holmes, K. V., & Mason, R. J. (2013). Innate immune response of human alveolar type ii cells infected with severe acute respiratory syndrome–coronavirus. American Journal of Respiratory Cell and Molecular Biology, 48(6), 742–748.

Qin, C., Zhou, L., Hu, Z., Zhang, S., Yang, S., Tao, Y., Xie, C., Ma, K., Shang, K., Wang, W., & Tian, D. S. (2020). Dysregulation of immune response in patients with coronavirus 2019 (COVID-19) in Wuhan, China. Clinical Infectious Diseases, 71(15), 762–768.

Rambaud-Althaus, C., Althaus, F., Genton, B., & D'Acremont, V. (2015). Clinical features for diagnosis of pneumonia in children younger than 5 years: A systematic review and meta-analysis. Lancet. Infectious Diseases, 15(4), 439–450.

Ramos-Lopez, O., Daimiel, L., Ramírez de Molina, A., Martínez-Urbistondo, D., Vargas, J. A., & Martínez, J. A. (2020). Exploring Host Genetic Polymorphisms Involved in SARS-CoV Infection Outcomes: Implications for Personalized Medicine in COVID-19. International Journal of Genomics, 2020, 6901217.

Rana, J. S., Khan, S. S., Lloyd-Jones, D. M., & Sidney, S. (2021). Changes in mortality in top 10 causes of death from 2011 to 2018. Journal of General Internal Medicine, 36(8), 2517–2518.

Regunath, H., & Oba, Y. (2021). Community-acquired pneumonia. In StatPearls

[Internet]. StatPearls Publishing.

Restrepo, B. J., Rabbitt, M. P., & Gregory, C. A. (2021). The effect of unemployment on food spending and adequacy: Evidence from coronavirus-induced firm closures. Applied Economic Perspectives and Policy, 43(1), 185-204.

Rezaei Javan, R., Van Tonder, A. J., King, J. P., Harrold, C. L., & Brueggemann, A. B. (2018). Genome sequencing reveals a large and diverse repertoire of antimicrobial peptides. Frontiers in Microbiology, 9, 2012.

Rokni, M., Ghasemi, V., & Tavakoli, Z. (2020). Immune responses and pathogenesis of SARS-CoV-2 during an outbreak in Iran: Comparison with SARS and MERS. Reviews in Medical Virology, 30(3), e2107.

Rosendahl Huber, S., van Beek, J., de Jonge, J., Luytjes, W., & van Baarle, D. (2014). T cell responses to viral infections–opportunities for peptide vaccination. Frontiers in immunology, 5, 171.

Rossi, E. D., Fadda, G., Mule, A., Zannoni, G. F., & Rindi, G. (2020). Cytologic and histologic samples from patients infected by the novel coronavirus 2019 SARS-CoV-2: An Italian institutional experience focusing on biosafety procedures. Cancer Cytopathology, 128(5), 317– 320.

Ruuskanen, O., Lahti, E., Jennings, L. C., & Murdoch, D. R. (2011). Viral pneumonia. Lancet, 377(9773), 1264–1275.

Sakuraba, A., Haider, H., & Sato, T. (2020). Population difference in allele frequency of HLA-C* 05 and its correlation with COVID-19 mortality. Viruses, 12(11), 1333.

Sambrook, J., & Russell, D. W. (2001). Molecular Cloning-Sambrook & Russel-

Vol. 1, 2, 3. Cold Springs Harbor Lab Press: Long Island, NY, USA.

Sanchez-Mazas, A., & Meyer, D. (2014). The relevance of HLA sequencing in population genetics studies. Journal of immunology research, 2014.

Santa Cruz, A., Mendes-Frias, A., Oliveira, A. I., Dias, L., Matos, A. R., Carvalho, A., ... & Silvestre, R. (2021). Interleukin-6 is a biomarker for the development of fatal severe acute respiratory syndrome coronavirus 2 pneumonia. Frontiers in immunology, 12, 613422.

Sarhan, A., Flaih, M., Hussein, T., & Hussein, K. (2020). Novel coronavirus (COVID-19) Outbreak in Iraq: The First Wave and Future Scenario. medRxiv.

Saulle, R., Minozzi, S., Amato, L., & Davoli, M. (2021). Impatto del distanziamento sociale per covid-19 sulla salute fisica dei giovani: una revisione sistematica della letteratura. Recenti Progressi in Medicina, 112(5), 347-359.

Schaefer, L. (2014). Complexity of danger: The diverse nature of damage-associated molecular patterns. Journal of Biological Chemistry, 289(51), 35237–35245.

Schuetz, P., Wirz, Y., Sager, R., Christ-Crain, M., Stolz, D., Tamm, M., ... & Mueller, B. (2018). Effect of procalcitonin-guided antibiotic treatment on mortality in acute respiratory infections: a patient level meta-analysis. The Lancet infectious diseases, 18(1), 95-107.

Schulien, I., Kemming, J., Oberhardt, V., Wild, K., Seidel, L. M., Killmer, S., Sagar, Daul, F., Salvat Lago, M., Decker, A., Luxenburger, H., Binder, B., Bettinger, D., Sogukpinar, O., Rieg, S., Panning, M., Huzly, D., Schwemmle, M., Kochs, G., . . . & Neumann-Haefelin, C. (2021). Characterization of pre-

existing and induced SARS-CoV-2-specific CD8+ T cells. Nature Medicine, 27(1), 78–85.

Schwartz, D. A., & Graham, A. L. (2020). Potential maternal and infant outcomes from coronavirus 2019-nCoV (SARS-CoV-2) infecting pregnant women: lessons from SARS, MERS, and other human coronavirus infections. Viruses, 12(2), 194.

Sette, A., & Crotty, S. (2021). Adaptive immunity to SARS-CoV-2 and COVID-19. Cell, 184(4), 861–880.

SeyedAlinaghi, S., Mehrtak, M., MohsseniPour, M., Mirzapour, P., Barzegary, A., Habibi, P., Moradmand-Badie, B., Afsahi, A. M., Karimi, A., Heydari, M., Mehraeen, E., Dadras, O., Sabatier, J. M., & Voltarelli, F. (2021). Genetic susceptibility of COVID-19: A systematic review of current evidence. European Journal of Medical Research, 26(1), 46.

Shi, Y., Huang, Y., Zhang, T. T., Cao, B., Wang, H., Zhuo, C., ... & Qu, J. M. (2019). Chinese guidelines for the diagnosis and treatment of hospital-acquired pneumonia and ventilator-associated pneumonia in adults (2018 Edition). Journal of Thoracic Disease, 11(6), 2581.

Siegel, R. J., Bridges Jr, S. L., & Ahmed, S. (2019). HLA-C: an accomplice in rheumatic diseases. ACR Open Rheumatology, 1(9), 571-579.

Speretta, G. F., & Leite, R. D. (2020). Covid-19: High rates of severity and death in elderly and patients with chronic diseases reinforces the importance of regular physical activity. Sport Sciences for Health, 16(3), 589–590.

Starke, S., Zhao, Y., Komura, T., & Zaman, K. (2020). Local motion phases for learning multi-contact character movements. ACM Transactions on Graphics (TOG), 39(4), 54-1.

References

Synowiec, A., Szczepański, A., Barreto-Duran, E., Lie, L. K., & Pyrc, K. (2021). Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2): A systemic infection. Clinical Microbiology Reviews, 34(2), e00133-20.

Tang, N., Bai, H., Chen, X., Gong, J., Li, D., & Sun, Z. (2020). Anticoagulant treatment is associated with decreased mortality in severe coronavirus disease 2019 patients with coagulopathy. Journal of Thrombosis and Haemostasis, 18(5), 1094–1099.

Taylor, C. J., Bolton, E. M., & Bradley, J. A. (2011). Immunological considerations for embryonic and induced pluripotent stem cell banking. Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences, 366(1575), 2312–2322.

Thierry, A. R., & Roch, B. (2020). SARS-CoV2 may evade innate immune response, causing uncontrolled neutrophil extracellular traps formation and multi-organ failure. Clinical Science, 134(12), 1295–1300.

Thomas, P., Baldwin, C., Bissett, B., Boden, I., Gosselink, R., Granger, C. L., ... & van der Lee, L. (2020). Physiotherapy management for COVID-19 in the acute hospital setting: recommendations to guide clinical practice. Pneumon, 33(1).

Tian, C., Hromatka, B. S., Kiefer, A. K., Eriksson, N., Noble, S. M., Tung, J. Y., & Hinds, D. A. (2017). Genome-wide association and HLA region fine-mapping studies identify susceptibility loci for multiple common infections. Nature communications, 8(1), 599.

Thüsen, J., & van der Eerden, M. (2020). Histopathology and genetic susceptibility in COVID-19 pneumonia. European Journal of Clinical Investigation, 50(7), e13259.

References

Tuttolomondo, A., Colomba, C., Di Bona, D., Casuccio, A., Di Raimondo, D., Clemente, G., ... & Pinto, A. (2018). HLA and killer cell immunoglobulin-like receptor (KIRs) genotyping in patients with acute viral encephalitis. Oncotarget, 9(25), 17523.

Ul Haque, A., Ghnai, S., & Saeed, M. (2022). Pneumonia Classification: A limited data approach for global understanding.

Valtierra-Alvarado, M. A., Castañeda-Delgado, J. E., Lugo-Villarino, G., Dueñas-Arteaga, F., Rivas-Santiago, B., Enciso-Moreno, J. A., & Serrano, C. J. (2022). Increased frequency of CD14+ HLA-DR-/low cells in type 2 diabetes patients with poor glycemic control. Human Immunology, 83(11), 789-795.

Van Moorsel, C. H. M. (2018). Trade-offs in aging lung diseases: A review on shared but opposite genetic risk variants in idiopathic pulmonary fibrosis, lung cancer and chronic obstructive pulmonary disease. Current Opinion in Pulmonary Medicine, 24(3), 309–317.

Vollmers, S., Lobermeyer, A., & Körner, C. (2021). The new kid on the block: HLA-C, a key regulator of natural killer cells in viral immunity. Cells, 10(11), 3108.

Wan, Y., Graham, R., Baric, R., & Li, F. (2020). An analysis based on decade-long structural studies of SARS 3, JVI Accepted Manuscript Posted Online 29 January 2020. Journal of Virology, 94(7), e00127-20.

Wang, D., Hu, B., Hu, C., Zhu, F., Liu, X., Zhang, J., Wang, B., Xiang, H., Cheng, Z., Xiong, Y., Zhao, Y., Li, Y., Wang, X., & Peng, Z. (2020). Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus–infected pneumonia in Wuhan, China. JAMA, 323(11), 1061–1069.

Wang, W., Zhang, W., Zhang, J., He, J., & Zhu, F. (2020). Distribution of HLA

allele frequencies in 82 Chinese individuals with coronavirus disease- 2019 (COVID- 19). Hla, 96(2), 194-196.

Wang, X., & Ding, Y. Q. (2020). From SARS to COVID-19: Pathogens, receptor, pathogenesis and principles of the treatment. Zhonghua Bing Li Xue Za Zhi Chinese Journal of Pathology, 49(6), 647–652.

Wang, Y., Grunewald, M., & Perlman, S. (2020). Coronaviruses: an updated overview of their replication and pathogenesis. Coronaviruses: Methods and Protocols, 1-29.

Wen, W., Su, W., Tang, H., Le, W., Zhang, X., Zheng, Y., ... & Wang, H. (2020). Immune cell profiling of COVID-19 patients in the recovery stage by single-cell sequencing. Cell discovery, 6(1), 31.

Weiner 3rd, J., Suwalski, P., Holtgrewe, M., Rakitko, A., Thibeault, C., Mueller, M., ... & Heidecker, B. (2021). Increased risk of severe clinical course of COVID-19 in carriers of HLA-C* 04: 01. EClinicalMedicine, 40, 101099.

Wieczorek, M., Abualrous, E. T., Sticht, J., Álvaro-Benito, M., Stolzenberg, S., Noé, F., & Freund, C. (2017). Major histocompatibility complex (MHC) Class I and MHC Class II Proteins: Conformational Plasticity in Antigen Presentation. Frontiers in Immunology, 8, 292.

Winn, J., & Shotton, J. (2006, June). The layout consistent random field for recognizing and segmenting partially occluded objects. In 2006 IEEE Computer Society Conference on Computer Vision and Pattern Recognition (CVPR'06) (Vol. 1, pp. 37-44). IEEE.

Wolters, F., van de Bovenkamp, J., van den Bosch, B., van den Brink, S., Broeders, M., Chung, N. H., Favié, B., Goderski, G., Kuijpers, J., Overdevest,

I., Rahamat-Langedoen, J., Wijsman, L., Melchers, W. J., & Meijer, A. (2020). Multi-center evaluation of Cepheid xpert® Xpress SARS-CoV-2 point-of-care test during the SARS-CoV-2 pandemic. Journal of Clinical Virology, 128, 104426.

Wong, S. H., Lui, R. N., & Sung, J. J. (2020). Covid-19 and the digestive system. Journal of Gastroenterology and Hepatology, 35(5), 744–748.

Wu, C., Chen, X., Cai, Y., Xia, J., Zhou, X., Xu, S., Huang, H., Zhang, L., Zhou, X., Du, C., Zhang, Y., Song, J., Wang, S., Chao, Y., Yang, Z., Xu, J., Zhou, X., Chen, D., . . . Song, Y. (2020). Risk factors associated with acute respiratory distress syndrome and death in patients with coronavirus disease 2019 pneumonia in Wuhan, China. JAMA Internal Medicine, 180(7), 934–943.

Wysocki, T., Olesińska, M., & Paradowska-Gorycka, A. (2020). Current understanding of an emerging role of HLA-DRB1 gene in rheumatoid arthritis–from research to clinical practice. Cells, 9(5), 1127.

Xu, J. W., Lin, Z. R., Zhou, Y. W., Lee, R., Shen, H. M., Sun, X. D., ... & Zhou, H. N. (2021). Intensive surveillance, rapid response and border collaboration for malaria elimination: China Yunnan's ''3+ 1''strategy. Malaria Journal, 20, 1-9.

Yang, L., Liu, J., Zhang, R., Li, M., Li, Z., Zhou, X., Hu, C., Tian, F., Zhou, F., & Lei, Y. (2020). Epidemiological and clinical features of 200 hospitalized patients with corona virus disease 2019 outside Wuhan, China: A descriptive study. Journal of Clinical Virology, 129, 104475.

Yu, F., Du, L., Ojcius, D. M., Pan, C., & Jiang, S. (2020). Measures for diagnosing and treating infections by a novel coronavirus responsible for a

pneumonia outbreak originating in Wuhan, China. Microbes and Infection, 22(2), 74–79.

Yuki, K., Fujiogi, M., & Koutsogiannaki, S. (2020). COVID-19 pathophysiology: A review. Clinical immunology, 215, 108427.

Zakharova, M. Y., Belyanina, T. A., Sokolov, A. V., Kiselev, I. S., & Mamedov, A. E. (2019). The contribution of major histocompatibility complex class II genes to an association with autoimmune diseases. Acta Naturae , 11(4 (43)), 4–12.

Zakharova, M. Y., Belyanina, T. A., Sokolov, A. V., Kiselev, I. S., & Mamedov, A. E. (2019). The contribution of major histocompatibility complex class II genes to an association with autoimmune diseases. Acta Naturae, 11(4), 4–12-12.

Zanoni, I. (2021). Interfering with SARS-CoV-2: Are interferons friends or foes in COVID-19? Current Opinion in Virology, 50, 119–127.

Zhang, F., Gan, R., Zhen, Z., Hu, X., Li, X., Zhou, F., Liu, Y., Chen, C., Xie, S., & Zhang, B. (2020a). Adaptive immune responses to SARS-CoV2 infection in severe versus mild individuals. Signal Transduction and Targeted Therapy, 5, 1–11.

Zhang, L., Li, R., Song, G., Scholes, G. D., & She, Z. S. (2021). Impairment of T cells' antiviral and anti-inflammation immunities may be critical to death from COVID-19. Royal Society Open Science, 8(12), 211606.

Zhang, Q., Bastard, P., COVID Human Genetic Effort, Cobat, A., & Casanova, J. L. (2022). Human genetic and immunological determinants of critical COVID-19 pneumonia. Nature, 603(7902), 587–598.

Zhang, R., Ouyang, H., Fu, L., Wang, S., Han, J., Huang, K., Jia, M., Song, Q.,

& Fu, Z. (2020). CT features of SARS-CoV-2 pneumonia according to clinical presentation: A retrospective analysis of 120 consecutive patients from Wuhan city. European Radiology, 30(8), 4417–4426.

Zhou, D., Borsa, M., & Simon, A. K. (2021). Hallmarks and detection techniques of cellular senescence and cellular ageing in immune cells Aging Cell, 20(2), e13316.

Zhou, F., Yu, T., Du, R., Fan, G., Liu, Y., Liu, Z., ... & Cao, B. (2020). Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. The lancet, 395(10229), 1054-1062.

Zhou, W., Xu, X., Chang, Z., Wang, H., Zhong, X., Tong, X., Liu, T., & Li, Y. (2020). Dynamic changes of antibody against SRAS-CoV-2. In COVID- 19 patients (pp. 2456–2943).

Zinzula, L., Basquin, J., Bohn, S., Beck, F., Klumpe, S., Pfeifer, G., ... & Baumeister, W. (2021). High-resolution structure and biophysical characterization of the nucleocapsid phosphoprotein dimerization domain from the Covid-19 severe acute respiratory syndrome coronavirus 2. Biochemical and biophysical research communications, 538, 54-62.

Zubieta-Calleja, G., & Zubieta-DeUrioste, N. (2021). Pneumolysis and “silent hypoxemia” in COVID-19. Indian Journal of Clinical Biochemistry, 36(1), 112-116.