Republic of Iraq
Ministry of Higher Education and Scientific Research
Department of Chemistry and Biochemistry

Significance of Nano-formulated Artemisinin on Serum Levels of Interleukin-4 and Chemokine (Ligand-21) in Rheumatoid Arthritis Patients with/without T2DM

A Thesis

Submitted to the Council of the College of Medicine, University of Kerbala, in Partial Fulfillment of the Requirements for the Master Degree in **(Clinical Chemistry)**

By

Haneen Madlool Amanah

B.Sc. in Chemistry - College of Science, University of Al-Qadisiyah

(2014-2015)

Supervised by

Professor

Dr. Fadhil Jawad Al-Tu'ma

College of Medicine

University of Kerbala

Assistant Professor

Dr. Atheer Hameid Al-Ghanimi

College of Medicine

University of Kerbala

Sep., 2024 A.D.

Rabi' Al-Awwal, 1446 A.H.

فَتَعَالَى ٱللَّهُ ٱلْمَلِكُ ٱلْحَقُّ ۗ وَلَا تَعْجَلْ بِٱلْقُرْءَانِ مِن قَبْلِ أَن يُقْضَىٰٓ إِلَيْكَ وَحْيُهُۥ ۖ وَقُل رَّبِّ زِدْنِى عِلْمًا

صَدَقَ اللهُ العَليّ العَظيم

(سورة طه / الآية 114)

Supervisor Certification

We certify that this thesis entitled"

Significance of Nano-formulated Artemisinin on Serum Levels of Interleukin-4 and Chemokine (Ligand-21) in Rheumatoid Arthritis Patients with/without T2DM

was carried under our supervision at the College of Medicine, University of karbala , as a partial fulfillment for the requirement of the degree of Master of Science in Clinical Biochemistry.

Professor

Dr. Fadhil Jawad Al-Tu'ma

/ / 2024

Assistant Professor

Dr. Atheer Hameid Al-Ghanimi

/ / 2024

In a review of the available recommendations, I forward the present thesis for debate by the examining committee.

Assistant Professor. Dr. Atheer Hameid Al-Ghanimi

Head of Chemistry and Biochemistry Department

/ / 2024

Examination Committee Certification

We the examining committee, certify that we have read this M.Sc. thesis entitled:

(Significance of Nano-formulated Artemisinin on Serum Levels of Interleukin-4 and Chemokine (Ligand-21) in Rheumatoid Arthritis Patients with/without T2DM)

we have examined the student **"Haneen Madlool Amanah"** in its content and our opinion; it meets the standard of thesis for the Master's degree in **"Clinical Chemistry"**.

Signature
Prof. Dr. Haider Sobhi Sadiq
College of Medicine
University of Kerbala
/ / 2024
(Member)

Signature
Assist. Prof. Dr. Rana Majeed Hameed
College of Medicine
University of Kerbala
2 / 12 / 2024
(Member)

Signature
Prof. Dr. Fadhil Jawad Al-Tu'ma
College of Medicine
University of Kerbala
4 / 12 / 2024
(Member / Supervisor)

Signature
Assist. Prof. Dr. Atheer Hameid Al-Ghanimi
College of Medicine
University of Kerbala
/ / 2024
(Member / Supervisor)

Signature
Prof. Dr. Ferdous Abbas Jabir
College of Medicine - University of Al-Qadisiyah
/ / 2024
(Chairman)

Approved by the College of Medicine/ University of Kerbala

Signature
Prof. Dr. Riyadh Dayhood Mahdi Al-Zubaidi
Dean of Medicine College/ University of Kerbala
/ / 2024

Dedication

Thanks to Allah forever and always for everything.

To my first teacher with great wisdom and insight … My Father

To my great lady with the kindest heart .... My Mother

To the pure soul, my dear husband, who stood with me and supported me

To my dear sisters and brothers.

To my dear close friends, to all the reason for what I have become

today, thanks for your support and continuous care.

Haneen

Acknowledgments

First and foremost, I would like to thank Allah Almighty for giving me the strength, knowledge, ability, and opportunity to undertake this research study and to persevere and complete it satisfactorily. Without his blessings, this achievement would not have been possible.

My deep thanks are due to all the patients who helped me and participated in my study by the donation of their samples.

I acknowledge, with deep gratitude and appreciation, the inspiration, encouragement, valuable time, and guidance given to me by my supervisor **Prof. Dr. Fadhil Jawad Al-Tu'ma,** professor of clinical biochemistry molecular, College of Medicine, University of Karbala.

Special thanks to **Assist. Prof. Dr. Atheer Hameid Al-Ghanimi**, for being my second supervisor.

I would like to express my deep thanks to **Assist. Prof. Dr. Maher Abbood Mukheef**, and **Assist.Prof. Dr. Rana M. Hameed,** for their assessments.

I would like to extend my thanks and gratitude to all my friends for helping me get the thesis done.

Finally, thanks to all my classmate

HANEEN

Summary

Rheumatoid arthritis (RA) is a chronic inflammatory disease with increased morbidity and mortality. It may contribute to diabetes and type 2 diabetes. Nano-drug delivery systems, such as niosomes, offer potential solutions for RA and T2DM. Hyaluronic acid can enhance drug release and targeting specific cells. Artemisinin and its derivatives show anti-inflammatory, immunomodulatory, and anti-infective effects. Important genes like MMP2, TIMP2, and COX2 play a significant role in disease progression.

Aims The study explores the use of inflammatory/anti-inflammatory biomarkers in early RA detection and T2DM prevention, and the potential of HA-coated niosomes for RA treatment. It also investigates the antioxidant and anti-inflammatory effects of Hyalo-Nio-artemisinin NPs.

Methods A case-control study carried out in Al-Hindiyah General Hospital-Rheumatology Unit, Kerbala Health Directorates / Kerbala – Iraq and Marjan Teaching Hospital, Babylon Health Directorates / Babylon – Iraq from October 2023 to March 2024 by using out-patients visiting the orthopaedic consultant unit. A total of 125 individuals were enlisted, categorized into two primary factions: 74 instances of rheumatoid arthritis and 51 healthy controls. These patients were then segregated into 40 rheumatoid arthritis without type 2 diabetes mellitus (T2DM) and 34 rheumatoid arthritis cases with T2DM, aligning with the 2010 American College of arthritis criteria, ensuring parity in mean age and gender within the study cohorts. Subsequently, all participants completed surveys, and venous blood specimens were collected, and partitioned into a sodium citrate vial for erythrocyte sedimentation rate evaluation, nano-assays, and peripheral blood mononuclear cell (PBMC) isolation, and a gelatin vial for serum specimen Samples were isolated and preserved at -20°C in eight Eppendorf tube for experimental analysis.

The study examined the correlation between rheumatoid arthritis and various biochemical parameters, including IL-4, CCL21, HbA1c%, FBG, and insulin. It also investigated the structural properties of hyaluronic acid-loaded artemisinin Nio-NPs, which were tested for their potential as a treatment for inflammation and oxidative stress in rheumatoid arthritis patients.

Result The study showed an increase in blood levels of insulin, fasting blood glucose, and HbA1c% with highly significant differences and a significant increase in IL-4, and MMP2 tents as well as the group of patients suffering from T2DM compared to the control group.

The results also showed a significant decrease in the level of antioxidants vitamin D3, and Zinc patients compared to the control group. The performance of nanocomposites of artemisinin molecule coated with hyaluronic acid was performed to reduce the rate of inflammatory signs as well as reduce signs of oxidative stress in patients. It was found that artemisinin can reduce these signs in large proportions in patients.

Conclusion The study concluded that inflammatory, oxidative, and antioxidant markers are valuable biomarkers for diagnosing RA. Specifically, it highlighted the therapeutic efficacy of hyaluronic acid-coated artemisinin nanoparticles in significantly reducing inflammatory and oxidative markers while enhancing antioxidant markers. This underscores the potential of such nano-formulations in managing RA by addressing the underlying oxidative stress and inflammation associated with the disease.

List of Contents

No.	Title	Page
	Summary	I
	List of Contents	III
	List of Tables	VII
	List of Figures	IX
	List of Abbreviations	XII
Chapter One: Introduction and Literature Review		
1.	Introduction	1
1.1.	Rheumatoid Arthritis	1
1.1.1.	Epidemiology of Rheumatoid Arthritis	2
1.1.2.	Rheumatoid Arthritis Risk Factors	2
1.1.2.1.	Genetic Factors	2
1.1.2.2.	Environmental Factors	3
1.1.2.3.	Hormonal Factors	4
1.1.2.4.	Vitamin D3 Deficiency	5
1.1.2.5.	Obesity	5
1.1.2.6.	Age	6
1.1.2.7.	Family History	6
1.1.3.	Stages of Rheumatoid Arthritis	6
1.1.3.1	Stage1 (Early stage)	6
1.1.3.2.	Stage II (Moderate-stage)	6
1.1.3.3.	Stage III	7
1.1.3.4.	Stage IV	7
1.1.4.	Symptoms of Rheumatoid Arthritis	7
1.1.5.	pathogenesis of Rheumatoid Arthritis	8

1.1.6.	Diagnostic of Rheumatoid Arthritis	8
1.1.6.1.	Acute Phase Reactants of Rheumatoid Arthritis	9
1.1.6.1.1.	Rheumatoid Factor	10
1.1.6.1.2.	C-Reactive Protein	10
1.1.6.1.3.	Erythrocyte Sedimentation Rate	10
1.1.7.	Role of Cytokines and Interleukins in Rheumatoid Arthritis	11
1.1.8.	Inflammatory Markers in Rheumatoid Arthritis	12
1.1.8.1.	Chemokines CCL-21	12
1.1.9.	Anti-Inflammatory Cytokines in RA	13
1.1.9.1	Interleukin 4	13
1.1.10.	Role of Oxidant and Anti-oxidant in Rheumatoid Arthritis	14
1.1.10.1.	Anti-oxidant in Rheumatoid Arthritis	14
1	Artemisinin	14
2	Zinc	16
3	Vitamin D3	16
1.1.11.	Rheumatoid Arthritis and Inflammatory Anti- Inflammatory Balance	17
1.2.	Diabetes Mellitus	19
1.2.1.	Classification of Diabetes Mellitus	20
1.2.2.	Causes of Diabetes Mellitus	21
1.2.3.	Rheumatoid Arthritis and Type 2 Diabetes Mellitus	22
1.2.3.1.	Role of Sugar in the Rheumatic Diseases	23
1.2.3.2.	Role of Insulin in the Rheumatic Diseases	24
1.2.3.3.	Role of insulin resistance in the Rheumatic Diseases	24
1.2.3.4.	Role of Glycated Hemoglobin in RA	25
1.3.	Role Artimisinin with T2DM	25
1.4.	Nanotechnology and Nano medicine	26

1.4.1.	Applications of Nanotechnology in Medicine	27
1.4.2.	Drug Delivery Systems	27
1.4.3.	Niosomes	28
1.4.4.	Hyaluronic acid	29
1.4.5.	Artemisinin -Loaded Hyaluronic Acid-Coated Niosomal NPs	29
1.5.	Peripheral Blood Mononuclear Cell	30
1.6.	Gene Expression Detection in Artemisinin Coated with Hyaluronic Acid NPs	31
	Aims of the Study	33
Chapter Two: Materials and Methods		
2.	Materials and Methods	34
2.1	Subjects	34
2.1.1.	Study Design	34
2.1.2.	Ethical Approval	34
2.1.3.	Inclusion Criteria	34
2.1.4.	Exclusion Criteria	35
2.1.5.	Blood Samples Collection	36
2.2.	Materials	37
2.2.1.	Chemicals and Kits	37
2.2.2.	Instruments and Laboratory Tools	38
2.3.	Methods	39
2.3.1.	Anthropometric and Serological Tests Determinations	39
2.3.1.1.	Determination of Body Max Index	39
2.3.1.2.	Disease Activity Score	39
2.3.1.3.	Erythrocyte Sedimentation Rate	40
2.3.1.4.	Determination of C-Reactive Protein	40
2.3.2.	**Part I: (Clinical Chemistry Biomarkers Assays)**	41

2.3.2.1.	Determination of Fasting Blood Glucose	41
2.3.2.2.	Determination of Glycated Hemoglobin	41
2.3.2.3.	Determination of Serum Insulin Level	42
2.3.2.4.	Determination of Insulin Resistance	43
2.3.2.5.	Determination of Serum Chemokine Ligand-21 Level	43
2.3.2.6.	Determination Serum Interleukin-4 Levels	47
2.3.2.7.	Determination of Serum Zinc Concentration	47
2.3.2.8	Determination of Serum 25(OH)D3 Levels	49
2.3.3.	**Part II:(Synthesis and Characterization of Nanoparticles)**	52
2.3.3.1.	Peripheral Blood Mononuclear Cell Isolation and Culture	52
2.3.3.2.	Synthesis of Niosome Nanoparticles.	52
2.3.3.3.	Synthesis of Artemisinin Loaded Niosomal Nanoparticles	52
2.3.3.4.	Synthesis of Artemisinin Loaded Hyaluroinc Acid Coated Niosomal Nanoparticles	52
2.3.3.5.	Physiochemical, Size, and Morphology of Nanoparticles	53
2.3.3.6.	Niosomal NPs *in vitro* Drug Release	53
2.3.3.7.	Peripheral Blood Mononuclear Cells Proliferation	53
2.3.3.8.	C-C Motif Chemokine Ligand-21 and Interlukin-4	54
2.3.4.	**Part III: (Molecular Studies)**	55
2.3.4.1.	Real-time Polymerase Chain Reaction	55
2.3.4.2.	RNA Extraction	56
2.3.4.3	cDNA Synthesis	57
2.3.4.4	Real Time PCR for Gene Expression	57
2.4.	Statistical Analysis	59
Chapter Three : Results		
3.	Results	60
3.1.	Part I: (Biochemical and clinical result)	60

3.1.1	Demographic Data Characteristic	63
3.1.2.	Distribution of Anthropometric Parameters	63
3.1.3.	Results of Inflammatory and Anti-inflammatory Markers	63
3.1.4	Results of other Biomarker in sera of Rheumatiod Arthritis	64
3.1.5.	Zinc and Vitamin D3 Levels in Sera of RA with/without T2DM	65
3.1.6.	Correlation Studies	66
3.1.7.	Association of Biomarkers with RA with / without T2DM	67
3.1.8.	Receiver Operating Characteristic Analysis	70
3.2.	**Part II (Results of Nanoparticle Preparation and Characterization)**	72
3.2.1.	Artemisinin Nanoparticles Loaded with Hyaluronic acid	72
3.2.2.	Determination of IL-4- and CC-21 Level in Pure Untreated Artemisinin Nio-AR NPs, and HNio- AR treated PBMCs.	81
3.3.	**Part III (Molecular Studies and Gene Expression)**	82
Chapter Four: Discussion		
4.	Discussion	84
4.1.	**Part I (Clinical Chemistry discussion)**	84
4.1.1.	Demographic and Anthropometric Characteristics	84
4.1.2.	Biomarkers Studied in Sera of Rheumatoid Arthritis	84
4.1.3.	Antioxidants Status and Rheumatoid Arthritis.	87
4.1.4.	Association of Biomarkers with Rheumatoid Arthritis	88
4.1.5.	Correlation Coefficient between Biomarkers Various Groups.	93
4.2.	**Part II (Nano studies discussion)**	96
4.2.1.	Role of Nanoparticles in Rheumatoid Arthritis	96
4.2.2.	Interleukin-4 and CCL-21 of NPs in Rheumatoid Arthritis	100

4.3.	**Part III (Molecular studies discussion)**	101
4.3.1.	Gene Expression and NPs in Rheumatoid Arthritis	101
Conclusions and Recommendations		
References		
الغلاف العربي والخلاصة العربية		

List of Tables

No.	Title	Page
1.1	Classification Criteria for Rheumatoid Arthritis	11
1.2	Differentiate between Type 2 DM and Type 1 DM.	18
2.1	Chemicals and kits are used in this study and their supplies.	39
2.2	Instruments and laboratory tools used in this study	40
2.3	The ranges of (BMI) were categorized into groups	41
2.4	Disease Activity Score (DAS)	41
2.5	Indicates the component of CRP kit.	42
2.6	Kit component of CCL21	46
2.7	Standard kit component of CCL21	47
2.8	Ideal condition for Zinc determination	52
2.9	Kit component of vitamin D3	53
2.10	The forward and reverse primer sequences used for real-time PCR	59
2.11	Program implemented by Real-time PCR	62
3.1	Demographics characteristic of the study groups	64
3.2	Comparison between study groups in Age, BMI and family history	66
3.3	The mean ± SD difference of inflammatory marker for level CCL-21, IL-4, ESR, and CRP among patients group of RA, RA with T2DM compared to the control group DM), compared to control group	67
3.4	The mean ± SD difference of metabolic disorder marker level	68

	for HOMO-IR, Insulin, HbA1c, and FBS among patients group of RA,RA with T2DM compared to the control group	
3.5	The mean ± SD difference of antioxidant disorder marker level for Vit D3 & Zinc among patients group of RA, RA with T2DM compared to the control group	69
3.6	The correlation coefficient between (CCL-21 and IL-4) (pg/ml) with other biomarkers among the Patients group.	70
3.7	Estimation of the associated of the analysed factors (IL-4, CCL-21, CRP, and ESR) in RA patients (with and without T2DM) as compared with control group	71
3.8	Estimation of the Associated of the analyzed factors (Vit. D3 and Zinc) in RA patients (with & without DM) as compared with control group	73
3.9	AUC, optimal threshold, sensitivity, and specificity of CCL-21 and IL-4 for obtained by ROC curve in patients RA.	73
3.10	AUC, optimal threshold, sensitivity, and specificity of CCL-21 and IL-4 obtained by ROC curve in RA patients with T2DM.	75
3.11	The size, PDI, and zeta potential of blank niosomal NPs NPs, Nio-artemisinin NPs, and Hyalo-Nio-artemisinin NPs	76

List of Figures

No.	Title	Page
1-1	Factors contributing to rheumatoid arthritis (RA) development	5
1-2	Chemical structure of Artemisinin	16
1-3	Rheumatoid Arthritis and Inflammatory Anti- Inflammatory Balance	20
1-4	prevalence of impaired glucose tolerance in adults (20–79 years) in 2019, adapted from International Diabetes Federation	21
1-5	illustrates the relationship between genetics, epigenetics, and environment,	24
1-6	structure of niosomes.	31
1-7	Structure of the Hyaluronic acid	31
2-1	Study design	37
2-2	Standard curve of chemokine CCL21 determination	48
2-3	Standard curve of Interleukin- 4 determination	51
2-4	Standard curve of Zinc determination	52
2-5	Standard curve of vitamin determination	55
3-1	Baseline characteristics and demographic descriptive of the study population in disease and control groups the number of participants for age groups	64
3-2	Baseline characteristics and demographic descriptive of the study population in disease and control groups number of participants for BMI groups	65
3-3	Baseline characteristics and demographic descriptive of the study population in disease and control groups the number of participants for Family history	65
3-4	Receiver Operating Characteristic (ROC) curve of serum CCL-21 and IL-4 levels as discriminators of patients RAwithT2DM	74
3-5	Receiver Operating Characteristic (ROC) curve of serum CCL-21 and IL-4 levels as discriminators of patients RAwithoutT2DM.	75
3-6	The size and PDI value of fabricated Hyalo-Nio-artemisinin NPs acquired with DLS	76

3-7	The SEM images of Hyalo-Nio-artemisinin NPs revealed their spherical morphology.	77
3-8	AFM image of Hyalo-Nio-artemisinin NPs agrees with results of DLS and SEM images of Hyalo-Nio-artemisinin NPs.	77
3-9	FTIR analysis of (A) hyaluronic acid, and (B) artemisinin, show their characteristic bands in FTIR of (C) blank niosome NPs, and (D) Hyalo-Nio-artemisinin	78
3-10	The 96 h *invitro* release experiment of artemisinin from Nio-artemisinin NPs, and Hyalo-Nio- artemisinin NPs at 37°C and pH 7.4	79
3-11	The proliferation effects of pure artemisinin, Nio-artemisinin NPs, and Hyalo-Nio-artemisinin NPs on PBMCs.	81
3-12	A. Comparison of IL-4 levels in untreated and treated pure artemisinin, Nio-artemisinin NPs, and Hyalo-Nio-artemisinin NPs.	82
3-12	B Comparison of CCL-21 levels in untreated and treated pure artemisinin, Nio-artemisinin NPs, and Hyalo-Nio-artemisinin NPs.	83
3-13	Expression levels of the genes in PBMCs before and after treatment	84

List of Abbreviations

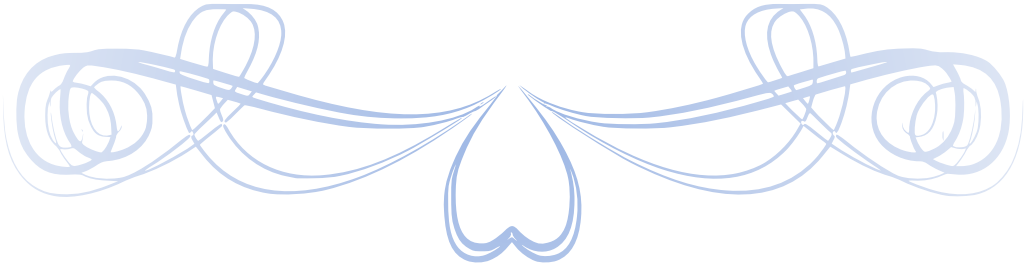
Abbreviations	Complete Names
ACCP	Anti-Cyclic Citrullinated Peptide
AFM	atomic force microscopy
ART	Artemisinin
APR	Acute phase reactants
bDMARDs	Biological disease-modifying anti-rheumatic medications
Nio NPs	niosomal nanoparticles
CO	Cholesterol Oxidase
CRP	C - Reactive protein
DAS	Disease Activity Score
DIP	Distal interphalangeal
DLS	Dynamic Light Scattering
DM	Diabetes mellitus
DNA	Deoxyribonucleic Acid
DSC	Differential Scanning Calorimeter
EBV	Epstein-Barr virus
EDTA	Ethylene Diamine Tetra Acetic Acid
ELISA	Enzyme Linked Immunosorbent Assay
ESR	Erythrocyte Sedimentation Rate
FBG	Fasting Blood Glucose
Fc	Fragment crystallize
FT- IR	Fourier-Transform Infrared Spectroscopy
GDM	Gestational diabetes mellitus
GOD	Glucose-oxidase enzyme
GTT	Glucose Tolerance Test
GWAS	Genome-wide association studies

HbA1c	Glycated Hemoglobin
HLA	Human Leukocyte Antigens
HLA-DRB1	(Major histocompatibility complex, class II, DRbeta1)
HOMA-IR	Homeostasis Model Assessment-Insulin Resistance
HPA	Hypothalamic-pituitary-adrenal
HRP	Horseradish Peroxidase
HSA	Human serum albumin
ICSH	International Committee for Standardization in Haematology
IDDM	Insulin-Dependent Diabetes Mellitus
IDF	International Diabetes Federation
IgE	Immunoglobulin E
IgG	Immunoglobulins type G
IL-4	Interleukin- 4
IR	Insulin Resistance
JAK/STAT	Janus kinase/Signal transducer and activator of transcription
LADA	Latent Autoimmune Diabetes In Adults
MENA	Middle East and North Africa
MP	Metacarpophalangeal
MMP2	Matrix metalloproteinase 2
MRI	Magnetic resonance imaging
NIDDM	Non-insulin dependent diabetes mellitus
NPs	Nanoparticles
NSAIDs	Non-steroidal anti-inflammatory drugs
OA	Osteoarthritis
PADs	Peptidylarginine Deiminases
PBMCs	Peripheral blood mononuclear
PBS	Phosphate-buffered saline
PCS	Photon Correlation Spectroscopy

PDI	poly dispersity index
PIP	Proximal interphalangeal
RA	Rheumatoid Arthritis
RF	Rheumatoid Factor
RNAs	Ribonucleic acids
SEM	scanning electron microscopy
SLE	Systemic lupus erythematosus
SNPs	Single nucleotide polymorphisms
T2DM	Type 2 diabetes mellitus
Th	T-helper
TEM	Transmission electron microscopy
TGFβ	Transforming Growth Factor-β
TNF	Tumor Necrosis Factor
WHO	World Health Organization

CHAPTER ONE

Introduction and Literature Review



1. Introduction

1.1. Rheumatoid Arthritis

Rheumatoid arthritis (RA), is an inflammatory chronic autoimmune disease, that demonstrates asymmetrical progression by initially targeting small joints and then advancing to larger joints, eventually impacting organs such as the skin, eyes, heart, kidneys, and lungs. It commonly results in damage to joint bone and cartilage, accompanied by deterioration of ligaments and tendons **(Bullock et al. 2019).** Rheumatoid arthritis is a condition characterized by deformities and bone erosion caused by joint degeneration, often affecting individuals aged 35-60. It is characterized by fatigue, fever, weight loss, and prolonged morning stiffness. It can also affect young individuals, particularly those under sixteen. **(Efthimiou *et al.* 2021).**

Rheumatoid arthritis has a complex etiology and prognosis, but advancements in therapies have improved outcomes. Current treatment focuses on clinical remission through prompt medication initiation and gradual escalation, guided by disease activity assessment. **(Aletaha *et al.*, 2010).** NSAIDs have lower anti-inflammatory efficacy, but corticosteroids are riskier, so they should be administered cautiously, especially during RA episodes, and used for localized inflammatory management **(Combe *et al.*, 2017).**Corticosteroids decrease inflammation by inhibiting phospholipid release and reducing eosinophil activity, but may cause side effects like immunosuppression, weight gain, diabetes, and osteoporosis. Calcium and vitamin D supplementation are recommended. Gradual dose reduction is advised, and autoantibody positivity may influence studies (**Padyukov 2022).** Rheumatoid arthritis (RA) is a complex autoimmune disorder with over 150 loci identified in recent genetic studies. These factors are common among other autoimmune disorders, enhancing our understanding of autoimmune pathways and paving the way for potential improvements in diagnostic criteria. **(Okada *et***

***al.* 2014; Amariuta-Bartell 2023).**In the progression towards an increased susceptibility to rheumatoid arthritis (RA), it was observed that the HLA alleles exhibited interactions with various environmental and genetic risk factors in multiple instances **(Diaz-Gallo *et al.* 2018).**

1.1.1.Epidemiology of Rheumatoid Arthritis

Rheumatoid arthritis affects 0.24–1.1% of the population, with a higher prevalence among women. Advancements in therapeutic strategies and drug regimens alleviate long-term effects, but the societal economic burden remains significant, as highlighted in the 2010 Global Burden of Disease Study **(Germano *et al*. 2021).**They are displaying a greater occurrence in the female demographic. Progressions in treatment approaches and pharmaceutical protocols mitigate enduring consequences, yet the substantial socioeconomic load on society persists, as underscored in the 2010 Global Burden of Disease Investigation **(Myasoedova *et al.* 2010).** Rheumatoid arthritis (RA) affects 40 cases per 100,000 individuals, with studies in the US and northern Europe revealing higher incidences and prevalence rates in certain populations, such as Pima Native people, which can be ten times greater than most other populations **(Venetsanopoulou *et al.* 2023).**Women have a higher likelihood of being impacted by RA compared to men, as the incidence and prevalence rates of the disease are twice as high in this demographic. The lifetime risk of developing RA is 1.7% for men and 3.6% for women alike **(Rajab *et al.* 2023).**

1.1.2. Risk Factors

1.1.2.1. Genetic Factors

Using single nucleotide polymorphisms (SNPs), genome-wide association studies have proposed that over 100 loci are linked to the development of RA **(Mikhaylenko *et al.* 2020).** HLA-DRB1 alleles account for nearly half of

genetic susceptibility among genes influencing RA development, as many loci are shared with other chronic inflammatory illnesses. **(Arleevskaya *et al.* 2016).**Research indicates that these HLA alleles can preferentially present specific peptide epitopes derived from pertinent RA autoantigens because they share amino acid sequences inside their peptide binding grooves **(Van Drongelen and Holoshitz, 2017).**Furthermore, a few of these HLA-DRB1 variants are linked to higher mortality rates and more aggressive bone degradation **(Wysocki, Olesińska, and Paradowska-Gorycka 2020).** When combined, these data point to a significant cell-dependent component in RA, with Th1 and Th17 T- cell subsets being the most common cell types involved in the pathomechanism of RA's inflamed synovial tissue **(Chemin, Gerstner, and Malmström, 2019).**Elevated mortality rates and more aggressive bone degradation are linked to HLA-DRB1 alleles **(Wysocki, Olesińska, and Paradowska-Gorycka, 2020)**. When combined, these data point to a significant T cell-dependent component in RA, with Th1 and Th17 T- cell subsets being the most common cell types in the inflammatory synovial region.

1.1.2.2. Environmental Factors.

Environmental factors, including smoking, are linked to a two-fold increase in RA incidence, particularly in individuals with ACCP antibodies, despite no single environmental factor being identified as a significant risk factor. **(Björk, Mofors, and Wahren-Herlenius, 2020).** There is inconsistent data to support the links between risk and the use of oral contraceptives, low vitamin D3 levels, and excessive alcohol and coffee use **(Afrin *et al.* 2021).** The environment, including infectious agents like bacteria and viruses, has been linked to an increased risk of RA. Patients with RA have higher levels of Epstein-Barr virus DNA, suggesting its involvement. The disease is linked to a reduction in EBV-specific T cell function. **(Hussein and Rahal, 2019)**. Clinical research supports the link between bacterial infections and RA, with animal models showing evidence of infections caused by

Porphyromonas gingivalis, Proteus mirabilis, and Streptococcus pyogenes, which can worsen the condition's symptoms**(Dieterich, Schink, and Zopf, 2018).** Additionally, it was discovered that Proteus species and Escherichia coli, two Gram-negative bacteria, were responsible for 23.5% of persistent UTIs in an Iraqi sample of RA patients **(Salih, 2022)**. Illness is influenced by both hereditary and environmental factors, such as smoking, obesity, vitamin D3 deficiency, and mucosal microbiota, with low concordance between twins indicating more significant environmental influences.; see Figure (1-1) **(Aslani *et al.*, 2018)**.

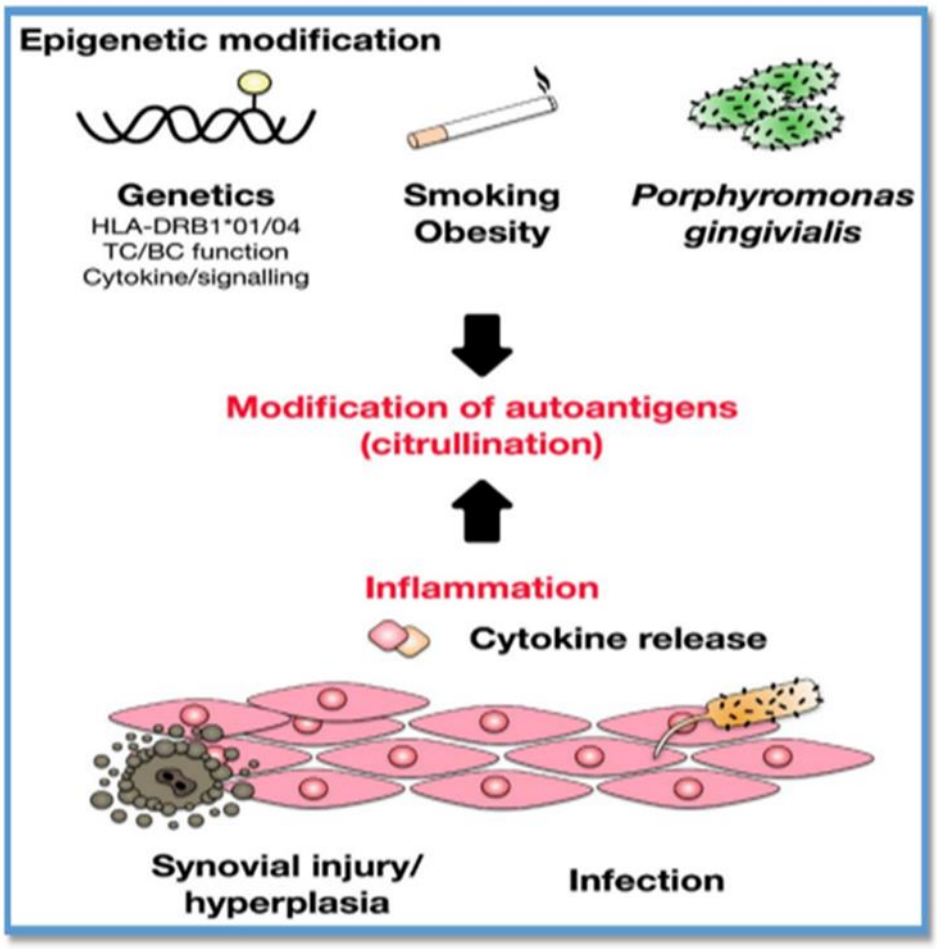


Fig. (1-1): Factors contributing to rheumatoid arthritis (RA) development (Lin, Anzaghe, and Schülke, 2020)

1.1.2.3. Hormonal Factors

Women are more likely to develop rheumatoid arthritis as compared with men, particularly during the premenopausal years; female sex hormones might be

responsible for these reasons **(Jackson, 2019)**.Sex hormones impact RA susceptibility, with men with RA having lower testosterone levels and more estrogens boosting the immune system, as women are more likely to develop the disease. **(Chaojie Yu *et al.* 2020).**Several studies have shown that oral contraceptives can prevent the development of rheumatoid arthritis in the future **(Li *et al*. 2023)**. Furthermore, RA is more common in women in the first year after giving birth than it was throughout pregnancy **(Aljary *et al.* 2020).** Age at menarche, age at menopause, and postmenopausal hormone use **(Kazmi, 2021).**

1.1.2.4. Vitamin D3 Deficiency

A fat-insoluble vitamin D3 acts as a hormone known for its influence on the immune system, traditionally linked to maintaining bone balance and overseeing immune responses various domains, such as managing autoimmune conditions like RA **(Malakooti *et al*., 2024).** A 11-year study on female patients found a higher susceptibility to RA due to inadequate Vitamin D3 intake. Global research suggests a potential inverse relationship between vitamin D3 deficiency and RA, suggesting a link between the two. **(Harrison *et al.*, 2020).**

Vitamin D3 influences T and B lymphocyte groups, regulating immune responses for disease prevention. RA synovium presents four disease phenotypes: lymphoid, myeloid, low-inflammatory, and fibroid. Myeloid type responds better to anti-TNFα treatment, suggesting vitamin D deficiency may impact different RA disease phenotypes. **(Athanassiou *et al*., 2023)**.

1.1.2.5. Obesity

Obesity increases the risk of autoimmune disorders and RA in women due to pro-inflammatory adipocytokines **(De Figueiredo, *et al.*, 2023).** High levels of adipokines, such as leptin, resistin, and visfatin, and low levels of anti-inflammatory adipokines, may increase inflammation and disease activity in obese patients with RA **(Guimarães, *et al.*, 2019).**

1.1.2.6. Age

The impact of age on rheumatoid arthritis (RA) holds notable importance. A study showed that age was associated with the non-achievement of clinical and functional remission criteria in RA patients **(Aoki *et al.*, 2020).** Individuals diagnosed with RA before age 50 had a significantly higher risk of fractures compared to matched controls, emphasizing the importance of bone health management in younger RA patients **(Erwin *et al*., 2021)**.

1.1.2.7. Family History

The study indicates that individuals with affected first-degree relatives are at a higher risk of developing seropositive RA **(Sitorukmi *et al.*,2020).** Influenced by family history, social support, and lifestyle choices **(Kim *et al.*, 2023).**

1.1.3. Stages of Rheumatoid Arthritis

1.1.3.1. Stage1 (Early-stage)

Stage 1 of RA is characterized by joint inflammation, causing stiffness, swelling, or soreness in joints. This stage typically disappears when the patient relocates. The synovium, the tissue lining the joint, increases with swelling, but bones remain unaffected. Symptoms may not always be clear, and doctors may struggle to diagnose the condition. However, if a medical professional determines the illness and provides appropriate treatment, the patient can recover. A strong likelihood that the illness will go into remission in 12 weeks (**Combe *et al*., 2017**).

1.1.3.2. Stage II (Moderate-stage)

Stage 2 of RA involves inflammation of the synovium, damaging bone and cartilage in joints. Degenerative cartilage can cause pain and limited range of motion, such as stiffness and difficulty bending fingers. Currently, blood tests may not reveal RA antibodies, even in severe cases, making it a rare condition. RA patients often have scro-negative antibodies, making it difficult to identify the

disease. The most typical course of events is the presence of RA antibodies years before symptoms appear. We refer to this as scro-negative RA **(Petrovská *et al.*, 2021).**

1.1.3.3. Stage III

RA is considered significant once it reaches stage 3, as the bone deteriorates and cartilage is damaged, causing rubbing between bones, edema, and pain. Some may lose muscle strength and become less mobile. Bones may change or degrade during creation, leading to permanent issues like bigger knuckles and bent fingers. Compressed tendons and signs of tendon rupture or carpal tunnel syndrome are also potential symptoms. Early diagnosis can help prevent extensor tendon rupture **(R1adu and Bungau, 2021).**

1.1.3.4. Stage IV

In stage four, RA causes joints to become non-functional, causing discomfort, oedema, stiffness, and restricted mobility. Patients may experience bone fusion (ankylosis) and joint damage, with potential loss of hand function or difficulty flexing hips or knees. Progressing through all four stages may take years, and ankylosis affects 0.8% of RA patients. Occasionally, no symptoms of RA activity may indicate a condition and a remission of RA **(Millier *et al*., 2022)**.

1.1.4. Symptoms of Rheumatoid Arthritis

Rheumatoid arthritis is a condition causing swollen, hot joints, exhaustion, fever, and appetite loss. It typically worsens in the morning and after sitting still. Smaller joints are affected, and symptoms extend to the shoulders, elbows, hips, knees, ankles, and wrists. In 40% of cases, affected areas include skin, eyes, lungs, heart-related kidneys, nervous tissue, and bone marrow vascular structures. Symptoms vary in intensity and cyclical nature, with periods of relative remission and flares. **(Pope, 2020).**

1.1.5. Pathogenesis of Rheumatoid Arthritis

The etiology of RA is intricate and diverse, encompassing a blend of environmental influences, hereditary elements, and disruptions in the immune system like the triggering of inflammasomes and inflammation mediated by cytokines **(Chen, Tang, and Chen, 2024).** It enmeshes numerous pathological processes, involving the activation of antigen-presenting cells (APC), the formation of self-reactive T cells, and the generation of autoantibodies targeting its cellular framework (such as rheumatoid factor (RF) and anticitrulline protein antibody (ACPA) **(Peng *et al.* 2024).** The mechanisms underlying RA are intertwined with the functions of B-lymphocytes, engaging in tasks such as presenting antigens, releasing cytokines, and synthesizing autoantibodies **(Wu *et al.* 2021).** The generation of ACPA is spurred by B-cells, initiating an immune reaction that commences with precise specificity and the spread of epitopes **(van Delft and Huizinga, 2020)**. Moreover, B cells showcase co-stimulatory components that aid in activating T-cells; these T-cells, along with various immune cells, migrate to the synovial membrane. Here, they unleash copious amounts of pro-inflammatory cytokines, engaging with synovial fibroblasts and macrophages, all contributing to the development of RA **(Mellado *et al*., 2015).**

1.1.6. Diagnosis of Rheumatoid Arthritis

According to Martinez **(Barberà *et al.* 2023).** Clinically, individuals with RA usually show up with abnormal lab findings, stiff joints in the morning, nonspecific symptoms of illness, and recently developed joint pain and swelling. **(Aletaha and Smolen, 2018).** As shown in the table (1.1), Diagnosing RA involves a combination of patient symptoms, physician examination, risk factors evaluation, medical history, ultrasound assessment, laboratory markers, Anti-cyclic citrulline peptide (ACCP), and elevated serum values. **(Burmester and Pope, 2017).** Both MRI and ultrasonography have been recommended for the aim of detecting and monitoring disease activity in individuals with RA **(Tang,**

Qu, and Yue, 2020). Inflamed joints can be seen by ultrasonic analysis, such as high-resolution musculoskeletal ultrasonography. By using grayscale and power analysis, it can detect synovial growth. Doppler can detect neoangiogenesis as well as active inflammation **(Prado *et al*., 2019).** Ultrasound can detect bone erosions and synovitis, contributing to radiographic sickness progression, making it frequently used in clinical practice and research for diagnosis and disease state monitoring. **(Lin, Anzaghe, and Schülke, 2020).**

Table (1.1): Classification criteria for rheumatoid arthritis

Classification Criteria for RA (Total Score ≥ 6 is Considered Satisfactory for the Diagnosis of RA)		Score
A. Joint involvement	1 joint	0
	2–10 large joints	1
	1–3 small joints	2
	4–10 small joints	3
	> 10 joints	5
B. Serology (at least one test result is needed)	Negative RF and negative ACPA	0
	Low-positive RF or low-positive ACPA	2
	High-positive RF or high-positive ACPA	3
C. Acute-phase reactants (at least one test result is needed)	Normal CRP and normal ESR	0
	Abnormal CRP or abnormal ESR	1
Duration of symptoms	<6 weeks	0
	>6 weeks	1

1.1.6.1. Acute Phase Reactants of Rheumatoid Arthritis.

Acute phase reactants (APRs) are proteins whose levels in the blood increase or decrease in response to inflammation, injury, or infection. In the case of

rheumatoid arthritis (RA), a chronic autoimmune disease, APRs play an important role in the body's inflammatory response. The major acute phase reactants that are typically elevated in RA include **(Almoallim and Cheikh, 2021)**.

1.1.6.1.1. Rheumatoid Factor

Rheumatoid factor (RF) serves as a prevalent autoantibody for the diagnosis and prognosis of RA, exhibiting a sensitivity range of 60-90% and a specificity of 85% in both autoimmune and healthy populations.**(Manivelavan and Vijayasamundeeswari, 2012)**. IgA RF levels are generally reported to be greater in males than in females, and around 60% of primary Sjögren's syndrome cases are RF-positive. RF can also exist in a positive state **(Frisell *et al.*, 2013).** Additionally, RF positivity has been linked to systemic lupus erythematous (SLE), with 40–50% of individuals with HCV infection having positive RF **(Ingegnoli, Castelli, and Gualtierotti, 2013).** The distribution of RF in the Caucasian population may be influenced by genetic and environmental factors, potentially leading to a favorable correlation between genders and young 4% **(Mosaad *et al.* 2014).**

1.1.6.1.2. C-Reactive Protein

The C-reactive protein (CRP), a liver-produced acute-phase reactant protein, is elevated during inflammation due to IL-6 action on the CRP transcription gene, exhibiting both pro- and anti-inflammatory effects. **(Ullah *et al.* 2021).** it aids in the identification and removal of foreign infections and damaged cells. The patient is particularly vulnerable to an inflammatory cytokine storm if the CRP level is higher than this cut-off **(Yuan *et al*. 2020).**

1.1.6.1.3. Erythrocyte Sedimentation Rate

The erythrocyte sedimentation rate (ESR) is a laboratory test used to measure red blood cell settlement in a blood specimen. High fibrinogen levels can cause red blood cell clotting in various conditions like inflammatory

processes, infections, autoimmune disorders, pregnancy, anemia, kidney conditions, and certain cancers **(Narang *et al.*, 2020).** Erythrocytes aggregate into "rouleaux" due to density, causing rapid tube sedimentation. ESR measurement methodology, endorsed by ICSH, relies on Westergren's century-old findings. **(Tishkowski and Gupta, 2022)**.

1.1.7. Role of Cytokines and Interleukins in Rheumatoid Arthritis

Cytokines, proteins generated by cells in response to inflammation and immune responses, are essential for activating and regulating other tissues and cells. They can be categorized into various types based on their structural features **(Marques-Rocha *et al*., 2015 ; Ferreira *et al*., 2018).** Cytokines are secreted by cells, affecting their behavior and potentially leading to self-release. They are produced by various cell types, including endothelial, fibroblast, stromal, and immune cells like mast cells, T lymphocytes, B lymphocytes, and macrophages. Different cells can generate specific cytokines **(Li, Dong, and Wang 2024).** Cytokines, also known as chemokines, are chemical switches that regulate immune cell types, playing a crucial role in immunological responses and inflammation **(Kumar and Barrett, 2022) .**

Cytokines targeting is now a viable treatment for rheumatoid arthritis (RA), a condition caused by an imbalance of pro- and anti-inflammatory cytokines, leading to chronic inflammation and joint destruction **(Ding *et al*. 2023).** The exact cause of RA is unknown, but inflammatory cytokines play a crucial role in its pathogenesis, as they contribute to the destruction of cartilage and bone in arthritic joints **(Molnar *et al.* 2021).** Anti-TNF-α, the first biological medication for RA treatment, exemplifies cytokine neutralization's role in reducing inflammation. Today, it is used for treating autoimmune and chronic inflammatory diseases, with TNF inhibition linked to decreased synovial joint inflammation **(Siegmund and Wajant, 2023).**

1.1.8. Inflammatory Markers in Rheumatoid Arthritis

Inflammatory markers play a crucial role in the diagnosis and management of rheumatoid arthritis (RA). In RA, interleukin-6 (IL-6) concentration in synovial fluid reflects joint inflammation activity, identifying predictive factors for RA development, such as autoantibodies and systemic inflammation markers **(Targońska-Stępniak *et al.* 2021).** It is crucial in managing patients at risk of developing RA. Early diagnosis and treatment initiation in patients with inflammatory arthritis at risk of progression can lead to optimal clinical responses and better long-term outcomes. Understanding and monitoring inflammatory markers are essential in the comprehensive management of RA **(Novella-Navarro *et al.* 2021).**

1.1.8.1. Chemokines (Ligand-21)

Genome-wide association studies (GWAS) have identified chemokines (Ligand-21) (CCL-21) polymorphisms associated with RA susceptibility **(Aslam *et al.* 2020).** Furthermore, the expression of CCR7 on RA monocytes and MΦs strongly corresponds with the patient's disease activity score **(Van Raemdonck, Umar, and Shahrara, 2020).** The CCL-21 and CCR7 are highly co-expressed on RA synovial tissue (ST) MΦs, FLS and blood vessels **(Sprangers *et al*., 2017)**. CCL-21 induces differentiation of RA monocytes into CD14+CD86+ M1 macrophages and TRAP+ osteoclasts. CCL-21-induced monokines promote Th17 cell differentiation, contributing to RA osteoclast formation. CCL21 activates CCR7+ endothelial cells, enhancing RA angiogenesis **(Sprangers *et al*., 2017)**. Joint neovascularization may be aggravated by pro-angiogenic factors from CCL-21-activated RA FLS and MΦ. CCL-21 is a multifaceted chemokine influencing RA pathology the significance of CCL-21 and its receptor CCR7 in RA pathogenesis. Prior research suggests that targeting the CCL-21/CCR7 disruption in RA and other conditions could offer substantial therapeutic potential.

1.1.9. Anti-Inflammatory cytokines in Rheumatoid Arthritis

The anti-inflammatory cytokines might effectively inhibit arthritis, either by affecting innate immune cells (for example, by skewing macrophage polarization), or by interfering with the activation of B cells or T- cells **(Chen *et al.*, 2019)**. Moreover, cytokines with anti-inflammatory qualities have been found in RA patients' joints. These include the cytokines IL-4 and IL-13 produced by T lymphocytes and IL-10, which is mostly produced by macrophages **(Ji et al. 2023).** In the meantime, pro-inflammatory cytokines TNF-α and IL-1 are not produced, which is how IL-4 and IL-10 work antagonistically to suppress the inflammatory response **(Gandhi *et al.* 2021).**

1.1.9.1. Interleukin-4

Allergy-related conditions are linked to innate cell recruitment to inflammatory areas, synthesis of immunoglobulin E (IgE), smooth muscle contraction, and mucus production **(Ricardo-Gonzalez *et al.* 2010).** These symptoms are closely linked to the expression of two essential cytokines (IL-4 and IL-13), both of which are crucial for type-2 immune responses in vivo **(Liang, Farh, and Farh, 2012).** Th2 cells and other innate immune cells, including natural killer T- cells, produce IL-4 and IL-13 during type-2 inflammation, playing a crucial role in allergic inflammation-prone organs **(Middendorp and Nieuwenhuis, 2009; Paget and Trottein, 2013).** According to a study, group 2 innate lymphoid cells are one possible source of innate IL-4 in vivo that may be used to identify the main cellular sources of IL-4 that trigger type II immune responses **(Lund, Walford, and Doherty, 2013).** The function of interleukin-4 (IL-4), which could be involved in the pathogenesis of rheumatoid arthritis, has been uncovered. It has been documented that IL-4 plays a role in the control of T cell activation, differentiation, proliferation, and the viability of various T- cell subgroups. Furthermore, IL-4 exhibits an immunomodulatory impact on B cells, mast cells, macrophages, and numerous

other cell varieties. An analysis of the literature concerning the roles of IL-4 in rheumatic conditions is provided **(Dong, 2021)**.

1.1.10. Oxidants and Antioxidants in Rheumatoid Arthritis

Oxidative stress is a pivotal player in the aggravation of chronic inflammatory joint disease. Both experimental models and assessments in patients showed, in addition to elevated Ractive Oxygen Species (ROS) and lipid peroxidation formation, a decrease in antioxidant defenses **(Smallwood, *et al*, 2018).** In this sense, antioxidant therapy may offer novel adjuvant/complementary treatment options aiming at better controlling disease activity **(Batooei, *et al*., 2018).**

1.1.10.1. Antioxidant in Rheumatoid Arthritis

The increase in ROS and RNS production or the decrease in antioxidant mechanisms generates a condition called oxidative and nitrosative stress, respectively, defined as the imbalance between pro- and antioxidants in favor of the oxidants **(Taysi, *et al*. 2019).** Antioxidants in foods help to prevent oxidative reactions, but their health effect depends on their systemic bioavailability, concentration, and function **(Ali, 2020).** The relationship between serum TAS and the quality and composition of the diet has not yet been studied in RA patients. Recently, it has been demonstrated that diet quality may contribute to the course and activity of RA **(Valencia, 2022).**

1. Artemisinin

Artemisinin (ART), a phytochemical compound from the sweet wormwood plant, has been shown to have therapeutic properties, including anti-tubercular, anti-inflammatory, and anti-cancer effects. A study investigating inflammatory markers in patients with rheumatoid arthritis and type 2 diabetes found a hazard ratio of 0.94 for developing diabetes in RA patients compared to matched controls. The global prevalence of diabetes was estimated at 8.3% in

2012, with an incidence rate of 6.3 per 1000 person-years in a British cohort **(Adewumi, Singh, and Singh, 2020).**

Artemisinin is a white needle-like crystal with mp 151–153 °C. Elementary analysis and mass spectra showed the figure (1-2) the molecular formula of C15H22O5. It is insoluble in water but dissolves in acetone, ethanol, ether, petroleum ether, and alkali solution. Sodium hydroxide titration of artemisinin consumes one equivalent. Qualitative analyses give positive color reactions in the oxidation of FeCl2 or NaI. It quantitatively reacts with triphenylphosphine to give one equivalent of triphenylphosphine oxide. These reactions indicated the existence of an oxidative group in its molecule **(Pu, Chen, and Cai, 1979).**

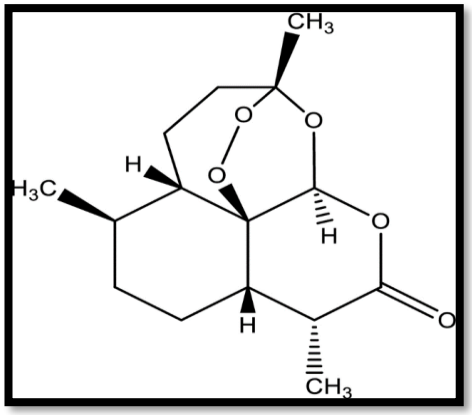


Fig. (1-2): Chemical formula of artemisinin

Artemisinin and its analogues have exhibited immunomodulatory effects on inflammatory and autoimmune disorders, such as rheumatoid arthritis (RA). RA is characterized as a persistent inflammatory condition leading to symptoms like joint pain, swelling, and restricted mobility. Research has illustrated the capacity of artemisinin to impede the movement and penetration of cells crucial in the development of RA **(Bao *et al.*, 2012)**. Artemisinin-based medications have shown potential in treating localized RA lesions by targeting synovial macrophages, which significantly impact the development of the disease. Their immunosuppressive effects alter the metabolic activities of proinflammatory

macrophages, indicating their potential effectiveness in managing RA symptoms and potentially decelerating the disease's progression **(Efferth and Oesch, 2021).**

2. Zinc

Zinc (Zn) has been studied for its potential role in rheumatoid arthritis (RA) due to its anti-inflammatory and antioxidant properties. Research has shown that zinc oxide nanoparticles-doped curcumin can assist in the recovery of RA by acting as an anti-arthritic and anti-inflammatory agent **(Qamar, John, and Bhatti, 2021)**. Additionally, the relevance of selenium status in RA has been explored, indicating a connection between antioxidant micronutrients, including zinc, and inflammatory markers in active RA patients. Furthermore, oxidative stress, which is a key process in the pathophysiology of RA, can be influenced by low intake of antioxidants, including zinc. Overall, incorporating zinc-rich foods into the diet may help in managing inflammation and oxidative stress in RA **(Gioia *et al.*, 2020)**.

One potential factor may involve a disruption in zinc metabolism, given zinc's essential roles in cellular and molecular functions within both innate and adaptive immunity, influencing susceptibility to infections **(Chasapis *et al*., 2020).** This paper explores the correlation between microbial infections and RA, examining whether low serum zinc is a pathophysiological outcome or a systemic zinc shortage affecting autoimmune features and disease pathogenesis **(Frangos and Maret, 2020).** Studies on individual immune cells reveal the impact of zinc deficiency or supplementation on B-cell and T-cell receptor signaling. The implications for diagnosing zinc-regulating substances in rheumatoid arthritis are crucial for disease management and treatment.

3. Vitamin D3

Vitamin D3 or the provitamin 25 (OH) D3 is a steroid hormone that regulates several other bodily processes in addition to its well-known roles in

calcium balance and bone metabolism **(Jun *et al.*, 2019).** The only vitamin that functions like a steroid hormone is vitamin D3. It is a fat-soluble vitamin with two distinct chemical configurations **(Webb *et al.*, 2021).** Vitamin D3 reduces inflammatory cytokines and immune responses, potentially reducing the production of inflammatory cytokines and promoting therapeutic benefits in the treatment of rheumatoid arthritis. **(Khorasanizadeh *et al.*, 2019).** Vitamin D3 is crucial for managing inflammatory diseases like rheumatoid arthritis, as it prevents inflammation and reduces pain. It acts as an anti-inflammatory and antioxidant, regulating the immune system and reducing inflammation **(Bishop *et al.*, 2021)**. It supports various bodily functions and can be incorporated into the diet or supplements for individuals with rheumatoid arthritis. Its anti-inflammatory and antioxidant properties make it a valuable addition to overall health **(Roy *et al.*, 2022)**.

The relationship between Vitamin D and Rheumatoid Arthritis (RA) is unclear, and it's unclear if Vitamin D supplementation can reduce RA recurrence, potentially leading to long-term remission and reducing the need for slow-acting drugs **(Yang *et al.*, 2015)**. A randomized clinical investigation found that lower levels of vitamin D3 in certain groups are a risk factor for the recurrence rate of RA.

1.1.11. Inflammatory and Anti-Inflammatory Balance in RA

Persistent synovitis, a primary symptom of rheumatoid arthritis, is caused by the continuous influx of immune cells into joints, triggering the production of pro-inflammatory cytokines, activating local synoviocytes, and causing bone and cartilage damage**(Kondo, Kuroda, and Kobayashi, 2021).** Synovitis is triggered by innate immune cells, including mast cells, neutrophils, and macrophages, releasing pro-inflammatory cytokines **(Chen *et al.*, 2019).** A complex array of cytokines facilitates the inflammatory pathway, with specific cytokines released into the bloodstream, observed in various inflammatory

conditions like RA **(Martínez-García and Hernández-Lemus, 2021)**. Their levels typically reflect the disease prognosis and severity. Cytokines are categorized as either pro-inflammatory (TNF-α, IFN-γ, IL-1, IL-2, IL-6, IL-8, IL-12, IL-15, IL-17, and IL-18) or anti-inflammatory (IL-4, IL-10) based on their functions **(Kany, Vollrath, and Relja, 2019).** The balance of pro- and anti-inflammatory cytokines in RA significantly influences inflammation, leading to various clinical implications and mediating tissue damage and chronic inflammation linked to arthritis pathophysiology. **(Bedeković *et al.*, 2023).** Joint imbalance leads to degeneration of articular cartilage, affecting joint biomechanics and other components, causing inflammation, degradation, and pain, ultimately affecting joint function **(Wojdasiewicz, Poniatowski, and Szukiewicz, 2014)**. As cartilage invasion and bone erosion progress, an imbalance between pro-inflammatory and anti-inflammatory factors emerges **(Haridas *et al.*, 2019).** This imbalance eventually triggers autoimmunity and chronic inflammation in individuals with RA **(Uttra *et al.* 2019).**

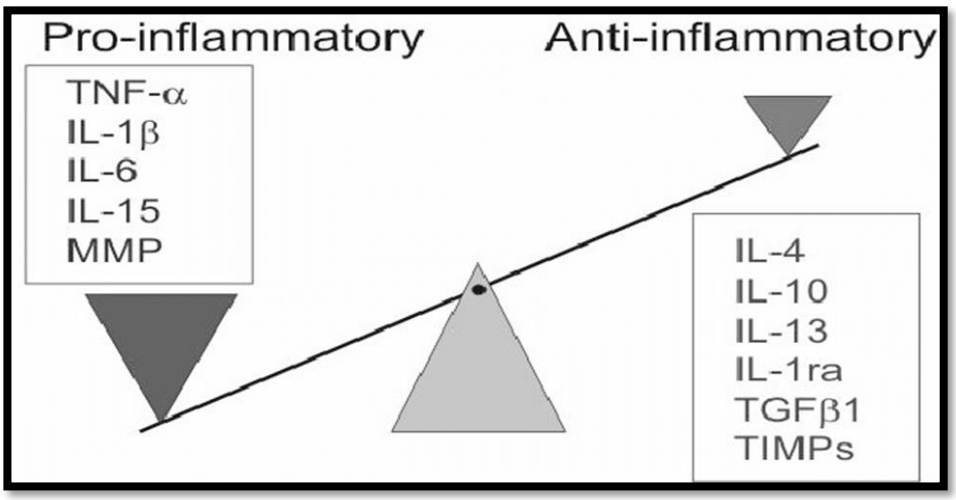


Fig. (1-3): The diseased joint contains inflammatory mediators. Rheumatoid arthritis patients have an imbalance between pro- and anti-inflammatory mediators. A chronic inflammatory environment brought on by an overabundance of pro-inflammatory mediators not only thins the cartilage but also produces clinical symptoms like joint pain and swelling. Pro-inflammatory mediator functional blockade enhances the overall health of the synovial junction (Kramer *et al.* 2003).

1.2. Diabetes Mellitus

Diabetes mellitus, a global disorder causing high glucose levels, is expected to affect a significant number of individuals by 2020, according to the International Diabetes Federation. **(Federation, 2020).** In the Middle East and North Africa region, including Iraq, the prevalence ranges from 373 to 557 cases **(Organization, 2019).** In 2018, the World Health Organization reported that 1.4 million Iraqi citizens were diagnosed with diabetes (**Redondo *et al*., 2018).** Diabetes mellitus is a disorder causing excessive urine production, leading to 32% of global fatalities. Type 2 diabetes, a chronic disorder, is a significant cause, with individuals under 60 accounting for 50% of these deaths **(Singh and Mug *et al,* 2021)**. Research indicates an expected increase in type 2 diabetes prevalence in the next two decades, particularly among individuals aged 45-64 **(Manu, Rogozea, and Cernea, 2021; Teufel *et al*., 2021).**

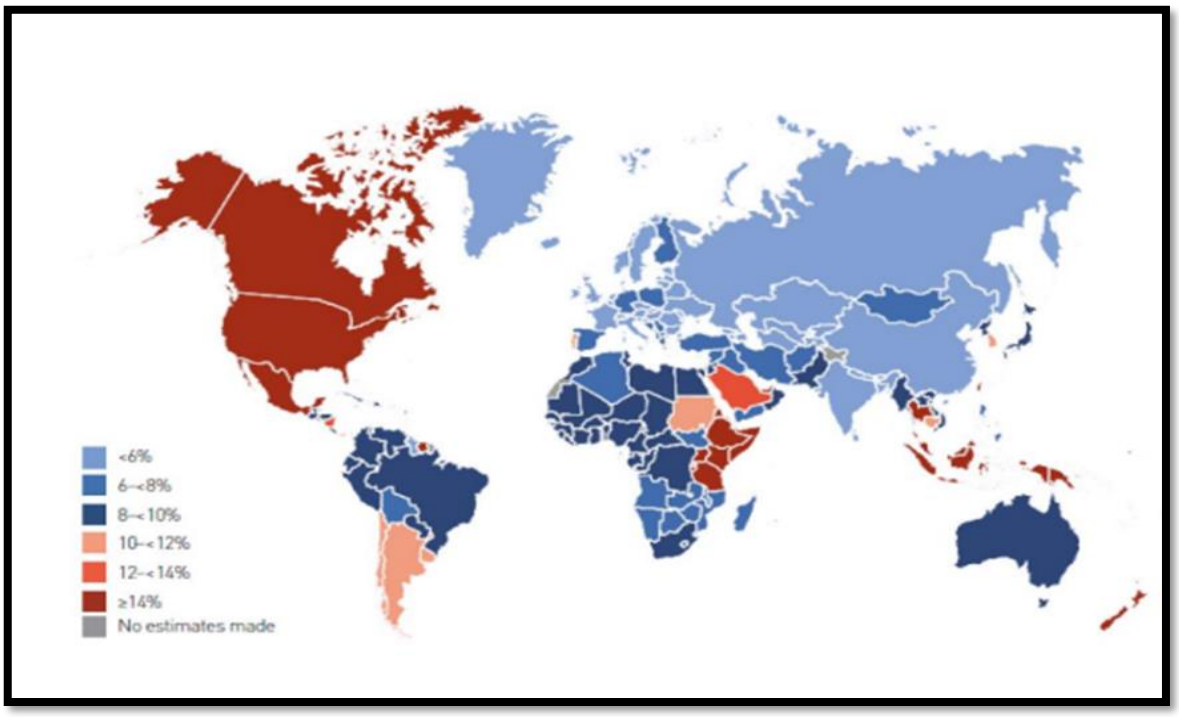


Fig. (1-4): Prevalence of impaired glucose tolerance in adults (20–79 years) in 2019, adapted from international Diabetes Federation (Federation 2020)**.**

1.2.1. Classification of Diabetes Mellitus

Diabetes mellitus (DM) is a condition characterized by various phenotypes, including Type 1 diabetes, a type typically diagnosed in juveniles, and insulin-dependent diabetes mellitus (IDDM), which is influenced by genetic predisposition, environmental triggers, and lifestyle choices **(Riaz, 2015).** Type 2 diabetes (T2DM) is a second type of diabetes mellitus that does not require insulin for treatment (NIDDM). Diabetes is categorized within the following general groupings:

A. Insulin-dependent diabetes mellitus (T1DM) is an autoimmune condition requiring continuous insulin therapy and frequent blood glucose monitoring due to an autoimmune attack on pancreatic β-cells. **(Mirazi, Rezaei, and Mirhoseini, 2016; Puchulu, 2018)**. Type 1 diabetes patients with polydipsia, polyphagia, weight loss, lethargy, eyesight loss, and ketoacidosis require insulin supplements for lifelong maintenance. (**Jakobsen and Szereday 2018)** .

B. Insulin-independent diabetes mellitus (IIDDM) or type 2 diabetes mellitus (T2DM) is the most common type of diabetes, affecting 85%-95% of cases and typically presenting after 40. **(Burtis and Bruns, 2014).** Diet is one of the most crucial aspects of managing type 2 diabetes. According to recent research, diabetes mellitus (DM) can be well managed with diet and exercise alone, negating the need for hypoglycemic medications**(Le Roux *et al.*, 2017)**.Furthermore, displayed in table (1.2) **(Oram *et al.*, 2016).**

C. Gestational diabetes mellitus (GDM), is condition characterized by varying levels of glucose intolerance identified for the first time during pregnancy through a range of diagnostic and ethnic parameters **(Puchulu *et a*l., 2018**

Table (1.2): Differences between T2DM and T1DM

Clinical features	T1DM	T2DM
Age of onset	Most 25 but can occur at any age (not before 6 months)	Usually 30 years
Weight	Usually thin	Overweight 90%
Islet autoantibody	Usually presents	Absent
C-Peptide	Undetectable low	Normal/high
Insulin production	Absent	Present
First line treatment	Insulin	Non-insulin – ant hyperglycemic agent
Family history of DM	Infrequent (5%-10%)	frequent (75-90%
Diabetic ketoacidosis (DKA)	Common	Rare

1.2.2. Causes of Diabetes

Obesity is the primary factor associated with T2DM predisposition. more precisely, genetic predisposition. Several studies have shown that mild to moderate chronic inflammation has a major role in the development of obesity and diabetes **(Kwon and Pessin, 2013; Shields *et al.*, 2015; Saltiel, Alan, *et al.*, 2017)**.While the projected incidence was almost 90%, the overall rate of overweight and obesity in 2014 was 52% **(Saltiel and Olefsky, 2017).**

T2DM is predicted to affect 10% to 15% of people over 65 and 20% of those aged 65-80, eight times more than the 2.4% prevalence among adults aged 18-44 due to aging-related changes in FBS. **(Huang *et al*., 2018).**

Hypertension and diabetes are both microvascular disorders, with type 2 diabetes and atherosclerosis being the main causes. Both conditions share a significant overlap in genesis and clinical processes. **(Frimpong and Nlooto, 2020).**

T2DM has a unique genetic basis, with dizygous twins having a higher concordance rate. First-grade family history is linked to a twice higher chance of future type 2 diabetes. Genetic research reveals several genes and unknown roles.. Figure (1-5) illustrates this relationship.**(Ali 2013; Hu and Jia, 2018).**

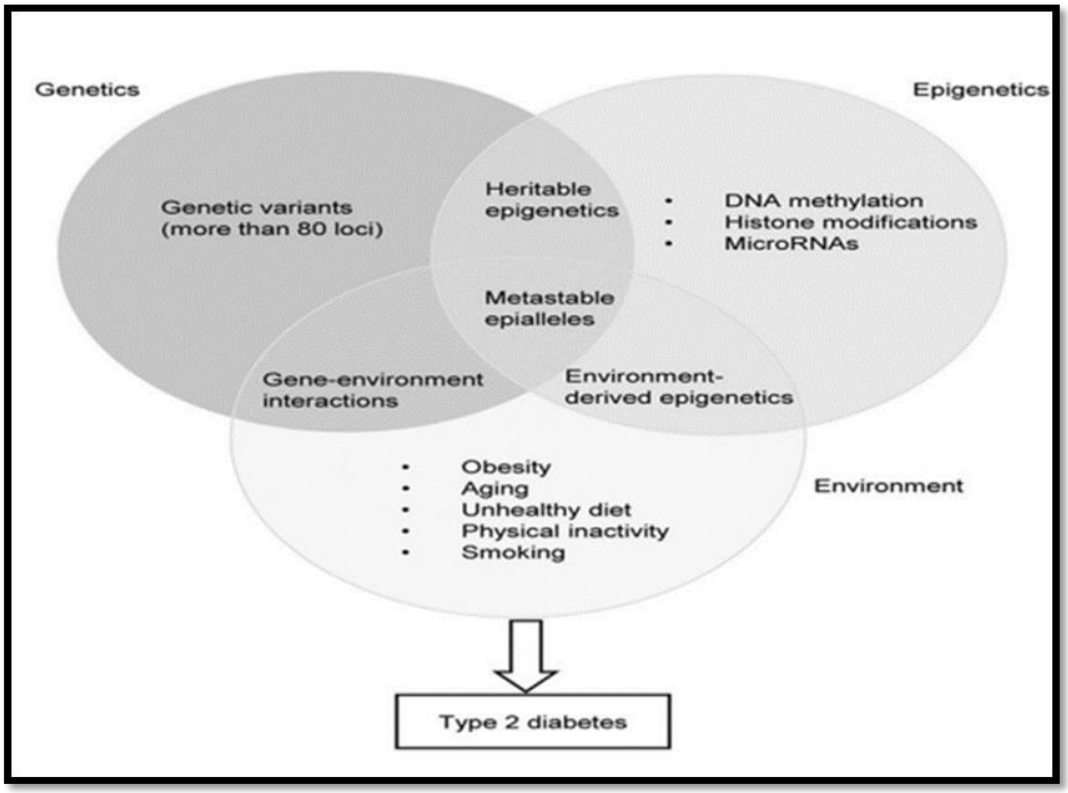


Fig. (1-5): Illustrates the relationship between genetics, epigenetics, and environment

1.2.3. Rheumatoid Arthritis and Type 2 Diabetes Mellitus

Despite advancements in pharmaceutical techniques for rheumatoid arthritis, numerous comorbidities continue to burden patients, increasing morbidity, death, and impairment **(Ferguson *et al*., 2019; Smolen *et al.*, 2023).** Because RA patients have higher rates of insulin resistance (IR) and type 2 diabetes (T2DM), a growing body of research has demonstrated a link between glucose dysregulation and the chronic inflammatory process**(Ruscitti *et al*., 2017)**. The Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) is a cost-effective and precise assessment of insulin resistance, which occurs

early in type 2 diabetes and during rheumatoid arthritis and may be influenced by pro-inflammatory pathways and pathogenic processes **(Donath and Shoelson, 2011).** In this context, past studies have documented the efficacy of immunomodulation therapies in people with co-occurring metabolic problems and RA **(Donath, Dinarello, and Mandrup-Poulsen, 2019; Di Muzio, Cipriani, and Ruscitti, 2022).** Interleukin's anti-inflammatory inhibition was inked to concomitant RA efficacy and concomitant glucose dysregulation **(Ruscitti *et al*. 2019; Genovese *et al*., 2020).** The glycemic indices, interleukin inhibition primarily, and the improvement of RA disease activity were observed following the administration of biological disease-modifying anti-rheumatic medications (bDMARDs) **(Giacomelli *et al.*, 2016)**. Due to the latter, RA patients with T2DM were able to meet the objectives of both disorders' treatments, leading to a benefit that was clinically significant **(Ruscitti *et al.*, 2019).** These clinical findings suggested possible therapy targets by revealing a possible amplification of T2DM's inflammatory processes in the context of RA **(Giacomelli *et al*., 2016).** Pro-apoptotic protein production is linked to β-cell apoptosis by pro-inflammatory cytokines, requiring the activation of Janus kinase/signal transducer and activator of transcription (JAK/STAT) **(Gurzov *et al*., 2016).** The in vivo metabolic role of the JAK/STAT signalling system suggests potential therapeutic benefits for glucose dysregulation, with mouse models showing effective characterization of this system **(Richard and Stephens, 2011; Gurzov *et al*., 2016).** JAK inhibitors, commonly used to treat RA, may also benefit T2DM patients by reducing glucose irregularities and inflammatory symptoms, according to previous studies **(Genovese *et al*., 2020)**.

1.2.3.1. Role of Sugar in the Rheumatic Diseases

In recent years, as more researchers have explored the relationship between high-sugar diet and inflammation, they have found that excessive sugar intake is closely associated with the development chronic inflammation and

(Bodur and NERGİZ ÜNAL 2019). Low-grade chronic inflammation has long been linked to obesity and increased body fat, and excess dietary sugar intake is a key contributor to obesity and weight gain. It has been revealed that dietary sugar is a key factor in inducing low-grade chronic inflammation, autoimmune diseases, and even neuron inflammation (**Ma *et al.*, 2022).**

1.2.3.2. Role of Insulin in the Rheumatic Diseases

Insulin is the main hormone regulating glucose homeostasis, and it acts through the transmembrane insulin receptors. The latter are expressed on multiple target cells, including hepatocytes, adipocytes, synoviocytes, and muscle cells. Intriguingly, these receptors may also be found on the surface membrane of immune cells **(Haeusler, McGraw, and Accili 2018).** These cells need glucose to produce energy, and through its receptors, insulin exerts its hypoglycaemic function and behaves as a growth-like factor as well as a cytokine regulator**(Yanfei Li *et al.*, 2023) .**

Therefore, this hormone may also exert immunomodulatory effects on the immune system in addition to well-known metabolic effects **(Van Niekerk *et al*. 2020).** Insulin signaling, similar to what is observed in T2DM, or metabolic syndrome, may be involved in the dysregulation of the immune response in inflammatory diseases. Epidemiological and laboratory studies reported a possible correlation between insulin resistance and rheumatoid arthritis (RA)**(Sánchez-Pérez *et al.*, 2017).** Insulin may decrease levels of CRP, reduce the ability of neutrophils to generate ROS, and suppress transcription of different Toll-like receptors (TLRs) on circulating mononuclear cells **(Tripolino *et al.*, 2021) .**

1.2.3.3. Role of insulin resistance in the Rheumatic Diseases

Insulin sensitivity occurs due to biological effects in the insulin responsive tissue, mainly adipose, liver, and striated muscle tissue. Decreased insulin sensitivity is also called insulin resistance (IR), and it is usually classified

as decreased suppression of hepatic glucose production, reduced lipolysis rate among adipose or fat tissue, and impaired clearance of glucose in striated muscle**(Koliaki and Roden 2016).**Insulin resistance in RA partially causes obesity by increasing fat mass, disease activities, and the occurrence of RF. Insulin resistance can also be linked significantly with some inflammation markers such as CRP and TNF. Insulin resistance is more serious and critical in people experiencing high disease activity than in people experiencing medium disease activity. Insulin is associated with all inflammatory cytokines **(Verma *et al.*, 2021).**

Insulin resistance can be evaluated using the homeostatic model assessment (HOMA). The idea was explained in a 1985 paper by David Matthews and colleagues **(Knutsson and Kempe, 2013).** Through mathematical derivation of the HOMA model, a link between FBS and fasting insulin was sought after. These correlations can be used to evaluate beta-cell function and insulin resistance **(HB, 2015)**. The following equations are applied:

HOMA-IR = (Fasting blood insulin × Fasting blood glucose) / 405

1.2.3.4. Role of Glycated Hemoglobin in RA

HbA1c is released into the bloodstream. Apart from indicating long-term blood glucose levels, free HbA1c can increase CRP, oxidative stress, and blood viscosity. These processes collectively contribute to damage to the endothelial cells lining the blood vessels **(Jingyang Chen, Yin, and Dou 2023).**

1.3. Role Artimisinin in T2DM

Artemisinin and its derivatives are crucial for managing type 2 diabetes and its complications. Comparing their characteristics and distribution in different tissues can better understand their therapeutic effects and guide future research. Artemisinin may reduce cognitive damage caused by diabetes **(Zeng, Xu, and Zheng, 2017; Albasher *et al.*, 2020).** These qualities make artemether, dihydroartemisinin, and artesunate worthy of more investigation to ascertain

their impact on cognitive impairment in diabetics. Additionally, artemisinin can cross placental and blood barriers **(Jiang *et al*., 2020)** and have the potential to accumulate in testicular tissue;thus, we must be mindful of these two medications' potential harm to reproduction. There are still numerous gaps in our knowledge of artemisinin and its derivatives' pharmacological properties **(Na-Bangchang *et al*., 2005; Gautam *et al*., 2011).** Artemisinins can reduce type 2 diabetes by attenuating insulin resistance and regaining islet cell activity, addressing the interplay between inflammation, insulin resistance, and obesity **(Jiang *et al*., 2020).** Research indicates that artemisinin and its derivatives can prevent type 2 diabetes by protecting islet ß cells, promoting insulin secretion, and achieving islet α-cell to ß-cell transdifferentiation.

1.4. Nanotechnology and Nano medicine

Nanotechnology, a significant scientific advancement in the 21st century, involves the control and utilization of materials below 100 nm. Its applications span environmental studies, agriculture, food sciences, biotechnological advancements, biomedical research, pharmaceuticals, and wastewater remediation **(Zahra *et al.* 2020).** Nanoparticles (NPs) are crucial for technology advancement due to their superior performance and flexibility. Traditionally, hazardous reducing agents convert metal ions into uncharged nanoparticles. However, recent initiatives focus on environmentally friendly technology using natural resources. Green synthesis uses biological methods, such as algae, yeast, plants, bacteria, actinomycetes, fungi, and algae, due to their ease of use, affordability, safety, cleanliness, and productivity **(Altammar, 2023)**. According to projections, nanobiotechnology—which is associated with data science and cognitive capacities—will create new opportunities in several industries over the next few decades, including various economic sectors, agriculture, the environment, and medical**(Odda, Alhaideri, and Jasima 2022).**The use of nanotechnology in medicine is known as nanomedicine,which

is the application of nanotechnology to health issues, utilizing the unique physical, chemical, and biological properties of nanoscale materials. It aims to provide early, precise diagnosis, treatment, and disease prevention by utilizing molecules and our understanding of the human body to prevent, diagnose, treat, and relieve pain while maintaining and enhancing human health **(Zahin *et al.*, 2020).**

1.4.1. Applications of Nanotechnology in Medical Research

Nanotechnology plays a crucial role in nanomedicine by making the use of dangerous drugs safer, increasing treatment efficacy, reducing adverse effects, and addressing targeted therapeutic administration issues. Its applications span vaccine manufacturing, drug delivery, imaging, diagnostic instruments, antimicrobial products, and the development of advanced diagnostic methods, non-medical devices, and specific drug administration mechanisms **(Haleem *et al.*, 2023).** Nanoparticles have the ability to connect with particular biomarkers for the purpose of improving various imaging techniques like magnetic resonance imaging (MRI), computerized tomography (CT) scans, and positron emission tomography (PET) scans, thereby increasing their sensitivity, precision, and specificity **(Singh and Amiji, 2022).**

1.4.2. Drug Delivery Applications

Nanotechnology has led to significant advancements in drug delivery systems, primarily focusing on adsorbed polymer matrices and nanoparticles containing therapeutic medications. The primary objectives of these systems are to extend injectable drug half-life and improve targeted tissue delivery bioavailability, enhancing the effectiveness of nanodrug systems **(Sur *et al.*, 2019)**.Drug encapsulation holds great promise for the advancement of nano medicine. Modern encapsulation techniques also have a number of advantages over conventional medical practices, such as the ability to target and penetrate

particular tissue at the molecular level. Because the medications or small molecules are encapsulated, dosage requirements and medication toxicity are decreased **(Montané *et al*., 2020).** Polymers are gaining popularity due to their biocompatibility and ease of manufacturing. However, nanomedicines require consideration of biological barriers such as extended circulation, efficient accumulation, successful penetration into tissues, and selective uptake of nanoparticles into cells **(Li *et al*., 2022).**

1.4.3. Niosomes

Niosomes are vesicles formed by the self-assembly of non-ionic amphiphilic surfactants. Cholesterol, and occasionally, charged molecules, are added to provide rigidity to the bilayers and to enhance the stability of the system. Niosomes share structure similarities with liposomes, and they were introduced as an alternative to overcome limitations associated with stability, sterilization, and large-scale production of liposomes **(Thabet, Elsabahy, and Eissa 2022)**. The structure of niosomes is shown in figures (1-6) **(Mishra *et al.* 2020).**

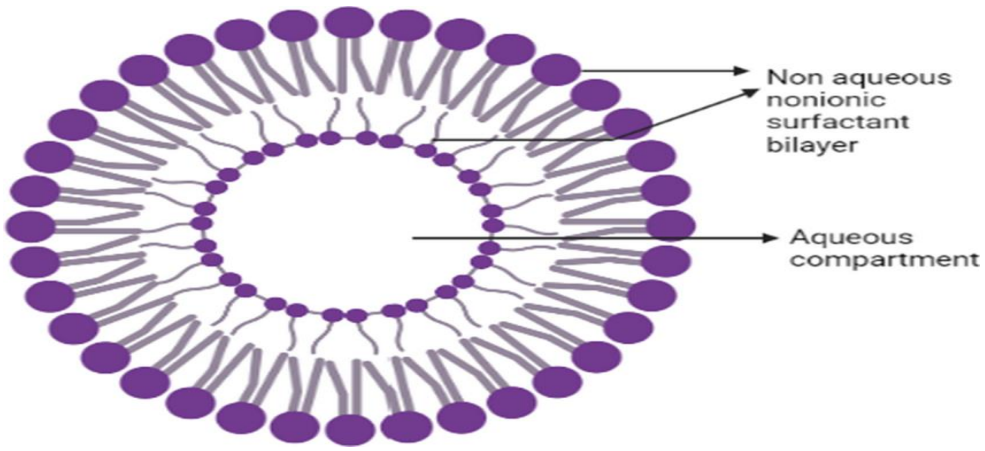


Fig (1-6): structure of niosomes.

1.4.4. Hyaluronic acid

Hyaluronic acid is a polysaccharide with characteristics that play a pivotal role in various fields such as medicine, cosmetics, and materials science for biomedical and cosmetic uses. With its water retention capacity, HA is essential for skin hydration and wound healing **(X. Li *et al.*, 2022).**

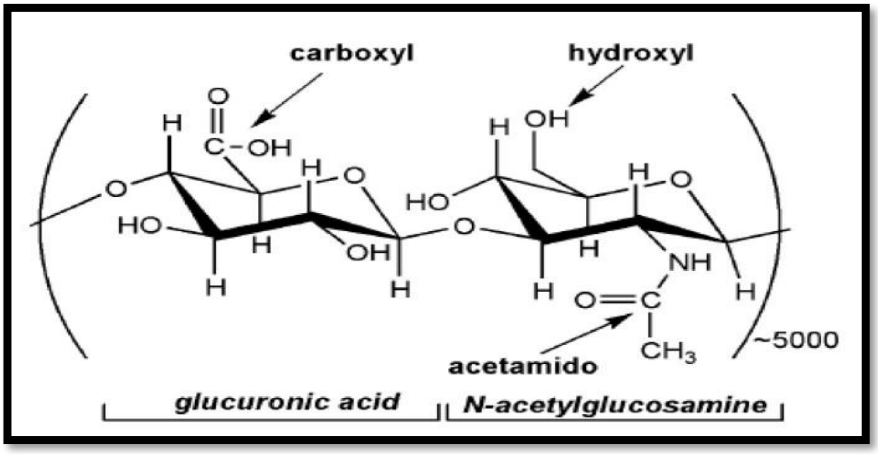


Fig. (1-7) Structure of the Hyaluronic acid (Machado *et al.*, 2022)

1.4.5. Artemisinin-Loaded Hyaluronic Acid-Coated Niosomal NPs

Nanomedicine is a ground breaking method that has surfaced for the management, detection, and accurate focus on rheumatoid arthritis by employing NPs **(Liu *et al.*, 2021)**. In order to augment the effectiveness of niosomes, which are characterized as non-ionic surfactant vessels akin to liposomes, various methodological strategies can be implemented in accordance with empirical research. A particular strategy involves the alteration of the composition, dimensions, and classification of niosomes to affect the permeation and administration of pharmaceuticals **(Choi and Maibach, 2005)**. Additionally, smart nanocarriers, including enzyme-responsive nanomaterials, can improve controlled drug delivery by integrating stimuli-responsive features into the niosome framework. **(Hu, Katti, and Gu, 2014)**. Targeted drug delivery via nanomaterial vehicles demonstrates potential in chronic disease management. Researchers aim to enhance drug delivery efficacy by tailoring niosomes with

specific ligands for organ or tissue targeting. For instance, PEGylated liposomes have been investigated for drug delivery to the retina, potentially improving therapeutic outcomes **(Vighi *et al.,* 2018)**. Hyaluronic acid (HA) has gained attention in treating arthritis (RA) due to its ability to biodegradability (break down naturally), biocompatibility (compatibility with the body), and minimal immunogenicity (low potential for causing reactions) **(Vighi *et al.,* 2018)**. Research indicates that HA can target CD44 receptors on activated macrophages in inflamed joints, showing promise as a treatment option for RA **(Wang *et al.,* 2020; Chen *et al.,* 2024)**. Hyaluronic acid has also been included in formulations such as HA coated nanoparticles designed for drug delivery and therapy in RA **(Changhui Yu *et al.,* 2019; Li *et al.,* 2021)**.

1.5. Peripheral Blood Mononuclear Cell.

Peripheral Blood Mononuclear Cells (PBMCs) play a crucial role in rheumatoid arthritis (RA) pathogenesis. Studies have shown that PBMCs from RA patients exhibit altered expression of reactive oxygen species (ROS), inflammatory cytokines, and adhesion molecules, contributing to the inflammatory response of fibroblast-like synoviocytes (FLSs) in the joints **(Lee *et al.,* 2021)**. Regulatory T cells in RA patients show increased mitochondrial ROS expression, while CEACAM1 expression on peripheral blood neutrophils is elevated in patients with active RA **(Masoumi *et al.,* 2021).** Additionally, changes in cell types, protein levels, and gene expression in PBMCs have been associated with methotrexate treatment response in RA patients. Furthermore, abnormal expression of T cell immunoglobulin and immune receptor tyrosine inhibitory motif domain (TIGIT) affects cytokine secretion function of CD56 T cells in RA **(Zhu *et al.,* 2023).** These findings highlight the importance of PBMCs in RA pathophysiology and treatment response, providing insights for potential therapeutic strategies targeting PBMC regulation to suppress the inflammatory response in RA **(Brynedal *et al.,* 2023).**

1.6. Gene Expression Detection in Artemisinin Coated with Hyaluronic Acid Nanoparticles

A gene is the basic physical and functional unit of heredity. Genes are made up of DNA. Some genes act as instructions to make molecules called proteins, which are needed for the body to function. However, many genes do not code for proteins; instead, they help control other genes **(Nicholl 2023).**

Gene expression involves the conversion of genetic information into functional products. This process primarily occurs through RNA transcription, producing either protein-coding or non-coding RNA. Gene expression functions as both a regulatory mechanism for the timing and location of RNA and protein synthesis and a determinant of the quantity produced. **(Mattick and Amaral 2023).** Gene expression regulation varies with conditions and cell types. Gene products, particularly RNA and proteins, modulate the expression of other genes. Gene expression can be evaluated through the functional activity of gene products or associated phenotypes.**(Gil and Ulitsky 2020).**

Matrix metallopeptidase-2 (MMP-2) and tissue inhibitor metalloproteinases-2 (TIMP-2) genes play a role in rheumatoid arthritis. The MMP-2 gene is associated with medullary thyroid carcinoma and can be regulated by prostaglandin E2 (PGE2) in rheumatoid arthritis. TIMP-2 inhibits BGF-induced endothelial cell proliferation in arthritis. Additionally, MMP-2 and MMP9 regulate T cell activation**(Ahmad *et al.*, 2019),** while TIMP2 is associated with rheumatoid arthritis. Furthermore, COX-2 inhibitors, diabetes mellitus , can attenuate joint inflammation in arthritis **(Janovits *et al.*, 2021).** Rhus verniciflua Stokes extract can suppress MMP and TIMP gene expression, as well as COX-2 gene expression, by inhibiting NF-kappaB activation. Overall, MMPs, TIMPs, and COX-2 genes are involved in the inflammatory response and joint abnormalities seen in rheumatoid arthritis**(Bedoui *et al.*, 2021).**

A recent exploration unveiled that the MMP-2 enzyme is excessively released within the joints of individuals battling rheumatoid arthritis, assuming a crucial role in both inflammation and immunity **(Caiwei Yu *et al.* 2022).** Within the joint capsule, this enzyme is synthesized by monocytes and macrophages, with chondrocytes contributing minimally to its production. The action of tissue inhibitor MMP (TIMP) is to avert uncontrolled degradation of ECM components; the heightened proteolytic activity of MMPs arises from an imbalance between their proteolytic functions and the inhibitory effects of both MMPs and TIMP **(Cabral-Pacheco *et al.,* 2020)**. TIMPs are responsible for breaking down the extracellular matrix, eliminating molecules from cell surfaces, and responding to inflammation by facilitating the proteolytic maturation of cytokines. During this process, TIMPs bind non-covalently to the active site of the target MMP in a 1:1 ratio to impede MMP activity **(Mukherjee and Das, 2024).**

The involvement of specific amino acids in the interaction between MMP-2 and artemisinin in rheumatoid arthritis has been directly addressed in the provided search results. However, MMP-2 is known to play a role in the pathogenesis of rheumatoid arthritis by modulating gene expression related to amino acid metabolism **(Xueling Liu *et al*., 2022).** Additionally, artemisinin has been studied for its potential therapeutic effects in rheumatoid arthritis, possibly through its interaction with MMP-2. Further research is needed to elucidate the specific amino acids involved in this interaction and their impact on the pathogenesis and treatment of rheumatoid arthritis **(Efferth and Oesch, 2021)**.

Aims of the Study:

The proposal of the presented work constitutes generally three parts (I, II, and III), they aim to:

Part / I: Clinical Chemistry Investigations and Analysis

1. The study aims to assess the serum levels of inflammatory and anti-inflammatory markers CCL-21 and IL-4 in RA patients and T2DM patients, as well as oxidation markers like vitamin D and zinc. It also investigates the relationship between IL-4 and CCL-21, vitamin D, zinc, FBS, insulin, and HbA1c in RA patients using ROC

Part / II: Nanoparticle Synthesis and Characterization:

2. Formulation, characterization, and evaluation of the effect of H-Nio-artemisinin nanoparticles to target peripheral blood cells *in vitro* derived from rheumatoid arthritis patients and evaluation of different agents for these cells and study their applications as anti-inflammatory and antioxidant agents.

Part / III: Gene Expression Detection

3. Detection of gene expressions including MMP2, TIMP2 and COX2 using reverse transcriptase polymerase chain reaction in niosomes after treatment with artemisinin nanoparticles *in vitro*

CHAPTER TWO

Materials and Methods

2. Materials and Methods

2.1. Subjects

2.1.1. Study Design

A case-control study design, for a total of 125 Subjects was collected throughout the period from October, 2023 to March, 2024; all the subjects included in this study are women, and the proposal application was carried out at the department of chemistry and biochemistry, college of medicine, university of Kerbala.

A total of 125 participants took part in this research, and they have been divided into three groups as illustrated in scheme (2-1). Their ages ranged between 30 to 70 years, and they were obtained from the rheumatology consultant clinic at Al-Hindiyah Teaching Hospital, Kerbala Health Directorates, Kerbala, Iraq, as well as Al-Sadiq and Marjan Teaching Hospital, Babylon Health Directorates, Babylon, Iraq. The initial and second groups consist of 74 patients with rheumatoid arthritis without T2DM and rheumatoid arthritis with T2DM, respectively, while the third group comprises 51 samples collected from apparently healthy women as a control

2.1.2. Ethical Approval

The ethical approval for the application of the project proposal was obtained from the ethical committee team, the college of medicine, the university of Kerbela, and the Kerbela Health Directorates (Kerbela, Iraq); see Appendices.

2.1.3. Inclusion Criteria

The rheumatologist diagnosed the patient with rheumatoid arthritis based on a thorough clinical examination and laboratory tests to meet the criteria for inclusion in the ACR/EULAR-2010 guidelines **(Aletaha *et al*., 2010).** Patients with RA and T2DM were chosen based on fasting blood glucose and HbA1c%, body mass index ≥ 29 kg/m^2, and their age was ranged between 30 and 70) years. Selection of patients was also based on disease severity criteria, including DAS28

≥ 5.1 (severe) and elevated levels of erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and rheumatoid factor (RF).

2.1.4. Exclusion Criteria

The exclusion criteria of the subjects studied include:

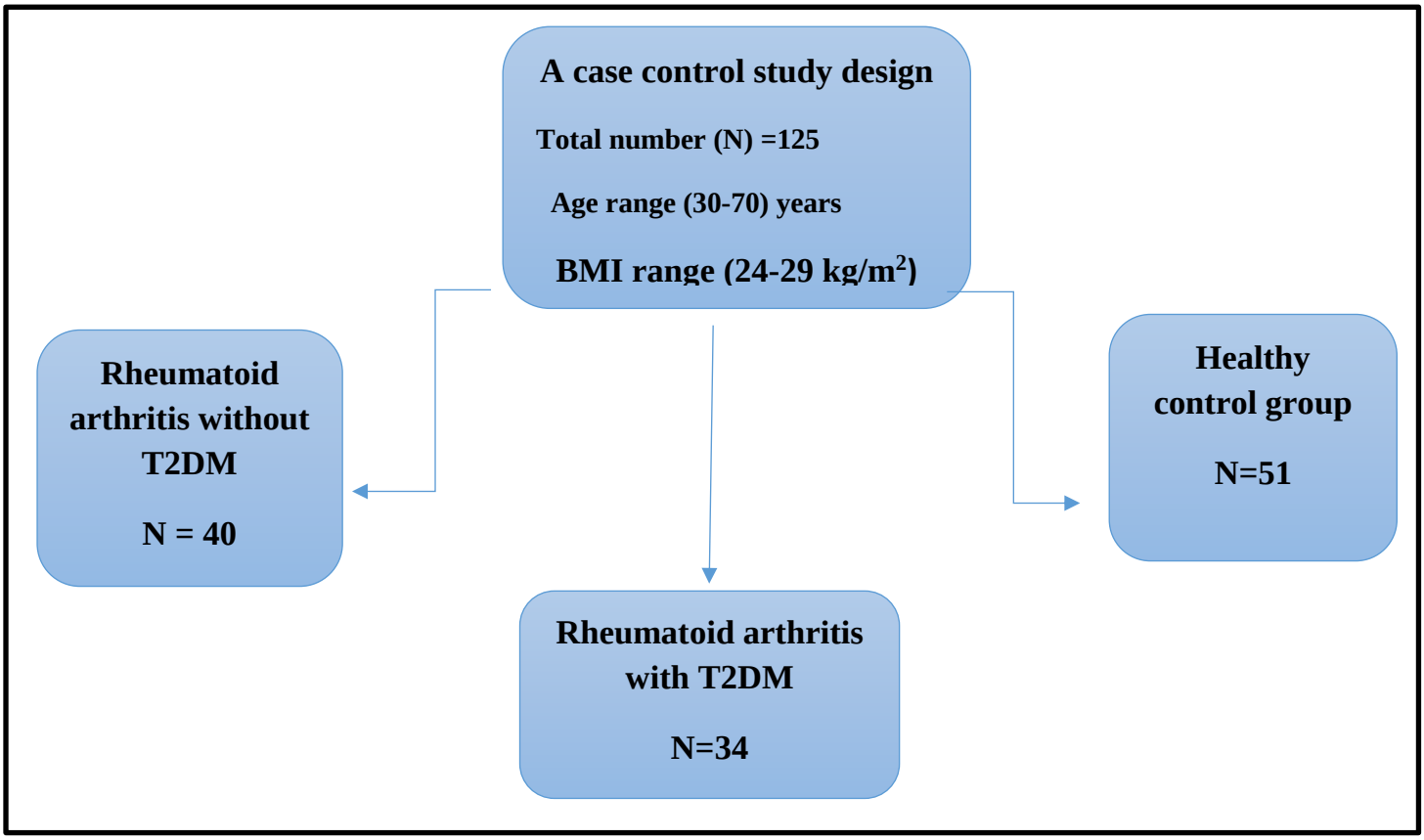
a. Pregnant and breast feeding women.

b. Patients with malignancy

c. Thyroid disorders.

d. Other rheumatology diseases.

e. All other autoimmune disorders and infectious disease.



scheme (2-1): Study design.

2.1.5. Blood Samples Collection

Blood samples (6 ml) were obtained from each RA patient and healthy control groups by vein puncture. Each sample obtained will be divided into two parts:

A.The tube contained 2 ml of blood, set in gel tubes. Next, it stayed for 10-15 minutes at room temperature for clotting.

Then centrifuged at 2000 x g for serum separation, the obtained sera were divided into 4 parts and put in Eppendorf tubes, then stored at -20 ˚C until the day of investigation or analysis of various biomarkers included in this study, and the other 1 ml were preserved in a heparin tube for subsequent erythrocyte sedimentation rate (ESR) analysis and glycated hemoglobin (HbA1c).

B. Three ml of whole blood samples were used after separating blood components using Ficoll and extracting peripheral blood cells, and the structural properties of artemisinin-loaded nanoparticles coated with hyaluronic acid as anti-inflammatory and antioxidant agents were investigated and their effects on the study markers of peripheral blood cells of rheumatoid arthritis patients were proven. The properties of the nanoparticles were measured using scanning electron microscopy (SEM), Fourier transform infrared (FTIR), AFM, MTT and various DLS techniques. Then the effect of artemisinin-loaded nanoparticles coated with hyaluronic acid on the gene expression of different genes including MPP2, TIMP2 and COX2 was evaluated. The work on the nanoparticle and gene part and the detection of the medical properties of rheumatoid arthritis patients before and after artemisinin treatment were carried out at Tabriz University of Medical Sciences

2.2. Materials

2.2.1 Chemicals and Kits

The kits used in this study are summarized in Table (2.1) listed below:

Table (2.1): Chemicals and kits are used in this study and their supplies.

No.	Chemicals and Kits	Company and country
1	Interleukin-4 kit	Iran /Karmania Pars Gene
2	Cytokine ligand-21 (CCL-21) kit	Iran /Karmania Pars Gene
3	Insulin kit	Snibe/Germany
4	Fasting blood sugar kit	Monarch (British)
5	Hemoglobin A1C	Lifotronic H8 /china
6	C-Reactive protein (CRP) kit	Hipro Biotechnology/China
7	Erythrocyte sedimentation rate (ESR) kit	AFCCO/Jordan
8	Vitamin D3 [25(OH)D3] kit	Dragon /China

2.2.2. Instruments and Laboratory Tools

The instruments, and laboratory tools used were summarized in table (2-2) listed below:

Table (2.2): Instruments and laboratory tools used in this study

No.	Instrument	Company supplied
1	Centrifuge,	HETTICH / Germany
2	Deep freezer,	COOLTECH /USA
3	Vortex mixer,	Clay Adams/ Germany
4	Water path,	Memmert/ Germany
5	ELISA reader, ELX800	U.S.A
6	Atomic Absorption Spectrometry,	SHIMADZU /Japan
7	Centrifugation over Histopaque,	Sigma, Germany
8	Dynamic light scattering system (DLS), ZS 90	Malvern Instruments Ltd, UK
9	Scanning electron microscopy (SEM)	PerkinElmer, Fremont, CA, USA
10	FT- IR spectrophotometer,	Shimadzu Kyoto/Japan
11	Ultraviolet spectrophotometry	PerkinElmer, Fremont, CA, USA
12	Atomic force microscopy (AFM)	Nanowizard II; JPK, Germany
13	Ultra violet spectrophotometry	Perkin-Elmer, Fremont CA/USA
14	Dimethyl – thiazolyl (MTT)	Sigma/ Germany
15	Shaker	Taiwan\ Taiwan
17	ESR tube class(Westergren)fast detector	Mheco\ China
18	High-Performance Liquid Chromatography (HPLC)	
20	Absorbance reader	Bio Tek/USA
21	Becker 500 ml	Mheco\ China
25	Auto – chemistry analyzer (CS-480)	Geno Lab TEK\USA
26	Lifrotronic H8	MEDI\\China

2.3. Methods

2.3.1. Anthropometric and Serological Tests Determinations

2.3.1. 1. Determination of Body Max Index

The body mass index (BMI) was determined for all subjects using a mathematical formula that involves the ratio of weight to height. This calculation entails dividing weight in kilograms by the square of height in meters, with the findings categorized accordingly **(Chang, *et al.*, 2020).**

$$\textbf{BMI (kg/m}^2\textbf{) = Weight (kg) / Height (m}^2\textbf{)}$$

The ranges of (BMI) were categorized into groups; see Table 2.3.

Table (2.3): The ranges of (BMI) were categorized into groups

Weight status	BMI (kg/m^2)
Underweight	≤ 18.5
Normal weight	18.5 - 24.9
Overweight	25.2-29.9
Obese	≥ 30

2.3.1.2. Disease Activity Score

Disease activity score (DAS) was calculated by an online DAS calculator when using CRP titer or ESR level.

Table (2.4): Disease Activity Score (DAS)

DAS (0-3.19)	Remission and Low activity(Mild)
DAS (3.20-5.1)	Moderate activity(Moderate)
DAS (>5.1)	High activity(Sever)

2.3.1.3. Erythrocyte Sedimentation Rate

A. Principle of assay

The ESR is the measurement of the sedimentation of red cells in diluted blood after standing for 1 hour in an open-ended glass tube of 30 cm in length mounted vertically on a stand**.**

B. Procedure of Test

1. The tube was placed upright on the ESR detector. The tubular blue graduation line is consistent with the top of the shelf**.**

2. Wait for 30 minutes, and we aligned the concave of the plasma in the ESR tube with the zero scales of the ESR detector.

3. The data was read by aligning the upper surface of the red blood cells with the scale on the ESR detector.

4. Normal Value:

Male: ≤15 mm/hr; Female**:** ≤ 20 mm/hr; Child**:** ≤10 mm/h

2.3.1.4. Determination of C-Reactive Protein Titter

A. Principle

The immune complexes formed by CRP and antibodies lead to light scattering, correlating with CRP levels. The CRP concentration is assessed by comparing sample turbidity against a standard *via* a protein analyzer that quantifies scattered light intensity.

B. Reagents

Table (2-5) Indicates the component of CRP kit.

Reagent1	**R1**	**Tries buffer**	**20mmol/l**
Reagent2	**R2**	**Anti-Human CRP AB**	**Appropriate**

C. CRP Assay Procedure

1. Reagent 1 and reagent 2 is ready-to-use liquid reagent. Gently shake reagent 2 to avoid bubbling.

2. Add 320 μl of R1 to 2 μl of the sample, and then wait for 300 seconds.

3. Then added 80 µl of R2 to the above mixture and left it for 24 seconds, and measured the first absorption at a wavelength of 546 nm, after that waited for 276 seconds, and measured the second absorption at 546 nm.

2.3.2. Part /I: (Clinical Chemistry Biomarkers Investigations)

2.3.2.1. Determination of Fasting Blood Glucose

Glucose-oxidase enzyme (GOD) oxidizes glucose to gluconate and hydrogen peroxide, according to the following equation

Glucose + H_2O + O_2 -----**Glucose oxidase**------► Gluconic acid + H_2O_2

H_2O_2 +Phenol + 4-Amino-antipyrine ------**Peroxidase**------►Quinonimin +4H_2O

The absorbance of the coloured complex was measured at 505 nm.

Calculation:

Glucose (mg/dl) = Abc(Assay) /Abc (Standard) x Standard concentration

2.3.2.2. Determination of Glycated Hemoglobin

The fully automated lifotronic H8 hemoglobin A1c analyzer offers a fast throughput of HbA1c results in 13 U seconds, with Hb variant detection, providing an outstanding solution for quick and reliable diabetic monitoring. No sample preparation and very little hands-on time by the operator are required for the H8 analyzer.

Sample Volume: 5µl; Diluted Blood: 750µl (Dilution Ratio 15:1500µl)

Reagent Pack: Eluent A, Eluent B, Eluent C, Hemolysin L.

HPLC Methodology

High-Performance Liquid Chromatography (HPLC) separates HbA1c directly with measuring the absorbance points continually to form a chromatogram. Using a normal distribution curve-fitting auto-iterative algorithm to get precise HbA1c testing results, excluding interference of variant and unstable hemoglobins like HbF. Standard Analysis Mode will report HbA1a、HbA1b、HbF、HbA1c、P3 HbA0 peak areas and ratio. And the result also includes IFCC, NGSP, and ADAG values for diverse client needs.

Eluents A B and C

Three eluates at different concentrations were used, forming an ascending gradient of ion concentration. Due to the difference in the charges carried by the target proteins, the adsorption capacity against impurities varies. Therefore, the concentration of the eluent gradually increases the target proteins HbA1a and HbA1b. HbF, LA1c, HbA1c, and HbA0 are removed sequentially.

The hemolytic agent has been used to dissolve red blood cells and release sugar hemoglobin molecules**.**

2.3.2.3. Determination of Serum Insulin Level

A-Principle of the Test

The insulin test is a chemiluminescence immunoassay. The sample (or calibrator/control), buffer, and magnetic microbeads coated with monoclonal antibody and β-insulin and monoclonal antibody ABEl isbiedanti-Insulin were mixed well and incubated, forming a sandwich of immunocomplexes. After sedimentation in a magnetic field, the supernatant is decanted, and then a wash cycle is performed. Next, I've added Starter1+2 to indicate checking the chemicals. The optical signal is measured by a photomultiplier as relative optical units (RLUs), which are proportional to the concentration of insulin present in the sample (or calibrator/control).

B- Preparation of the Reagent

The magnetic beads were automatically re-suspended when the kit was loaded successfully, ensuring that the magnetic beads suspended completely homogenously before use.

C- Dilution

Dilute samples with concentrations above the measurement range. The recommended dilution is 1:19. After manual dilution, the result was multiplied by the dilution factor.

2.3.2.4. Determination of Insulin Resistance

Homeostatic model assessment (HOMA) is a method used for the evaluation of insulin resistance and β-cell function. A software program was used to solve the equations so that the estimation of insulin resistance and β-cell function by using fasting glucose and insulin concentration:

HOMA- IR = [Glucose (mg/dl) x Insulin (µU/ml)] / 405.

2.3.2.5. Determination of Serum Chemokine Ligand-21 Level

Principle

The quantitative sandwich enzyme immunoassay technique is used to detect CCL-21. Antibody is pre-coated onto a microplate, and samples are pipetted into the wells. The immobilized antibody bounds any CCL-21 present. A biotin-conjugated antibody is added, followed by avidin-conjugated horseradish peroxidase. A substrate solution is added, and color develops proportionally to the bound CCL-21. The intensity is measured.**Components of the kit used for CCL-21 determination**

Table (2.6): Kit component of CCL21

Item	Catalog	Volume
10 x washing buffer	KPG -WB	40 ml
Detection Ab	KPG- HCL21D	5.5 ml
Dilution HRP	KPG -DH	100 µl
HRP	HAA	22 µl
HRP-Avidin buffer	KPG- HA	5.5 ml
Human anti- HCL-21 coated plate	KPG –HCL21P	96 vials
Standards	KPG –HCL21SN1- 4	200 µl
Stopping	KPG -ST	3.5 ml
Substrate	KPG -SU	5.5ml

A- Preparation of solution for [CCL-21]

1- Washing buffer

Dilute solution with distilled water 10 time

2- HRP – Avidin

. spin the HRP vial using a microfuge.

. Added 500 microliters of HRP-Avidin vial to the HRP vial.

overmixing

. Added all content to HRP. -Avidin vial

. Added 25 microliters of dilution HRP vial to the HRP-Avidin vial.

. shake by hand for 3 minutes to mix well.

Standard to CCL-21

Table (2.7): Standard kit component of CCL-21

Standard	CN	Pg\ml	OD
Standard 4	KPG- HCL21S4	200 pg/ml	2.2-1.7
Standard 3	KPG- HCL21S3	100 pg/ml	1.2-0.7
Standard 2	KPG-HCL21S2	50 pg/ml	0.5-0.3
Standard 1	KPG-HCL21S1	5 pg/ml	0.05-0.1
Blank	-	0 pg/ml	0.08-0.05

B-Procedure

1. Removed the plate from the foil pouch and the blank, standard, and sample wells were marked.
2. Fifty µL were added as standard (S1, S2, S3, and S4) and concentrations were (200, 100, 50, and 5 pg/mL) respectively to the first to fourth wells.
3. Fifty µL of the samples were added to the rest of the well and incubated for 60 minutes on a 200 RPM shaker at room temperature.
4. The ELISA plate wells were washed 3 times using the washing solution, incubated the plates for approximately 1 minute at room temperature and then drained.
5. Fifty µLof the conjugated antibody was added to all wells and incubated for 60 minutes on a 200 RPM shaker at 37 °C.
6. The ELISA plate wells were washed 3 times using the washing solution.
7. The HRP-Avidin solution was added 50 µL to all wells and incubated for 30 minutes on a shaker at least at RPM 200.
8. The ELISA plate wells were washed 5 times using the washing solution.
9. All wells received 50µL of substrate to all walls and incubated the plate at 37 °C for 15 minutes.
10. Stop Solution was added 25µL to all wells and the color of the wells changed from blue to yellow.
11. Absorbance was read at 450 nm using an ELISA reader.

C-Calculation

Concentration standards were plotted on the x-axis and OD values on the y-axis. Construct the standard curve on graph paper. Determine sample concentration using the OD value; Alternatively, a linear regression equation was derived from the standard concentrations and OD values. Later, use the sample's OD value to calculate its concentration.

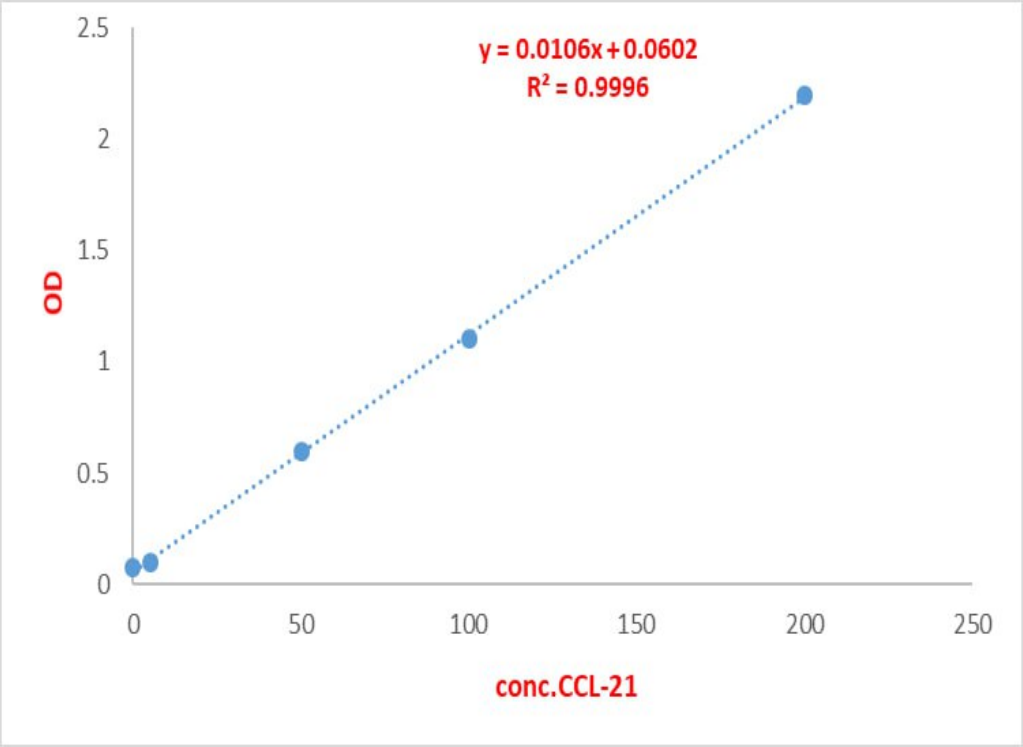


Fig. (2-2): Standard curve of chemokine CCL-21 determination

2.3.2.6. Determination Serum Interleukin-4 Levels

* Principle, procedure, and Calculation of Results are mentioned in chemokine CCL-21 ELISA Test section 2.3.2.5.

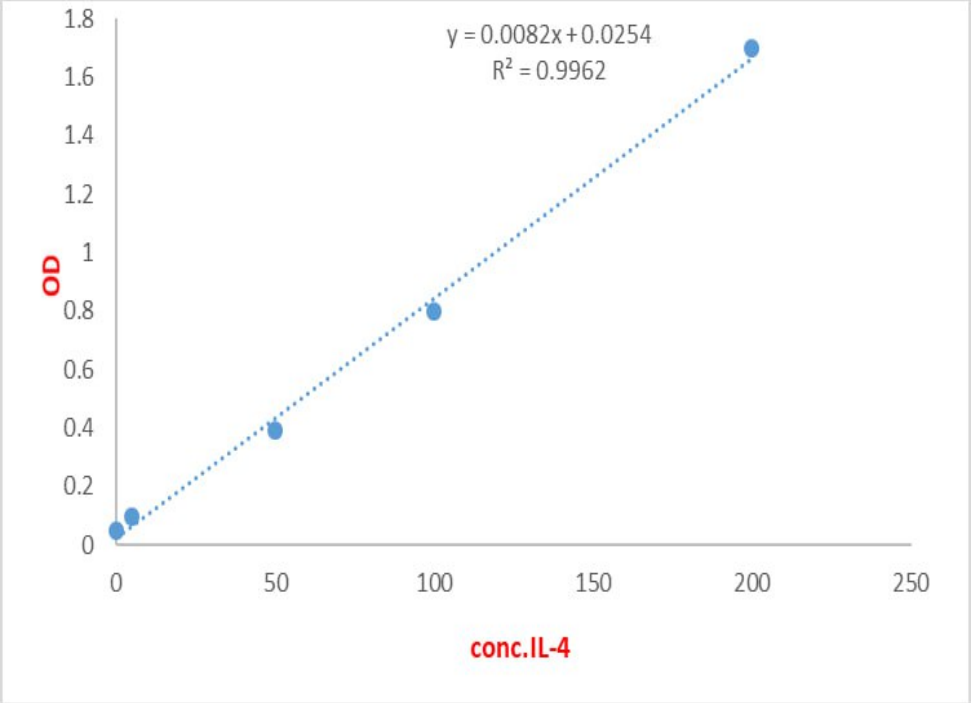


Fig. (2-3): Standard curve of Interleukin- 4 determination

2.3.2.7. Determination of Serum Zinc Concentration

Four standard solutions (2.5, 5, 7.5, and 10) were prepared for calibration curve analysis as depicted in Figures (2-4). Procedures involved injecting 20 µl of samples into a graphite tube, which was heated to vaporize and atomize the analyte. The concentrations of zinc in samples were continuously measured against the calibration curve derived from standard solutions. The conditions for selenium determination are detailed in Table 2.8

Table (2.8): Ideal condition for Zinc determination

Variable	Ideal condition
Atomizer	Graphite Furnace
Fuel	Argon gas
Lamp current	mA35
Lighting mode	BGC-D2
Sample size	20 µl
Slit width	0.7 nm
Wavelength	196 nm

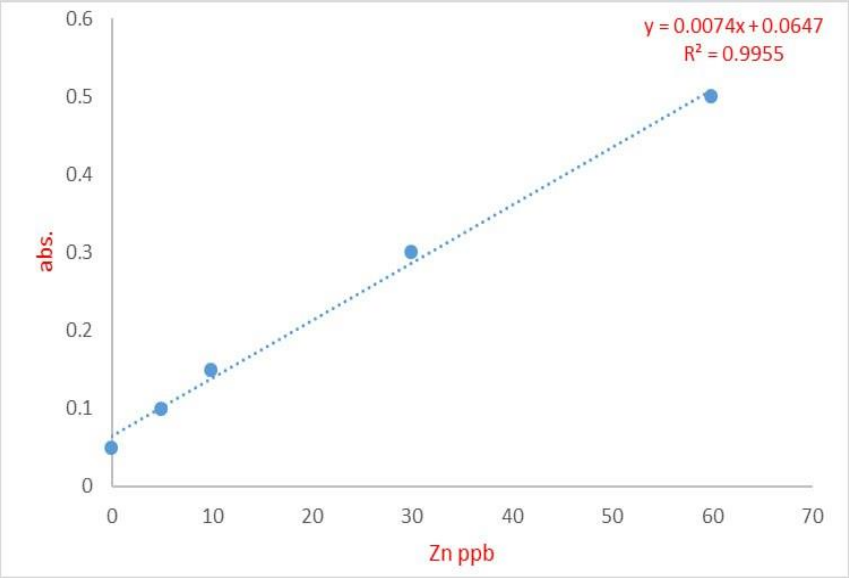


Fig. (2-4): Standard curve of Zinc determination

2.3.2.8. Determination of Serum 25(OH)D3 Levels

Table (2.9): Kit component of vitamin D3

Item	Volume(96 vial)
Assay Diluent	24 ml
Biotinylated 25(OH)D Reagent	0.55 ml
Microwell plate coated with anti-Vitamin D	12
Stop Solution	12 ml
Streptavidin-HRP	23 ml
TMB Substrate	12 ml
Vitamin D Control	0.5 ml
Vitamin D Standard	0.5 ml
Wash Concentrate	25 m

A- **Principle**

The last is a solid phase enzyme-linked immunoassay (ELISA). Antivitamin D3 antibody-coated wells are subjected to incubation with various vitamin D3 components for a duration of 90 minutes at room temperature. During this period, biotin-labeled vitamin D3 competes with endogenous vitamin D3 for binding sites on the antivitamin D3 antibody. After washing, the bound vitamin D3-Biotin is quantified using Streptavidin HRP, with a decrease in SA-HRP conjugate correlating to increased vitamin D3 concentration. Following another wash, TMB reagent is introduced and incubated for 30 minutes, leading to color development. The color reaction is halted with a stop solution, and absorbance is measured at 450 nm spectrophotometrically. A standard curve is constructed by correlating standard concentrations with their respective absorbance values. The intensity of color is inversely related to the concentration of 25-OH vitamin D3 in the sample. The assay quantifies both 25-OH vitamin D2 and D3 forms. The total duration of the assay procedure is 2.5 hours. **(Bikle, 2010).**

B- Preparation of the Reagent

Before running the test, prepare the following:

1. Standards and Reagents: Standards are serum-based solutions that are stable when stored at 2-8°C, protected from light, until the expiry date on the label.

2. 51X Biotin Conjugate: Prepare 1X working solution immediately before use with assay diluent (e.g., add 0.1 mL of 51X Concentrated Vitamin D3-Biotin Conjugate to 5 mL of assay diluent). The remaining assay diluent should be stored at 2-8°C in a dark, tightly covered place.

3. Add the contents of the bottle (25 ml, 20X) to 475 ml of distilled or deionized water. Store at a temperature of 20-25 degrees Celsius.

C- Procedure of Vitamin D3

1. 10 µl of 25-OH vitamin D standards, controls, and samples were dispensed into each well, as required.

2. Dispense 200 µL of working solution of reagents, 25(OH)D3, into each well.

3. The contents in the wells were carefully mixed for 20 s using a plate shaker at 200-400 xg (or equivalent motion).

4. The plate was incubated for 90 minutes at room temperature.

5. We shake the contents of the wells lightly into the waste tank.

6. Washing: 300 µL of 1X wash solution was dispensed into each well, repeated 2 additional times for a total of 3 washes.

7. Dispense 200 µL of enzyme conjugate (Streptavidin-HRP) into each well. Incubation #2: Incubate for 30 minutes at room temperature.

8. Incubate for 30 minutes, at room temperature.

10. We repeat the sixth point.

11. Using a multichannel pipette, 200 µL of TMB substrate was dispensed into each well.

12. Incubate for 30 minutes at room temperature, preferably in the dark.

13. Stop: 50 µl of stop solution was dispensed into each well to stop the enzymatic reaction. We carefully mix the contents of the plate for 20–30 seconds.

14. Read the absorbance on an ELISA reader at 450 nm within 10 minutes of adding the stop solution.

D- Calculation

Concentration standards were plotted on the x-axis and OD values on the y-axis. Construct the standard curve on graph paper. Determine sample concentration using the OD value; Alternatively, regression equation was derived from the standard concentrations and OD values. Later, use the sample's OD value to calculate its concentration.

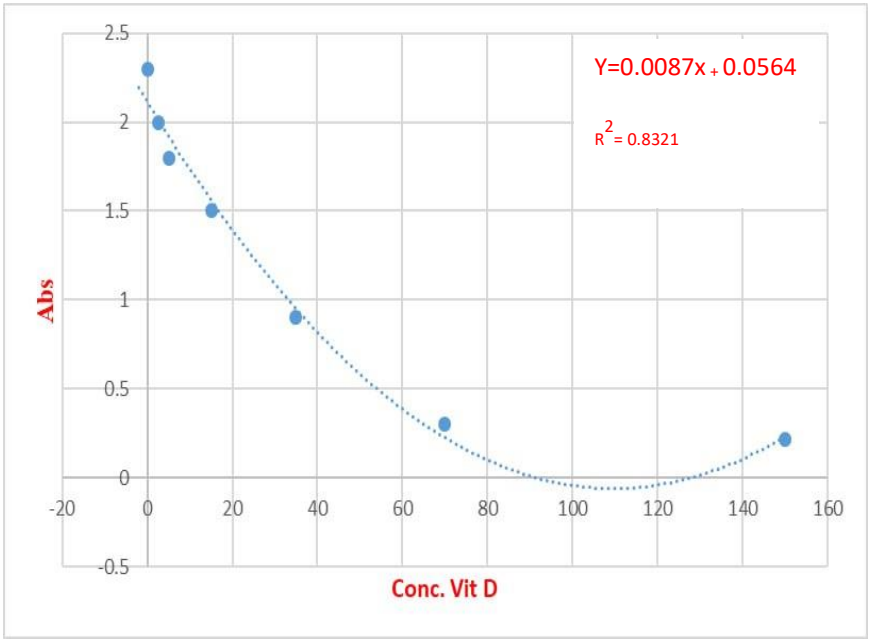


Fig. (2-5): Standard curve of vitamin D3 determination

2.3.3. Part /II: (Synthesis and Characterization of Nanoparticles)

2.3.3.1. Peripheral Blood Mononuclear Cell Isolation and Culture

Peripheral blood mononuclear cells (PBMCs) were isolated via Histopaque 1077 density gradients using centrifugation at 1500 xg for 5 minutes. The PBMCs underwent three washes with phosphate-buffered saline (PBS, pH 7.4) and were re-suspended at a concentration of 1×10^6 cells/ml in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS), 10 U/mL penicillin, and 10 μg/mL streptomycin. The cell suspensions were incubated at 37 °C in 5% CO_2 until achieving 90% confluency.

2.3.3.2. Synthesis of Niosome Nanoparticles.

The thin-film hydration method was used for the synthesis of niosomal nanoparticles (NPs). Cholesterol (6 mg) and Span 60 (36 mg) were dissolved in methanol (6 ml) and chloroform (3 ml). This mixture was moved into a rotary evaporator at 55-60 °C and 0.46 atm for 1 hour to mix the substances and remove the solvents, forming a lipid film. Subsequently, the film was hydrated with 10 ml of PBS by using the rotary evaporator at 55-60 °C and 0.9 atm for another hour. The final product was ultrasonicated for 30 minutes at 24 °C to reduce the size of the synthesized niosomal NPs.

1.3.3.3.Synthesis of Artemisinin Loaded Niosomal Nanoparticles

The artemisinin-loaded niosomal NPs (Nio-artemisinin NPs) were synthesized with the same method as above with the addition of 2.28 mg artemisinin to chloroform and methanol along with span 60 and cholesterol.

2.3.3.4. Synthesis of artemisinin Loaded Hyaluronic Acid Coated with Niosomal Nanoparticles

To synthesize artemisinin-loaded hyaluronic acid-coated niosomal NPs (H-Nio- artemisinin NPs), 10 mL of normal saline containing 0.1% (w/v) hyaluronic

acid solution was added dropwise to blank Nio-artemisinin NPs, while the mixtures were stirring at ambient temperature for one hour to reform the NPs and coating the hyaluronic acid onto the NPs surface

2.3.3.5. Physiochemical, Size, and Morphology of Nanoparticles

The niosomal nanoparticles were characterized through various analytical methods. FT-IR spectroscopy analyzed the compounds' spectral characteristics pre- and post-nanoparticle synthesis within the 400-4000 cm-1 range at a 4 cm^{-1} resolution. The Zeta sizer dynamic light scattering system assessed the size distribution, polydispersity index (PDI), and zeta potential of the niosomal nanoparticles. Moreover, scanning electron microscopy and atomic force microscopy (AFM) were utilized to examine the nanoparticles' surface morphology.

2.3.3.6. Niosomal NPs in *vitro* Drug Release

To assess the drug release characteristics of artemisinin from niosomal nanoparticles, a dialysis method was utilized: 5 ml of Hyalo-Nio-artemisinin NPs were placed in a dialysis membrane tube (12 kDa) and magnetically stirred at 120 xg in PBS (pH = 7.4) at 37 °C. At predetermined intervals (12, 24, 36, 48, 72, 84, 96 hours), 2 ml of the immersion solution was substituted with an equivalent volume of fresh PBS. The absorbance of the liberated artemisinin was quantified at λmax of artemisinin using ultraviolet spectrophotometry.

2.3.3.7. Peripheral Blood Mononuclear Cells Proliferation

The effect of varying doses of free artemisinin, blank Nio NPs, Nio-artemisinin NPs, and H- Nio-artemisinin NPs on the viability of PBMCs was determined by performing an MTT reduction assay. An MTT assay is a colorimetric assay that detects the color change from yellow of the tetrazolium dye to purple due to the formation of formazan in the presence of

viable cells with active metabolism (**Oh *et al.*, 2022**). An MTT reduction assay determined the effect of various doses of free artemisinin, Nio-artemisinin NPs, and H- Nio-artemisinin NPs on the viability of PBMCs.

1.Firstly, 5×10^3 cells were seeded in each well of 96-well plates and incubated for 24 hours at 37 °C with 5% CO_2.

2.The cells were treated with free artemisinin (2.5-30 μM), Nio-artemisinin NPs (2.5-30 μM), and H- Nio-artemisinin NPs (2.5-30 μM) at 37 °C with 5% CO_2.

3.After 48 hours, the medium containing treatment substances was replaced with 200 μL of MTT solution and incubated for 4 hours at 37 °C and in dark conditions.

4.The MTT solution was excluded from wells, and 200 μL of dimethyl sulfoxide (DMSO) which is a chemical that dissolves many organic and inorganic substances was added to each well, followed by shaking on a plate shaker for 20 minutes.

5.The optical density of wells was measured at 570 nm using Microplate Absorbance Reader, and the cell viability effects of free artemisinin, Nio-artemisinin NPs, and H- Nio-artemisinin NPs were calculated using GraphPad Prism 8.4 software.

2.3.3.8. C-C Motif Chemokine Ligand-21 and Interlukin-4

1.Peripheral blood mononuclear cells (1×10^5) were seeded in a 6-well plate and incubated for 24 at 37 °C with 5% CO_2 to attach the plates.

2. The PBMCs cells were treated with IC50 concentration of pure artemisinin, Nio-artemisinin NPs, and H- Nio-artemisinin NPs for 48 hours at 37 °C with 5% CO_2. A group of cells remained untreated as a control.

3. The CCL-21 and IL-4 levels in treated and untreated PBMCs were measured by an enzyme immunoassay using the human ELISA Kit **(Johnson and Jackson, 2010).**

2.3.4. Part / III: (Molecular Studies – Gene Expression)

2.3.4.1. Real-time Polymerase Chain Reaction

Real-time PCR was conducted with a Bio-Rad IQ5 instrument. The reaction included specific components such as DNA-Master, SYBR Green I, primers, cDNA, and $MgCl_2$. The amplification protocol featured initial denaturation, followed by 40 cycles with precise temperatures for MMP2, TIMP2, and COX2 detection. GAPDH was utilized as the reference gene for normalization of target mRNA expression levels. Primer sequences for quantitative PCR are detailed in Table 2.11 SYBR Green I fluorescence was monitored post-amplification cycles to assess PCR product accumulation. Melting curve profiles were analyzed post-run to verify transcript amplification specificity. Fluorescence readings were quantified using the second derivative maximum method via Roche Molecular Biochemicals software. Standard curves for GAPDH and other primers were created through serial cDNA dilution. All concentrations were reported relative to their respective standards.

Table (2.10): The forward and reverse primer sequences used for real-time PCR

Genes	Forward	Reverse
MMP2	AATGCCATCCCCGATAACCTG	CTCAGCAGCCTAGCCAGTCG
TIMP2	CCCCTCCAACCCATATAACACC	CACCCGGCTCTTCTTAACCTG
COX2	GTCCTCTATATCATCTCGCTA	TTCTATTGGCAGAACGACT
GAPDH	ATCCTGGGCTACACTGAGCAC	CCTGTTGCTGTAGCCAAATTCGT

2.3.4.2. RNA Extraction

RNase Free microtubes, tubes and falcons were used for RNA extraction. First, the flask cells were separated by trypsin and counted. About 1 x 10^6 cells along with 2 ml of RPMI medium containing 10% FBS were transferred to each well of a 6-well plate and the plate was incubated for 24 hours at 37 °C and 5% carbon dioxide for the cells to adhere to the wells. After 24 hours, the medium of the wells was emptied and each well was washed twice with PBS. Then the plates were incubated for 48 hours in an incubator at 37 °C and 5% carbon dioxide. After 48 hours, the contents of the wells were emptied and 500 microliters of Trizol was added to each well. After 10 minutes, the trizol was collected from the wells and transferred to a microtube, and 200 µL of chloroform was added to each microtube. Next, the microtubes were transferred to a refrigerated centrifuge and centrifuged for 10 minutes at 12,000 xg and 4 °C. After centrifugation, 3 phases were formed inside the microtubes: the upper phase (transparent colour) containing RNA, the middle phase (dark and white color) containing DNA, and the lower phase (pink colour) containing protein. The supernatant phase of each microtube was collected and transferred to other microtubes. 200 microliters of isopropanol were added to each microtube and the microtubes were incubated for 10 minutes at 4 °C and centrifuged for 10 minutes at 12000 xg with a refrigerated centrifuge at 4 °C. Next, the supernatant was removed and 1 ml of 75% alcohol was added to each microtube. After centrifugation at 7500 rpm for 5 minutes at 4°C and adding 10 microliters of DEPC water to the samples using NanoDrop device (ND-1000, Thermo, USA) in the OD260/OD280 ratio, the quality of the extracted RNA samples was measured.

2.3.4.3. cDNA Synthesis

The synthesis of DNA from the RNA template is called cDNA, which is done by the reverse transcription enzyme. Reverse transcription enzyme requires primers for cDNA synthesis., two types of random hexamers and oligodt primers are used to make cDNA. The difference between these two types of primers is that dT oligo is attached to the polyadenine tail of mRNA, while random hexamers are randomly attached to different parts of mRNA. After connecting the primers, the reverse transcription enzyme synthesizes cDNA from template RNA.

cDNA synthesis was done according to the method mentioned by the kit manufacturer. In this way, 5 microliters of extracted RNA were mixed with 1 microliter of random primer and 14 microliters of cDNA master mix, and the samples were incubated for 30 minutes at 50°C. In order to inactivate the reverse transcriptase enzyme, the samples were incubated at 90°C for 5 minutes. After cDNA synthesis, the samples were transferred to -20-degree refrigerator until use.

2.3.4.4. Gene Expression and Real Time-PCR

The mRNA expression level of the aforementioned genes was investigated by RT-PCR test. SYBER Green qPCR Master Mix kit was used to perform the RT-PCR (MIC) test. All the steps of the test were done on ice and to prevent contamination, all the work was done under the laminar hood. The principles of performing the Real-Time PCR method are based on the fluorescence property. SYBER Green dye is the most widely used RT-PCR method. The SYBER green dye binds to the small groove of double-stranded DNA and emits fluorescence light, and the more the reaction proceeds and the more products is produced, the more this dye binds to DNA and produces more fluorescence. To normalize the level of gene expression in the comparison of the two control groups and the treated group, genes whose expression levels are constant in the cell are used. In this project, ß-actin and GAPDH genes were used to normalize the results. The

melting curve was used to evaluate the quality of RT-PCR. With this analysis, expression intensity, product concentration, and the degree of purity of the amplified product can be obtained in each sample. If the peak of the graph is seen at low temperatures (about 65 to 75 degrees Celsius), it indicates the presence of a primer dimer because SYBER Green colour non-specifically binds to the primer dimer in the reaction vessel, which causes false positive results. Also, the dimer sequence length of the primers is small and around 20 bp, which separate at low temperature and cause false positive results. The device draws the curve after completing cycle number 40. In this stage, the temperature of the device gradually increases from 60 to 95 degrees Celsius, during this temperature increase, the two-stranded products are separated from each other based on the length of the organic bases and the percentage of CG, which causes the intensity of the SYBER Green fluorescence colour to decrease. The melting curve shows this reduction and determines the presence of primer dimer or the presence of any non-specific product by creating different peaks, which confirms the accuracy and validity of the reaction and the obtained product. To perform the RT-PCR test, 7 microliters of SYBR Green qPCR Master Mix2x, 0.3 microliters of a mixture of forward and reverse primers, 2 microliters of synthesized cDNA, and 4.7 microliters of DEPC water were combined and according to the List of primers used in table 2-21 is mentioned.

Table (2.11) Program implemented by Real-time PCR

Cycle	Time	Temperature	Level
1	**5 min**	**95**	**Primary denaturation**
36	**10 sec**	**95**	**Denaturation**
36	**35 sec**	**55**	**Primer binding**
36	**20 sec**	**72**	**Lengthening**
1	**5 sec**	**65-95**	**Melting**

2.4. Statistical Analysis

The data analysis for this work was generated using the Statistical Package for the Social Sciences software, version 22.0 (IBM, SPSS, Chicago, Illinois, USA). Values were illustrated by n (%) for categorical, Scale variables were presented by mean ± standard deviation for normal data. The distribution of the data was checked using the Shapiro-Wilk test as a numerical means of assessing normality. Biomarkers were compared using the one-way analysis of variance (ANOVA) was done to compare the means of different groups. Significant differences in categorical variables among the parameters were confirmed through analytical statistical tests. Results of all hypothesis tests with p-values $\leq$ 0.05 (two-tail) were considered to be statistically significant. The optimal threshold with high specificity and sensitivity for critical cases was detected using receiver operating characteristic (ROC) analysis. It was found that all the values of P were two-sided, and a $P \leq 0.05$ was considered to be statistically significant.

CHAPTER THREE

Results

3. Results

3.1. Part I (Biochemical and clinical result)

3.1.1. Demographic Characteristics

Table (3.1) summarizes the baseline demographic characteristics of the study population and provides a preliminary overview of the disease duration (T2DM and RA) among the study population. The total patients number (N=74) and the healthy control groups (N=51) were involved in this study. A total of 125 participants were also divided into subgroups based on age, family history, and BMI groups. The distribution of age groups is fairly similar across both groups, with a slight trend towards older ages in the patient group. More than of patients (27%) fall within this age which ranged between 30-39 years as compared with controls (19.60%). Similar distribution for the age ranged between 40-49 years with a slightly higher percentage in the patient group (40.57%) as compared with controls (29.40%). A slightly higher percentage of controls (33.40%) are in the age range between 50-59 years as compared to patients group (12.16%) while about (20.27%) of patients were in the age range between 60-70 years as compared to controls (17.60%) as shown in Figure 3-1.

The results demonstrated that the patient group appears to have a higher prevalence of overweight and obesity as compared to the control group, about (32.44%) of patients were classified as normal weight as compared to controls (58.8%). A higher percentage of patients (67.56%) were overweight as compared to controls (41.20%), whereas, all patients (100%) in the obesity category belong to the patient group, with none in the control group, as presented in Figure 3-2.

Regarding the family history characteristic, the results shown a difference between the groups studied. All patients (100%) reported a family history of the RA condition, as shown in Figure 3-3. The majority of patients. (33.80%) do not have a family history as compared to only (66.20%) of patients.

Table 3.1 Demographics characteristic of the study groups

Variable	Groups	RA Patient N = 74	N%	Control N = 51	N%	*P* value
Age Groups Years	30-39	20	27%	10	19.60%	**0.565**
	40-49	30	40.57%	15	29.40%	
	50-59	9	12.16%	17	33.40%	
	60-70	15	20.27%	9	17.60%	
BMI, kg/m^2 Groups	Normal weight	24	32.44%	30	58.8%	**0.001***
	Over weight	50	67.56%	21	41.20%	
Family History	Yes	49	66.20%	19	37.25%	
	No	25	33.80%	32	45.10%	

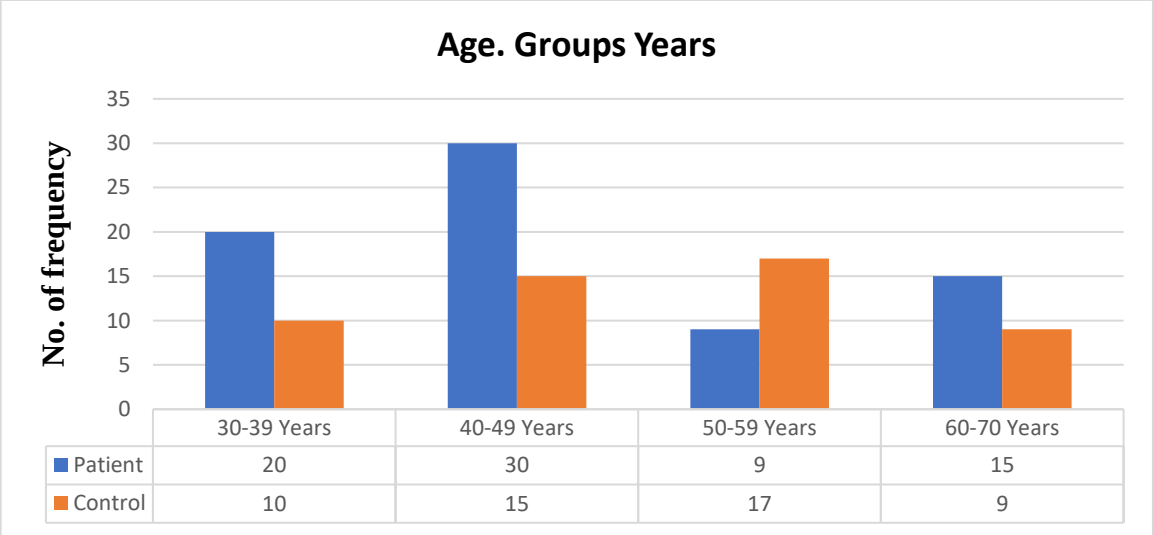


Fig. (3-1): Baseline characteristics and demographic descriptive of the study population in disease and control groups the number of participants for age groups

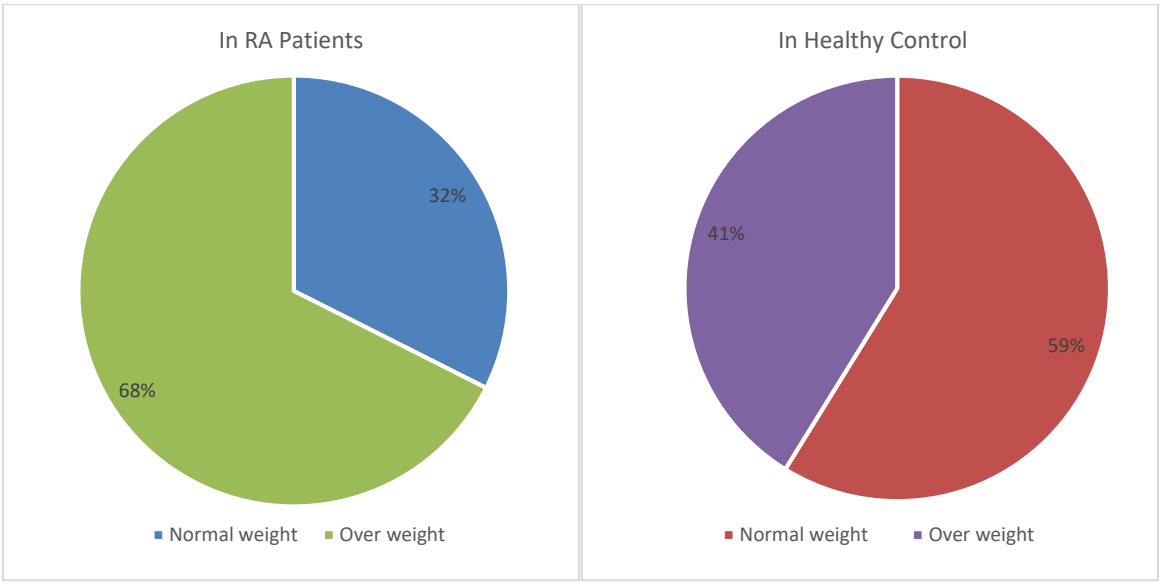


Fig. (3-2): Baseline characteristics and demographic descriptive of the study population in disease and control groups number of participants for BMI groups

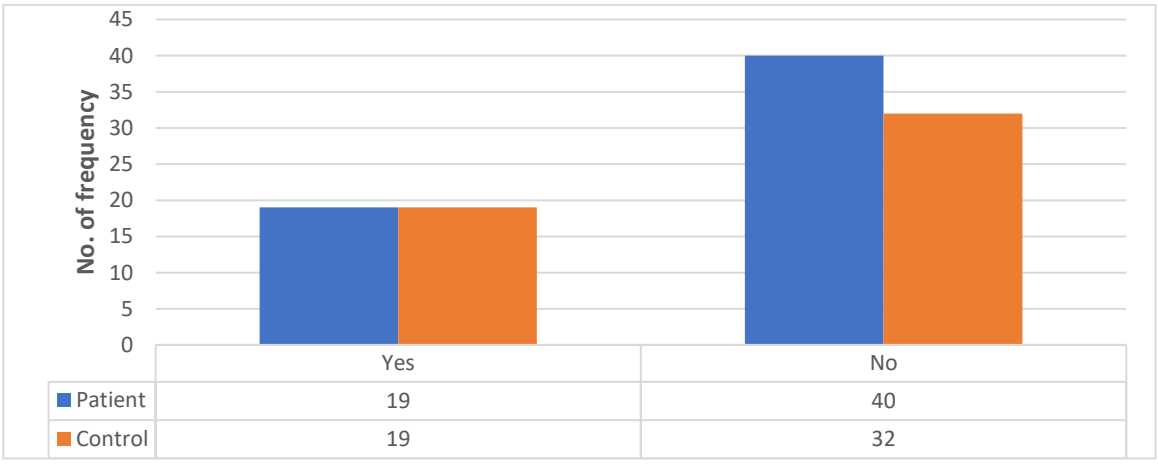


Fig. (3-3): Baseline characteristics and demographic descriptive of the study population in disease and control groups the number of participants for Family history

3.1.2. Distribution of Anthropometric Parameters

Table (3.2) showed no statistically significant age differences between the rheumatoid arthritis group and the control group (P = 0.565), indicating a homogeneous age distribution in the sample. Statistically significant differences were observed in BMI between the patient group and the control group ($P \leq 0.001$).

Table (3.2): Comparison between study groups in Age, BMI and family history

Groups	RA N =74	Control N=51	*P* value
Age (year)	46.93 ± 10.36	48.54 ± 11.26	0.565
BMI (kg/m^2)	26.45 ± 3.89	24.58 ± 3.08	≤0.001

3.1.3. Results Inflammatory Biomarkers in Sera of Rheumatoid Arthritis Patients

Table 3.3 The mean ± SD difference of inflammatory marker for level CCL-21, IL-4, ESR, and CRP among patients group of RA, RA with T2DM compared to the control group

Biomarkers	RA Mean ± SD N = 40	RA ± T2DM Mean ± SD N = 34	Control Mean ± SD N = 51	*P* value
CCL-21, pg/ml	98.80 ± 22.16 **a**	85.98 ±27.18 **b**	40.87 ±13.20 **c**	**<0.001 [S]**
IL-4, pg/ml	15.89 ± 1.98 **a**	14.76 ± 2.89 **a**	7.90 ±1.39 **b**	**<0.001 [S]**
ESR, mmol/hr	36.17 ±3.89 **c**	53.18 ± 15.03 **a**	14.17± 4.84 b	**<0.001 [S]**
CRP, mg/l	6.08 ±1.91 **b**	11.79 ± 4.23 **a**	4.69 ± 6.72 **c**	**<0.001 [S]**

ANOVA -test was *: significant at p ≤ 0.05; SD: standard deviation; S: significant; NS= Non-significant; Classifications in which the same letter shares the same class. There are no significant differences according to the Duncan-Duncan test

The observed mean ± SD results regarding cytokine (ligand-21) (CCL-21) in both RA groups (with and without T2DM) indicated a significantly higher levels (85.98 ±27.18 pg/ml) , (98.80 ± 22.16 pg/ml) than that found in sera of control group (40.87 ±13.2 pg/ml) respectively, (P<0.001), whereas, the IL-4 levels in sera of RA +

T2DM patient group displayed the significant highest levels (14.76 ± 2.89 pg/ml) followed by RA without T2DM group (15.89 ± 1.98 pg/ml) as compared with that observed in sera of control group (7.90 ±1.39 pg/ml) , (*P*<0.001[S]),

The mean ± SD level of each of CRP observed in sera of both RA (with and without T2DM) groups had significantly elevated levels (11.79 ± 4.23 mg/l), (6.08 ±1.91 mg/l) as compared with that found in healthy control group (4.69 ± 6.72 mg/l) respectively, (*P*<0.001), while the mean ± SD level of ESR observed in sera of RA with T2DM groups had significantly elevated levels (53.18 ± 15.03 mmol/hr) than that found in each of RA without T2DM (36.17 ± 3.89 mmol/hr) and healthy control group (14.17± 4.84 mmol/hr) respectively, (*P*<0.001). as shown in Table (3.3).

3.1.4. Results of other Biomarkers in Sera of Rheumatoid Arthritis Patients

The other parameters investigated in sera of all subjects studied include insulin and insulin resistance (homeostatic model assessment of insulin resistance, HOMA-IR). The mean ± SD level of insulin in RA group with and without T2DM indicate a significant lower results (11.37 ± 1.85 μU/ml ; 11.51 ± 2.14 μU/ml) than that found in control group (13.18 ± 2.28 μU/ml), (*P*<0.001), while the insulin resistance (HOMA-IR) result found in sera of RA with T2DM was significantly higher (8.41 ± 2.56) than that found in each of RA without T2DM (3.04 ± 0.62) and control group (3.15 ± 0.59), (*P*<0.001).

The mean ± SD level of fasting blood glucose (FBS) was significantly elevated in sera of RA with T2DM and reach to (298.54 ± 76.05 mg/dl) more than that found in sera of RA without T2DM (106.94 ± 8.23 mg/dl), as compared with control group (96.92 ± 8.83 mg/dl), (*P*<0.001), while the mean ± SD of HbA1c% in RA with T2DM was significantly elevated and reached to (11.18 ± 3.20) as compared with control group (6.35 ± 1.69), while its level in sera of RA group without T2DM was (5.56 ± 0.62), (*P*<0.001) as presented in Table(3.4).

Table 3.4: The mean ± SD difference of metabolic disorder marker level for HOMO-IR, Insulin, HbA1c, and FBS among patients group of RA,RA with T2DM compared to the control group

Biomarkers	RA Mean ± SD N = 40	RA ± T2DM Mean ± SD N = 34	Control Mean ± SD N = 51	*P* value
HbA1c%	5.56 ± 0.62c	11.18 ± 3.20a	6.35 ± 1.69b	<0.001[S]
FBS, mg/dl	106.94 ± 8.23b	298.54 ± 76.05a	96.92 ± 8.83c	<0.001[S]
Insulin, µU/ml	11.51 ± 2.14a	11.37 ± 1.85a	13.18 ± 2.28b	<0.001[S]
HOMA-IR	3.04±0.62b	8.41±2.56a	3.15±0.59b	<0.001 [S]

ANOVA -test was *: significant at p ≤ 0.05; SD: standard deviation; S: significant; NS= Non-significant; Classifications in which the same letter shares the same class. There are no significant differences according to the Duncan-Duncan test

3.1.5. Zinc and Vitamin D3 Levels in Sera of RA with/without T2DM

Table (3.5): Results indicated a highly statistically significant difference in Zinc and Vit D were significant between RA (with and without) T2DM with the control group ($p \leq 0.05$), but not statistically significant between RA with T2DM and RA without T2DM group, the mean level and standard deviation (7.40 ± 1.06 mg/dl), (7.93 ± 1.25 mg/dl), and (35.51 ± 9.19 mg/dl) respectively for Vit D and (37.63 ± 7.66 mcg/L), (66.62±12.88 mcg/L), and (38.49 ± 8.1 mcg/L) respectively for Zinc.

Table 3.5 The mean ± SD difference of antioxidant disorder marker level for Vit D3 & Zinc among patients group of RA, RA with T2DM compared to the control group

Biomarkers	RA Mean ± SD N = 40	RA ± T2DM Mean ± SD N = 34	Control Mean ± SD N = 51	*P* value
Zinc, mg/dl	38.49 ± 8.10 b	37.63 ± 7.66 b	78.03 ±6.90 a	<0.001 [S]
VitD3, mg/dl	7.93 ± 1.25 b	7.40 ± 1.06 b	35.51 ± 9.19 a	<0.001 [S]

ANOVA -test was *: significant at p ≤ 0.05; SD: standard deviation; S: significant; NS= Non-significant; Classifications in which the same letter shares the same class. There are no significant differences according to the Duncan-Duncan test

3.1.6. Correlation Studies

Table 3.6: The correlation coefficient between (CCL-21 and IL-4) (pg/ml) with other biomarkers among the Patients group.

Biomarkers	CCL-21 pg/ml		IL-4 (pg/ml)	
	(r)	*P value*	*(r)*	*P value*
CCL-21 pg/ml	1	-	0.162	0.221[NS]
IL-4 (pg/ml)	0.162	0.221[NS]	1	-
ESR mmol/h	-0.240	0.067[NS]	-0.218	0.096[NS]
CRP mg/l	-0.336**	0.009[S]	-.189	0.152[NS]
HbA1c	-0.289*	0.027[S]	-0.242	0.065[NS]
FBS	-0.132	0.317[NS]	-0.312*	0.016[S]
Insulin (µU/ml)	-0.008	0.954[NS]	.115	0.386[NS]
HOMAIR	-0.158	0.231[NS]	-0.271*	0.038[S]
Zinc mg/dl	-0.051	0.704[NS]	0.010	0. 938[NS]
Vit D3 ng/ml	0.048	0.716[NS]	0.357	0.005[S]

p<0.05 considered significantly different, [S]= Significant, [NS]= Non significant ®:Correlation Coefficient

Considering the important role of the measured biomarkers, the spearman rank test analysis of RA with or without T2DM was used to analyse the response relationship between various biomarkers studied. These correlations show many significant results among the measured parameters (table 3.6). The most important correlations were between RA with T2DM levels which were positively related to the IL-4, CCL-21, CRP, ESR, FBS, Zinc, HOMA-IR, HbA1c%, and 25(OH)D3 levels. The table summarizes the correlation coefficients (r) and (*P)* values between various biomarkers in patients with rheumatoid arthritis. A statistically significant correlation is considered $P < 0.05$ and is indicated by [S], while non-significant correlations are denoted by [NS].

The IL-4 shows a significant positive correlation with CRP (r = 0.5, $P < 0.05$) and HOMA-IR (r = 0.5, $p < 0.05$), suggesting a potential link between IL-4, inflammation, and insulin resistance, while not indicated a significant correlation with other biomarkers (CCL-21, ESR, FBG, zinc, HbA1c, vitamin D3), while CCL-21 was indicated a non-significant correlation with most biomarkers, except for a weak positive correlation with zinc (r = 0.4, $P < 0.05$), as presented in table (3.6).

CRP and HOMA-IR displayed significant positive correlations with FBG and HbA1c (markers of blood sugar control). Finally, CRP and HOMA-IR also had negative correlations with CCL-21, suggesting a potential inverse relationship.

3.1.7. Association of Biomarkers with RA with/without T2DM

The study utilized binary logistic regression and forward logistic regression to analyze results, utilizing the correlation coefficient to determine linear relationships among biochemical markers in patient and control groups. It was found that all biomarkers IL-4, CCL-21, CRP, and ESR HbA1c were risk factors and had highly statistically significant difference

An odd ratio of more than one indicates an increased event occurrence, while an odd ratio of less than one indicates a decreased event (protective exposure). As shown in Table (3.7). Table (3.8) presented the odds ratios (OR) and *P*-values for the association between various biomarkers and the presence of rheumatoid arthritis (RA) with or without T2DM as compared to that found in control group. The control group serves as the reference category (OR = 1). Statistically significant associations ($P < 0.05$) are indicated by [S].

Table 3.7: Estimation of the associated of the analysed factors (IL-4, CCL-21, CRP, and ESR) in RA patients (with and without T2DM) as compared with control group

Variable	Groups	OR (Lower–upper)	*P* value
CCL-21			
	Control	1[a]	-
	RA	1.151 (1.089-1.216)	<0.001[S]
	RA + T2DM	1.125 (1.067-1.186)	<0.001[S]
IL-4			
	Control	1[a]	-
	RA	121.64 (0.645-1.9674)	<0.001[S]
	RA + T2DM	92.542 (0.532-1.2357)	<0.001[S]
CRP			
	Control	1[a]	-
	RA	1.075 (0.964-1.199)	<0.001[S]
	RA + T2DM	1.342 (1.173-1.535)	<0.001[S]
ESR			
	Control	1[a]	-
	RA	1.960 (0.895-1.029)	<0.001[S]
	RA + T2DM	1.170 (1.081-1.266)	<0.001[S]
HbA1c			
	Control	1[a]	-
	RA	0.535 (0.370-0.775)	<0.001[S]
	RA + T2DM	1.063 (0.954-1.184)	<0.001[S]
p <0.05 considered significantly different, [S]= Significant, [NS]= Non Significant 1[a]: reference category is control			

The observed results indicated that IL-4, CCL-21, CRP, and ESR biomarkers showed a significantly increased odds ratio for patients having RA as compared to controls (*P*-value < 0.001). This suggests a strong association between elevated levels of these markers and the presence of RA, regardless of T2DM status.

Interestingly, the OR values for IL-4, CRP, and ESR were nearly identical for both RA without T2DM and RA with T2DM groups. This suggests that the presence of T2DM may not significantly alter the association of these markers with RA.

The obtained results were also shown that a non-significant association between HbA1c% and RA (with or without T2DM) as compared to controls. These findings were highlighted the potential role of IL-4, CCL21 CRP, and ESR as markers for RA, therefore, the presence of T2DM may not significantly affect the association between these specific markers with RA.

Table (3.8). presented the odds ratios (OR) and *P*-values for the association between vitamin D3 and zinc levels and the presence of rheumatoid arthritis (RA) with or without T2DM as compared to a control group.

Both RA without T2DM and RA with T2DM groups showed significantly decreased odds of having RA as compared to controls (*P*-value < 0.001). This translates to an OR of approximately 5.5 for RA without T2DM and 4.6 for RA with T2DM, indicating that lower vitamin D3 levels are associated with an increased risk of RA, regardless of T2DM status.

In contrast, zinc level in both RA without T2DM and RA with T2DM groups had significantly decreased odds ratio in RA patients as compared to controls (*P* < 0.001) with OR values around 0.2. This suggests that lower zinc levels are also associated with an increased risk of RA, and the presence of T2DM does not alter this association. These findings might confirm that vitamin D3 and zinc are generally expected to be protective against inflammatory conditions as found in RA, see Table (3.8).

Table 3.8: Estimation of the associated analysed factors (vit. D3 and zinc) in RA patients (with without DM) as compared with control group

Variable	Groups	OR (Lower–upper)	*P* value
Zinc			
	Control	1[a]	-
	RA	0.078 (0.073-0.083)	<0.001[S]
	RA + T2DM	0.077 (0.077-0.077)	<0.001[S]
Vit. D3			
	Control	1[a]	-
	RA	0.048 (0.030-0.075)	<0.001[S]
	RA + T2DM	0.032 (0.032-0.032)	<0.001[S]
***P* <0.05 considered significantly different, [S]= Significant, [NS]= Non Significant 1[a]: reference category is control**			

3.1.8. Receiver Operating Characteristic Analysis

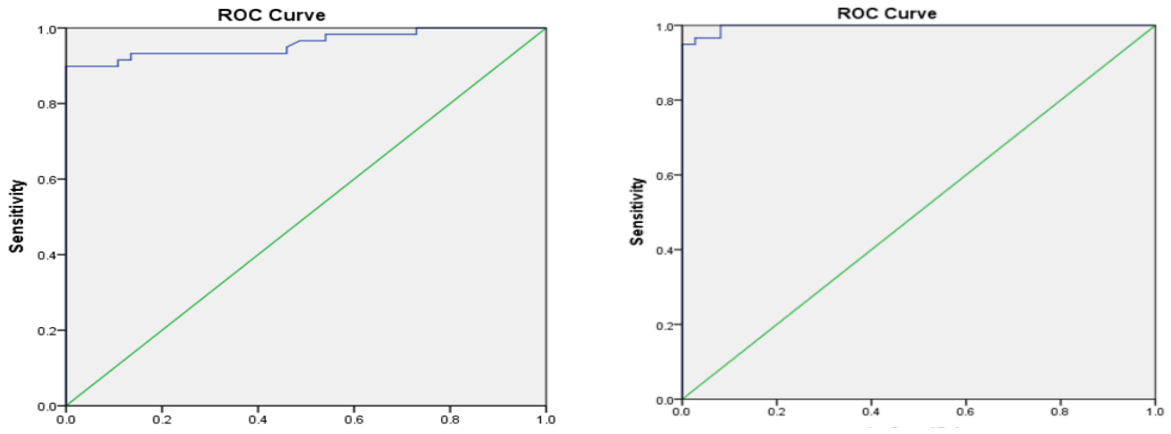
3.1.8.1. ROC curve and AUC analysis for the CCL-21 and IL-4 for RA (without T2DM) compared to the healthy groups

Results of the receiver operating curve (ROC) curve and AUC analysis for the Insulin besides as possible diagnostic parameters.CCL-21 and IL-4 showed good diagnostic performance for predication of the RA (without T2DM) compared to the fate groups between discharge and passed; data are presented in Table (3.9).

For CCL-21 levels: (sensitivity 90 %, specificity 94 %) at a level = 70.09, IL-4 levels: (sensitivity 95 %, specificity 93 %) at a level = 11.23, the p-values of the AUC were <0.05 and statistically significant. Youden's J statistics of the parameters in Figure (3-4) confirm these results.

Table (3.9): AUC, optimal threshold, sensitivity, and specificity of CCL-21 and IL-4 for obtained by ROC curve in patients RA.

Parameters	Cut-off	Sensitivity	Specificity	AUC	P-value	95% CL	
CCL-21, (pg/ml)	≥70.09	90%	94%	**0.986**	<0.001	0.958	1.000
IL-4, (pg/ml)	≥11.23	95%	93%	**1.000**	<0.001	1.000	1.000
AUC= Area under curve, CI= confidence interval							



A **(B)**

Fig (3-4): Receiver operating characteristics (ROC) curve analysis

A- CCL-21 in patients' RA (without T2DM) disease patients

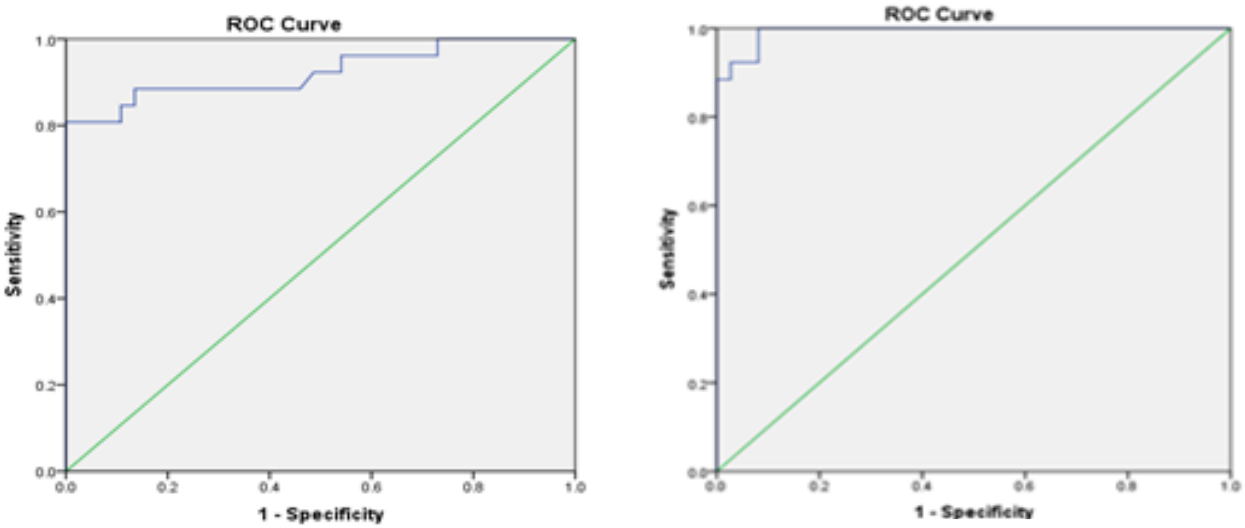
A- IL-4 in patients' RA (without T2DM) disease patients

3.1.8.2. ROC curve and AUC analysis for the CCL-21 and IL-4 for RA (with T2DM) compared to the healthy groups

Results of the receiver operating curve (ROC) curve and AUC analysis for the insulin besides as possible diagnostic parameters.CCL-21 and IL-4 showed good diagnostic performance for predication of the RA (with T2DM) compared to the fate groups between discharge and passed; data are presented in Table (3.10). For CCL-21 levels: (sensitivity 81%, specificity 88%) at a level = 66.42, IL-4 levels: (sensitivity 90%, specificity92%) at a level = 10.075, the p-values of the AUC were <0.05 and statistically significant. Youden's J statistics of the parameters in Figure (3-5) confirm these results.

Table (3.10): AUC, optimal threshold, sensitivity, and specificity of CCL-21 and IL-4 obtained by ROC curve in RA patients with T2DM.

Parameters	Cut-off	Sensitivity	Specificity	AUC	P-value	95% CL	
CCL-21, (pg/ml)	≥66.42	81%	88%	**0.924**	<0.001	0.847	1.000
IL-4, (pg/ml)	≥10.075	90%	92%	**0.993**	<0.001	0.980	1.000



(A) **(B)**

Fig (3-5): Receiver operating characteristics (ROC) curve analysis

A- CCL-21 in patients' RA (with T2DM) disease patients

B- IL-4 in patients' RA (with T2DM) disease patients

3.2. Part / II: (Results of Nanoparticle Synthesis and Characterization)

3.2.1. Artemisinin Nanoparticles Loaded with Hyaluronic acid

(Table 3.11) listed the size, PDI, and zeta potential of fabricated NPs with DLS. The average diameter of the blank niosome NPs was 109 ± 8.11 nm as shown in (Table 3.11).

Table 3.11: The size, PDI, and zeta potential of blank niosomal NPs, Nio-artemisinin NPs, and Hyalo-Nio-artemisinin NPs

Groups	Size (nm)	Polydispersity Index	Zeta potential (mV)
Niosome	109 ± 8.11	0.377	−23.7 ± 4.1
Nio-artemisinin NPs	137 ± 9.27	0.829	−19.1 ± 2.6
Hyalo-Nio-artemisinin NPs	144 ± 6.07	0.627	−21 ± 5.3

The fabricated nanoparticles, the Hyalo-Nio-artemisinin NPs exhibited the largest average diameter of 144 ± 6.07 nm.

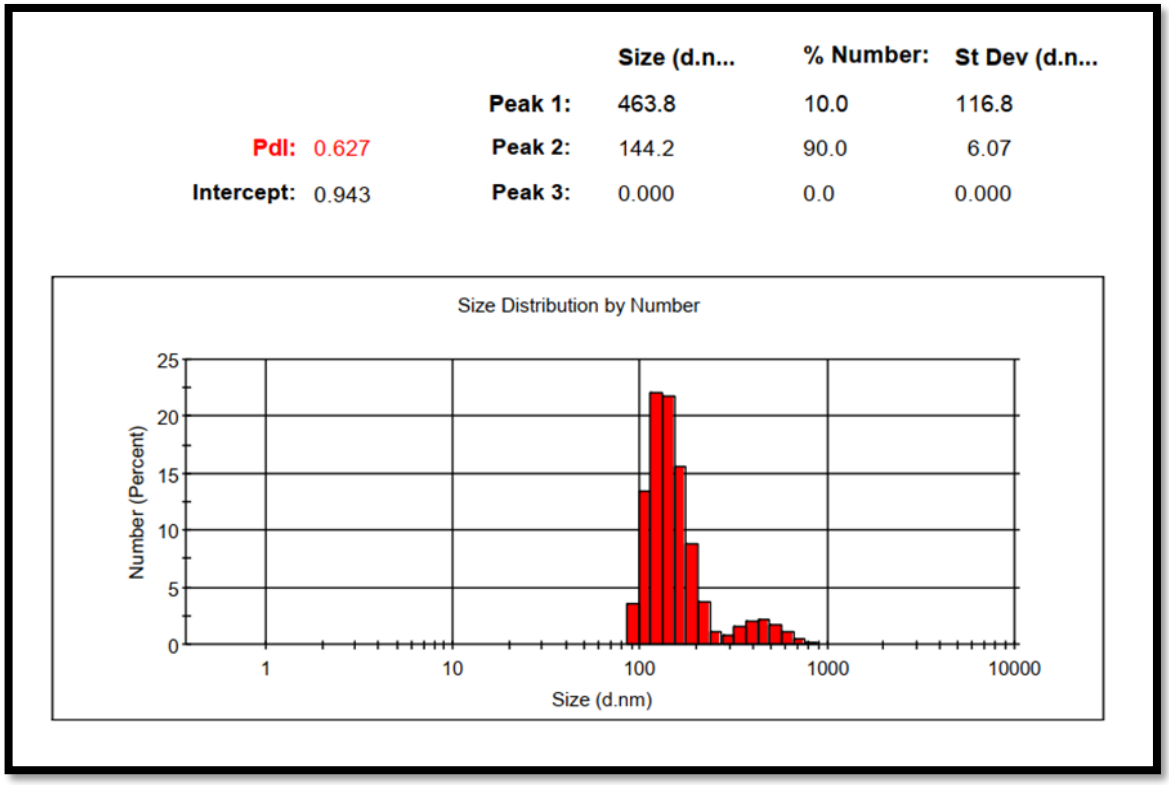


Fig. (3-6): The size and PDI value of fabricated Hyalo-Nio-artemisinin NPs acquired with DLS.

The SEM images of fabricated niosomal NPs show the same results (Figure 3-7). The results of AFM revealed the presence of nanoparticles, (Figure 3-8) with artemisinin size of 110 nm.

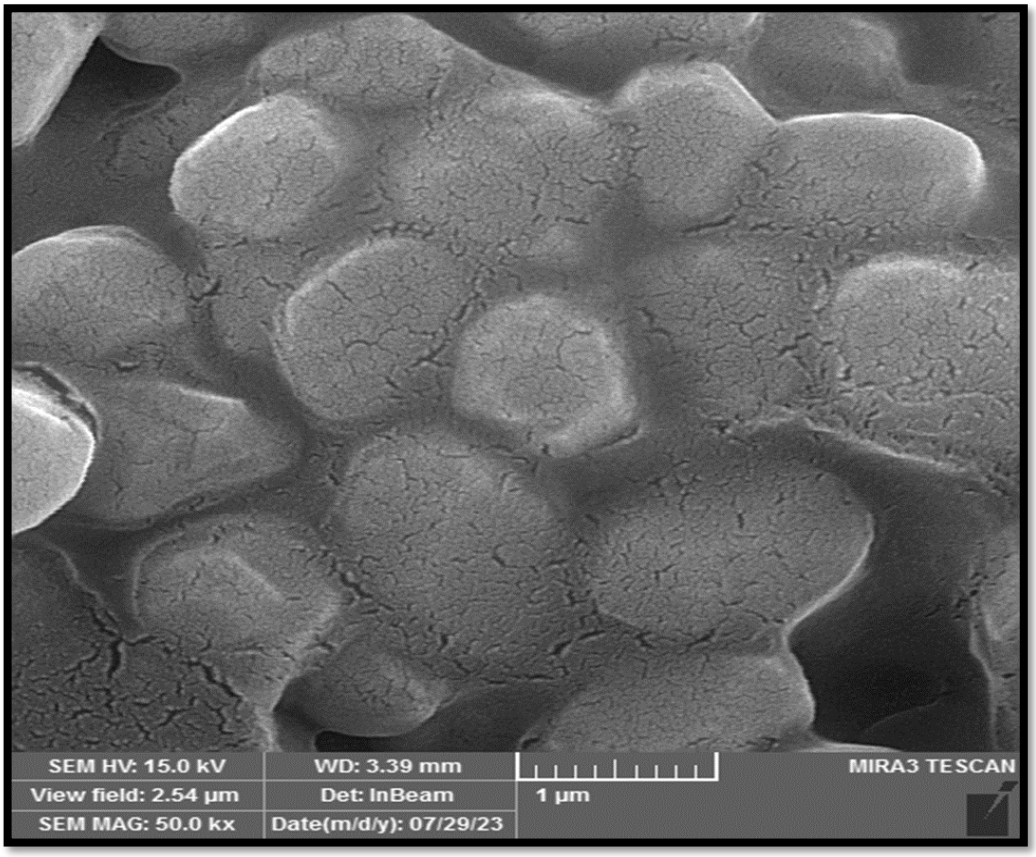


Fig. (3-7): The SEM images of Hyalo-Nio-artemisinin NPs revealed their spherical morphology.

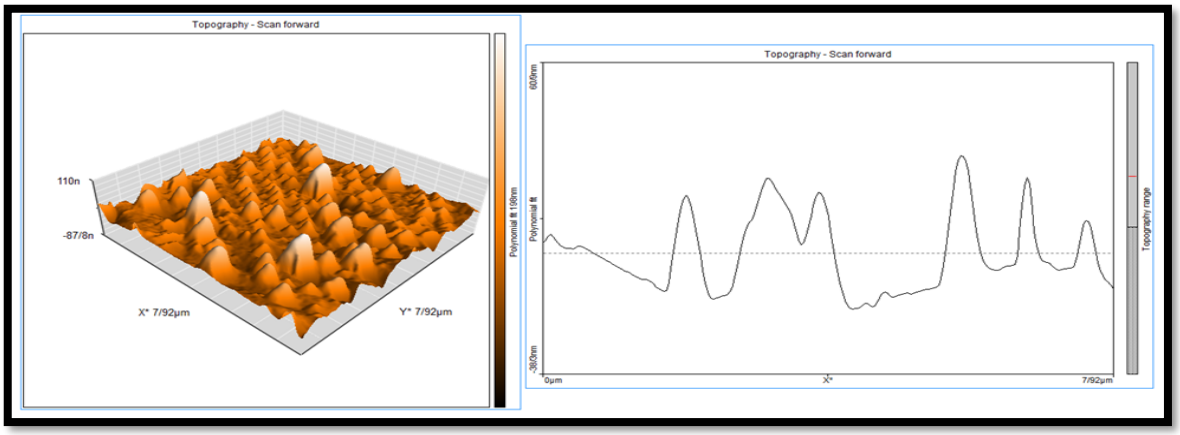


Fig. (3-8): AFM image of Hyalo-Nio-artemisinin NPs agrees with results of DLS and SEM images of Hyalo-Nio-artemisinin NPs.

FTIR is an effective analytical method used for detecting functional chemical groups and characterizing covalent bonding information. The FTIR spectra of the components involved in the niosome formation provided insight into the chemical interactions facilitating niosome formation. In the FTIR spectrum of Span 60, peaks at 3389, 2916, and 1736 cm^{-1} were identified, indicative of OH stretching, carbonyl dimer, and C=O stretching, respectively (Figure 3-9). Cholesterol exhibited a prominent peak at 3435 cm^{-1}, signifying OH stretching. The spectrum of blank niosomes revealed the OH stretching peak from Span 60 at 3388 cm^{-1}, with the carbonyl dimer peak shifting to 2918 cm^{-1} and the C=O stretching peak to 1737 cm^{-1}.

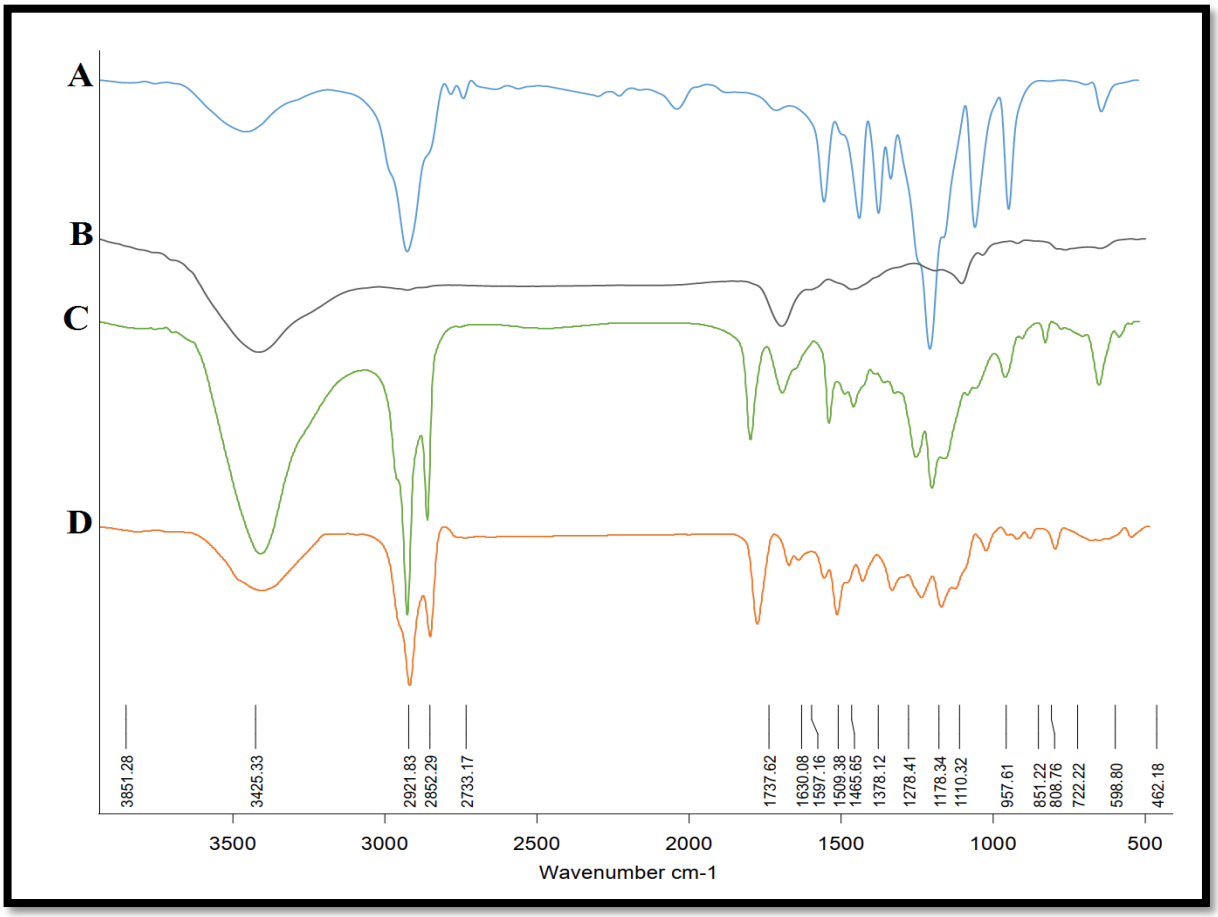


Fig. (3-9): FTIR analysis of (A) hyaluronic acid, and (B) artemisinin, show their characteristic bands in FTIR of (C) blank niosome NPs, and (D) Hyalo-Nio-artemisinin

Figure 3-10 illustrates the 96-hour release pattern of artemisinin from Nio-met and Hyalo-Nio-artemisinin NPs at 37 °C. Both Nio-artemisinin and Hyalo-Nio-artemisinin NPs exhibited a biphasic release pattern. The maximum release rates were achieved within the first 12 hours of the experiment.

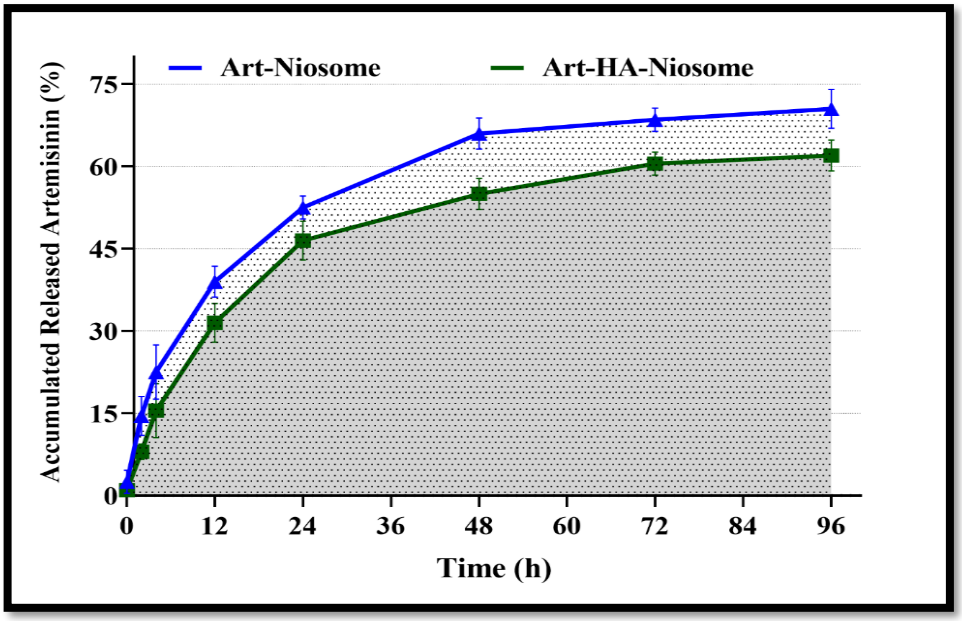


Fig. (3-10): The 96 h *invitro* release experiment of artemisinin from Nio-artemisinin NPs, and Hyalo-Nio- artemisinin NPs at 37°C and pH 7.4

Figure 3-11 illustrates the inhibitory effects of pure artemisinin, Nio-artemisinin NPs, and Hyalo-Nio-artemisinin NPs on PBMCs at various concentrations.150µM**.** Niosomal nanoparticles enhance drug treatment efficacy by improving solubility and bioavailability.

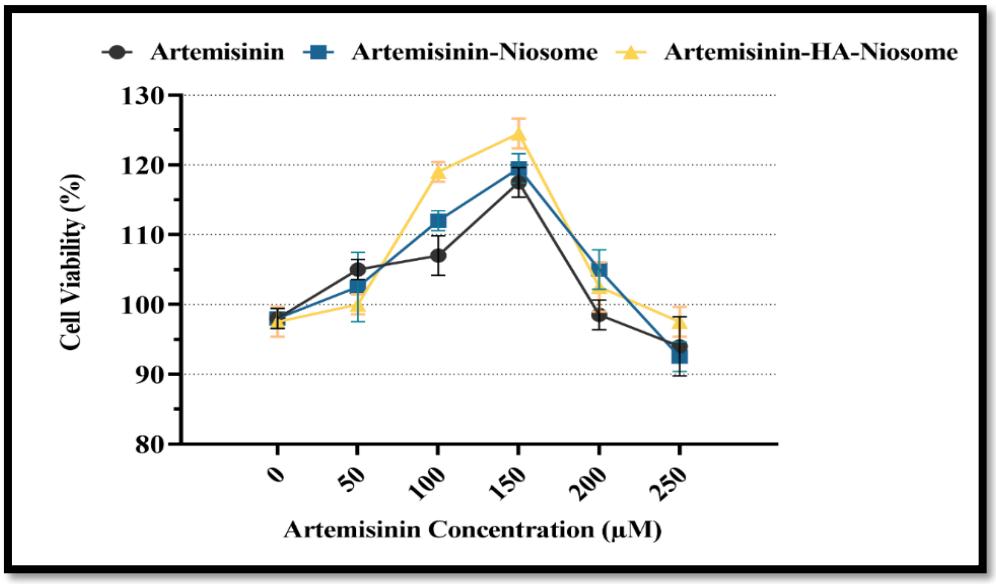


Fig. (3-11): The proliferation effects of pure artemisinin, Nio-artemisinin NPs, and Hyalo-Nio-artemisinin NPs on PBMCs.

3.2.2. Determination of IL-4- andCC-21 Level in Pure Untreated Artemisinin Nio-AR NPs, and HNio- AR treated PBMCs.

Figure 3-12A show the IL-4 level changes in PBMCs treated with pure artemisinin, Nio-artemisinin NPs, and Hyalo-Nio-artemisinin NPs The peak point of IL-4 levels in comparison to the control group was reached through the application of H-Nio-AR NPs treatment.

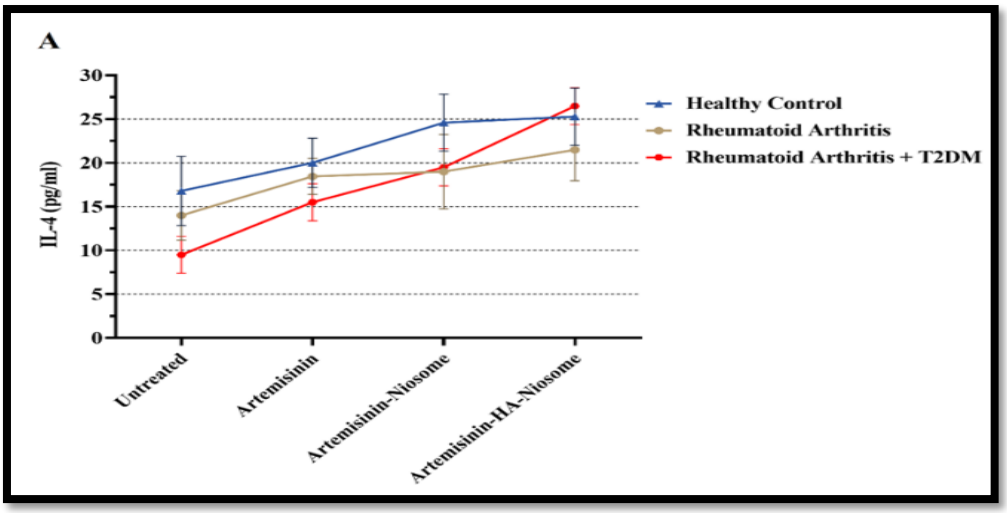


Fig. (3-12 A): Comparison of IL-4 levels in untreated and treated pure artemisinin, Nio-artemisinin NPs, and Hyalo-Nio-artemisinin NPs.

Figure 3-12B show the CCL-21, level changes in PBMCs treated with pure artemisinin, Nio-artemisinin NPs, and Hyalo-Nio-artemisinin NPs. The highest point of reduction in CCL-21 levels compared to the control group was reached by applying H-Nio-AR NPs treatment.

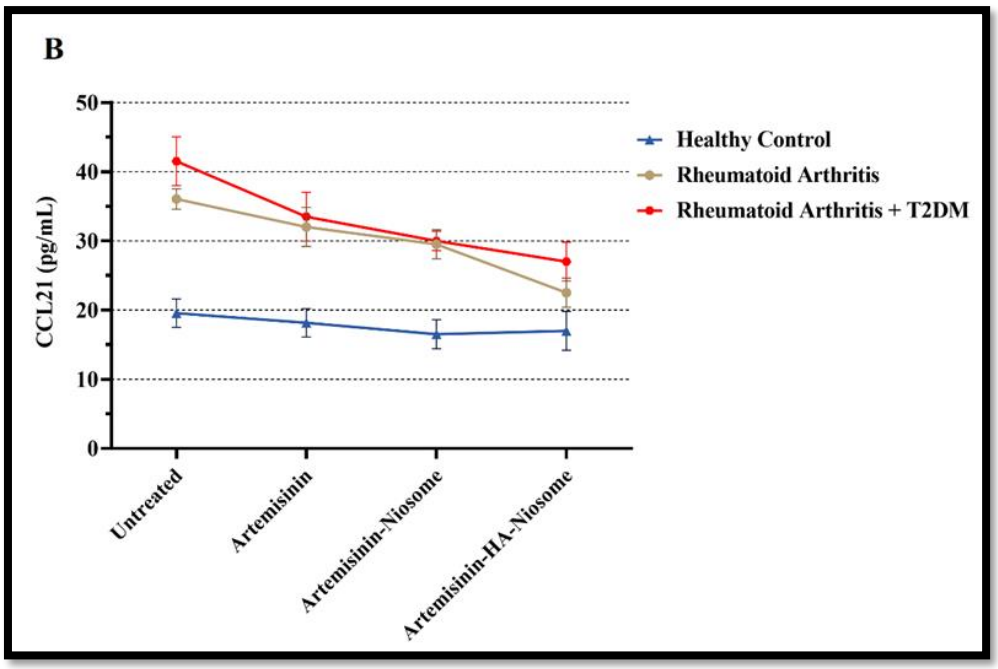


Fig. (3-12 B): Comparison of CCL-21 levels in untreated and treated pure artemisinin, Nio-artemisinin NPs, and Hyalo-Nio-artemisinin NPs.

3.3. Part III (Molecular Studies and Gene Expression)

Figure 3-13 for both RA patients and RA patients with T2DM. There is no significant change in COX-2 gene expression following treatment with artemisinin in RA patients. Same results are repeated for TIMP2 expression level in RA patients with T2DM. According to the results all treatment groups have better impact on RA patients compared to RA patients with T2DM. The most significant gene expression change belongs to TIMP2 gene in RA patients treated with Hyalo-Nio-artemisinin NPs, this treatment groups also shows a significant result compared to pure artemisinin, and Nio-artemisinin NPs treatment groups.

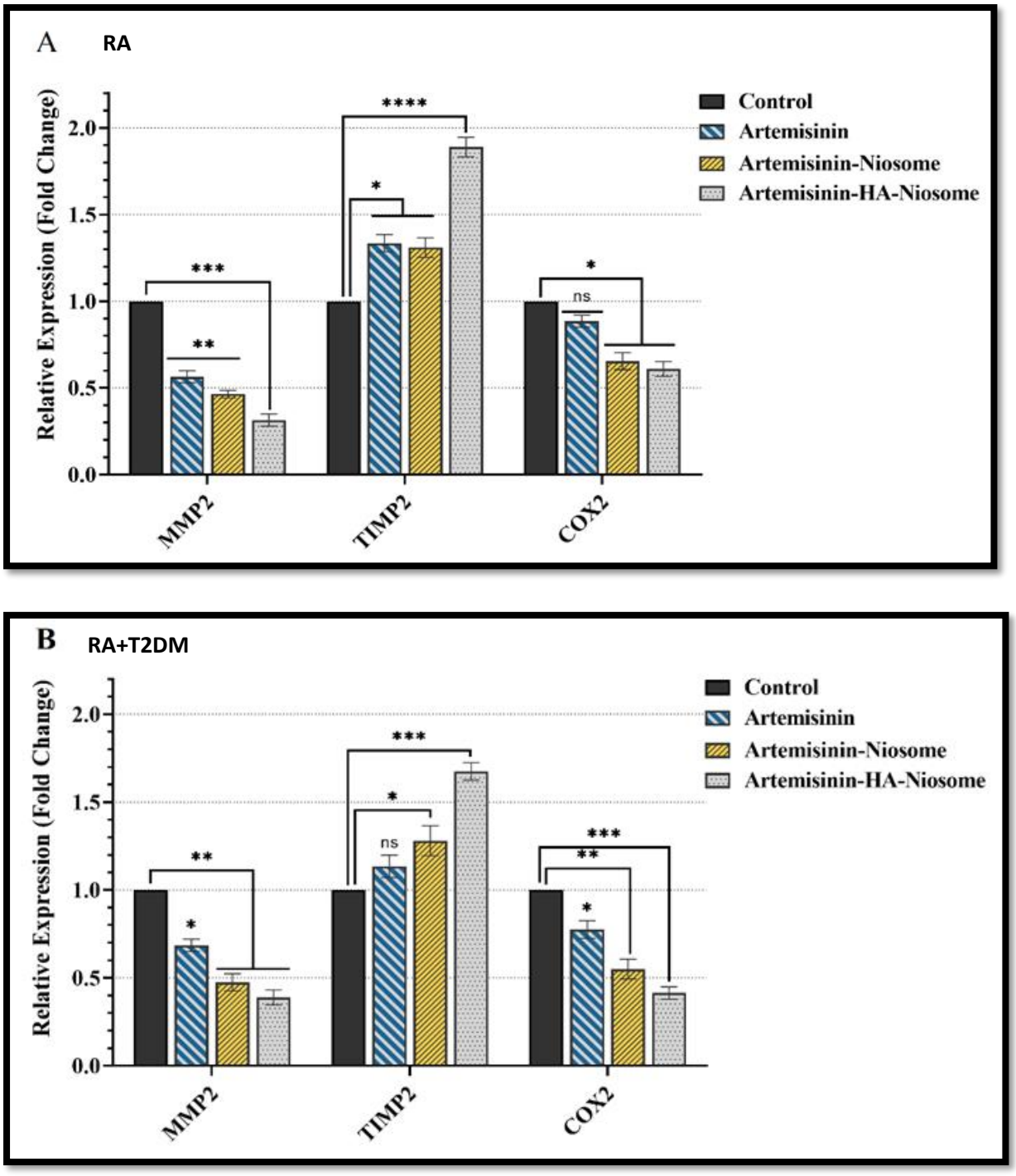


Fig. (3-13): The effect of the pure artemisinin, Nio-artemisinin NPs, and Hyalo-Nio-artemisinin NPs on expression of MMP2, TIMP2, and COX-2 in the PBMCs isolated from RA patients (A) and RA patients with T2DM (B) (p value < 0.001 *, *p* value < 0.01 **, and *p* value < 0.1 *).**

CHAPTER FOUR

Discussion

4. Discussion

Rheumatoid arthritis (RA) is a chronic inflammatory condition marked by polyarthritis, joint damage, disability, immunological irregularities, systemic inflammation, higher co-morbidity, and early mortality. Autoimmune diseases like RA arise from a sustained imbalance between pro- and anti-inflammatory immune responses, resulting in chronic inflammation.**(VD 2020).** Inflammatory mechanisms are integral to the etiology of rheumatoid arthritis (RA). Indicators of inflammation, including C-reactive protein, erythrocyte sedimentation rate (ESR), and interleukins, are critical in this context **(Schett *et al.* 2013)** .

4.1. Part / I: (Clinical Chemistry)

4.1.1. Demographic and Anthropometric Characteristics

The statistical data presented in table (3.1) indicate a total of 125 participants were meticulously divided into two distinct categories: individuals diagnosed with RA and a control group were depicted as mean ± SD of 46.93 ± 10.36 and 48.54 ± 11.26, respectively. Based on the findings, Table (3.1) and Figure (3-1) reveal that there is no significant correlation between age and RA, as well as the control group. These results align with previous studies performed by others **(R. Ahmad and Zgair 2021).** RA may onset at any age, but the peak age is 40–49 years in women, again with a geographic difference**(Silva-Fernández *et al*. 2020)**.

4.1.2. Biomarkers Studied in Sera of Rheumatoid Arthritis

In this study, there was a significant increase in the level of all inflammatory markers. Serum CCL-21 level was elevated and reached 2.2 times higher than that found in RA patients as compared with control group, whereas serum IL-4 level was elevated and reached to nearly double times than that observed in RA patients as compared to controls.

The observed ESR level was slightly higher than that found in RA patients as compared to control, and CRP levels were almost double times higher than that in RA patients as compared to control. These findings were confirmed with other observations from previous studies that the serum concentrations of inflammatory markers are elevated in the majority of patients with RA. The previous findings confirmed an active inflammatory response in patients with RA. This aligns with the established understanding of RA as an autoimmune disease characterized by chronic joint inflammation **(Xiang *et al*. 2023).**

The elevated serum CCL-21 levels play a pathological role in recruiting the immune cells to inflamed tissues **(Li, Wu, and Zhao, 2022).** This significant finding suggests its involvement in the inflammatory processes within RA pathogenesis in joints **(Van Raemdonck, Umar, and Shahrara, 2020)**.While typically association with Th2 (T helper cell type 2) responses, the IL-4 can also contribute with chronic inflammation in RA **(Mellado *et al.* 2015)**. Its elevation might indicate a complex immune system involvement in the RA disease. These findings indicate that IL-4 is a crucial mediator of inflammation in rheumatoid arthritis (RA) and significantly contributes to disease development and progression. It can stimulate synovial cells and chondrocytes to produce prostaglandins, reactive oxygen species, and neutral proteinases such as collagenases and stromelysin, which leads to the degradation of proteoglycans and collagen, ultimately causing cartilage destruction. **((Iwaszko, Biały, and Bogunia-Kubik 2021; Mukherjee and Das, 2024)**.

For each of the ESR and CRP biomarkers, they act as established inflammatory markers that are routinely used in RA diagnosis and monitoring disease activity **(Shapiro 2021)**. Their significant increase supports their utility in this study. The RA is characterized by chronic inflammation and hypertrophy

of the synovial membranes, therefore, inflammation of the joints occurs in response to the production of growth factors, cytokines, and chemokines by many different types of cells present in synovium and cartilage, in addition to infiltrating cells from the peripheral blood**(Edwards III *et al*. 2012)** .

Cytokines and acute phase proteins modulate one another's expression and functions, forming an intercellular communication network among fibroblasts, macrophages, lymphocytes, and hepatocytes. The activation of this network induces inflammation and the gradual deterioration of joints, as well as systemic symptoms associated with rheumatoid arthritis (RA). **(Shrivastava *et al.* 2015).**

In this study no significant difference was observed in HbA1c levels between RA patients and controls. Fasting blood glucose (FBG) levels were significantly higher in RA patients compared to controls. This reflects the potential impaired blood sugar regulation in RA patients. Both insulin levels and HOMA-IR were significantly higher in RA patients compared to controls. The significant increasing in RA patients compared to controls indicates a potential impaired blood sugar regulation and insulin resistance. These findings were in line with other research who reported that chronic inflammation associated with RA can disrupt insulin signaling pathways, leading to insulin resistance and elevated blood sugar levels **(Tripolino *et al.*, 2021).**

Increased IR, estimated by the homeostatic assessment model (HOMA-IR), has been reported in RA patients compared with healthy controls (**Giles *et al.* 2015)**, but the cause is still unclear. Some RA studies revealed a significant correlation of HOMA-IR with markers of inflammation **(Shahin *et al.*, 2010)** which was not the case in other investigations **(Gallagher *et al.*, 2020)**.

The mechanisms of altered glucose metabolism in RA remain partially elucidated. In the general population, obesity, characterized by low-grade chronic inflammation due to proinflammatory cytokines from adipose tissue,

significantly influences HOMA-IR. **(Bastard *et al.,* 2006).** In RA patients, the association of HOMA-IR index with markers of inflammation was demonstrated in some studies **(Shahin *et al.* 2010)** but not confirmed with the others (**Gallagher *et al.* 2020)**. Dessein and Joffe indicated that RA patients with high-grade inflammation were more insulin resistant than those with low-level inflammation, but this difference disappeared after adjustment for waist circumference**(Dessein and Joffe, 2006).**

Glucose intolerance was revealed in two studies with untreated patients, indicating, at least partly, the impact of inflammation on IR in the early and active RA **(den Uyl *et al.,* 2012)**.

Chronic systemic inflammation also contributes to defective insulin secretion. As insulin sensitivity and secretion are interrelated, β-cell response is crucial to maintaining euglycemia. In response to decreased insulin sensitivity, β-cells augment insulin release, inducing an increase in the HOMA2-%B index. Therefore, the rising HOMA2-%B index does not mean the amelioration of β-cell function but merely compensates for decreased insulin sensitivity **(Ristić *et al.* 2021)** .

4.1.3. Antioxidants Status and Rheumatoid Arthritis.

The observed results show significantly lower levels of both antioxidants determined in RA patients as compared to controls. Low antioxidant levels (zinc and vitamin D3) could contribute to increased oxidative stress in RA patients. Oxidative stress is an imbalance between free radicals and antioxidants, which can damage cells and tissues and worsen inflammation. Deficiencies in these specific antioxidants might play a role in the development or progression of RA **(Duarte *et al.,* 2022)**.

Oxidative stress (OS) is an important process related to the pathophysiology of rheumatoid arthritis and can be increased by the low intake of antioxidants. Zinc metal (Zn) is an important antioxidant trace-element for

human health and the assessment of the nutritional status of this micronutrient in these patients is of relevance. Aim This study aimed to evaluate Zn nutritional status in rheumatoid arthritis patients and its relation to OS. In this context, it was confirmed that rheumatoid arthritis patients were zinc and vit D deficient and have increased OS **(Duarte *et al*. 2022)**.

Other findings was revealed a significant inverse relationship between the serum zinc level and RA severity **(Rajaee *et al.*, 2018).** Several studies have examined the roles of trace elements in the etiology and pathogenesis of RA and have identified decreased plasma zinc levels in patients with RA. Evidence suggests that the zinc distribution in the body is influenced by inflammation. Previous studies have suggested that there is a correlation between the extent of inflammation and serum zinc depletion, and some other studies have indicated a protective role of zinc in RA. Several studies have reported that serum zinc concentrations decrease during the aging process **(Duarte *et al*., 2022).**

On the other hand, a decrease in serum levels of vitamin. D3 has been observed previously **(Ioannidou *et al.* 2024)**.and they are thought to have immunomodulatory and anti-inflammatory properties; their deficiency in RA patients may contribute to the progression or severity of the disease. Low levels of vitamin D3 may be associated with increased immune activation, as noted in several studies **(Meena *et al*., 2022)**, but one of these showed that patients with high disease activity had lower vitamin D3 levels than those with moderate or low disease activity **(Attar, 2012)**.

4.1.4. Association of Biomarkers with Rheumatoid Arthritis

In the current study, there was a potential correlation between serum level of CCL-21 and rheumatoid arthritis (RA). This indicates a potential role for CCL-21 in the disease process. The possible explanation might be due to immune cell recruitment. CCL-21 is a chemokine known to attract immune

cells, specifically T cells, to inflamed tissues. In RA, this could contribute to the chronic inflammation in the joints. Some studies suggest that CCL-21 might play a role in the development of new blood vessels (angiogenesis) within the inflammed joint lining (synovium) in RA **(Elshabrawy *et al.*, 2015**). This increased blood vessel formation can worsen inflammation and tissue damage. Other studies have reported its role in osteoclast genesis, in which the CCL-21 might also be involved in stimulating the formation and activity of osteoclasts, cells responsible for bone breakdown. This could contribute to the bone erosion characteristic of RA. **(Lee *et al.*, 2017)**.

The association of interleukin-4 (IL-4) with rheumatoid arthritis (RA) was studied and complex and not fully understood. It could be involved by the potential pro-inflammatory role. Initially, IL-4 was primarily associated with Th2 (T helper cell type 2) responses, promoting anti-inflammatory effects. However, in RA, the picture seems different. Studies have shown elevated IL-4 levels in the synovial fluid (joint fluid) of RA patients compared to healthy individuals **(Lubberts and van den Berg, 2013)**. Some theories suggest that IL-4 in RA might contribute to inflammation by: stimulating the production of pro-inflammatory mediators from certain immune cells and promoting the survival and function of B cells, which can contribute to autoantibody production in RA **(Mueller *et al.*, 2021)**.

C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) are well-established and widely used biomarkers for rheumatoid arthritis (RA). These results were confirmed by other research that reported that in a patient with inflammatory arthritis, the presence of a rheumatoid factor, elevated C-reactive protein level, or erythrocyte sedimentation rate suggests a diagnosis of rheumatoid arthritis. Many interleukins drive production of the acute-phase reactant C-reactive protein (CRP) following an inflammatory event **(Jones *et al.* 2018)**. While CRP is an important biomarker of systemic inflammation in

RA, its comprehensive role and relationship with comorbidities remain underexplored. Generally, CRP is crucial for host defense against infections and mediates the inflammatory response. **(Sproston and Ashworth 2018)**. A significant correlation has been seen between serum CRP levels and tissue inflammation scores from knee synovium biopsy samples in patients with RA. **(Orr *et al*. 2018)**.

Regarding the potential association between HbA1c and RA, various mechanisms may be involved. These encompass inflammation, wherein hyperglycemia can trigger a pro-inflammatory state marked by increased pro-inflammatory cytokines, such as interleukins, integral to RA pathogenesis. Heightened inflammation influences immune cells and fibroblast-like synoviocytes (FLS).**(Koedderitzsch *et al*., 2021).**

Another mechanism was oxidative stress; high glucose levels can lead to increased production of reactive oxygen species (ROS), which may cause oxidative stress and tissue damage. Oxidative stress has been implicated in RA pathogenesis, as it can promote inflammation, synovial hyperplasia, and joint destruction**(Khan *et al*. 2020).**

Furthermore, altered immune cell function may be influenced by hyperglycemia. Elevated glucose levels can disrupt the equilibrium between pro-inflammatory Th17 cells and anti-inflammatory regulatory T cells, which may facilitate the onset of seronegative RA. Furthermore, hyperglycemia might affect macrophage polarization and activity, fostering a pro-inflammatory phenotype that exacerbates synovial inflammation and joint damage **(Nekoua *et al*., 2016)**.

Finally, high glucose levels induce non-enzymatic glycation of proteins, forming advanced glycation end-products (AGEs). AGEs interact with RAGE on immune cells, leading to inflammation and tissue damage. The accumulation of AGEs in the synovium may worsen inflammation and joint degradation, thereby aiding the progression of seronegative RA. **(Monu, Agnihotri, and Biswas, 2022)**. The results of the current study have practical implications for understanding the relationship between HbA1c levels and the risk of RA.

Finally, regarding the potential association between Zn, Vit D3, and RA, their results were also consistent with other research for the Zn level, The obtained results indicated that the zinc levels were lower in RA patients as compared to healthy individuals. In patients with rheumatoid arthritis, it has been found that there may be an impairment of enzymatic or non-enzymatic antioxidant systems, including low serum zinc levels **(Quiñonez-Flores *et al*., 2016)**.

Zinc deficiency lead to oxidative stress, impaired binding activity and non-proper functioning of *p53*, interfering with *p53* functions required in the repair of damaged DNA **(Ho and Ames 2002).** Thus, zinc deficiency causes impaired DNA integrity through several mechanisms which may lead to DNA damage by oxidative stress directly and/or may impair the repair system of damaged DNA **(Ho, 2004).** DNA damage may be a result of disturbances between cellular antioxidant networks and repair systems **(Simone *et al.*, 2008)**.Shown that lower zinc levels cause oxidative DNA damage as an independent risk factor **(Mahmoud, Ali, and Al-Timimi, 2021).** Oxidative stress is a pathogenic hallmark in patients with rheumatoid arthritis due to the high cellular production of reactive oxygen species (ROS) **(Altindag *et al*., 2007).** It has been confirmed that oxidative DNA damage is linked to low serum zinc levels in rheumatoid arthritis patients, which may contribute to the high

cellular production of ROS in patients compared to healthy individuals, suggesting that oxidative DNA damage mediated by ROS and inflammation is a pathogenic hallmark. Free radicals can directly cause joint damage by attacking cartilage and its proteoglycan and inhibit its synthesis **(Hassan, 2024)**. Similar findings were observed and reported by others in which the mean serum vitamin D3 level was significantly lower in RA patients as compared to the normal participants **(Cen *et al.*, 2015).**

Other investigations were confirmed that vitamin D3 have a central role in modulating RA disease activity and is already known to be important in osteoporosis and falls the fractures, which are common in RA. The antiproliferative, immunomodulatory, and anti-inflammatory properties of vitamin D3 could be exploited to treat a variety of autoimmune rheumatic diseases (**Adorini and Penna, 2008**). Various studies done so far suggest that vitamin D3 deficiency increases the risk of developing autoimmune diseases such as RA. Vitamin D3 has immunoregulatory activity which is mediated through VDRs present on antigen presenting cells, activated T-lymphocytes, and activated B-lymphocytes. Vitamin D3 seems to interact with the immune system through its actions on the regulation and differentiation of cells such as lymphocytes, macrophages, and natural killer cells, besides interfering in the production of cytokines **(Meena *et al.*, 2018)**.

Merlino *et al*. demonstrated an inverse association between vitamin D3 and RA risk. Greater intake of vitamin D3 was inversely associated with risk of RA **(Harrison *et al*., 2020)**. Studies conducted by other investigators found significantly lower vitamin D3 levels in patients with RA as compared to the control population, thus supporting the possible role of vitamin D3 in the pathogenesis,activity, and treatment of various autoimmune diseases**(Ibrahim, Bakheet, and Abdel-Sater, 2014; Yağız *et al.* 2015 ; Elshabrawy *et al.* 2015)**,

whereas another study found that 90% of RA patients were either vitamin D3 deficient or insufficient. The mean serum vitamin D3 level of RA patients was significantly low in comparison to healthy controls. Levels of vitamin D3 in patients with high disease activity were significantly lower compared to those in patients with moderate- and low-disease activity, and vitamin D3 level had a significant negative correlation with the DAS28 score**(Sharma *et al*. 2014).** Another meta-analysis study of RA patients and controls showed that the prevalence of vitamin D3 deficiency was significantly higher in the RA group than in the control group, and the mean serum vitamin D3 level in the RA group was also significantly lower than that in the control group. This meta-analysis also showed a significant inverse correlation between the vitamin D3 levels and DAS28 **(Lee and Bae, 2016)** .

4.1.5. Correlation Coefficient between Biomarkers Various Groups.

The correlation study showed many significant correlations among the measured parameters, the objective of the conducted research was to ascertain the correlation coefficient to elucidate the linear relationships among various biomarkers between cohorts of rheumatoid arthritis (RA) patients without type 2 diabetes mellitus (T2DM) and those with T2DM. The findings of this investigation underscore a plausible association between interleukin-4 (IL-4), C-reactive protein (CRP) related to inflammation, and homeostasis model assessment for insulin resistance (HOMA-IR) among individuals suffering from RA and its comorbidity with T2DM. This observation indicates that elevated levels of IL-4 are concomitant with heightened inflammatory markers (CRP) and augmented insulin resistance (HOMA-IR). IL-4 is recognized for its significant role in facilitating inflammatory responses within autoimmune disorders such as RA **(Tian *et al.*, 2016).** Persistent inflammation has the potential to disturb insulin signaling pathways, ultimately culminating in

insulin resistance **(Soltani *et al.*, 2018).** The positive correlation identified between IL-4, CRP, and HOMA-IR insinuates a possible interaction among these variables in RA patients with T2DM. A plausible interpretation is that increased levels of IL-4 may be instrumental in exacerbating the inflammatory mechanisms that underlie RA. The correlation coefficient pertaining to IL-4 levels was highly significant and positive (*P*-value < 0.05); however, it may be influenced by the limited sample size in this study, resulting in a feeble correlation of insulin levels among the patient groups (both T2DM and non-T2DM). Furthermore, the heightened risk of developing T2DM may be attributable to an inflammatory state correlated with RA, corroborating findings from other scholarly reports **(Fabio *et al.*, 2023; Baker *et al.*, 2021).**

The result found in glucose level was highly significant in RA patients with T2DM (*P*-value <0.001) because of a dynamic interaction between glucose and insulin levels to predict insulin sensitivity or β cell production, these results agreed and consistent with another study **(Rasouli, *et al.*, 2024).**

Inflammation and insulin resistance chronic inflammation can disrupt insulin signaling, leading to insulin resistance and impaired blood sugar control. This could explain the positive correlation between each other. CRP and serves as an indicator of systemic inflammation. The association between IL-4 and CRP reinforces the hypothesis of a pronounced inflammatory condition in patients with Rheumatoid Arthritis (RA) who also have Type 2 Diabetes Mellitus (T2DM), particularly among those exhibiting elevated IL-4 concentrations **(Janet, *et al.*, 2020).** This investigation also illuminates the potential interactions among inflammation, insulin resistance, and glycemic regulation in individuals suffering from Rheumatoid Arthritis (RA) alongside comorbid Type 2 Diabetes Mellitus (T2DM). Noteworthy positive correlations were identified between CRP, an established marker of inflammation, and

Insulin Resistance (HOMA-IR) in conjunction with Fasting Blood Sugar (FBG) and Hemoglobin A1c (HbA1c).

This indicates that increased levels of inflammation and insulin resistance are concomitant with suboptimal glycemic control, as evidenced by elevated FBG and HbA1c values. Ineffectively managed blood sugar (high FBG and HbA1c) can inherently contribute to a pro-inflammatory milieu, potentially engendering a detrimental feedback loop. Curiously, CRP and HOMA-IR exhibited inverse correlations with chemokine ligand-21 (CCL-21). This observation implies that heightened levels of inflammation and insulin resistance may be linked to diminished concentrations of CCL-21 **(Chen, *et al.*, 2023).**

Vitamin D3, recognized as a critical lipophilic antioxidant, is conveyed within the serum alongside serum lipids. Previous investigations have indicated that the concentrations of vitamin D3 in the serum of patients diagnosed with rheumatoid arthritis (RA) are markedly diminished in comparison to those in control populations. This finding has been corroborated by subsequent research endeavors. The diminished levels of this antioxidant may be associated with the pathogenesis of RA, either through direct mechanisms or in conjunction with other etiological factors **(Sil *et al.*, 2014).** It has been established that low levels of antioxidants constitute a significant risk factor for the onset of RA. It is plausible that vitamin D3 is utilized in the process of neutralizing free radicals. A reduction in plasma vitamin D3 levels has also been documented in cases of juvenile rheumatoid arthritis. Vitamin D3 possesses lipophilic characteristics that enable it to function as a chain-breaking agent in the context of lipid peroxidation. Additionally, vitamin D, which is integrated within cellular membranes, plays a pivotal role in the mitigation of lipid hydroperoxides and is likely regarded as the most effective hydrophobic scavenger known to date. **(Vasanthi, *et al*., 2009).**

The correlation of zinc levels has also been consistent with prior investigations, which have indicated that a reduction in zinc concentration is associated with a modest elevation in the concentration of C-reactive protein (CRP). Similarly, the systemic inflammatory response has been linked to a decrease of as much as 48% in plasma zinc concentration concomitant with an increase in CRP **(Ghashut *et al.* 2017).** The diminished levels of Se may lead to a depletion of circulating antioxidants, thereby intensifying the inflammatory condition of the disease through unregulated production of reactive oxygen species (ROS) **(Heyland *et al.* 2017).**

4.2. Part II (Nano studies discussion)

4.2.1. Role of Nanoparticle in Rheumatoid Arthritis

Nanoparticles of artemisinin have unveiled promising healing abilities across various health ailments, such as cancer **(Alven and Aderibigbe 2020)** and epilepsy, while there is direct information about the role of artemisinin nanoparticles in treating rheumatoid arthritis (RA) **(Du *et al*. 2022)** Nanoparticles have become known for their effectiveness, in delivering drugs offering benefits like penetration into cells controlled release of medications and increased availability, at specific locations or organs **(Syam Kumar *et al.*, 2023)** . They can tackle the challenges of drug delivery by overcoming obstacles and delivering treatments with precision. A wide range of nanoparticles, including dendrimers, nanocrystals, nano polymer NPs, liposomes and niosomes have been created for drug delivery purposes**(Jadid et al. 2023; Manasa and Shivananjappa, 2023)**. Niosomes are non-ionic nanoparticles that protect drugs from degradation, enhance stability and water solubility, and improve drug uptake by cells and tissues **(Jadid *et al.* 2023)**. These nanocarriers can be tailored to target specific sites, thereby reducing adverse effects on non-target cells and increasing treatment efficiency**(Cheng,**

Xie, and Sun, 2023) Hyaluronic acid-coated nanoparticles demonstrate affinity for CD44-expressing cells like macrophages. This research involved the synthesis of hyaluronic acid-coated niosomal nanoparticles for targeting rheumatoid cells. Nanoparticle size critically affects material properties and biomedical performance, with an optimal range of 1-100 nm for diverse applications, including drug delivery systems. **(Odeniyi *et al.*, 2018)** Table 3.12 listed the size, PDI, and zeta potential of fabricated NPs with DLS. The average diameter of the blank niosome NPs was 109±8.11 nm (Table 3.12). However, encapsulating artemisinin within these nanoparticles increased their average diameter to 137±9.27 nm. Among the fabricated nanoparticles, the Hyalo-Nio-artemisinin NPs exhibited the largest average diameter of 144±6.07 nm, attributed to the coating with hyaluronic acid and the encapsulation of artemisinin.

Zeta potential is another crucial surface parameter in the characterization of NPs. It is measured to estimate the surface charge and stability of nanomaterials, as changes in these characteristics directly influence the biological activity of the NPs **(Balika 2022)**. (Table 3.12) lists the zeta potential and PDI values of the niosomal NPs. A zeta potential value between +30 mV and -30 mV is essential for preventing particle aggregation, thereby maintaining nanoparticle stability **(Umbarkar *et al.* 2020)** figure (3.7). PDI provides information about the size distribution and uniformity of NPs. A low PDI value indicates a narrow size distribution, which is essential for ensuring consistent nanoparticle performance in terms of solubility, drug release, dissolution, and cellular uptake **(Kunasekaran and Krishnamoorthy 2015 ; Danaei *et al.* 2018)**. The PDI values of fabricated NPs in this study were in acceptable. Figure (3.7)The interaction of nanoparticles with biological systems is influenced by various factors, including size, shape, and surface properties **(Kenry *et al.* 2020)** Their morphology significantly affects

interactions with biological materials such as cells and tissues **(Liebert *et al.* 2011)**. Specifically, the morphology of nanoparticles can impact their biological activities, including cell membrane penetration, and interactions with proteins **(Xiang Liu *et al.* 2012; Ge *et al*. 2015)**. Previous studies collectively indicate that niosomes typically exhibit a spherical morphology **(Hajizadeh *et al.*, 2019; Barani *et al.* 2021)**. Figure (3.10) These shifts in the carbonyl groups' peaks suggest hydrogen bonding between Span 60 and cholesterol, indicative of niosome formation **(Alkilani *et al.*, 2022)**. The FTIR spectra of pure artemisinin showed characteristic bands at 3379 cm^{-1} (O-H stretching vibrations), 2947 cm^{-1} (Fermi resonance of the symmetric CH_3 stretch with overtones of the methyl bending modes), 1093 cm^{-1} (C-O stretching), 890-820 cm^{-1} (O-O-C stretching in boat/twist form), 825 cm^{-1} (O-O stretching in boat/twist form), and 1420-1300 cm^{-1} (C=O stretching) **(Lawal *et al.*, 2012)**. In the spectrum of Nio-artemisinin NPs, significant peaks were observed at 3370, 2920, and 1738 cm^{-1}, likely representing OH stretching, carbonyl dimer, and C=O stretching. These shifts are similar to those seen in blank niosomes and point to interactions that facilitate niosomal formation **(Alkilani *et al.*, 2022)** . Additionally, the Fourier Transform Infrared (FTIR) spectrum of cholesterol demonstrated a prominent band at 3432 cm^{-1}, signifying the occurrence of hydroxyl (OH) stretching vibrations. The symmetric and asymmetric stretching vibrations corresponding to the methylene (CH_2) groups within the alkyl chains were detected at 2989 cm^{-1} and 2882 cm^{-1}, respectively. A notable band at 1716 cm^{-1} was ascribed to the presence of a double bond situated within the second ring of the cholesterol molecular structure. **(Hanafy *et al.* 2023)** .

Upon the coatation of HA onto the Nio-artemisinin NPs, additional characteristic peaks were observed. The presence of a band at 1048.92 cm^{-1} is attributed to the C–O–C stretching vibration of hyaluronic acid **(Niu *et al.***

2022). Moreover, a discernible peak at 1655 cm^{-1} corresponding to the amide group emerged, affirming the successful incorporation of HA into the final structure. These observations confirm the successful encapsulation of the drug within the niosomes and the formation of niosomal structures through interactions between Span 60, cholesterol, and hyaluronic acid. The shifts in the characteristic peaks support the presence of hydrogen bonding and other interactions, which are crucial for the stability and formation of niosomes.

Figure (3.10) Drug release patterns play key role in the effectiveness of drug therapies. Scientists have created various controlled release to control how drugs are released to make them more effective systems **(Uhrich *et al.* 1999)**. Size of NPs, surface characteristics and their structure significantly influence the drug release profiles from them **(Slowing *et al*. 2007 ; Odeniyi *et al*. 2018 ; Ways *et al.*, 2020)** . For medical applications, it is highly desirable for drugs to be released steadily from nanoparticles ***(*Javaid *et al*. 2021).**

Niosomes, made from materials that break down naturally and are unlikely to cause reactions can trap both amphiphilic and lipophilic medications making them ideal for drug delivery applications **(Rao *et al*. 2018 ; Hazira and Reddy 2023)**. Studies have shown that niosomes can release drugs slowly over time, such as α-tocopherol and dexamethasone with varying levels of release reaching, up to 70% in a biphasic release process **(Mavaddati, Moztarzadeh, and Baghbani, 2015 ; Basiri, Rajabzadeh, and Bostan, 2017)**. The peak release rates occurred during the initial 12 hours, followed by a decline. This initial elevation in release rate is ascribed to the weak adsorption of artemisinin on the niosomal nanoparticle surface, rather than complete encapsulation.

Figure (3.11) MTT is a widely used method for assessing cytotoxicity, viability, and proliferation studies in cell biology**(Wang *et al.* 2012)** . This is based on the ability of mitochondrial enzymes in viable cells to reduce the MTT

yellow tetrazolium salt to purple formazan crystals **(Hirsch *et al.*, 2015)**. Artemisinin hrysin is known for its cytotoxicity against cancer cells while sparing normal cells**(Kiani *et al.*, 2020)**. The Hyalo-Nio-artemisinin NPs are formulated with cholesterol and hyaluronic acid, which are natural components found in the human body and have been confirmed as safe for normal cells **(Ge *et al.*, 2015)**.As previously delineated, niosomal nanoparticles have demonstrated the capability to augment treatment efficacy through enhanced drug solubility and bioavailability. Furthermore, the incorporation of hyaluronic acid on their surface promotes targeted drug delivery near PBMCs, thereby increasing the probability of cellular uptake and improving therapeutic outcomes.

4.2.2. Interleukin-4 and CCL-21 of NPs in Rheumatoid Arthritis

Interleukin-4 (IL-4) is a multifunctional cytokine that plays a crucial role in modulating immune responses. It is secreted by activated T cells and has pleiotropic effects on both B and T lymphocytes **(Lewis *et al.*, 1988).** IL-4 is involved in regulating antibody production, hematopoiesis, inflammation, and the development of effector T-cell responses **(Martirosyan *et al.*, 2022).** According to results treatment with pure artemisinin, Nio-artemisinin NPs, and Hyalo-Nio-artemisinin NPs could increase IL-4 level in both RA patients and RA patients with T2DM (Figure 3.12A). The use of Hyalo Nio NPs resulted in an increase compared to pure artemisinin, as well, as Nio-artemisinin NPs treatment across all groups.

CCL-21, also referred to as C C motif chemokine 21 play vital role in recruiting and moving immune cells. It functions by activating the G protein coupled receptor CCR7 guiding cells to lymph nodes and promoting interactions among cells, in lymphoid tissues **(Love *et al.* 2012 ; Cui *et al.* 2020).** Studies have highlighted the involvement of CCL21 in angiogenesis

within the context of RA, where it acts as an angiogenic mediator **(Pickens *et al.*, 2012).** CCL21 has been found to be upregulated in RA synovial fluid, primarily secreted by synovial tissue macrophages and fibroblasts **(Van Raemdonck *et al*. 2022).** The dysregulation of CCL21 has been observed in RA fibroblasts and macrophages, indicating a novel role for CCL21 in promoting angiogenesis in RA **(Pickens *et al.*, 2011) .** The treatment with pure artemisinin, Nio-artemisinin NPs, and Hyalo-Nio-artemisinin NPs leads to a decrease in CCL 21 level in all groups (Figure 3.12.B). PBMCs derived from healthy individuals showed the least sensitivity and change in the level of CCL 21 to the treatment.

4.3. Part / III: (Molecular studies)

4.3.1. Gene Expression and NPs in Rheumatoid Arthritis

Figure (3.13) The expression of various genes plays a role in RA, so the expression of some of these genes was investigated by real-time PCR. Matrix metalloproteinase 2 (MMP2) plays an important role in rheumatoid arthritis progression, specifically in angiogenesis and invasion of tumor progression **(Zhou *et al.*, 2014)**. The serum levels of MMP2 are significantly higher in RA patients compared to healthy individuals **(Giannelli *et al.* 2004)**.Studies has indicated that MMP2 can stimulate the invasion spread and growth of cancer cells **(Ni *et al.* 2014)** . MMP2 expression in RA synoviocytes is elevated via the NFκB/HIF 1α pathway. **(G. Li *et al*. 2013)**. Additionally MMP2 is linked to the movement and invasion of RA cells with its decrease hindering these processes **(Zhang *et al*. 2018)**. In the context of arthritis TIMP2 expression plays a role, in disease development **(Gaafar *et al.* 2020)** .

Research has demonstrated that MMP2 possesses the capacity to enhance the invasive spread and proliferation of neoplastic cells **(Siddhartha, *et al.*, 2021).** A multitude of investigations has revealed that matrix

metalloproteinases (MMPs) are implicated in the initiation and advancement of rheumatic autoimmune disorders. These disorders exhibit critical features such as abnormalities in extracellular matrix metabolism, vascular inflammatory damage, and significant infiltration of inflammatory cells **(Lerner, *et al.*, 2018).** It has been noted that under certain conditions, the expression of MMP2 is elevated in rheumatoid arthritis (RA) synoviocytes via the NF κB/HIF 1α signaling pathway **(Yang, *et al.*, 2022).** Furthermore, MMP2 is associated with the motility and invasion of RA.

The principal endogenous modulators of matrix metalloproteinase (MMP) activities within the tissue microenvironment are tissue inhibitors of metalloproteinases (TIMPs), which possess the capacity to modify cellular functionality and govern matrix turnover **(Alpoim-Moreira, *et al.*, 2022).** Within the framework of arthritic conditions, the expression of TIMP2 is integral to the progression of the disease **(Cabral-Pacheco, *et al.*, 2020).** The elevated levels of TIMP2 observed in patients with systemic sclerosis accompanied by arterial hypertension accentuate its importance as a biomarker for inflammation and vascular damage in this context **(Hasselbalch, *et al.*, 2023 ;Costa, *et al.*, 2024).** Metalloproteinases represent a subclass of endopeptidases that are linked to extracellular processes and possess the ability to cleave internal peptide bonds within polypeptide chains. Among these, matrix metalloproteinases (MMPs) are the most significant, as they are involved in the pathogenesis of various diseases, including rheumatoid arthritis (RA). The levels of MMPs are frequently found to be markedly elevated in association with lesions of the synovial joints, thereby indicating a strong correlation between MMPs and the onset of RA. In recent decades, this assertion has been consistently corroborated **(Martu, *et al.*, 2021 ; Meng, *et al.*, 2022).**

The heightened presence of TIMP2 in systemic sclerosis patients with arterial hypertension underscores its significance as an inflammation and

vascular injury marker in this scenario **(Pendergrass *et al*., 2010)**. COX2 is an inducible enzyme that is involved in pathophysiological processes such as pain, inflammation, and fever **(Cottrell and O'Connor, 2010)** . COX2, found in the synovial cells of individuals, with arthritis (RA) plays a role in the inflammation process in the joints **(Kawai, 1998)**.Selective COX2 inhibitors have been used for treating RA due to their effectiveness and lower risk of side effects compared to traditional non selective nonsteroidal anti-inflammatory drugs **(Sundy 2001 ; Lazzaroni and Bianchi Porro, 2004)** . shown in (Figure 3.13) for both RA patients and RA patients with T2DM. There is no significant change in COX2 gene expression following treatment with artemisinin in RA patients. Same results are repeated for TIMP2 expression level in RA patients with T2DM. According to the results all treatment groups have better impact on RA patients compared to RA patients with T2DM. The most significant gene expression change belongs to TIMP2 gene in RA patients treated with Hyalo-Nio-artemisinin NPs, this treatment groups also shows a significant result compared to pure artemisinin, and Nio-artemisinin NPs treatment groups.

Conclusions and Recommendations

1. Conclusions

According to the observed data, we can conclude the following:

1. The results of this study showed an increase in levels of biomarkers which are important for monitoring and diagnosis of developing disease Biomarkers of IL-4 may be useful for diagnosis of RA and monitoring to prevent RA from developing to T2DM as a cause of increase in Insulin level that leading to insulin resistance. In addition to other routine biomarker can be assist evaluating of progress of disease.

2. The current study identified significant differences in antioxidant levels, particularly vitamin D3 and Zinc, in rheumatoid arthritis (RA) patients versus healthy individuals.

3. artemisinin-loaded HA-coated nanoparticles showed a significant reduction in oxidant levels and suppression of inflammatory cytokine activity, as well as suppression of MMP2 gene expression and increased TIMP2 gene expression in patients. Taken together, these results suggest the potential therapeutic efficacy of artemisinin-loaded HA-coated nanoparticles in alleviating inflammation and modulating immune response in RA patients.

2. Recommendations

1. Engaging in comprehensive research initiatives concerning rheumatoid arthritis to avert its progression to type 2 diabetes, which is notably prevalent among the Iraqi populace.

2. The significant impact of hyperglycemia on patients with rheumatoid arthritis is very clear, so the research recommends making glucose analysis an approved method for those with a family history of the disease and monitoring the diabetes of those with the disease to reduce the progress and development of RA.

3. Further prospective studies with larger sample sizes are needed to confirm our findings.

4. More research is necessary to understand the precise and complete effect of artemisinin on markers of inflammation and oxidation in patients with rheumatoid arthritis. And also to explore its effects on people with diabetes as a risk factor for the disease. Proposing it as a new therapeutic approach and improving its therapeutic potential by taking advantage of nano-based development.

5. More research and experiments are needed to study the effect of nano-artemisinin on the gene expression of genes associated with rheumatoid arthritis more broadly and to use blood serum in the analyses instead of PBMCs.

6. More studies are needed to know the effect of artemisinin on cells in vivo and its ability to adapt to body conditions.

7. Further research is needed to ensure safety and regulatory NPs compliance.

References

Reference

Adewumi, Odunayo Ayodeji, Vijender Singh, and Gunjan Singh. **(2020)**. "Chemical Composition, Traditional Uses and Biological Activities of Artemisia Species." *Journal of Pharmacognosy and Phytochemistry* 9(5): 1124–40.

Adorini, Luciano, and Giuseppe Penna. **(2008)**. "Control of Autoimmune Diseases by the Vitamin D Endocrine System." *Nature clinical practice Rheumatology* 4(8): 404–12.

Afrin, Sadia et al. **(2021).** "Diet and Nutrition in Gynecological Disorders: A Focus on Clinical Studies." *Nutrients* 13(6): 1747.

Ahmad, Bashir et al. **(2019).** "Natural Gypenosides: Targeting Cancer through Different Molecular Pathways." *Cancer management and research*: 2287–97.

Ahmad, RusulH, and AyaidK Zgair. **(2021).** "Immunological and Biological Manifestation of Rheumatoid Arthritis Patient in Iraq." *Indian Journal of Forensic Medicine & Toxicology* 15(4): 1344–50.

Albasher, Gadah et al. **2020.** "Evaluation of the Neuro-protective Effect of Artemisia Judaica Extract in a Murine Diabetic Model." *Journal of Food Biochemistry* 44(8): e13337.

Aoki, T., Ito, H., Ogura, T., Hirata, A., Nishiwaki, Y., & Kameda, H. **(2020).** Association of age with the non-achievement of clinical and functional remission in rheumatoid arthritis. Scientific reports, 10(1), 15277.

Aletaha, Daniel et al. **(2010)**. "2010 Rheumatoid Arthritis Classification Criteria: An American College of Rheumatology/European League Against Rheumatism Collaborative Initiative." *Arthritis & rheumatism* 62(9): 2569–81.

Aletaha, Daniel, Josef Smolen, and Michael M Ward. **(2006)**. "Measuring Function in Rheumatoid Arthritis: Identifying Reversible and Irreversible Components." *Arthritis & Rheumatism* 54(9): 2784–92.

Ali, Omar. **(2013)**. "Genetics of Type 2 Diabetes." *World journal of diabetes* 4(4): 114.

Ali, S. S., Ahsan, H., Zia, M. K., Siddiqui, T., & Khan, F. H. **(2020).** Understanding oxidants and antioxidants: Classical team with new players. Journal of food biochemistry, 44(3), e13145.

Aljary, Hissah, Nicholas Czuzoj-Shulman, Andrea R Spence, and Haim A Abenhaim. **(2020)**. "Pregnancy Outcomes in Women with Rheumatoid Arthritis: A Retrospective Population-Based Cohort Study." *The Journal of Maternal-Fetal & Neonatal Medicine* 33(4): 618–24.

Alkilani, Ahlam Zaid et al.**(2022)**. "Formulation and Evaluation of Azithromycin-Loaded Niosomal Gel: Optimization, in Vitro Studies, Rheological Characterization, and Cytotoxicity Study." *ACS omega* 7(44): 39782.

Allalou, Amina et al. **(2016)**. "A Predictive Metabolic Signature for the Transition from Gestational Diabetes Mellitus to Type 2 Diabetes." *Diabetes* 65(9): 2529–39.

Almoallim, Hani, and Mohamed Cheikh. **(2021).** "Skills in Rheumatology."

Altammar, Khadijah A. **(2023)**. "A Review on Nanoparticles: Characteristics, Synthesis, Applications, and Challenges." *Frontiers in Microbiology* 14: 1155622.

Altindag, Ozlem et al. **(2007).** "Increased DNA Damage and Oxidative Stress in Patients with Rheumatoid Arthritis." *Clinical Biochemistry* 40(3–4): 167–

71.

Alven, Sibusiso, and Blessing Atim Aderibigbe. **(2020)**. "Nanoparticles Formulations of Artemisinin and Derivatives as Potential Therapeutics for the Treatment of Cancer, Leishmaniasis and Malaria." *Pharmaceutics* 12(8): 748.

Amariuta-Bartell, Tiffany. **(2023)**. "Trans-Ancestry Genome-Wide Association Study Identifies Novel Genetic Mechanisms in Rheumatoid Arthritis."

Arleevskaya, Marina I et al. **(2016)**. "How Rheumatoid Arthritis Can Result from Provocation of the Immune System by Microorganisms and Viruses." *Frontiers in microbiology* 7: 179811.

Aslam, Muhammad Muaaz et al. **(2020)**. "Investigating the GWAS-Implicated Loci for Rheumatoid Arthritis in the Pakistani Population." *Disease markers* 2020.

Aslani, Saeed, Ramazan Rezaei, Ahmadreza Jamshidi, and Mahdi Mahmoudi. **(2018).** "Genetic and Epigenetic Etiology of Autoimmune Diseases: Lessons from Twin Studies." *Rheumatology Research* 3(2): 45–57.

Athanassiou, Lambros, Ifigenia Kostoglou-Athanassiou, Michael Koutsilieris, and Yehuda Shoenfeld. **(2023).** "Vitamin D and Autoimmune Rheumatic Diseases." *Biomolecules* 13(4): 709.

Attar, Suzan M.**(2012)**. "Vitamin D Deficiency in Rheumatoid Arthritis." *Saudi Med J* 33(5): 520–25.

Azad, M B et al. **(2017).** "Diabetes in Pregnancy and Lung Health in Offspring: Developmental Origins of Respiratory Disease." *Paediatric respiratory reviews* 21: 19–26.

Balika, S D. **(2022).** "Determination of Zeta-Potential of Nanofluids Based on

Electrolyte Solutions from the Measurements by the Methods of Electrical Spectroscopy and Laser Correlation Spectroscopy." *arXiv preprint arXiv:2210.16054*.

Bao, Fei et al. **(2012)**. "Nitric Oxide-Driven Hypoxia Initiates Synovial Angiogenesis, Hyperplasia and Inflammatory Lesions in Mice." *PloS one* 7(3): e34494.

Barani, Mahmood et al. **(2021).** "In Vitro and in Vivo Anticancer Effect of PH-Responsive Paclitaxel-Loaded Niosomes." *Journal of Materials Science: Materials in Medicine* 32: 1–13.

Basiri, Ladan, Ghadir Rajabzadeh, and Aram Bostan. **(2017).** "α-Tocopherol-Loaded Niosome Prepared by Heating Method and Its Release Behavior." *Food chemistry* 221: 620–28.

Bastard, Jean-Philippe et al.**(2006).** "Recent Advances in the Relationship between Obesity, Inflammation, and Insulin Resistance." *European cytokine network* 17(1): 4–12.

Batooei, M., Tahamoli-Roudsari, A., Basiri, Z., Yasrebifar, F., Shahdoust, M., Eshraghi, A., ... & Ataei, S. **(2018).** Evaluating the effect of oral N-acetylcysteine as an adjuvant treatment on clinical outcomes of patients with rheumatoid arthritis: a randomized, double blind clinical trial. Reviews on recent clinical trials, 13(2), 132-138.

Bedeković, Dražen et al. **(2023).** "Role of Inflammatory Cytokines in Rheumatoid Arthritis and Development of Atherosclerosis: A Review." *Medicina* 59(9): 1550.

Bedoui, Yosra et al. **(2021)**. "Robust COX-2-Mediated Prostaglandin Response May Drive Arthralgia and Bone Destruction in Patients with Chronic Inflammation Post-Chikungunya." *PLoS Neglected Tropical Diseases*

15(2): e0009115.

Bikle, Daniel D. **(2010)**. "Vitamin D and the Skin." *Journal of bone and mineral metabolism* 28: 117–30.

Björk, A, J Mofors, and M Wahren-Herlenius. **(2020)**. "Environmental Factors in the Pathogenesis of Primary Sjögren's Syndrome." *Journal of internal medicine* 287(5): 475–92.

Bodur, Mahmut, and REYHAN NERGİZ ÜNAL. **(2019)**. "The Effects of Dietary High Fructose and Saturated Fatty Acids on Chronic Low Grade Inflammation in the Perspective of Chronic Diseases." *Cukurova Medical Journal* 44(2).

Bragazzi, Nicola L et al. **(2017).** "Vitamin D and Rheumatoid Arthritis: An Ongoing Mystery." *Current opinion in rheumatology* 29(4): 378–88.

Brynedal, Boel et al. **(2023).** "Molecular Signature of Methotrexate Response among Rheumatoid Arthritis Patients." *Frontiers in Medicine* 10: 1146353.

Bullock, Jacqueline et al. **(2019)**. "Rheumatoid Arthritis: A Brief Overview of the Treatment." *Medical Principles and Practice* 27(6).

Burtis, Carl A, and David E Bruns. **(2014)**. *Tietz Fundamentals of Clinical Chemistry and Molecular Diagnostics-E-Book: Tietz Fundamentals of Clinical Chemistry and Molecular Diagnostics-E-Book*. Elsevier Health Sciences.

Cabral-Pacheco, Griselda A et al. **(2020)**. "The Roles of Matrix Metalloproteinases and Their Inhibitors in Human Diseases." *International journal of molecular sciences* 21(24): 9739.

Cen, Xiaomin et al. **(2015)**. "Association between Serum 25-Hydroxyvitamin D Level and Rheumatoid Arthritis." *BioMed Research International* 2015(1):

913804.

Chasapis, Christos T, Panagoula-Stamatina A Ntoupa, Chara A Spiliopoulou, and Maria E Stefanidou. **(2020)**. "Recent Aspects of the Effects of Zinc on Human Health." *Archives of toxicology* 94: 1443–60.

Chemin, Karine, Christina Gerstner, and Vivianne Malmström. **(2019).** "Effector Functions of CD4+ T Cells at the Site of Local Autoimmune Inflammation—Lessons from Rheumatoid Arthritis." *Frontiers in immunology* 10: 428082.

Chen, Mengyao, Dong. **(2024).** "Advantages of Chinese Herbal Medicine in Treating Rheumatoid Arthritis: A Focus on Its Anti-Inflammatory and Anti-Oxidative Effects." *Frontiers in Medicine* 11: 1371461.

Chen, Jingyang, Dong Yin, and Kefei Dou. **(2023)**. "Intensified Glycemic Control by HbA1c for Patients with Coronary Heart Disease and Type 2 Diabetes: A Review of Findings and Conclusions." *Cardiovascular Diabetology* 22(1): 146.

Chen, Junjie et al. **(2024)**. "A Multifunctional Nanoaggregate-based System for Detection of Rheumatoid Arthritis via Optoacoustic/NIR-II Fluorescent Imaging and Therapy via Inhibiting JAK-STAT/NF-κB/NLRP3 Pathways." *Aggregate* 5(1): e419.

Chen, Po-Ku, Kuo-Tung Tang, and Der-Yuan Chen. **(2024)**. "The NLRP3 Inflammasome as a Pathogenic Player Showing Therapeutic Potential in Rheumatoid Arthritis and Its Comorbidities: A Narrative Review." *International Journal of Molecular Sciences* 25(1): 626.

Chen, Zhu, Aline Bozec, Andreas Ramming, and Georg Schett. **(2019)**. "Anti-Inflammatory and Immune-Regulatory Cytokines in Rheumatoid Arthritis." *Nature Reviews Rheumatology* 15(1): 9–17.

Cheng, Xiaoxiao, Qirong Xie, and Yang Sun. **(2023).** "Advances in Nanomaterial-Based Targeted Drug Delivery Systems." *Frontiers in bioengineering and biotechnology* 11: 1177151.

Cheung, Bernard M Y, and Chao Li. **(2012).** "Diabetes and Hypertension: Is There a Common Metabolic Pathway?" *Current atherosclerosis reports* 14: 160–66.

Choi, M J, and H I Maibach. **(2005)**. "Liposomes and Niosomes as Topical Drug Delivery Systems." *Skin pharmacology and physiology* 18(5): 209–19.

Combe, Bernard et al. **(2017)**. "2016 Update of the EULAR Recommendations for the Management of Early Arthritis." *Annals of the rheumatic diseases* 76(6): 948–59.

Cottrell, Jessica, and J Patrick O'Connor. **(2010)**. "Effect of Non-Steroidal Anti-Inflammatory Drugs on Bone Healing." *Pharmaceuticals* 3(5): 1668–93.

Cui, Kai et al.**(2020)**. "Gene Expression Profiles in Chemokine (CC Motif) Ligand 21-Overexpressing Pancreatic Cancer Cells." *Pathology & Oncology Research* 26: 201–8.

Danaei, MRMM et al. **(2018)**. "Impact of Particle Size and Polydispersity Index on the Clinical Applications of Lipidic Nanocarrier Systems." *Pharmaceutics* 10(2): 57.

van Delft, Myrthe A M, and Tom W J Huizinga. **(2020).** "An Overview of Autoantibodies in Rheumatoid Arthritis." *Journal of autoimmunity* 110: 102392.

Dessein, Patrick H, and Barry I Joffe. **(2006)**. "Insulin Resistance and Impaired Beta Cell Function in Rheumatoid Arthritis." *Arthritis & Rheumatism* 54(9): 2765–75.

de Figueiredo, I. R., Taulaigo, A., de Carvalho, M. V., & Silva-Nunes, J. **(2023).** Nutritional status in rheumatoid arthritis. Nutrition, Metabolism and Cardiovascular Diseases, 33(2), 369-375.

Diaz-Gallo, Lina-Marcela et al. **(2018)**. "Systematic Approach Demonstrates Enrichment of Multiple Interactions between Non-HLA Risk Variants and HLA-DRB1 Risk Alleles in Rheumatoid Arthritis." *Annals of the rheumatic diseases* 77(10): 1454–62.

Díaz, Ana et al. **(2019)**. "Sex Differences in Age-Associated Type 2 Diabetes in Rats—Role of Estrogens and Oxidative Stress." *Oxidative medicine and cellular longevity* 2019.

Dieterich, Walburga, Monic Schink, and Yurdagül Zopf. **(2018)**. "Microbiota in the Gastrointestinal Tract." *Medical Sciences* 6(4): 116.

Ding, Qian et al. **(2023).** "Signaling Pathways in Rheumatoid Arthritis: Implications for Targeted Therapy." *Signal transduction and targeted therapy* 8(1): 68.

Donath, Marc Y, Charles A Dinarello, and Thomas Mandrup-Poulsen. **(2019).** "Targeting Innate Immune Mediators in Type 1 and Type 2 Diabetes." *Nature Reviews Immunology* 19(12): 734–46.

Donath, Marc Y, and Steven E Shoelson. **(2011).** "Type 2 Diabetes as an Inflammatory Disease." *Nature reviews immunology* 11(2): 98–107.

Dong, Chen. **(2021).** "Cytokine Regulation and Function in T Cells." *Annual review of immunology* 39: 51–76.

Van Drongelen, Vincent, and Joseph Holoshitz. **(2017).** "Human Leukocyte Antigen–Disease Associations in Rheumatoid Arthritis." *Rheumatic Disease Clinics* 43(3): 363–76.

Du, Yawei et al. **(2022).** "In Situ-Activated Phospholipid-Mimic Artemisinin Prodrug via Injectable Hydrogel Nano/Microsphere for Rheumatoid Arthritis Therapy." *Research* 2022: 3.

Duarte, Graziela Biude Silva et al. **(2022)**. "Evaluation of Biomarkers Related to Zinc Nutritional Status, Antioxidant Activity and Oxidative Stress in Rheumatoid Arthritis Patients." *Nutrition and Health* 28(2): 257–64.

Duzenli, Ozgun Firat, and Sezer Okay. **(2020)**. "Promoter Engineering for the Recombinant Protein Production in Prokaryotic Systems." *AIMS Bioengineering* 7(2): 62–81.

Edwards III, Carl K et al. **(2012).** "Combined Anti-Tumor Necrosis Factor-α Therapy and DMARD Therapy in Rheumatoid Arthritis Patients Reduces Inflammatory Gene Expression in Whole Blood Compared to DMARD Therapy Alone." *Frontiers in immunology* 3: 366.

Efferth, Thomas, and Franz Oesch. **(2021)**. "The Immunosuppressive Activity of Artemisinin-type Drugs towards Inflammatory and Autoimmune Diseases." *Medicinal research reviews* 41(6): 3023–61.

Efthimiou, Petros et al. **(2021)**. "Adult-Onset Still's Disease in Focus: Clinical Manifestations, Diagnosis, Treatment, and Unmet Needs in the Era of Targeted Therapies." In *Seminars in Arthritis and Rheumatism*, Elsevier, 858–74.

Elshabrawy, Hatem A et al. **(2015).** "The Pathogenic Role of Angiogenesis in Rheumatoid Arthritis." *Angiogenesis* 18: 433–48.

Erwin, J., Enki, D. G., & Woolf, A. D. **(2021)**. Younger people with rheumatoid arthritis are at increased risk of fracture even before age 50 years: a population-based cohort study. Osteoporosis International, 32, 1651-1659.

Reference

Federation, I D. **(2020)**. "IDF DIABETES ATLAS 9th 2019." *International Diabetes Federation, Brussels, Belgium*.

Ferguson, Lyn D, Stefan Siebert, Iain B McInnes, and Naveed Sattar. **(2019)**. "Cardiometabolic Comorbidities in RA and PsA: Lessons Learned and Future Directions." *Nature Reviews Rheumatology* 15(8): 461–74.

Ferreira, Vinicius L et al. **(2018)**. "Cytokines and Interferons: Types and Functions." *Autoantibodies and cytokines* 13.

Frangos, Theoharris, and Wolfgang Maret. **(2020)**. "Zinc and Cadmium in the Aetiology and Pathogenesis of Osteoarthritis and Rheumatoid Arthritis." *Nutrients* 13(1): 53.

Frimpong, Ebenezer, and Manimbulu Nlooto. **(2020)**. "Management of Diabetes and Hypertension among Tswana and Zulu Traditional Health Practitioners: A Comparative Cross-Sectional Study Using a Mixed-Methods Approach." *African Journal of Biomedical Research* 23(2): 135–46.

Frisell, Thomas et al. **(2013)**. "Familial Risks and Heritability of Rheumatoid Arthritis: Role of Rheumatoid Factor/Anti–Citrullinated Protein Antibody Status, Number and Type of Affected Relatives, Sex, and Age." *Arthritis & Rheumatism* 65(11): 2773–82.

Gaafar, Nada A G et al. **(2020)**. "The Oral Administration Effect of Drug Mannuronic Acid (M2000) on Gene Expression of Matrix and Tissue Inhibitor of Metalloproteinases in Rheumatoid Arthritis Patients." *Current Drug Discovery Technologies* 17(5): 704–10.

Gallagher, Lorna et al. **(2020)**. "Insulin-resistant Pathways Are Associated with Disease Activity in Rheumatoid Arthritis and Are Subject to Disease Modification through Metabolic Reprogramming: A Potential Novel Therapeutic Approach." *Arthritis & Rheumatology* 72(6): 896–902.

Gandhi, Gopalsamy Rajiv et al. **(2021)**. "Anti-Inflammatory Natural Products as Potential Therapeutic Agents of Rheumatoid Arthritis: A Systematic Review." *Phytomedicine* 93: 153766.

Gautam, R et al. **(2011).** "Accumulation of Aerosols over the Indo-Gangetic Plains and Southern Slopes of the Himalayas: Distribution, Properties and Radiative Effects during the 2009 Pre-Monsoon Season." *Atmospheric Chemistry and Physics* 11(24): 12841–63.

Ge, Cuicui et al. **(2015)**. "Towards Understanding of Nanoparticle–Protein Corona." *Archives of toxicology* 89: 519–39.

Genovese, Mark C et al. **(2020)**. "Interleukin-6 Receptor Blockade or TNFα Inhibition for Reducing Glycaemia in Patients with RA and Diabetes: Post Hoc Analyses of Three Randomised, Controlled Trials." *Arthritis Research & Therapy* 22: 1–12.

Germano, Jaqueline L et al. **(2021)**. "Prevalence of Rheumatoid Arthritis in South America: A Systematic Review and Meta-Analysis." *Ciência & Saúde Coletiva* 26: 5371–82.

Giacomelli, Roberto et al. 2016. "IL-1β at the Crossroad between Rheumatoid Arthritis and Type 2 Diabetes: May We Kill Two Birds with One Stone?" *Expert Review of Clinical Immunology* 12(8): 849–55.

Giannelli, Gianluigi et al. **(2004)**. "MMP-2, MMP-9, TIMP-1 and TIMP-2 Levels in Patients with Rheumatoid Arthritis and Psoriatic Arthritis." *Clinical and experimental rheumatology* 22(3): 335–38.

Gil, Noa, and Igor Ulitsky. **(2020)**. "Regulation of Gene Expression by Cis-Acting Long Non-Coding RNAs." *Nature Reviews Genetics* 21(2): 102–17.

Giles, Jon T et al. **(2015)**. "Insulin Resistance in Rheumatoid Arthritis: Disease-

related Indicators and Associations with the Presence and Progression of Subclinical Atherosclerosis." *Arthritis & Rheumatology* 67(3): 626–36.

Gurzov, Esteban N et al. **(2016)**. "The JAK/STAT Pathway in Obesity and Diabetes." *The FEBS journal* 283(16): 3002–15.

Guimarães, M. F. B. D. R., Rodrigues, C. E. M., Gomes, K. W. P., Machado, C. J., Brenol, C. V., Krampe, S. F., ... & Kakehasi, A. M. **(2019)**. High prevalence of obesity in rheumatoid arthritis patients: association with disease activity, hypertension, dyslipidemia and diabetes, a multi-center study. Advances in Rheumatology, 59, 44.

Haeusler, Rebecca A, Timothy E McGraw, and Domenico Accili. **(2018)**. "Biochemical and Cellular Properties of Insulin Receptor Signalling." *Nature reviews Molecular cell biology* 19(1): 31–44.

Hajizadeh, Mohammad Reza et al. **(2019)**. "In Vitro Cytotoxicity Assay of D-Limonene Niosomes: An Efficient Nano-Carrier for Enhancing Solubility of Plant-Extracted Agents." *Research in pharmaceutical sciences* 14(5): 448–58.

Haleem, Abid et al. **(2023)**. "Applications of Nanotechnology in Medical Field: A Brief Review." *Global Health Journal* 7(2): 70–77.

Hanafy, Nemany A N, Rehab Fouad Sheashaa, Eman A Moussa, and Magdy E Mahfouz. **(2023)**. "Potential of Curcumin and Niacin-Loaded Targeted Chitosan Coated Liposomes to Activate Autophagy in Hepatocellular Carcinoma Cells: An in Vitro Evaluation in HePG2 Cell Line." *International journal of biological macromolecules* 245: 125572.

Haridas, Vikram et al. **(2019)**. "Reciprocal Regulation of Pro-Inflammatory Annexin A2 and Anti-Inflammatory Annexin A1 in the Pathogenesis of Rheumatoid Arthritis." *Molecular biology reports* 46(1): 83–95.

Harrison, Stephanie R et al. **(2020)**. "Vitamin D and Early Rheumatoid Arthritis." *BMC rheumatology* 4: 1–7.

Harrison, Stephanie R et al. **(2020)**. "Vitamin D, Autoimmune Disease and Rheumatoid Arthritis." *Calcified tissue international* 106: 58–75.

Hassan Sr, Wahid M. **(2024)**. "Oxidative DNA Damage and Zinc Status in Patients With Rheumatoid Arthritis in Duhok, Iraq." *Cureus* 16(1).

Hazira, Rahiman Mohammed Noor, and M Sunitha Reddy. **(2023)**. "Niosomes: A Nanocarrier Drug Delivery System." *GSC Biological and Pharmaceutical Sciences* 22(2): 120–27.

HB, Irfan Ahamed. **(2015)**. "Insulin Resistance and Its Significant Co-Morbidities in Young Individuals-Including Homa (Homeostatic Model Assessment)."

Hepkema, Jacob et al. **(2023)**. "Predicting the Impact of Sequence Motifs on Gene Regulation Using Single-Cell Data." *Genome Biology* 24(1): 189.

Hirsch, Gabriela E et al. **(2015)**. "Γ-oryzanol Reduces Caveolin-1 and PCGEM1 Expression, Markers of Aggressiveness in Prostate Cancer Cell Lines." *The Prostate* 75(8): 783–97.

Ho, Emily. **(2004)**. "Zinc Deficiency, DNA Damage and Cancer Risk." *The Journal of nutritional biochemistry* 15(10): 572–78.

Ho, Emily, and Bruce N Ames.**(2002)**. "Low Intracellular Zinc Induces Oxidative DNA Damage, Disrupts P53, NFκB, and AP1 DNA Binding, and Affects DNA Repair in a Rat Glioma Cell Line." *Proceedings of the National Academy of Sciences* 99(26): 16770–75.

Hu, Cheng, and Weiping Jia. **(2018)**. "Diabetes in China: Epidemiology and Genetic Risk Factors and Their Clinical Utility in Personalized

Medication.” *Diabetes* 67(1): 3–11.

Hu, Quanyin, Prateek S Katti, and Zhen Gu. **(2014)**. “Enzyme-Responsive Nanomaterials for Controlled Drug Delivery.” *Nanoscale* 6(21): 12273–86.

Huang, Xingjun, Guihua Liu, Jiao Guo, and Zhengquan Su. **(2018)**. “The PI3K/AKT Pathway in Obesity and Type 2 Diabetes.” *International journal of biological sciences* 14(11): 1483.

Hussein, Hadi M, and Elias A Rahal. **(2019)**. “The Role of Viral Infections in the Development of Autoimmune Diseases.” *Critical reviews in microbiology* 45(4): 394–412.

Ibrahim, Mohamed Hashem, Mohamad Sayyed Bakheet, and Khaled A Abdel-Sater. **(2014)**. “Relationship between Vitamin D and Disease Activity in Some Rheumatic Diseases.” *British Journal of Biology Health and Medical Research* 1(1): 1–21.

Ingegnoli, Francesca, Roberto Castelli, and Roberta Gualtierotti.**(2013)**. “Rheumatoid Factors: Clinical Applications.” *Disease markers* 35: 727–34.

Ioannidou, Stavroula et al. **(2024)**. “Evaluation of Biochemical and Oxidative Stress Markers in the Early Stages of Rheumatoid Arthritis in a Comparative Study of Two Different Therapeutic Approaches.” *Oxygen* 4(3): 253–65.

Iwaszko, Milena, Sylwia Biały, and Katarzyna Bogunia-Kubik. **(2021)**. “Significance of Interleukin (IL)-4 and IL-13 in Inflammatory Arthritis.” *Cells* 10(11): 3000.

Jackson, Gabrielle. **(2019)**. *Pain and Prejudice: A Call to Arms for Women and Their Bodies*. Hachette UK.

Jadid, Mahdi Farhoudi Sefidan et al. **(2023)**. “Enhanced Anti-Cancer Effect of

Curcumin Loaded-Niosomal Nanoparticles in Combination with Heat-Killed Saccharomyces Cerevisiae against Human Colon Cancer Cells." *Journal of Drug Delivery Science and Technology* 80: 104167.

Jakobsen, Odin Andreas Johan, and Laszlo Szereday. **(2018)**. "The 'Three Amigos' Lurking behind Type 1 Diabetes: Hygiene, Gut Microbiota and Viruses." *Acta microbiologica et immunologica Hungarica* 65(4): 421–38.

Janovits, Priscila Motta, Elbio Leiguez, Viviane Portas, and Catarina Teixeira. **(2021)**. "A Metalloproteinase Induces an Inflammatory Response in Preadipocytes with the Activation of COX Signalling Pathways and Participation of Endogenous Phospholipases A2." *Biomolecules* 11(7): 921.

Javaid, Sana et al. **(2021)**. "Cefotaxime Loaded Polycaprolactone Based Polymeric Nanoparticles with Antifouling Properties for In-Vitro Drug Release Applications." *Polymers* 13(13): 2180.

Ji, XuLing et al. **(2023)**. "Effect of Tumor-Associated Macrophages on the Pyroptosis of Breast Cancer Tumor Cells." *Cell Communication and Signaling* 21(1): 197.

Jiang, Ya-yi et al. **(2020)**. "The Potential Roles of Artemisinin and Its Derivatives in the Treatment of Type 2 Diabetes Mellitus." *Frontiers in Pharmacology* 11: 585487.

Johnson, Louise A, and David G Jackson. **(2010)**. "Inflammation-Induced Secretion of CCL21 in Lymphatic Endothelium Is a Key Regulator of Integrin-Mediated Dendritic Cell Transmigration." *International immunology* 22(10): 839–49.

Jones, Simon A et al. **(2018)**. "Interleukin 6: The Biology behind the Therapy." *Considerations in Medicine* 2(1): 2–6.

Jun, Jae Chang et al. **(2019)**. "The Effect of Vitamin D Administration on Inflammatory Markers in Patients with Inflammatory Bowel Disease." *Intestinal research* 17(2): 210.

Kany, Shinwan, Jan Tilmann Vollrath, and Borna Relja. **(2019)**. "Cytokines in Inflammatory Disease." *International journal of molecular sciences* 20(23): 6008.

Kawai, S. **(1998)**. "Cyclooxygenase Selectivity and the Risk of Gastro-Intestinal Complications of Various Non-Steroidal Anti-Inflammatory Drugs: A Clinical Consideration." *Inflammation Research* 47: 102–6.

Kazmi, Zehra. **(2021).** "The Influence of Early Menopause in Women with Rheumatoid Arthritis; a Systematic Review." *The Healer Journal of Physiotherapy and Rehabilitation Sciences* 1(1): 27–37.

Kenry et al. **(2020)**. "Mechanistic Understanding of the Biological Responses to Polymeric Nanoparticles." *ACS nano* 14(4): 4509–22.

Khan, Mohd W A et al. **(2020)**. "Glycation and Oxidative Stress Increase Autoantibodies in the Elderly." *Molecules* 25(16): 3675.

Khorasanizadeh, Mir Hojjat, Mahsa Eskian, Carlos A Camargo, and Nima Rezaei. **(2019)**. "Vitamin D and the Immune System." *Nutrition and Immunity*: 15–51.

Kiani, Bushra Hafeez et al. **(2020).** "Artemisinin and Its Derivatives: A Promising Cancer Therapy." *Molecular Biology Reports* 47: 6321–36.

Kim, Kyoung-Woon et al. **(2015)**. "Role of C-Reactive Protein in Osteoclastogenesis in Rheumatoid Arthritis." *Arthritis research & therapy* 17: 1–12.

Kim, H. J., Swan, H., Kazmi, S. Z., Hong, G., Kim, Y. S., Choi, S., ... & Ahn,

H. S. **(2023)**. Familial risk of seropositive rheumatoid arthritis and interaction with smoking: a population-based cohort study. Rheumatology, 62(9), 3006-3013.

Knutsson, Anders, and Anders Kempe. **(2013)**. *Systematiska Kunskapsöversikter; 4. Diabetes Och Arbete*. Arbets-och miljömedicin, Göteborgs universitet.

Koedderitzsch, Kathrin et al. **(2021)**. "TNF Induces Glycolytic Shift in Fibroblast like Synoviocytes via GLUT1 and HIF1A." *Scientific reports* 11(1): 19385.

Koliaki, Chrysi, and Michael Roden. **(2016)**. "Alterations of Mitochondrial Function and Insulin Sensitivity in Human Obesity and Diabetes Mellitus." *Annual review of nutrition* 36(1): 337–67.

Kondo, Naoki, Takeshi Kuroda, and Daisuke Kobayashi. **(2021)**. "Cytokine Networks in the Pathogenesis of Rheumatoid Arthritis." *International journal of molecular sciences* 22(20): 10922.

Kramer, IJsbrand, Auragun Wibulswas, Daniel Croft, and Elisabeth Genot. **(2003)**. "Rheumatoid Arthritis: Targeting the Proliferative Fibroblasts." *PROGRESS IN CELL CYCLE RESEARCH.* 5: 59–70.

Kumar, Vijay, and James E Barrett. **(2022)**. "Toll-like Receptors (TLRs) in Health and Disease: An Overview." *Toll-like Receptors in Health and Disease*: 1–21.

Kunasekaran, Viveksarathi, and Kannan Krishnamoorthy. **(2015).** "Experimental Design for the Optimization of Nanoscale Solid Lipid Particles Containing Rasagiline Mesylate." *Journal of Young pharmacists* 7(4): 285.

Kwon, Hyokjoon, and Jeffrey E Pessin. **(2013)**. "Adipokines Mediate Inflammation and Insulin Resistance." *Frontiers in endocrinology* 4: 71.

L Bishop, Emma et al. **(2021)**. "Vitamin D and Immune Regulation: Antibacterial, Antiviral, Anti-Inflammatory." *Journal of Bone and Mineral Research plus* 5(1): e10405.

Lawal, A et al.**(2012)**. "FTIR and UV-Visible Spectrophotometeric Analyses of Artemisinin and Its Derivatives." *Journal of Pharmaceutical and Biomedical Sciences* 24(24): 6–14.

Lazzaroni, M, and G Bianchi Porro. **(2004)**. "Gastrointestinal Side-effects of Traditional Non-steroidal Anti-inflammatory Drugs and New Formulations." *Alimentary pharmacology & therapeutics* 20: 48–58.

Lee, Ha-Reum et al. **(2021)**. "Reduction of Oxidative Stress in Peripheral Blood Mononuclear Cells Attenuates the Inflammatory Response of Fibroblast-like Synoviocytes in Rheumatoid Arthritis." *International Journal of Molecular Sciences* 22(22): 12411.

Lee, Jiyeon et al. **(2017).** "Stimulation of Osteoclast Migration and Bone Resorption by C–C Chemokine Ligands 19 and 21." *Experimental & molecular medicine* 49(7): e358–e358.

Lee, Young Ho, and Sang-Cheol Bae. **(2016).** "Vitamin D Level in Rheumatoid Arthritis and Its Correlation with the Disease Activity: A Meta-Analysis." *Clin Exp Rheumatol* 34(5): 827–33.

Lewis, David B et al. **(1988).** "Restricted Production of Interleukin 4 by Activated Human T Cells." *Proceedings of the National Academy of Sciences* 85(24): 9743–47.

Li, Baosheng, Jigang Dong, and Hongrui Wang. **(2024).** "Effect of Platelet-Rich

Plasma (PRP) Binding CTRP9 Mediated PUM1-TLR4 on Chondrocyte Proliferation, Apoptosis and Oxidative Stress in Osteoarthritis."

Li, Chenxi et al. **(2021).** "Intra-Articular Administrated Hydrogels of Hyaluronic Acid Hybridized with Triptolide/Gold Nanoparticles for Targeted Delivery to Rheumatoid Arthritis Combined with Photothermal-Chemo Therapy." *Res. Sq*.

Li, Guoqing et al. **(2013)**. "Interleukin-17A Promotes Rheumatoid Arthritis Synoviocytes Migration and Invasion under Hypoxia by Increasing MMP2 and MMP9 Expression through NF-KB/HIF-1α Pathway." *Molecular immunology* 53(3): 227–36.

Li, Hongyi, Min Wu, and Xia Zhao. **(2022)**. "Role of Chemokine Systems in Cancer and Inflammatory Diseases." *MedComm* 3(2): e147.

Li, Wenchang et al. **(2023).** "Causal Effects of Reproductive Behaviors on the Risk of Osteoarthritis and Rheumatoid Arthritis: A Mendelian Randomization Study."

Li, Xiaoyun et al. **(2022).** "Design of Smart Size-, Surface-, and Shape-switching Nanoparticles to Improve Therapeutic Efficacy." *Small* 18(6): 2104632.

Li, Yanfei et al. **(2023)**. "Helical-Like Assembly of Nateglinide as Coating for Oral Delivery of Insulin and Their Synergistic Prevention of Diabetes Mellitus." *Advanced Science* 10(29): 2301879.

Li, Yanping et al. **(2015)**. "Birth Weight and Later Life Adherence to Unhealthy Lifestyles in Predicting Type 2 Diabetes: Prospective Cohort Study." *bmj* 351.

Liang, Jian, Crystal I C Farh, and Jiing-Lih Farh. **(2012)**. "Psychological

Antecedents of Promotive and Prohibitive Voice: A Two-Wave Examination." *Academy of Management journal* 55(1): 71–92.

Liebert, Tim, Marc Kostag, Jana Wotschadlo, and Thomas Heinze. **(2011)**. "Stable Cellulose Nanospheres for Cellular Uptake." *Macromolecular bioscience* 11(10): 1387–92.

Lin, Yen-Ju, Martina Anzaghe, and Stefan Schülke. **(2020)**. "Update on the Pathomechanism, Diagnosis, and Treatment Options for Rheumatoid Arthritis." *Cells* 9(4): 880.

Liu, Xiang et al. **(2012)**. "Determination of Monolayer-Protected Gold Nanoparticle Ligand–Shell Morphology Using NMR." *Nature communications* 3(1): 1182.

Liu, Xueling et al. **(2022)**. "Natural Medicines of Targeted Rheumatoid Arthritis and Its Action Mechanism." *Frontiers in Immunology* 13: 945129.

Liu, Yan et al. **(2021)**. "Magnetic Nanoparticles: A New Diagnostic and Treatment Platform for Rheumatoid Arthritis." *Journal of Leucocyte Biology* 109(2): 415–24.

Livingstone, Shona J et al. **(2015).** "Estimated Life Expectancy in a Scottish Cohort with Type 1 Diabetes, 2008-2010." *Jama* 313(1): 37–44.

Love, Melissa et al. **(2012)**. "Solution Structure of CCL21 and Identification of a Putative CCR7 Binding Site." *Biochemistry* 51(3): 733–35.

Lubberts, Erik, and Wim B van den Berg. **(2013)**. "Cytokines in the Pathogenesis of Rheumatoid Arthritis and Collagen-Induced Arthritis." *Madame Curie Bioscience Database [Internet]*.

Lund, Sean, Hannah H Walford, and Taylor A Doherty. **(2013)**. "Type 2 Innate Lymphoid Cells in Allergic Disease." *Current immunology reviews* 9(4):

214–21.

Lyssenko, Valeriya et al. **(2008)**. "Clinical Risk Factors, DNA Variants, and the Development of Type 2 Diabetes." *New England Journal of Medicine* 359(21): 2220–32.

M. Ways, Twana Mohammed, Keng Wooi Ng, Wing Man Lau, and Vitaliy V Khutoryanskiy. **(2020**). "Silica Nanoparticles in Transmucosal Drug Delivery." *Pharmaceutics* 12(8): 751.

Ma, Xiao et al. **(2022)**. "Excessive Intake of Sugar: An Accomplice of Inflammation." *Frontiers in immunology* 13: 988481.

Machado, V., Morais, M., & Medeiros, R. **(2022).** Hyaluronic acid-based nanomaterials applied to cancer: where are we now?. Pharmaceutics, 14(10), 2092.

Mahmoud, Hivi M, Ardawan F Ali, and Dhia J Al-Timimi. **(2021)**. "Relationship between Zinc Status and DNA Oxidative Damage in Patients with Type 2 Diabetes Mellitus." *Biological Trace Element Research* 199(4): 1276–79.

Malakooti, Shahdi K et al. **(2024)**. "Higher Vitamin D Levels before Methotrexate Therapy Initiation Are Associated with Lower Subsequent Mortality in Patients with Rheumatoid Arthritis." *Nutrients* 16(3): 401.

Manasa, R, and Mahesh Shivananjappa. **(2023)**. "Role of Nanotechnology-Based Materials in Drug Delivery." *Advances in Novel Formulations for Drug Delivery*: 279–307.

Manivelavan, D, and C K Vijayasamundeeswari. **(2012)**. "Anti-Cyclic Citrullinated Peptide Antibody: An Early Diagnostic and Prognostic Biomarker of Rheumatoid Arthritis." *Journal of Clinical and Diagnostic*

Research: JCDR 6(8): 1393.

Marques-Rocha, José Luiz et al. **(2015)**. "Noncoding RNAs, Cytokines, and Inflammation-related Diseases." *The FASEB Journal* 29(9): 3595–3611.

Martínez-García, Mireya, and Enrique Hernández-Lemus. **(2021)**. "Periodontal Inflammation and Systemic Diseases: An Overview." *Frontiers in physiology* 12: 709438.

Martirosyan, Danik et al. **(2022)**. "Inflammatory Factors and Immunoglobulins Alterations in Subjects with Type 2 Diabetes Mellitus Treated with Squalene." *Functional Food Science-Online ISSN: 2767-3146* 2(8): 181–97.

Masoumi, Maryam et al. **(2021)**. "Destructive Roles of Fibroblast-like Synoviocytes in Chronic Inflammation and Joint Damage in Rheumatoid Arthritis." *Inflammation* 44: 466–79.

Mattick, John, and Paulo Amaral. **(2023)**. *RNA, the Epicenter of Genetic Information*. Taylor & Francis.

Mavaddati, Mohammad Amin, Fathollah Moztarzadeh, and Fatemeh Baghbani. **(2015)**. "Effect of Formulation and Processing Variables on Dexamethasone Entrapment and Release of Niosomes." *Journal of Cluster Science* 26: 2065–78.

Meena, Narendra et al. **(2018)**. "Assessment of Vitamin D in Rheumatoid Arthritis and Its Correlation with Disease Activity." *Journal of natural science, biology, and medicine* 9(1): 54.

Meena, Vijay Singh et al. **(2022)**. "Underutilized Fruit Crops of Indian Arid and Semi-Arid Regions: Importance, Conservation and Utilization Strategies." *Horticulturae* 8(2): 171.

Mellado, Mario et al. **(2015)**. "T Cell Migration in Rheumatoid Arthritis."

Frontiers in immunology 6: 384.

Middendorp, S, and E E S Nieuwenhuis. **(2009)**. "NKT Cells in Mucosal Immunity." *Mucosal immunology* 2(5): 393–402.

Mikhaylenko, Dmitry S et al. **(2020)**. "Genetic Polymorphisms Associated with Rheumatoid Arthritis Development and Antirheumatic Therapy Response." *International journal of molecular sciences* 21(14): 4911.

Millier, Melanie J et al. **(2022).** "Plasma Interleukin-23 and Circulating IL-17A+ IFNγ+ Ex-Th17 Cells Predict Opposing Outcomes of Anti-TNF Therapy in Rheumatoid Arthritis." *Arthritis Research & Therapy* 24(1): 57.

Mirazi, Naser, Maryam Rezaei, and Mahmoud Mirhoseini. **(2016)**. "Hypoglycemic Effect of Satureja Montanum L. Hydroethanolic Extract on Diabetic Rats." *J HerbMed Pharmacol* 5(1): 17–22.

Mishra, Vijay et al. **(2020)**. "Niosomes: Potential Nanocarriers for Drug Delivery." *J Pharm Clin Res* 11(03): 389–94.

Molnar, Vilim et al. **(2021)**. "Cytokines and Chemokines Involved in Osteoarthritis Pathogenesis." *International journal of molecular sciences* 22(17): 9208.

Montané, Xavier et al. **(2020)**. "Current Perspectives of the Applications of Polyphenols and Flavonoids in Cancer Therapy." *Molecules* 25(15): 3342.

Monu, Prachi Agnihotri, and Sagarika Biswas. **(2022)**. "AGE/Non-AGE Glycation: An Important Event in Rheumatoid Arthritis Pathophysiology." *Inflammation* 45(2): 477–96.

Mosaad, Youssef M et al. **(2014)**. "Vitamin D Receptor Gene Polymorphism as Possible Risk Factor in Rheumatoid Arthritis and Rheumatoid Related Osteoporosis." *Human immunology* 75(5): 452–61.

Mueller, Anna-Lena et al. **(2021)**. "Recent Advances in Understanding the Pathogenesis of Rheumatoid Arthritis: New Treatment Strategies." *Cells* 10(11): 3017.

Mukherjee, Anwesha, and Bodhisatwa Das. **(2024).** "The Role of Inflammatory Mediators and Matrix Metalloproteinases (MMPs) in the Progression of Osteoarthritis." *Biomaterials and Biosystems*: 100090.

Di Muzio, Claudia, Paola Cipriani, and Piero Ruscitti. **(2022)**. "Rheumatoid Arthritis Treatment Options and Type 2 Diabetes: Unravelling the Association." *BioDrugs* 36(6): 673–85.

Myasoedova, Elena et al. **(2010)**. "Is the Incidence of Rheumatoid Arthritis Rising?: Results from Olmsted County, Minnesota, 1955–2007." *Arthritis & Rheumatism* 62(6): 1576–82.

Na-Bangchang, Kesara, Aurathai Thanavibul, Pongsri Tippawangkosol, and Juntra Karbwang. **(2005).** "Pharmacokinetics of the Four Combination Regimens of Dihydroartemisinin/Mefloquine in Acute Uncomplicated Falciparum Malaria." *Southeast Asian J. Trop. Med. Public Health* 36: 23–33.

Nanayakkara, Natalie et al. **(2018)**. "Age-Related Differences in Glycaemic Control, Cardiovascular Disease Risk Factors and Treatment in Patients with Type 2 Diabetes: A Cross-Sectional Study from the Australian National Diabetes Audit." *BMJ open* 8(8): e020677.

Narang, Vikram et al. **(2020).** "Comparative Analysis of Erythrocyte Sedimentation Rate Measured by Automated and Manual Methods in Anaemic Patients." *Journal of laboratory physicians* 12(04): 239–43.

Navaratnam, Visweswaran et al. **(2000).** "Pharmacokinetics of Artemisinin-Type Compounds." *Clinical pharmacokinetics* 39: 255–70.

Nekoua, Magloire Pandoua et al. **(2016)**. "Modulation of Immune Cells and Th1/Th2 Cytokines in Insulin-Treated Type 2 Diabetes Mellitus." *African health sciences* 16(3): 712–24.

Ni, Xiaojian et al. **(2014)**. "Downregulation of MiR-106b Induced Breast Cancer Cell Invasion and Motility in Association with Overexpression of Matrix Metalloproteinase 2." *Cancer science* 105(1): 18–25.

Nicholl, Desmond S T. **(2023)**. *An Introduction to Genetic Engineering*. Cambridge University Press.

Van Niekerk, Gustav, Claudia Christowitz, Daleen Conradie, and Anna-Mart Engelbrecht. **(2020)**. "Insulin as an Immunomodulatory Hormone." *Cytokine & growth factor reviews* 52: 34–44.

Niu, Jiangxiu et al. **(2022)**. "Hyaluronic Acid Micelles for Promoting the Skin Permeation and Deposition of Curcumin." *International Journal of Nanomedicine* 17: 4009.

Novella-Navarro, Marta, Chamaida Plasencia-Rodríguez, Laura Nuño, and Alejandro Balsa. **(2021)**. "Risk Factors for Developing Rheumatoid Arthritis in Patients with Undifferentiated Arthritis and Inflammatory Arthralgia." *Frontiers in Medicine* 8: 668898.

Odda, Atheer Hameid, Amer Fadhil Alhaideri, and Ibrahim Haidar Jasima. **(2022)**. "Heavy Metals in the Neurological Disease: Toxic Effects and Prospective Detoxification Approaches." *NeuroQuantology* 20(8): 542.

Odeniyi, Michael Ayodele, Omobolanle A Omoteso, Adewale O Adepoju, and Kolawole T Jaiyeoba. **(2018)**. "Starch Nanoparticles in Drug Delivery: A Review." *Polim Med* 48(1): 41–45.

Okada, Yukinori et al. **(2014)**. "Genetics of Rheumatoid Arthritis Contributes to

Biology and Drug Discovery." *Nature* 506(7488): 376–81.

Oram, Richard A et al. **(2016)**. "A Type 1 Diabetes Genetic Risk Score Can Aid Discrimination between Type 1 and Type 2 Diabetes in Young Adults." *Diabetes care* 39(3): 337–44.

Orr, Carl K et al. **(2018)**. "The Utility and Limitations of CRP, ESR and DAS28-CRP in Appraising Disease Activity in Rheumatoid Arthritis." *Frontiers in medicine* 5: 185.

Padyukov, Leonid. **(2022)**. "Genetics of Rheumatoid Arthritis." In *Seminars in Immunopathology*, Springer, 47–62.

Paget, C, and F Trottein. **(2013)**. "Role of Type 1 Natural Killer T Cells in Pulmonary Immunity." *Mucosal immunology* 6(6): 1054–67.

Peng, Yanhui et al.**(2024)**. "Associations between Rheumatoid Arthritis and Intestinal Flora, with Special Emphasis on RA Pathologic Mechanisms to Treatment Strategies." *Microbial Pathogenesis*: 106563.

Petrovská, Nora et al. **(2021).** "The Pre-Clinical Phase of Rheumatoid Arthritis: From Risk Factors to Prevention of Arthritis." *Autoimmunity Reviews* 20(5): 102797.

Pickens, Sarah R et al. **(2011).** "Characterization of CCL19 and CCL21 in Rheumatoid Arthritis." *Arthritis & Rheumatism* 63(4): 914–22.

———. 2012. "Role of the CCL21 and CCR7 Pathways in Rheumatoid Arthritis Angiogenesis." *Arthritis & Rheumatism* 64(8): 2471–81.

Pope, Janet E. **(2020)**. "Management of Fatigue in Rheumatoid Arthritis." *RMD open* 6(1): e001084.

Pu, J L, R G Chen, and J L Cai. **(1979)**. "The Structur Edetermination of

Artemisinin Derivatives." In *Proceedings of 2nd Annual Meeting*, , 82.

Puchulu, Félix Miguel. **(2018)**. "Definition, Diagnosis and Classification of Diabetes Mellitus." *Dermatology and Diabetes*: 7–18.

Qamar, Naila, Peter John, and Attya Bhatti. **(2021)**. "Emerging Role of Selenium in Treatment of Rheumatoid Arthritis: An Insight on Its Antioxidant Properties." *Journal of Trace Elements in Medicine and Biology* 66: 126737.

Quiñonez-Flores, Celia María, Susana Aideé González-Chávez, Danyella Del Río Nájera, and César Pacheco-Tena. **(2016).** "Oxidative Stress Relevance in the Pathogenesis of the Rheumatoid Arthritis: A Systematic Review." *BioMed research international* 2016(1): 6097417.

Radu, Andrei-Flavius, and Simona Gabriela Bungau. **(2021)**. "Management of Rheumatoid Arthritis: An Overview." *Cells* 10(11): 2857.

Van Raemdonck, Katrien et al. **(2022)**. "Metabolic Reprogramming of Macrophages Instigates CCL21-induced Arthritis." *Immunology and cell biology* 100(2): 127–35.

Van Raemdonck, Katrien, Sadiq Umar, and Shiva Shahrara. **(2020)**. "The Pathogenic Importance of CCL21 and CCR7 in Rheumatoid Arthritis." *Cytokine & growth factor reviews* 55: 86–93.

Rajab, Basem, Abdulhamid Alkout, Alaa Lagha, and Hadeel Elbouhali. **(2023)**. "Sensitivity and Specificity of Some Laboratory Diagnostic Test in Rheumatoid Arthritis." *AlQalam Journal of Medical and Applied Sciences*: 822–28.

Rajaee, Elham et al. **(2018)**. "The Relationship between Serum Zinc Levels and Rheumatoid Arthritis Activity." *Frontiers in Biology* 13: 51–55.

Rao, N Narasimha et al. **(2018)**. "Niosomes: A Vesicular Drug Delivery System." *Research Journal of Pharmacy and Technology* 11(8): 3731–36.

Rawshani, Aidin et al. **(2017)**. "Mortality and Cardiovascular Disease in Type 1 and Type 2 Diabetes." *New England journal of medicine* 376(15): 1407–18.

Ricardo-Gonzalez, Roberto R et al. **(2010)**. "IL-4/STAT6 Immune Axis Regulates Peripheral Nutrient Metabolism and Insulin Sensitivity." *Proceedings of the National Academy of Sciences* 107(52): 22617–22.

Richard, Allison J, and Jacqueline M Stephens. **(2011)**. "Emerging Roles of JAK–STAT Signaling Pathways in Adipocytes." *Trends in Endocrinology & Metabolism* 22(8): 325–32.

Ristić, Gorica G et al**. (2021)**. "Impact of Disease Activity on Impaired Glucose Metabolism in Patients with Rheumatoid Arthritis." *Arthritis Research & Therapy* 23: 1–11.

Le Roux, Carel W et al. **(2017)**. "3 Years of Liraglutide versus Placebo for Type 2 Diabetes Risk Reduction and Weight Management in Individuals with Prediabetes: A Randomised, Double-Blind Trial." *The Lancet* 389(10077): 1399–1409.

Roy, Anirban et al. **(2022)**. "Anti-Inflammatory Effects of Different Dietary Antioxidants." In *Plant Antioxidants and Health*, Springer, 1–25.

Ruscitti, Piero et al. **(2017)**. "Poor Clinical Response in Rheumatoid Arthritis Is the Main Risk Factor for Diabetes Development in the Short-Term: A 1-Year, Single-Centre, Longitudinal Study." *PLoS One* 12(7): e0181203.

———. 2019. "Anti-Interleukin-1 Treatment in Patients with Rheumatoid Arthritis and Type 2 Diabetes (TRACK): A Multicentre, Open-Label, Randomised Controlled Trial." *PLoS medicine* 16(9): e1002901.

Salh, Khonaw K. **(2022)**. "Antimicrobial Resistance in Bacteria Causing Urinary Tract Infections." *Combinatorial chemistry & high throughput screening* 25(7): 1219–29.

Saltiel, Alan R, and Jerrold M Olefsky. **(2017)**. "Inflammatory Mechanisms Linking Obesity and Metabolic Disease." *The Journal of clinical investigation* 127(1): 1–4.

Sánchez-Pérez, Hiurma et al. **(2017)**. "Insulin Resistance in Systemic Lupus Erythematosus Patients: Contributing Factors and Relationship with Subclinical Atherosclerosis." *Clin Exp Rheumatol* 35(6): 885–92.

Schett, Georg et al. **(2013)**. "How Cytokine Networks Fuel Inflammation: Toward a Cytokine-Based Disease Taxonomy." *Nature medicine* 19(7): 822–24.

Shahin, Dina, Ehab Eltoraby, Abeer Mesbah, and Maha Houssen. **(2010)**. "Insulin Resistance in Early Untreated Rheumatoid Arthritis Patients." *Clinical biochemistry* 43(7–8): 661–65.

Shapiro, Samantha C. **(2021)**. "Biomarkers in Rheumatoid Arthritis." *Cureus* 13(5).

Sharma, Rajeev et al. **(2014)**. "Estimation of Vitamin D Levels in Rheumatoid Arthritis Patients and Its Correlation with the Disease Activity." *J Assoc Physicians India* 62(8): 678–81.

Shields, Beverley M et al. **(2015).** "Can Clinical Features Be Used to Differentiate Type 1 from Type 2 Diabetes? A Systematic Review of the Literature." *BMJ open* 5(11): e009088.

Shrivastava, Amit Kumar et al. **(2015)**. "Inflammatory Markers in Patients with Rheumatoid Arthritis." *Allergologia et immunopathologia* 43(1): 81–87.

Siegmund, Daniela, and Harald Wajant. **(2023).** "TNF and TNF Receptors as Therapeutic Targets for Rheumatic Diseases and Beyond." *Nature Reviews Rheumatology* 19(9): 576–91.

Silva-Fernández, Lucía et al. **(2020**). "The Prevalence of Rheumatoid Arthritis in Spain." *Scientific Reports* 10(1): 21551.

Simmons, David. **(2015).** "Prevention of Gestational Diabetes Mellitus: Where Are We Now?" *Diabetes, Obesity and Metabolism* 17(9): 824–34.

Singh, Amit, and Mansoor M Amiji.(**2022)**. "Application of Nanotechnology in Medical Diagnosis and Imaging." *Current opinion in biotechnology* 74: 241–46.

Sirdah, Mahmoud M, and N Scott Reading. **(2020)**. "Genetic Predisposition in Type 2 Diabetes: A Promising Approach toward a Personalized Management of Diabetes." *Clinical Genetics* 98(6): 525–47.

Slowing, Igor I, Brian G Trewyn, Supratim Giri, and VS-Y Lin. **(2007).** "Mesoporous Silica Nanoparticles for Drug Delivery and Biosensing Applications." *Advanced Functional Materials* 17(8): 1225–36.

Smolen, Josef S et al. **(2023)**. "EULAR Recommendations for the Management of Rheumatoid Arthritis with Synthetic and Biological Disease-Modifying Antirheumatic Drugs: 2022 Update." *Annals of the rheumatic diseases* 82(1): 3–18.

Smallwood, M. J., Nissim, A., Knight, A. R., Whiteman, M., Haigh, R., & Winyard, P. G. **(2018)**. Oxidative stress in autoimmune rheumatic diseases. Free Radical Biology and Medicine, 125, 3-14.

Sprangers, Ben et al. **(2017)**. "Origin of Enriched Regulatory T Cells in Patients Receiving Combined Kidney–Bone Marrow Transplantation to Induce

Transplantation Tolerance." *American Journal of Transplantation* 17(8): 2020–32.

Sproston, Nicola R, and Jason J Ashworth. **(2018)**. "Role of C-Reactive Protein at Sites of Inflammation and Infection." *Frontiers in immunology* 9: 754.

Sundy, John S. **(2001)**. "COX-2 Inhibitors in Rheumatoid Arthritis." *Current Rheumatology Reports* 3(1): 86–91.

Sur, Srija et al. **(2019).** "Recent Developments in Functionalized Polymer Nanoparticles for Efficient Drug Delivery System." *Nano-Structures & Nano-Objects* 20: 100397.

Syam Kumar, Valli N, Swetha Sunkar, Karthick Raja Namasivayam Selvaraj, and Jayshree Nellore. **(2023)**. "Nano Drug Delivery Systems: A Mini-Review." *Nanoscience & Nanotechnology-Asia* 13(3): 68–81.

Targońska-Stępniak, Bożena et al. **(2021)**. "Systemic Inflammatory Parameters in Patients with Elderly-Onset Rheumatoid Arthritis (EORA) and Young-Onset Rheumatoid Arthritis (YORA)—an Observational Study." *Journal of Clinical Medicine* 10(6): 1204.

Tasliyurt, Turker et al.**(2013)**. "The Frequency of Antibodies against Cyclic Citrullinated Peptides and Rheumatoid Factor in Healthy Population: A Field Study of Rheumatoid Arthritis from Northern Turkey." *Rheumatology international* 33: 939–42.

Thabet, Yasmeena, Mahmoud Elsabahy, and Noura G Eissa. **(2022).** "Methods for Preparation of Niosomes: A Focus on Thin-Film Hydration Method." *Methods* 199: 9–15.

Tishkowski, K, and V Gupta. **(2022)**. "Erythrocyte Sedimentation Rate.[Updated 2022 May 8]." *StatPearls [Internet]. Treasure Island (FL):*

StatPearls Publishing.

Tripolino, Cesare et al. **(2021)**. "Insulin Signaling in Arthritis." *Frontiers in immunology* 12: 672519.

Ullah, Irfan et al.**(2021).** "Role and Perspectives of Inflammation and C-Reactive Protein (CRP) in Psychosis: An Economic and Widespread Tool for Assessing the Disease." *International Journal of Molecular Sciences* 22(23): 13032.

Umbarkar, Mahesh G et al. **(2020)**. "Formulation and Evaluation of Polymeric Nanoparticle by Nano-Precipitation Method." *Journal of Drug Delivery and Therapeutics* 10(5-s): 136–42.

Uttra, Ambreen Malik et al. **(2019)**. "Ribes Orientale: A Novel Therapeutic Approach Targeting Rheumatoid Arthritis with Reference to pro-Inflammatory Cytokines, Inflammatory Enzymes and Anti-Inflammatory Cytokines." *Journal of ethnopharmacology* 237: 92–107.

den Uyl, Debby et al. **(2012)**. "Metabolic Effects of High-dose Prednisolone Treatment in Early Rheumatoid Arthritis: Balance between Diabetogenic Effects and Inflammation Reduction." *Arthritis & Rheumatism* 64(3): 639–46.

Vandenbon, Alexis. **(2021)**. "Guidance for RNA-Seq Co-Expression Estimates: The Importance of Data Normalization, Batch Effects, and Correlation Measures." *bioRxiv* 11.

Valencia, M. **(2022)**. Sex and Gender in Rheumatoid Arthritis: Considering a Risk Factor Hierarchy. Cornell Undergraduate Research Journal, 1(1), 23-29.

VD, Nimesh Sand Ashwlayan. **(2020)**. "Journal of Bone Biology and

Osteoporosis Cutting Edge of Herbal Drugs over Allopathic Drugs in Clinical Treatment of Rheumatoid Arthritis."

Venetsanopoulou, Aliki I, Yannis Alamanos, Paraskevi V Voulgari, and Alexandros A Drosos. **(2023)**. "Epidemiology and Risk Factors for Rheumatoid Arthritis Development." *Mediterranean Journal of Rheumatology* 34(4): 404.

Verma, Amit K et al. **(2021)**. "Association of Rheumatoid Arthritis with Diabetic Comorbidity: Correlating Accelerated Insulin Resistance to Inflammatory Responses in Patients." *Journal of Multidisciplinary Healthcare*: 809–20.

Vieira Borba, Vânia, Kassem Sharif, and Yehuda Shoenfeld. **(2018)**. "Breastfeeding and Autoimmunity: Programing Health from the Beginning." *American Journal of Reproductive Immunology* 79(1): e12778.

Vighi, Eleonora et al. **(2018)**. "Combination of CGMP Analogue and Drug Delivery System Provides Functional Protection in Hereditary Retinal Degeneration." *Proceedings of the National Academy of Sciences* 115(13): E2997–3006.

Wang, Ting-Ting, Shao-Kang Wang, Gui-Ling Huang, and Gui-Ju Sun. **(2012)**. "Luteolin Induced-Growth Inhibition and Apoptosis of Human Esophageal Squamous Carcinoma Cell Line Eca109 Cells in Vitro." *Asian Pacific journal of cancer prevention* 13(11): 5455–61.

Webb, Ann R, Rehab Alghamdi, Richard Kift, and Lesley E Rhodes. **(2021)**. "100 YEARS OF VITAMIN D: Dose–Response for Change in 25-Hydroxyvitamin D after UV Exposure: Outcome of a Systematic Review." *Endocrine Connections* 10(10): R248–66.

Wojdasiewicz, Piotr, Łukasz A Poniatowski, and Dariusz Szukiewicz. **(2014).**

"The Role of Inflammatory and Anti-Inflammatory Cytokines in the Pathogenesis of Osteoarthritis" ed. Chuanju Liu. *Mediators of Inflammation* 2014: 561459. https://doi.org/10.1155/2014/561459.

Wu, Fengping et al. **(2021)**. "B Cells in Rheumatoid Arthritis： Pathogenic Mechanisms and Treatment Prospects." *Frontiers in immunology* 12: 750753.

Wysocki, Tomasz, Marzena Olesińska, and Agnieszka Paradowska-Gorycka. 2020. "Current Understanding of an Emerging Role of HLA-DRB1 Gene in Rheumatoid Arthritis–from Research to Clinical Practice." *Cells* 9(5): 1127.

Xiang, Yu et al. **(2023)**. "The Role of Inflammation in Autoimmune Disease: A Therapeutic Target." *Frontiers in Immunology* 14: 1267091.

Yağız, Abdullah Erman et al. **(2015)**. "Association of Vitamin D with Disease Activity in Rheumatoid Arthritis and Ankylosing Spondylitis."

Yang, Junxia et al. **(2015)**. "Effect of Vitamin D on the Recurrence Rate of Rheumatoid Arthritis." *Experimental and therapeutic medicine* 10(5): 1812–16.

Yu, Caiwei et al. **(2022)**. "Dextran Sulfate-Based MMP-2 Enzyme-Sensitive SR-A Receptor Targeting Nanomicelles for the Treatment of Rheumatoid Arthritis." *Drug Delivery* 29(1): 454–65.

Yu, Changhui et al. **(2019)**. "Hyaluronic Acid Coated Acid-Sensitive Nanoparticles for Targeted Therapy of Adjuvant-Induced Arthritis in Rats." *Molecules* 24(1): 146.

Yu, Chaojie et al. **(2020)**. "Gender Differences in Rheumatoid Arthritis: Interleukin-4 Plays an Important Role." *Journal of immunology research* 2020.

Yuan, Xia, Sen Cui, Yan Liu, and TaiJi Song. **(2020)**. "Analysis of Serum Rheumatoid Factors in Patients with Rheumatoid Arthritis in Han, Tibetan and Hui Nationalities in Qinghai." *International Immunopharmacology* 83: 106380.

Zahin, Nuzhat et al. **(2020)**. "Nanoparticles and Its Biomedical Applications in Health and Diseases: Special Focus on Drug Delivery." *Environmental Science and Pollution Research* 27(16): 19151–68.

Zahra, Zahra, Zunaira Habib, Sujin Chung, and Mohsin Ali Badshah. **(2020).** "Exposure Route of TiO2 NPs from Industrial Applications to Wastewater Treatment and Their Impacts on the Agro-Environment." *Nanomaterials* 10(8): 1469.

Zeng, Zhiwen, Jinying Xu, and Wenhua Zheng. **(2017)**. "Artemisinin Protects PC12 Cells against β-Amyloid-Induced Apoptosis through Activation of the ERK1/2 Signaling Pathway." *Redox biology* 12: 625–33.

Zhang, Haiyan et al. **(2018)**. "Tetrandrine Suppresses Cervical Cancer Growth by Inducing Apoptosis in Vitro and in Vivo." *Drug design, development and therapy*: 119–27.

Zhou, Meng et al. **(2014)**. "Immunolocalization of MMP-2 and MMP-9 in Human Rheumatoid Synovium." *International journal of clinical and experimental pathology* 7(6): 3048.

Zhu, Xiaoxu et al. **(2023)**. "Expression and Effect of TIGIT in Peripheral Blood CD56+ T Cells of Patients with Rheumatoid Arthritis." *Xi bao yu fen zi Mian yi xue za zhi= Chinese Journal of Cellular and Molecular Immunology* 39(11): 1010–18.

APPENDICES

University of Kerbala – College of Medicine

Department of Chemistry and Biochemistry

Student Name: Haneen Madlool Amanah

Supervisors:

Prof. Dr. Fadhil Jawad Al-Tu'ma - College of Medicine – University of Kerbala

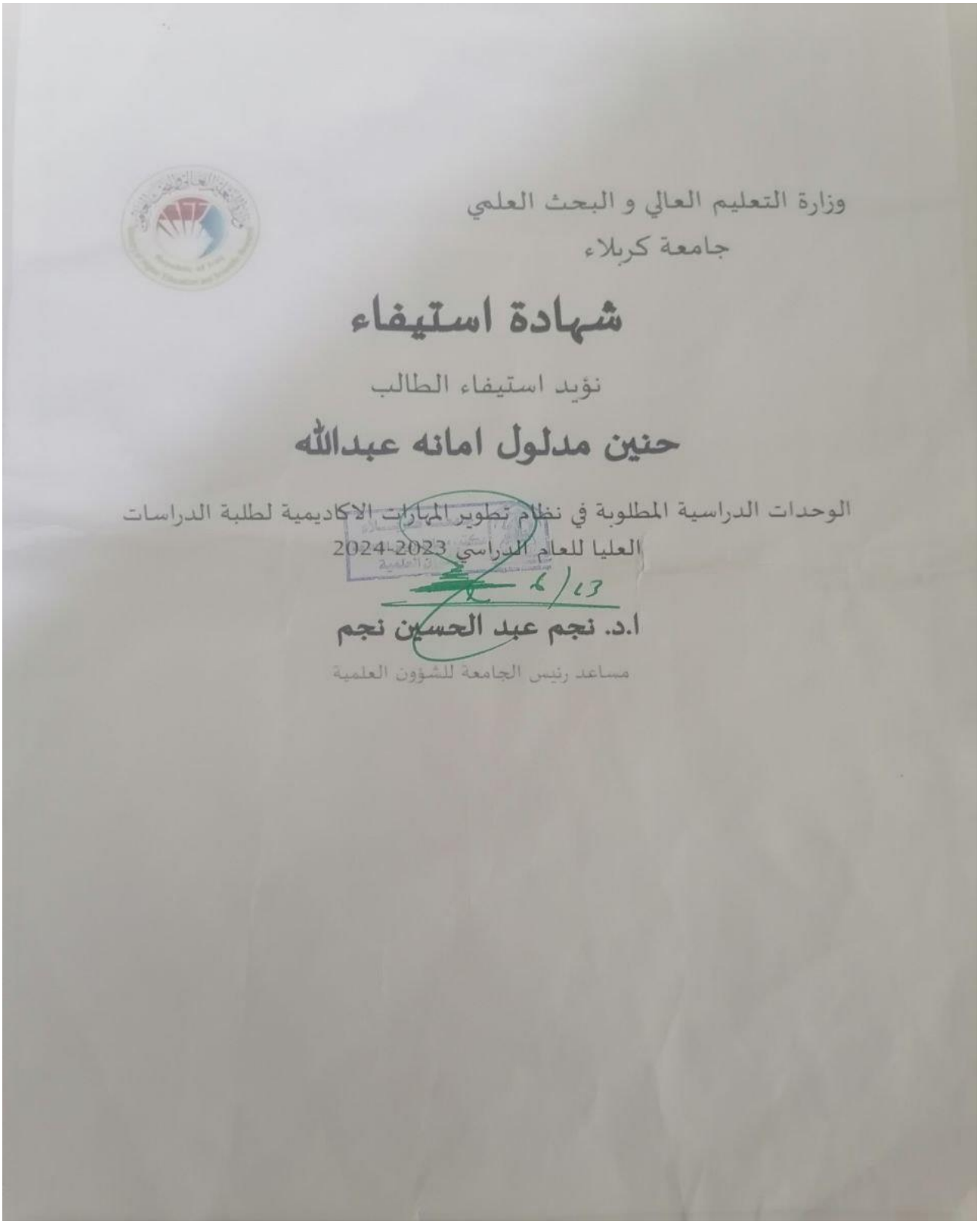
Dr.Atheer Al-Ghanimi – College of Medicine – University of Karbala

((Experimental Data))

Significance of Nano-formulated Artemisinin on Sera Levels of Interleukin-4 and Chemokine (Ligand-21) in Rheumatoid Arthritis Patients with / without T2DM

Sample NO:
Type of study: a case-control/cross-sectional
Inclusion Criteria: Rheumatoid arthritis RA, and rheumatoid arthritis with T2DM
Exclusion Criteria: Kidney disease, liver disease, cancer, and any acute or chronic inflammatory disease., all other autoimmune disorders, and infectious diseases.
Rheumatoid arthritis description : severe(chronic) , moderate , slightly
Age:
Family History:
Duration of RA : ( ) years
Type of Treatment:

Clinical Biochemical Markers :	
C-reactive protein CRP	
ESR mmol/h	
IL-4 pg/ml	
CCL-21pg/ml	
HbA1c mmol/mol	
FBS mg/dl	
Insulin Mu/ml	
HOMA IR %	
Zinc mg/dl	
Vit D mg/dl	
Height:	Weight:
BMI:	
Nanoparticles Studies	
Synthesis and characterization of hyaluronic acid-decorated loaded NPs and evaluation of MMP2, TIMP2 and COX2 gene expression in rheumatoid arthritis patients.	



وزارة التعليم العالي و البحث العلمي

جامعة كربلاء

شهادة استيفاء

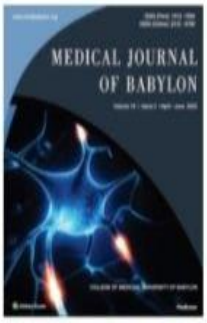
نؤيد استيفاء الطالب

حنين مدلول امانه عبدالله

الوحدات الدراسية المطلوبة في نظام تطوير المهارات الاكاديمية لطلبة الدراسات العليا للعام الدراسي 2023-2024

ا.د. نجم عبد الحسين نجم

مساعد رئيس الجامعة للشؤون العلمية



Medical Journal of Babylon

Open access and peer reviewed international journal

The journal is indexed in DOAJ, EBSCO, Scimago Journal Ranking, SCOPUS

Website: https://www.medjbabylon.org , Email: WKHLRPMedknow_editorial@wolterskluwer.com

Editor-in-Chief: Mufeed J. Ewadh, **Publisher:** Wolters Kluwer Medknow, **EISSN:** 2312-6760

Indexed by Scopus

Acceptance of article for publication in Medical Journal of Babylon

Dear Dr.

Date: 28-07-2024

Haneen Madlool Amanah*, Fadhil Jawad Al-Tu'ma and Atheer Hameid Odda

I am pleased to inform you that your manuscript (**MJBL_644_24**) titled as:

Association between Interleukin-4 and Insulin Resistance in Sera of Rheumatoid Arthritis with Type 2 Diabetes of Iraqi Women

has been accepted for publication in Medical Journal of Babylon.

We have received the payment for publication. So, you will receive the galley proof within 4-5 weeks. You must have to solve the query if we point out any in the galley proof.

After correction of galley proof, your article will be published online at https://www.medjbabylon.org

Best Regards

Prof. Mufeed J. Ewadh
Editor-in-Chief-MJBL

Corresponding author: Haneen Amanah. Department of Chemistry and Biochemistry, College of Medicine, University of Kerbala / Karbala – Iraq.
E. mail: haneen.madlool@s.uokerbala.edu.iq

Republic of Iraq
Ministry of Higher Education & Scientific Research
University of Karbala
College of Pharmacy
Kerbala Journal of Pharmaceutical Sciences

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة كربلاء
كلية الصيدلة
مجلة كربلاء للعلوم الصيدلانية

Number: 503
Date: 28/7/2024

Acceptance Letter

Dear Sir/Madam,

I am pleased to inform you that your manuscript title" **Association betweeen Interleukin-4 and Chemokine CCL-21 Levels with the Pathogenesis of Rheumatoid Arthritis in Iraqi Women** " authored by " **Haneen Madlool Amanah ; Fadhil Jawad Al-Tu'ma and Atheer Hameid Odda** " has been accepted for publication in the Kerbala Journal of Pharmaceutical Sciences. After careful consideration and peer review, we believe that your work makes a significant contribution to the field and will be of great interest to our readers.

Your manuscript will be an excellent addition to our upcoming issue.

Additionally, please ensure that you have completed and returned all necessary copyright forms and any other documentation required for publication.

Once again, congratulations on your acceptance, and thank you for submitting your outstanding work to Kerbala Journal of Pharmaceutical Sciences. We look forward to publishing your article and hope to receive more of your valuable research in the future.

Thanks,

Prof. Dr. Ahmed Salih Sahib

Editor in Chief

Email:kerbala.jps@uokerbala.edu.iq

العراق- محافظة كربلاء- مكتب بريد كربلاء- ص ب 1125

الخلاصة

التهاب المفاصل الروماتويدي (RA)هو مرض التهابي مزمن يتميز بالتهاب الغشاء المفصلي، وتلف الغضروف، وتآكل العظام، والذي يرتبط بزيادة معدلات الإصابة بالأمراض والوفيات مقارنة مع عامة السكان. قد يساهم الالتهاب الجهازي المرتبط بالتهاب المفاصل الروماتويدي في خطر الإصابة بمرض السكري في المستقبل. يرتبط التهاب المفاصل الروماتويدي بالالتهاب المزمن، والذي قد يؤدي، جزئيًا على الأقل، إلى تطور مرض السكري من النوع 2 .(T2DM)تقدم أنظمة توصيل الأدوية النانوية حلولاً واعدة لقيود العلاجات التقليدية لالتهاب المفاصل الروماتويدي ((RA)و .(T2DM)من بين الجسيمات النانوية، تعتبر النيوسومات فعالة بشكل خاص بسبب ثباتها وسهولة تحضيرها وقدرتها على تقليل السمية الجهازية. أنها توفر إطلاقًا خاضعًا للرقابة للأدوية وتعزز قابلية ذوبان واستقرار المركبات الصيدلانية. يمكن أن يؤدي دمج حمض الهيالورونيك (HA)في النيوسومات إلى تعزيز فعاليتها من خلال استهداف خلايا معينة، وتحسين توصيل الأدوية، وزيادة العلاج. لقد أظهر الأرتيميسينين ومشتقاته نتائج واعدة في علاج التهاب المفاصل الروماتويدي من خلال إظهار تأثيرات مضادة للالتهابات ومعدلة للمناعة ومضادة للعدوى. تلعب الجينات المهمة، مثل MMP2 وTIMP2 وCOX2، دورًا مهمًا في تطور المرض.

تهدف هذه الدراسة إلى تحديد المؤشرات الحيوية الالتهابية/المضادة للالتهابات للكشف المبكر عن التهاب المفاصل الروماتويدي والتشخيص لمنع تطور T2DM، وهو ارتباط بين علامات المتغيرات التي ترتبط بالمرض، واستخدام مادة الأرتيميسينين، وهو دواء شائع، يتم تقديمه بشكل فعال باستخدام مادة HA المغلفة تم إجراء النيوسومات لإثبات إمكانات هذا النهج في علاج RA وT2DM بواسطة تقنية ترطيب الطبقة الرقيقة. وبالإضافة إلى ذلك، فإنه يدرس تطبيق Hyalo-Nio-artemisinin NPs كمضاد للأكسدة ومضاد للالتهابات في مرضى التهاب المفاصل الروماتويدي. وإظهار تأثير Hyalo-Nio-artemisinin NPs على MMP-2 وTIMP-2 وCOX2 في المرضى الذين يعانون من .RA

دراسة الحالات والشواهد أجريت في مستشفى الهندية العام – وحدة الروماتيزم مديرية صحة كربلاء / كربلاء – العراق ومستشفى مرجان التعليمي مديرية صحة بابل / بابل – العراق من تشرين الأول 2023 إلى آذار 2024 باستخدام المرضى الخارجيين الذين يزورون قسم العظام وحدة استشارية. تم اخذ مجموعه 125 فردًا، تم تصنيفهم إلى فصيلين رئيسيين: 74 حالة من التهاب المفاصل الروماتويدي و51 حالة تحكم قوية. تم بعد ذلك تقسيم هؤلاء المرضى إلى 40 حالة من التهاب المفاصل الروماتويدي بدون داء السكري من النوع 2 (T2DM)و34 حالة من حالات التهاب المفاصل الروماتويدي مع T2DM، بما يتماشى مع معايير الكلية الأمريكية لالتهاب المفاصل لعام 2010، مما يضمن التكافؤ في متوسط العمر والجنس داخل

مجموعات الدراسة. بعد ذلك، أكمل جميع المشاركين المسوحات، وتم جمع عينات الدم الوريدي، وتقسيمها إلى قارورة سترات الصوديوم لتقييم معدل ترسيب كريات الدم الحمراء، وفحوصات النانو، وعزل خلايا الدم وحيدة النواة (PBMC)، وقنينة الجيلاتين لعينة المصل. معزولة وحفظها عند -20 درجة مئوية في ثمانية قوارير إيبندورف للتحليل التجريبي.

تضمن الاستكشاف تقييم تراكيز الإنترلوكين -4 (IL-4)، والكيموكين (C-C) ليجند-21(CCL21)، والهيموجلوبين السكري (HbA1c%)، وجلوكوز الدم الصائم (FBG)، والأنسولين وارتباطها بالتهاب المفاصل الروماتويدي. تم أيضًا فحص الميول التشخيصية للعلامات في هذا البحث كمعلمة كيميائية حيوية في RA مع T2DM من خلال تحليل .ROCبالإضافة إلى ذلك، قمنا بصياغة ودراسة السمات الهيكلية لـ Hartemisinin Nio-NPs المحملة بحمض الهيالورونيك وقمنا بتقييم استخدامات هذه الجسيمات النانوية كعلاج للالتهاب والإجهاد التأكسدي لدى الأفراد المصابين بالتهاب المفاصل الروماتويدي.

تم تقييم مستويات IL-4 وCCL-21، ثم تم إنشاء مركب نانوي يحتوي على مادة الأرتيميسينين وحمض الهيالورونيك. تم تقييم خصائص الجسيمات النانوية من خلال المجهر الإلكتروني الماسح (SEM) ومطياف الأشعة تحت الحمراء لتحويل فورييه (FTIR)، إلى جانب طرق مختلفة بما في ذلك MTT وDLS و .AFMتم إجراء التحليل الإحصائي باستخدام الحزمة الإحصائية للعلوم الاجتماعية الإصدار 21.

أظهرت الدراسة زيادة في مستويات الأنسولين وسكر الدم الصائم ونسبة HbA1c%مع وجود فروق معنوية عالية وزيادة معنوية في مستويات IL-4 وMMP2 وكذلك مجموعة المرضى الذين يعانون من T2DM مقارنة بالمجموعة الضابطة.

كما أظهرت النتائج انخفاضاً معنوياً في مستوى مضادات الأكسدة فيتامين د3، والزنك لدى المرضى مقارنة بمجموعة السيطرة. تم إجراء أداء المركبات النانوية من جزيء الأرتيميسينين المطلي بحمض الهيالورونيك لتقليل معدل علامات الالتهاب وكذلك تقليل علامات الإجهاد التأكسدي لدى المرضى. وقد وجد أن مادة الأرتيميسينين يمكن أن تقلل من هذه العلامات بنسب كبيرة لدى المرضى.

وفي الختام، لخصت الدراسة إلى أن علامات الالتهابات والأكسدة ومضادات الأكسدة هي مؤشرات حيوية قيمة لتشخيص التهاب المفاصل الروماتويدي. وعلى وجه التحديد، سلط الضوء على الفعالية العلاجية لجسيمات الأرتيميسينين النانوية المغلفة بحمض الهيالورونيك في تقليل علامات الالتهابات والأكسدة بشكل كبير مع تعزيز علامات مضادات الأكسدة. وهذا يؤكد إمكانات مثل هذه التركيبات النانوية في إدارة التهاب المفاصل الروماتويدي من خلال معالجة الإجهاد التأكسدي الأساسي والالتهابات المرتبطة بالمرض.

جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامعة كربلاء - كلية الطب

فرع الكيمياء والكيمياء الحياتية

أهمية الأرتيميسينين المحضر على شكل نانو على الإنترلوكين-4 والكيموكين (ليغاند-21) في مصل مرضى التهاب المفاصل الروماتويدي مع/بدون مرض السكر من النوع الثاني

رسالة ماجستير

مقدمة إلى مجلس كلية الطب/ فرع الكيمياء والكيمياء الحياتية/ جامعة كربلاء كجزء من متطلبات نيل درجة الماجستير في (الكيمياء السريرية)

من قبل

حنين مدلول امانه

بكالوريوس علوم كيمياء- كلية العلوم – جامعة القادسية 2014-2015

بأشراف

الاستاذ الدكتور

فاضل جواد ال طعمة

الأستاذ المساعد الدكتور

أثير حميد عودة الغانمي

2024م

1446هـ