

بسم الله الرحمن الرحيم





شبكة المعلومات الجامعية التوثيق الالكتروني والميكرو فيلم



جامعة عين شمس

التوثيق الإلكتروني والميكروفيلم

قسم

نقسم بالله العظيم أن المادة التي تم توثيقها وتسجيلها
علي هذه الأقراص المدمجة قد أعدت دون أية تغيرات



يجب أن

تحفظ هذه الأقراص المدمجة بعيدا عن الغبار





MicroRNA-146a Expression as a Potential Biomarker for Rheumatoid Arthritis in Egypt

Thesis

Submitted to Faculty of Medicine, Ain Shams University for Partial Fulfillment of M.D Degree in Medical Microbiology and Immunology

By

Heba Mohamed Abdelkader Elsayed

MBB Ch, M Sc

Faculty of Medicine, Ain shams university

Under Supervision of

Dr. Maha Salah El-din Hamdy

Professor of Medical Microbiology and Immunology

Faculty of Medicine, Ain Shams University

Dr. Ayman Asaad Ibrahim

Professor of Medical Microbiology and Immunology

Faculty of Medicine, Ain Shams University

Dr. Walaa Shawky Khater

Assistant Professor of Medical Microbiology and Immunology

Faculty of Medicine, Ain Shams University

Dr. Nashwa Ali Morshedy

Assistant Professor of Rheumatology and Internal Medicine

Faculty of Medicine, Ain Shams University

Faculty of Medicine

Ain Shams University

2021

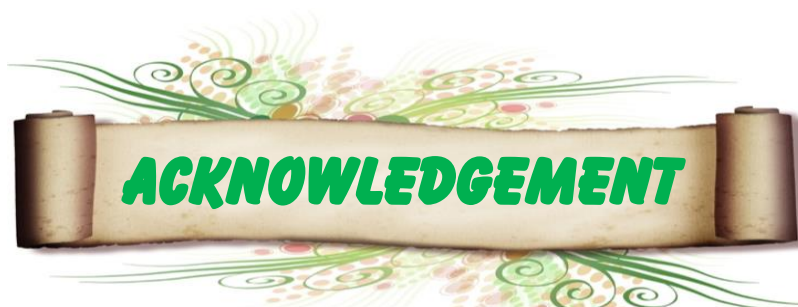
بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

قالوا

سببناك لا تعلم لنا
إلا ما علمتنا إنك أنت
العليم العظيم

صدق الله العظيم

سورة البقرة الآية: ٣٢



First of all, thanks to Allah whose magnificent help was the main factor in completing this work.

*Words cannot express my deepest gratitude to **DR. FATEN MOSTAFA ALI**, Professor and head of Medical Microbiology and Immunology, Faculty of Medicine, Ain Shams University, for her encouragement and support to complete such work,*

*Words cannot express my deepest gratitude to **DR. MAHA SALAH EL-DIN HAMDY**, Professor of Medical Microbiology and Immunology, Faculty of Medicine, Ain Shams University, who helped me throughout this work by training, precious instructions, valuable advice, and scientific knowledge to accomplish such work,*

*It has been a great honor for me to work under his generous supervision. Great words really needed to express my gratitude, sincere appreciation and respect to **DR. AYMAN ASAAD IBRAHIM**, Professor of Medical Microbiology and Immunology, Faculty of Medicine, Ain Shams University, for his great help, continuous support, and sincere advice during this work,*

*I would also like to thank **DR. WALAA SHAWKY KHATER**, Assistant Professor of Medical Microbiology and Immunology, Faculty of Medicine, Ain Shams University for her continuous encouragement, guidance, and support.*

*I would also like to thank **DR. NASHWA ALI MORSHEDY**, Assistant Professor of Rheumatology and Internal Medicine,*

Faculty of Medicine, Ain Shams University for her continuous guidance and support she gave me throughout the whole work,

Finally, my deepest thanks to all my family and colleagues who helped me in the production of this work,

 **HEBA MOHAMED ABDELKADER.**

Contents

Subject	Page NO.
List of abbreviations.....	II
List of figures.....	VI
List of tables.....	VIII
Introduction	1
• Aim of the Work	5
• Review of Literature	
♦ Chapter (1): Rheumatoid Arthritis	6
♦ Chapter (2): MicroRNAs	24
♦ Chapter (3): MicroRNA-146 Family	47
♦ Chapter (4): Therapeutic Potential of miRNAs	46
• Subjects and Methods	53
• Results	60
• Discussion	74
• Summary	111
• Conclusion	114
• Recommendations	115
• References	116
• Arabic Summary	

List of Abbreviations

Abbreviation : Full-term	
ACPAs / anti-CCP	: Anti-Citrullinated Protein/Peptide Antibodies.
ACR	: American College of Rheumatology.
AGO2	: Argonaute 2.
ARA	: American Rheumatism Association.
ASUH	: Ain Shams University Hospitals.
AUC	: Area Under the Curve.
BCR	: B-Cell Receptor.
CBC	: Complete Blood Count.
CDAI	: Clinical Disease Activity Index.
cDNA	: Complementary DNA.
CI	: Confidence Interval.
COX-1	: Cyclooxygenase-1.
COX- 2	: Cyclooxygenase-2.
CRP	: C-Reactive Protein.
DAS	: Disease Activity Scale.
DAS 28	: Modified Disease Activity Scale 28.
DGCR8	: Di George syndrome Critical Region 8.
DMARDS	: Disease-Modifying Anti-Rheumatic Drugs.
DNA	: Deoxyribo-Nucleic Acid.
DUSP	: Dual Specificity Protein Phosphatase.
EGA	: Evaluator Global Assessment.
ESR	: Erythrocyte Sedimentation Rate.
EULAR	: European League Against Rheumatism.
FLS	: Fibroblast-Like Synoviocytes.
FO	: Follicular.
GCs	: Glucocorticoids.

List of Abbreviations

GTP	: Guanosine Triphosphate.
Hb	: Haemoglobin.
HLA	: Human Leukocyte Antigen.
HS	: Highly Significant.
IFN-γ	: Interferon- gamma.
IL	: Interleukin.
IRAK	: Interleukin-1 Receptor-Associated Kinase.
IRF-5	: Interferon Regulatory Factor-5.
lin-14	: Lineage gene defective-14.
LPS	: Lipopolysaccharide.
LSD	: Least Significant Difference.
MAPK	: Mitogen activated protein kinase.
miR or miRNA	: MicroRNA.
miRNPs	: MiRNA-containing Ribonucleo-Protein Particles.
mRNA	: Messenger RNA.
MZ	: Marginal Zone.
NF-κB activated B cells.	: Nuclear Factor kappa-light-chain-enhancer of
NGS	: Next Generation Sequencing.
NO	: Nitric oxide.
NPV	: Negative predictive Value.
NS	: Non-Significant.
NSAIDs	: Non-Steroidal Anti-Inflammatory Drugs.
nt	: Nucleotide.
PBMC	: Peripheral Blood Mononuclear Cell.
PCR	: Polymerase Chain Reaction.
PGA	: Patient Global Assessment.
PI3K	: Phosphatidyl-Inositol-3 kinase.
Piwi	: P-element-induced wimpy testes.
Pol II	: Polymerase II.

List of Abbreviations

Pol III	: Polymerase III.
Poly (A) tail	: Poly-Adenylation tail.
PPV	: Positive Predictive Value.
Pre-miRNA	: Precursor miRNA.
Pri-miRNAs	: Primary miRNA.
PTEN	: Phosphatase and Tensin Homolog.
PTPN22	: Protein Tyrosine Phosphatase, Non-receptor type 22.
PV	: Probability value.
qRT-PCR	: Quantitative Real-Time PCR.
RA	: Rheumatoid Arthritis.
RAFLS	: Rheumatoid Arthritis fibroblast-like synoviocytes.
RAN	: RAS-related Nuclear protein.
RF	: Rheumatoid Factor.
RIG-1	: Retinoic acid-Inducible Gene 1.
RISC	: RNA-Induced Silencing Complex.
RNA	: Ribonucleic acid.
RNAi	: RNA interference.
ROC	: Receiver Operator Characteristic.
ROS	: Reactive Oxygen Species.
rRNA	: Ribosomal RNA.
rpm	: Rounds per minute.
RQ	: Relative Quantification.
S	: Significant.
SD	: Standard Deviation.
SDAI	: Simplified Disease Activity Index.
SE	: Standard Error.
SHP-2	: SH2 domain containing tyrosine phosphatase.
shRNA	: Short hairpin RNA.
siRNA	: Small interfering RNA.

List of Abbreviations

SMARDs	: Symptom-Modifying Anti-Rheumatic Drugs.
snoRNA	: Small nucleolar RNA.
snRNA	: Small nuclear RNA.
SOCS1	: Suppressor of Cytokine Signaling 1.
SPSS	: Statistical Program for Social Science.
SS	: Single Stranded.
STAT-1	: Signal Transducer and Activator of Transcription 1.
stRNA	: Small temporal RNA.
TCR	: T-Cell Receptor.
Th1	: T helper 1.
Th2	: T helper 2.
Th17	: T helper 17.
TLR	: Toll-Like Receptor.
TNF-α	: Tumor Necrosis Factor-alpha.
TRAF 6	: TNF Receptor-Associated Factor 6.
TRBP	: Transactivating Response RNA Binding Protein.
tRNA	: Transfer RNA.
VAS	: Visual Analogue Scale.
XPO5	: Exportin 5.
WBCs	: White Blood Cells.

List of Figures

Figure NO.	Title	Page NO.
Figures in review		
1	Humoral and cellular immunity in pathogenesis of rheumatoid arthritis.	9
2	Hands deformation by rheumatoid arthritis.	16
3	Biogenesis of miRNA and gene silencing pathway.	28
4	RNA-Induced Silencing Complex (RISC) loading and activation.	30
5	MiRNAs controlling lymphocyte development.	37
6	Regulation of T regulatory cells by miRNAs.	40
7	Negative regulation of signal transduction pathways and NF- κ B by miRNA-146a.	48
8	The RNA interference pathway.	54
9M	The miRNeasy Mini Kit"	63
10M	Gyrozen centrifuge.	65
11M	The thermal cycler used in reverse transcription reaction (Biometra, Germany).	68
12M	Quantitative real time PCR.	69
Figures in results		
13R	Subgroups of patients divided according to disease activity by SDAI.	75
14R	Comparison between rheumatoid arthritis patients and controls regarding age.	77
15R	Comparison between rheumatoid arthritis patients and controls regarding gender.	77
16R	Comparison between rheumatoid arthritis patients and healthy controls regarding ESR.	80
17R	Comparison between rheumatoid arthritis patients and healthy controls regarding Hb.	80
18R	Comparison between different patient subgroups regarding RQ of miR-146-a.	82

List of Figures

Figure NO.	Title	Page NO.
19R	Comparison between different patient subgroups regarding ESR.	82
20R	Comparison between different patient subgroups regarding CRP.	83
21R	Comparison between different patient subgroups regarding Hb level.	83
22R	Comparison between different patient subgroups regarding RF.	84
23R	Comparison between different patient subgroups regarding anti-CCP.	84
24R	Comparison between different patient subgroups and controls regarding RQ of miR-146a expression.	86
25R	Comparison between different patient subgroups and controls regarding anti-CCP.	86
26R	Comparison between different patient subgroups and controls regarding ESR.	87
27R	Comparison between different patient subgroups and healthy controls regarding Hb level.	87
28R	Comparison between different patient subgroups and controls regarding RF.	88
29R	Scatter diagrams showing the correlation between RQ of miR-146a expression and ESR, CRP, Hb level and anti-CCP.	90
30R	The ROC curve analysis of RQ of miR-146a expression in patients versus controls.	91
31R	The ROC curve analysis of RF in patients versus controls.	91
32R	Roc curve of anti-CCP in RA patients versus controls.	92
33R	Combined ROC curve analysis of RQ of miR-146a expression, RF and anti-CCP.	94

List of Tables

Table NO.	Title	Page NO.
Tables in review		
1	The ACR/EULAR 2010 classification criteria for rheumatoid arthritis.	19
2	Different Activity Indices for rheumatoid arthritis.	20
3	Tissue-specific expression of miRNAs.	34
4	MiRNAs in rheumatoid arthritis.	41
Tables in results		
5R	Subgroups of rheumatoid arthritis patients divided according to disease activity by simplified disease activity index (SDAI).	75
6R	Demographic data of the study participants.	76
7R	Clinical and laboratory data of the rheumatoid arthritis patients' group (n=25).	78
8R	Comparison between rheumatoid arthritis patients and controls regarding results of routine laboratory tests and relative quantification (RQ) of miR-146a.	79
9R	Comparison between different rheumatoid arthritis patients' subgroups as regard laboratory findings using One Way ANOVA test.	81
10R	Comparison between rheumatoid arthritis patients' subgroups and controls as regard laboratory findings using One Way ANOVA test.	85
11R	Pearson correlation between relative quantification (RQ) of miR-146a expression and other laboratory findings in rheumatoid arthritis patients.	89