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College of Science

Department of Biology

Investigation of miRNA-146a in Patients with Rheumatoid Arthritis and its Association with Some Biomarkers

A Thesis

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بِسْمِ ٱللَّهِ ٱلرَّحْمَٰنِ ٱلرَّحِيمِ

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Dedication

To Allah my Lord,

My homeland Iraq, is the symbol of civilization and the country to I proudly belong, despite the depth of its wounds .

To my spiritual inspiration, my father, may his soul rest in peace

To my Family

I dedicate this work.

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summary

Patients with Rheumatoid arthritis disease was shown an increasing range level of the folding change of MicroRNA-146a when compared to the healthy control groups, a comparison of serum level of miRNA-146 a folding change. The mean level of Micro146 folding change in patients was (8.49) which was significantly higher than for the Control group (2.07), ($P \leq 0.001$). The study also included a comparison of serum level of miRNA-146a fold change in different BMI groups, the level of folding change showed a massive increase seen with increasing BMI. In overweight patients, the folding change was (11.2) while in normal weight was (2.1), p-value was 0.05. Interesting, male patients have shown a significantly higher level of Fold change (9.51) compared to female patients (7.46), p-value was 0.009. It was also found that microRNA-146a and folding change were shown highly significant risk factors in rheumatic arthritis disease .

Results of the receiver operating curve (ROC) curve and AUC analysis for the Fold change as a diagnostic parameter showed a good performance for prediction RA patients. Furthermore, results indicated a significant difference in IL-33 and Leptin . The mean level of IL-33 in patients was (950.80) which was significantly higher than for the Control group (297.10), ($p \leq 0.001$) and the mean level of Leptin. The hormone was (7.21) for the patient and (2.78) for the control .

It was found that both biomarkers showed highly significant differences in such disease and represented a risk factor. Leptin was illustrated to be a three-time risk factor for Rheumatoid arthritis disease than IL33. AUC analysis for IL-33 as a diagnostic parameter showed that IL-33 has a good performance for predicting such cases.

In conclusion, miRNAs 164a may be a good candidate for biomarkers in disease diagnosis, prognosis, treatment, and other clinical applications. despite only a few

miRNAs taking part in the pathogenesis of RA, researchers need paying more attention toward other types of miRNAs which might be a good step for better understand the gene regulatory networks implicated in the pathogenesis of RA.

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List of Abbreviations

Abbreviations	
ACPA	Anti-Citrullinated Protein Antibodies
ACR	American College Of Rheumatology
AREs	Uridine-Rich Elements
AUF1	Au-Rich Binding Factor 1
AUP	Area Under Pick
BMI	Body Mass Index
cDNA	Complementary Dna
Ct U6	Cycle Thresholds Rnu6b
DCt	Ct Average Of Mirna – Ct Average Of U6
DDCt	Dct – Average Of Dct Control
DNA	Deoxyribonucleic Acid
ECM	Extracellular Matrix
ELISA	Enzyme-Linked Immunosorbent Assay
FLSs	Fibroblast-Like Synoviocytes
G-CSF	Granulocyte Colony Stimulating Factor
gapdh	Glyceraldehyde-3-Phosphate Dehydrogenase
GH	Growth Hormone
HRP	Avidin-Horseradish Peroxidase
IgE	Immunoglobulin E
IGFBP5	Ulin-Like Growth Factor Binding Protein 5
IL	Interleukins
JAK/STAT	Janus Kinase/Signal Transducer And Activator Of Transcription
LIF	Leukemia-Inhibiting Factor
MCP	Modified Citrus Pecti
miRBase	Microrna Database
miRNAs	Micrornas
MMP	Matrix Metalloproteinase
MMPs	Matrix Metalloproteinases
MTP	Medical Termination Of Pregnancy
NPV	Negative Predictive Value
NS	Non-Significant
OD	Optical Density
OR	Odd Ratio
PBMC	Peripheral Blood-Derived Mononuclear Cells

PCR	Polymerase Chain Reaction
PIP	Positive Inspiratory Pressure
PPV	Positive Predictive Value
qPCR	Quantitative Polymerase Chain Reaction
qRT-PCR	Quantitative Reverse Transcriptase Polymerase Chain Reaction
qRT–PCR	Quantitative Reverse Transcriptase Polymerase Chain Reaction
RA	Rheumatoid Arthritis
RF	Rheumatoid Factor
ROC	Receiver Operating Curve
S	Significant
SD	Stander Deviation
SEMA3A	Regulates Semaphoring 3 A
SF	Synovial Fluid
SLE	Systemic Lupus Erythematosus
TLRs	Toll-Like Receptors
TNF-a	Tumor Necrosis Factor-Alpha
TRAF6	Tnf Receptor-Associated Factor 6
TTP	Tristetraprolin
U6	Rnu6b

Chapter One

Introduction

1.1 Introduction

Rheumatoid arthritis (RA) is a chronic, progressive, inflammatory autoimmune disease associated with articular, extra-articular, and systemic effects. It has been reported that RA affects 0.5-1% of the adult population of developed regions (***Kuo et al., 2013***).

Incidence and prevalence, the worldwide prevalence of RA has been estimated as 0.24 % based on the Global Burden of Disease. (***Cross et al., 2014***). In 2017, estimates of RA prevalence in the United States and Northern European countries were typically higher, usually between 0.5 to 1 % (***Hunter et al., 2017***). The annual incidence of RA in the United States and Northern European countries is estimated to be approximately 40 per 100,000 persons (Eriksson et al., 2013). As a result, epidemiologic estimates of RA and identification of risk factors come largely from these populations. The incidence and prevalence of RA are much greater in some populations, the prevalence of RA varies across different populations and regions (***Alamanos et al., 2005***).

In such cases, patients might have joint destruction, severe physical disability and multiple co-morbidities (***Plenge et al., 2009***). Mortality rates are more than twice as high in patients with RA as in the general population (***Sen et al., 2014***). T cells, B cells and the orchestrated interaction of pro-inflammatory cytokines play key roles in the pathophysiology of RA (***Smolen et al., 2023***). The cytokines most directly implicated in this process are TNF-a and IL-6; IL-1 and IL-17 may also play important roles in the disease process (***Smolen et al., 2023***).

It has been reported that female sex is a predictor of disability and that the progression of disability is three times faster in females than in males (***Karpouzas et al., 2012***). As women have lower muscular strength than men, the impact of RA in

the functional capacity is greater in this group. Sex hormones also play an important role in the differences between genders. The severity of RA correlates inversely with androgen levels, which is a possible explanation for the lower severity of the disease in men ***(Cutolo et al., 2009***). Testosterone interacts with the immune system suppressing the humoral and cellular response (***Cutolo et al., 2000***). The course of the disease varies, but most patients develop a chronic progressive disease leading to pain, joint destruction and disability (***Sokka et al., 2008***).

MicroRNAs (miRNAs), a class of endogenous single-stranded short noncoding RNAs, have emerged as vital epigenetic regulators of both pathological and physiological processes in animals. They direct fundamental cellular pathways and processes by fine-tuning the expression of multiple genes at the posttranscriptional level. Growing evidence suggests that miRNAs are implicated in the onset and development of rheumatoid arthritis (RA) (***Peng et al., 2013***),

Overall, it is clear that miR-146a plays a critical role in regulating inflammatory responses through a negative feedback pathway. Given this role, it is not surprising that miR-146a has been shown to be differentially expressed in a number of inflammatory autoimmune diseases including systemic lupus erythematosus (SLE) (***Wang et al., 2010***), and rheumatoid arthritis (RA). Specifically, it has been reported that miR-146a is highly expressed in synovial tissue and synovial fibroblasts of RA patients compared to normal individuals and osteoarthritis patients (***Nakasa et al., 2008***),

miRNA-146a is one of the most extensively investigated. In this regard, basal expression of miR-146a is elevated in the tissues of psoriasis and rheumatoid arthritis (***Sonkoly et al., 2007***), diseases that are associated with chronic inflammation. The expression of miR-146a in rheumatoid arthritis synovial fibroblasts was higher than

that in the fibroblasts from osteoarthritis (***Stanczyk et al., 2008***). In vitro studies also demonstrated that miR-146a expression was rapidly increased in response to IL-1ß and TNF-α in various types of cells including A549 cells, BEAS2B cells, primary human airway epithelial cells, primary human airway smooth muscle cells, and human rheumatoid arthritis synovial fibroblasts (***Liu et al., 2017***).

1.1.1 Study Hypothesis

Ongoing research on miRNAs has potential implications in the diagnosis and treatment of RA. Their different levels in peripheral blood and synovial fluid between RA patients and healthy population makes them candidates for being used as biomarkers of such disease, while targeting miRNAs may be a novel therapeutic strategy in such cases.

1.1.2 Aims of the study

To find out the role of microRNA as a biomarker in patients with arthritis and its correlation with certain physiological parameters, this can be achieved by the following :

1. Estimation of the level of hole blood miRNA-146 in RA cases.
2. Estimation of the level of serum IL-33 and leptin related to RA cases.
3. Studying the correlation of serum miRNA-146, IL-33 and leptin in such cases.
4. To evaluate the sensitivity and specificity of the Preselection miRNA, proposed biomarkers and their diagnostic value in RA cases.

Chapter Two

Literature Review

2.1 Literature Review

RA is a systemic disease characterized by a complex pathogenesis involving interactions between various cell types located in synovial compartments and peripheral blood, rather than resulting from a single pathogenic factor (***Mclnnes et al., 2011***). These cell populations, comprising fibroblast-like synoviocytes (FLSs), innate and adaptive immune cells, and bone-related cells, change in number, status, and behavior in response to the dynamic microenvironment, which perturbs cytokine secretion, intracellular signaling networks and homeostasis and consequently leads to corresponding pathology. Multiple dysfunctional cell types and the crosstalk between these pathogenic cells collectively contribute to the onset, progression and perpetuation of RA (***Mclnnes et al., 2017***), as shown in Figure (2.1)

Under normal physiological conditions, FLSs express components of the extracellular matrix (ECM) and synovial fluid to lubricate and nourish cartilage surfaces, thereby maintaining the homeostasis of joints. However, accumulating studies have identified FLSs as key players in many pathogenic events in the RA synovium. In pathological conditions, such as RA, FLSs increase rapidly in number and are redistributed in the synovium and joints, exhibiting heterogeneity across different locations within the synovium and across different joints (***Dakin et al., 2018***).. FLSs in RA display unique aggressive behavior that arises from their reduced rate of apoptosis; deregulated proliferation, migration, and invasion; and improved ability to secrete inflammatory mediators and matrix metalloproteinases (MMPs) into the synovial fluid (***Mousavi et al., 2021***).

The role of B lymphocytes (B cells) in RA pathogenesis goes beyond autoantibody production, antigen presentation and cytokine release. Toll-like receptors (TLRs) drive the differentiation of self-reactive B cells into plasma cells that synthesize

autoantibodies (***Wu et al., 2021***). B cells differentiate into plasma cells that mediate autoantibody release and immune complex formation. These immune complexes subsequently induce macrophages to produce proinflammatory cytokines and facilitate osteoclast-mediated bone resorption (***Negishi-Koga et al., 2015).***

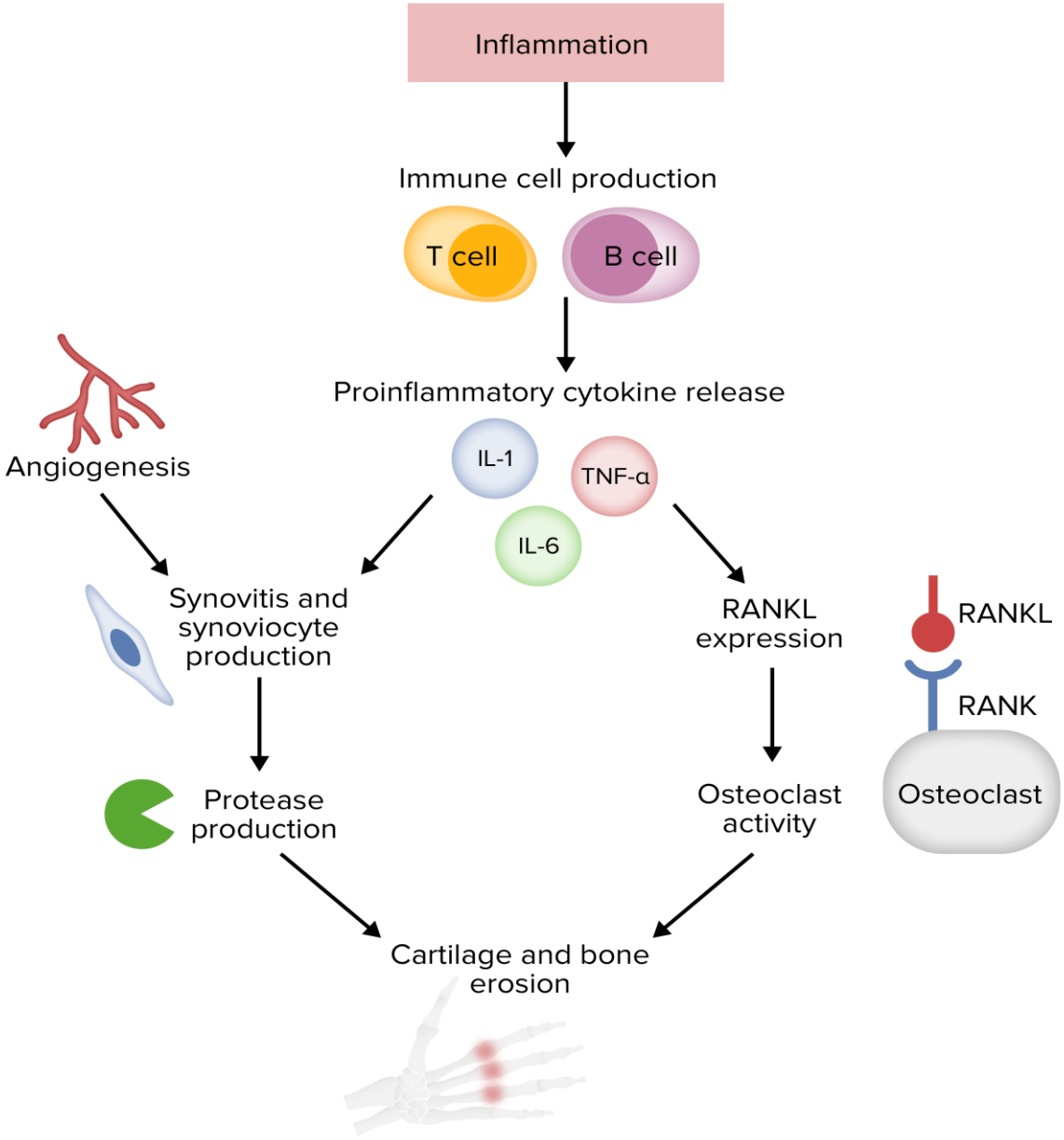


Figure 2.1: Adaptive and Innate Immune Processes in Rheumatoid Arthritis (*Firestein et al., 2020)*

2.1.2 Risk Factors for Rheumatoid Arthritis

The Risk factors for developing RA can be generically divided into host- and environment-related (Figure 2.2). Host factors that have been associated with RA development may be further grouped into genetic; epigenetic; hormonal, reproductive and neuroendocrine; and comorbid host factors. In turn, environmental risk factors include smoking and other airborne exposures; microbiota and infectious agents; diet; and socioeconomic factors (***Romão et al., 2021***).

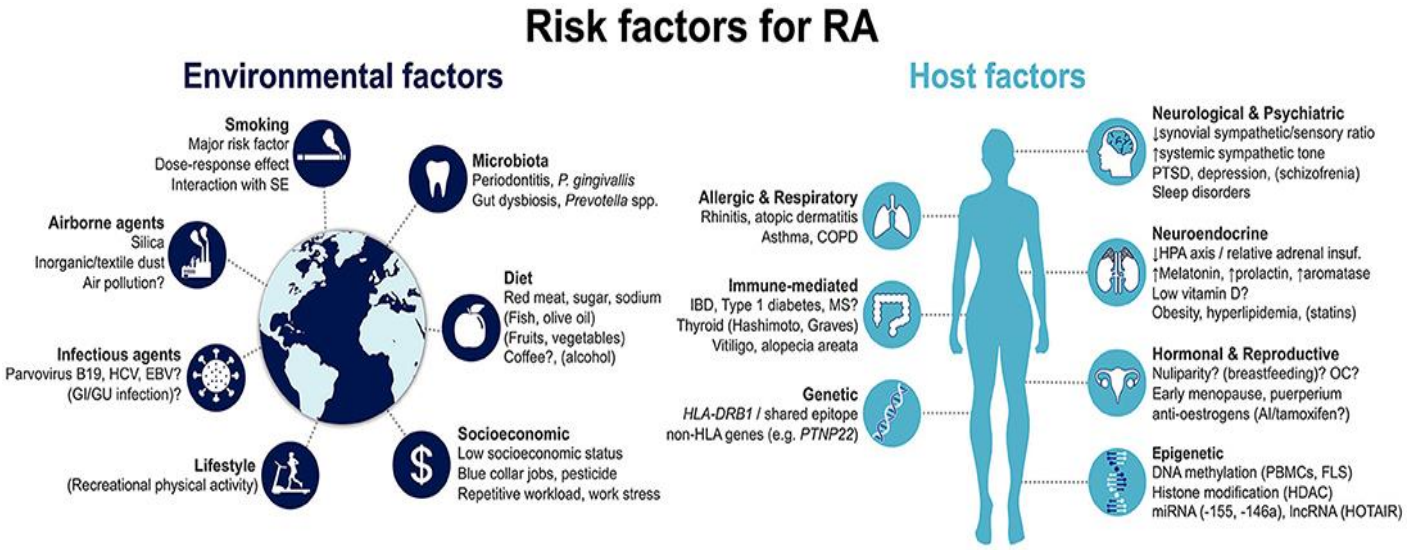


Figure 2.2: Risk factors of Rheumatoid Arthritis (***Romão et al., 2021***).

In the last decade, the role of epigenetics in RA development has started to be unraveled. Epigenetic mechanisms induce heritable variations in gene expression without actual changes in the deoxyribonucleic acid (DNA) sequence (***Nemtsova et al., 2019***).

Substantially less is known about the role of histone modification in RA. Histones can be modified by processes such as acetylation, methylation, phosphorylation, citrullination, and others, resulting in alterations of chromatin structure and, consequently, gene transcription (***Klein et al., 2018***).

Non-coding RNAs are yet another mode of epigenetic regulation and include microRNAs (miRNAs, around 22 nucleotides) and long non-coding RNAs (lncRNAs, over 200 nucleotides), both of which have been extensively studied in RA susceptibility, severity and treatment (***Li et al., 2018***). miRNAs are non-coding RNAs that bind messenger RNA (mRNA), leading to its destruction or blocking its translation. Due to this regulation effect on gene expression, they have been the object of significant attention in areas like oncology, metabolic diseases and inflammatory arthritides (***Vicente et al., 2016***). A wealth of miRNAs have been studied in RA, of which the most established in terms of relevance for RA pathogenesis include miRNA-155, miRNA-146a, miRNA-223 and miRNA124a (***Klein et al., 2018***).

2.1.3 Criteria for rheumatoid arthritis

In 1958, the American Rheumatism Association (ARA), nowadays called the American College of Rheumatology (ACR), suggested diagnostic criteria defining definite, possible, probable, and classical RA (***Ropes et al., 1958***). In 1987 the criteria were revised and intended for the classification purposes rather than diagnostic criteria as presented in Table (2.1) (***Arnett et al., 1988***).

Table 2.1: The 1987 revised ACR criteria for classification of RA. According to these, a patient can be classified as having RA if at least four criteria are satisfied (***Arnett et al., 1988***).

Criteria
1. Morning stiffness of 60 minutes*
2. Arthritis in three or more joint areas*
3. Arthritis in hand joints (wrist, MCP, PIP) *
4. Symmetrical arthritis (wrists, MCPs, PIPs, MTPs) *
5. Rheumatoid nodules
6. Positive rheumatoid factor test
7. Typical X-ray findings in hand- or wrist joints

* Criterion 1 - 4 must have been present for at least 6 weeks.

The 1987 revised ACR classification criteria, which are still used as the golden standard, were based on patients with established disease and are rather insensitive in patients with very early disease. In RA patients with very early arthritis (< 3 months), only 52% fulfilled 4 or more criteria (***Machold et al., 2002***). New tests for antibodies against citrullinated peptides/proteins (ACPA) have shown extremely high diagnostic specificity for RA and, apart from being as sensitive as rheumatoid factor (RF), the new generation of tests for antibodies to cyclic citrullinated peptides (anti-CCP) enable early diagnosis of RA with high accuracy (***Söderlin et al., 2004 ; Avouac et al., 2006)***.

2.1.4 The Progression of Rheumatoid Arthritis:

As RA progresses, the body changes. Some changes you can see and feel, while others you cannot. Each stage of RA comes with different treatment goals.

- **Stages of RA:**

Early stage RA stage I: Is characterized by synovitis, or an inflammation of the synovial membrane, causing swelling of involved joints and pain upon motion. During this stage, there is a high cell count in synovial fluid as immune cells migrate to the site of inflammation. However, there is generally no x-ray evidence of joint destruction, with the exception of swelling of soft tissues and possibly evidence of some bone erosion (***Al-Rubaye et al., 2017***). Many people feel joint pain, stiffness, or swelling. During stage 1, there's inflammation inside the joint. The tissue in the joint swells up. There's no damage to the bones, but the joint lining, called the synovium, is inflamed (***Peng et al., 2023)***.

Stage II: In moderate RA, stage II, there is a spread of inflammation in synovial tissue, affecting joint cavity space across joint cartilage. This inflammation will gradually result in the destruction of cartilage, accompanied by a narrowing of the

joint (***Al-Rubaye et al., 2017***). Stage II is moderate-stage RA. In this stage, the synovium's inflammation causes damage to the joint cartilage. Cartilage is tissue that covers the end of bones at the site of joints. When cartilage is damaged, people may experience pain and loss of mobility. The range of motion in the joints may become limited(***Peng et al., 2023)***.

Stage III. Severe RA, stage III, is marked by the formation of pannus in the synovium. Loss of joint cartilage exposes bone beneath the cartilage. These changes will become evident on x-rays, along with erosions around the margins of the joint. Joint deformities may also become evident (***Al-Rubaye et al., 2017)***. Once RA has progressed to stage 3, it's considered severe. At this point, damage extends not only to the cartilage but also to the bones themselves. Since the cushion between bones is worn away, they'll rub together. There may be more pain and swelling. Some people may experience muscle weakness and more mobility loss. The bone can be damaged (erosion), and some deformities may occur (***Peng et al., 2023)***.

Stage IV: Is called terminal or end stage RA. The inflammatory process has subsided and the formation of fibrous tissue and or fusing of bone results in ceased joint function. This stage may be associated with the formation of subcutaneous nodules (***Al-Rubaye et al., 2017)***.

At stage 4, there's no longer inflammation in the joint. This is end-stage RA, when joints no longer work. In end-stage RA, people may still experience pain, swelling, stiffness, and mobility loss. There may be reduced muscle strength. The joints may become destroyed, and the bones become fused together (ankylosis). Progression through all four stages can take many years, and some people don't progress through all stages within their lifetime. Some people have periods of no RA activity. In some cases, this may mean that RA has gone into remission (***Peng et al., 2023)***.

2.1.5 Micro RNA

MiR-146a has been shown to play an important role in the negative regulation of inflammatory innate immune responses, and to be differentially expressed in a number of human diseases including rheumatoid arthritis (RA) (***Pauley et al., 2011)***.

miR-146a was found to be one of several miRNAs that demonstrated increased expression in response to LPS and other inflammatory stimuli in the human monocytic cell line THP-1 (***Taganov et al., 2006)***. Furthermore, it was demonstrated that miR-146a targets TNF receptor-associated factor 6 (TRAF6) and IL-1 receptor-associated kinase 1 (IRAK1), two key adaptor molecules in the TLR and IL-1 receptor signaling pathways (***Bhaumik et al., 2009)***. Since then, miR-146a has been shown to suppress NF-κB activity, suppress the LPS-induced inflammatory response (***Pauley et al., 2011)***, and play a role in the development of endotoxin tolerance (***Nahid et al., 2011)***.

Overall, it is clear that miR-146a plays a critical role in regulating inflammatory responses through a negative feedback pathway. Given this role, it is not surprising that miR-146a has been shown to be differentially expressed in a number of inflammatory autoimmune diseases including systemic lupus erythematosus (SLE) (***Wang et al., 2010)***, and rheumatoid arthritis (RA). Specifically, it has been reported that miR-146a is highly expressed in synovial tissue and synovial fibroblasts of RA patients compared to normal individuals and osteoarthritis patients (***Nakasa et al., 2008)***.

2.1.5.1 MiRNA biogenesis and function

MiRNAs are a group of evolutionarily conserved endogenous noncoding RNA molecules 22 nucleotides in length. These small RNAs have tightly regulated expression patterns and serve as posttranscriptional regulators of gene expression (***Ha***

et al., 2014). Most miRNAs are expressed in a temporal and tissue-specific manner, meaning that miRNAs are differentially expressed in various stages of organism development and that the expression of identical miRNAs may vary in different organs and tissues (***Jf ., 2006)***.

Genes encoding miRNAs in mammals are generally localized within the introns of protein-coding genes. However, there are also some miRNAs that originate from intergenic regions and act as independent transcription units (***Di Leva et al., 2014)***. The process of miRNA biogenesis involves multiple steps (Figure 2.3) comprising a canonical pathway dependent on Drosha processing and a noncanonical pathway in which pre-miRNAs can be alternatively generated via spliceosome machinery (***Krol et al., 2010)***.

Mature miRNAs mediate the inhibition of gene expression at the posttranscriptional level, which is associated with cellular function maintenance. The efficiency and mode of gene silencing depend on whether the miRNA has sufficient complementarity to the target mRNA (***Bartel et al., 2004)***. A perfect match between a miRNA and the 3′ UTR of its target leads to irreversible mRNA decay, while imperfect complementarity promotes the repression of translation at the initiation or elongation stages (***Wilson et al., 2013)***.

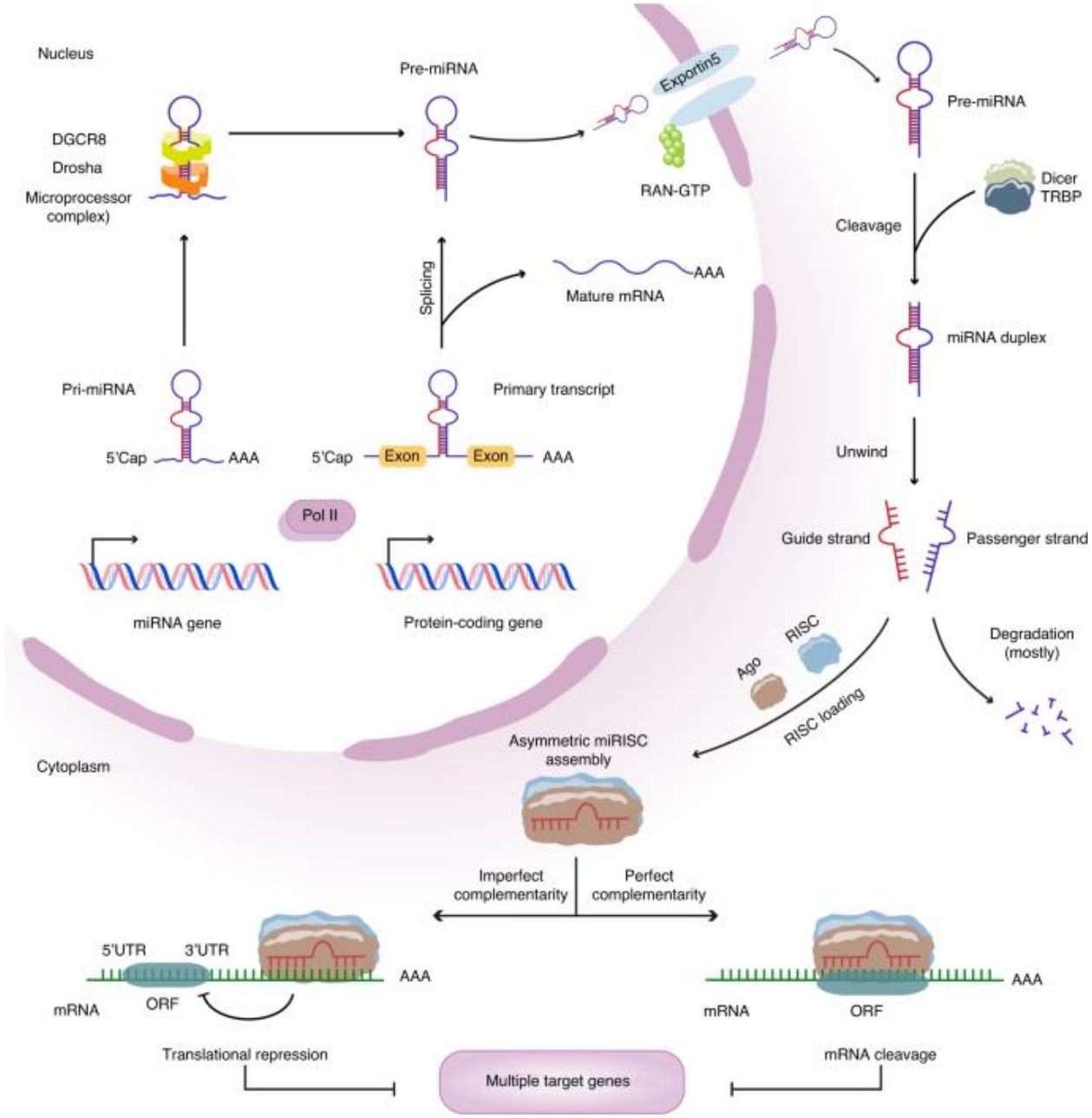


Figure 2.3: the biogenesis and mechanism of action of miRNAs (*Wilson et al., 2013)*.

2.1.5.2 miRNA Medical Applications

For the past few years, the field of miRNA biology has expanded considerably. MiRNAs have been shown to play essential roles in the developmental timing of stage-specific cell lineages and control multiple biological processes related to

development, differentiation, growth, and metabolism (***Bushati et al., 2007)***. The widely observed deregulation of miRNAs in diverse diseases, including inflammatory diseases (***Ali et al., 2021)***, has rendered miRNAs attractive candidate targets. In addition, the role of miRNAs in the development of RA has recently attracted attention, as a growing body of evidence has revealed the aberrant expression of numerous miRNAs in clinical samples and experimental models of RA. Studying deregulated miRNAs in RA will provide novel mechanistic into the progression of the disease (Figure 2.4)

This mechanism was as follows:

a. In normal synovial joints, many cell types serve as major sources of short noncoding RNAs, including miRNAs, which are transcribed from DNA. Precursor miRNAs are cleaved and processed into miRNAs, which further function within parental cells and neighboring functional cells and can be secreted into biological fluids such as blood and joint fluid as free molecules or extracellular vesicles.

b. In normal conditions in synovial joints, miRNAs are mainly produced by a variety of cells involved in the composition of bone remodeling, osteoimmunology and synovial systems. MiRNAs procedurally regulate well-organized bone remodeling, appropriate levels of immune responses and the secretion of normal synovial fluid. Conversely, persistent synovial hyperplasia, progressive inflammation, and subsequent destruction of affected joints are prominent characteristics of RA. The development, differentiation and homeostasis of diverse cell populations in the inflamed synovial compartment are dysregulated as a result of miRNA deregulation. Interactions between these abnormal cell types with disrupted function ultimately contribute to RA pathogenesis. miRNA microRNA, APC antigen-presenting cell, RANKL

receptor activator of NF-κB ligand, FLS fibroblast-like synoviocyte, MMP matrix metalloproteinase (***Peng et al., 2023)***.

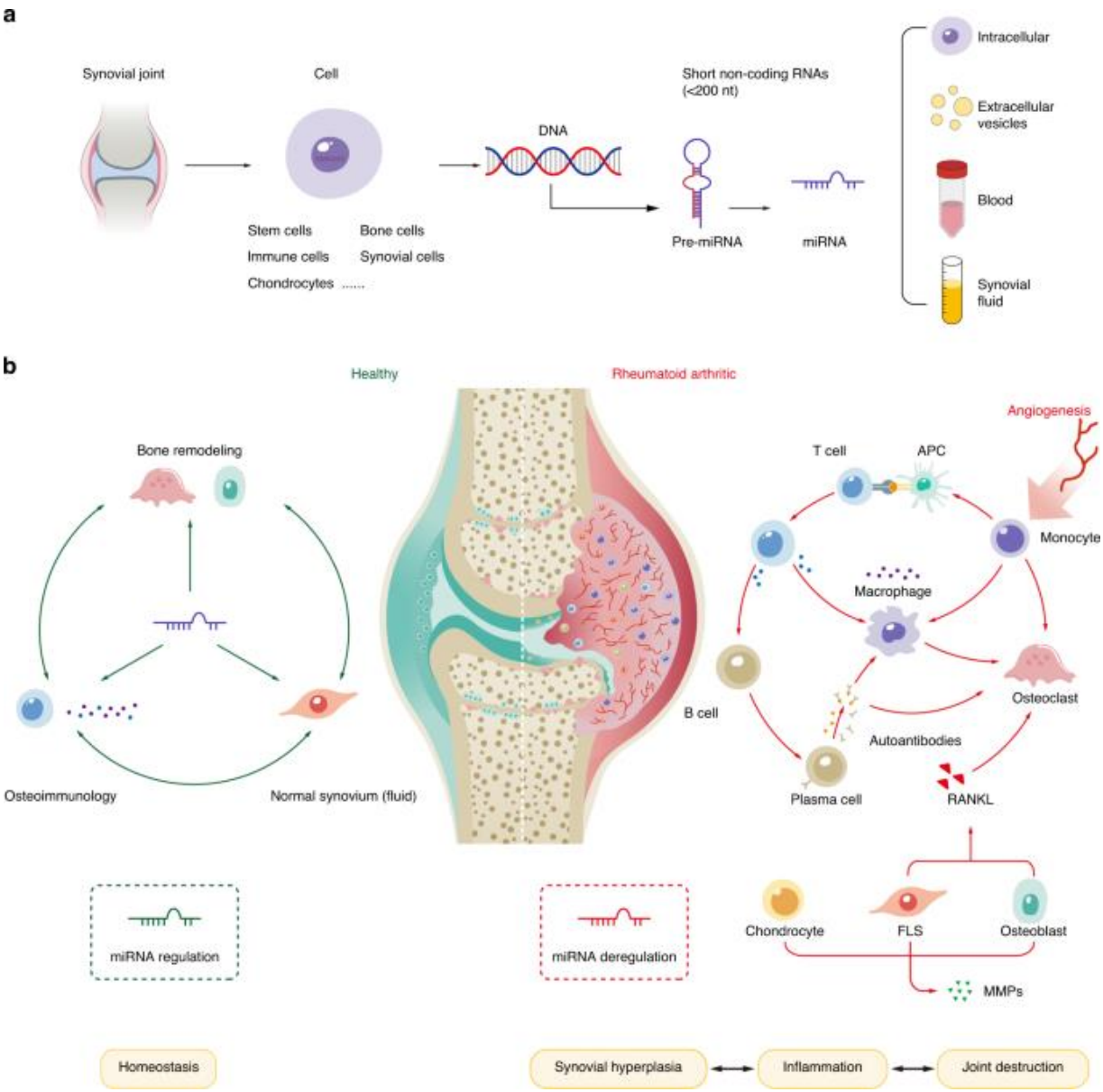


Figure 2.4: Schematic diagram of miRNAs in normal development, homeostasis, and RA progression (*Peng et al., 2023)*.

2.1.6 MicroRNA and Cytokines

Although the number of distinct mature miRNA in the Registry database (miRBase) continues to grow rapidly (***Saini et al., 2007)***. Only few miRNAs are directly associated with regulating cytokine production in that the 3' untranslated regions (3'UTRs) of many cytokines lack direct binding sites for a miRNA. Besides the direct binding of miRNA to its target 3'UTR seed region, production of many cytokines is also indirectly controlled by miRNAs through a mechanism of regulating a cluster of adenine and uridine-rich elements (AREs). The basic unit of the ARE is a pentamer of AUUUA or nonamer of UUAUUAUU. The ARE recruits several different ARE-binding proteins (ARE-BPs) that can positively or negatively regulate cytokine mRNA stability and/or translation (***Anderson et al., 2008)***. ARE-BPs such as Tristetraprolin (TTP), AU-rich binding factor 1 (AUF1), and members of Hu protein R (HUR) family are regulated by miRNAs. Currently, the ARE registry lists over 4000 genes as potential targets of post-transcriptional regulation (***Bakheet et al., 2006)***, and many cytokines contain ARE sites at their 3'UTR region (***Asirvatham et al., 2009)***.

While the cytokine expression can be regulated by miRNAs through the mechanisms of direct binding to the target or indirect controlling ARE-BPs, a number of cytokines can also regulate miRNA synthesis. For instance, IL-1ß and TNF-α are potent stimulators for induction of miR-146a and miR-155 in a variety of cells. MiR-146a is an inflammation-responsive miRNA and plays a role in regulating immune response, chronic inflammation, and cell proliferation and differentiation. Here I will focus on the role of miR-146a in regulating chronic inflammation and potential connection to the development of cancer.

2.1.7 Role of microRNA-as a mediated dysfunction in FLSs

Dysregulation of several miRNAs is responsible for the aberrant biological behaviors of FLSs in RA. Various intracellular pathways are related to the altered expression of miRNAs, and the most significantly implicated pathways are the Wnt, NF-κB, Janus kinase/signal transducer ,and activator of transcription (JAK/STAT) and TLR pathways. The first two miRNAs found to be differentially expressed in RA-FLSs were miR-155 and miR-146a (***Stanczyk et al., 2008)***. The expression of miR-155 is prominently upregulated in RA-FLSs when they are stimulated with tumor necrosis factor (TNF)-α. In addition to its widely reported proinflammatory role in immune cells, miR-155 also upregulates the secretion of inflammatory factors from RA-FLSs (***Xie et al., 2018)***. Of interest, miR-155 is assumed to protect against the acquisition of a destructive phenotype by RA-FLSs by repressing the expression of MMP3 and MMP1 (***Liu et al., 2017).*** In this regard, further research is warranted to dissect the precise role of miR-155 in RA-FLSs and identify its direct target genes and pathways. MiR-146a, a well-described epigenetic regulator in immune cells, is also expressed at high levels in RA-FLSs upon stimulation with proinflammatory stimuli such as IL-1β (***Stanczyk et al., 2008)***. It was proposed that miR-146a orchestrates the inflammatory response, suppressing the proliferation, changing the metabolic state, and restricting the osteoclastogenic potential of RA-FLSs. These actions are elicited by targeting TNF receptor associated factor 6 (TRAF6), an important component of the NF-κB pathway, and disrupting the receptor activator of the NF-κB ligand (RANKL)/osteoprotegerin (OPG) ratio. MiR-146a deficiency has been demonstrated to be responsible for aggravated inflammatory joint damage in a model of TNF-driven arthritis (***Saferding et al., 2017)***. Likewise, miR-146a potently inhibits the secretion of inflammatory factors as well as connective tissue growth factor (CTGF) by RA-FLSs. Downregulated CTGF expression limits the proliferation

of RA-FLSs and angiogenesis, thereby, to some extent, limiting pannus formation and attacking cartilage (***Sun et al., 2020).*** The levels of miR-143 and miR-145 are also augmented in RA-FLSs compared to OA-FLSs. Overexpression of miR-143 enhances TNFα-induced pro-inflammatory signals by downregulating insulin-like growth factor binding protein 5 (IGFBP5). MiR-145 regulates semaphorin 3 A (SEMA3A) to render RA-FLSs susceptible to vascular endothelial growth factor 165 (VEGF165) stimulation, contributing to cell migration and invasion (***Hong et al., 2017).*** In addition, it has been noted that miR-145-5p exacerbates RA progression by promoting nuclear translocation of p65 to activate NF-κB, which increases the levels of MMP-9 (***Wang et al., 2019)***.

2.1.8 MiRNA-based diagnostic strategies for RA

Early diagnosis and treatment of RA are the key to stopping the progression of the disease and reducing the rate of disability. Owing to the insufficient sensitivity and specificity of the early predictive indicators such as RF and ACPA, the diagnosis mainly depends on the integrated consideration of medical history, clinical manifestations, physical examination results, and radiological characteristics. In many cases, by the time a patient is conclusively diagnosed with RA, irreversible joint damage has already occurred, and the optimal time for treatment has been missed. Targeting characteristic miRNA expression profiles in RA may provide breakthroughs for early diagnosis and therapy.

As mentioned above, miRNAs that are formed and function inside cells can be released into circulating body fluids. Whether these miRNAs are retained or released depends on cell-type-specific miRNA sorting motifs (***Garcia-Martin et al., 2022***) Circulating miRNAs of distinct cellular sources are encapsulated in EVs such as exosomes and microvesicles and transferred between cells, thereby establishing

intercellular communication (***Alghamdi et al., 2022***). Likewise, some proteins, such as those in Ago2 complexes and lipoproteins, may serve as vesicle-free carriers that transfer extracellular miRNAs to recipient cells (Vickers). Because they are contained within these carriers as cargo, circulating miRNAs are resistant to degradation by endogenous RNases and thus present in body fluids in a stable form, making it relatively simple to noninvasively extract samples for detection (***Lindner et al., 2015***). Moreover, miRNA expression can be easily detected and accurately quantified by currently available techniques, including quantitative reverse transcriptase polymerase chain reaction (qRT–PCR), in situ hybridization, microarray, and small RNA sequencing. Accordingly, circulating miRNAs are attractive and promising biomarkers for clinical applications in RA, such as aiding early diagnosis, monitoring of the disease course, and prediction of treatment response.

2.1.9 Mir-146a and Chronic Inflammatory Disorders

To date, 721 human miRNAs have been identified, but only a few miRNAs have been reported that might be associated with chronic inflammation. Among these miRNAs associated with chronic inflammation, and miR-146a was found to localize at the CD68+ macrophages, CD3+ T-cells and CD79a+ B-cells at the superficial and sub-lining layers of the synovial tissue (***Nakasa et al., 2008***), These findings suggest that miR-146a is associated with not only chronic inflammation, but also innate immunity. Consistent with these reports, we have demonstrated that expression of miR-146a in response to inflammatory cytokine stimulation was significantly increased in primary human airway epithelial cells by microarray assay as well as by real time RT-PCR . Interestingly, however, miR-146a was not increased in tissues obtained from patients with other chronic inflammatory disorders such as lung biopsies from mild asthma or the skin from atopic eczema (***Sonkoly et al., 2007***).

2.1.10 Potential contribution of IL33 in rheumatoid arthritis

IL-33 was recently described as a new member of the IL-1 family, whose common characteristic is the pro-inflammatory activity (***Liew et al., 2012***). IL-33 plays an important immune role associated with Th2 response, significantly stimulating the secretion of IL-5 and IL-13 by Th2 polarized cells.

Basophils activated by immunoglobulin E (IgE) produce IL-33 and release histamine and, additionally, basophil migration also appears to be regulated by IL-33. These findings aid in the understanding of independent immune responses of antigens present in tissues that express the mRNA of IL-33, for instance, smooth muscle cells in bronchial tissue and epithelial cells of the airways (***Smithgall et al., 2008***).

Mast cells are very responsive to IL-33, which results in increased production of IL-6, IL-13, IL-1beta, TNF, prostaglandin D2 and MCP-1 (***Xu et al., 2008***). In addition, IL-33 promotes survival, adhesion, and cytokine production in human mast cells and also in mast cell progenitors (***Ali et al., 2007***).

Several studies using experimental models of arthritis have evaluated the participation of IL-33 in pictures of joint inflammation. Proposed mechanisms for joint inflammation induction by IL-33 were activation of mast cells, and therefore, the production of inflammatory cytokines; increased secretion of IL-6 and IL-1beta by activated mast cells; or CD4+ T cells stimulation that would lead to the production of IL-5 and IL-13. This latter mechanism would increase the activation of B cells and immunoglobulin production, worsening the joint inflammation process and stimulating mast cell degranulation and the formation of immune complexes with collagen. These authors also demonstrated that, in this experimental model, mast cells are important, albeit not essential for the development of arthritis (***Xu et al., 2008***).

2.1.11 Leptin and rheumatic diseases

Leptin is a 16 Kda protein discovered in 1994 by Friedman and collaborators It belongs to the class I helical cytokine family which includes growth hormone (GH), leukemia-inhibiting factor (LIF), granulocyte colony-stimulating factor (G-CSF), interleukins (IL) (***Zhang et al., 2005***).

Adipokine levels have been reported to be greatly increased in serum and synovial fluid (SF) of RA patients (***Chihara et al., 2020***). Furthermore, several studies outlined the implication of adipokines in the progression and severity of OA and the chronic inflammation in articular joints (***Hu et al., 2011***).

Leptin is the main adipokine secreted by adipose cells. It exerts its role by binding to the long isoform receptor Ob-Rb and transducing the signal through the Janus kinase/signal transducer and activator of the transcription (JAK/STAT) signaling pathway (***Wada et al., 2010***). In addition to its evident role in regulating energy homeostasis and food intake, it also has pleiotropic functions (***Kelesidis et al., 2010***). Leptin is implicated in both adaptive and innate immunity. Increasing evidence suggests that leptin exerts potent modulatory actions in the network of factors implicated in the pathophysiology of rheumatic diseases such as OA and RA (***Conde et al., 2010***).

Leptin and its receptor are associated with the stage of OA disease and related pain. Notably, high leptin concentrations in RA patients are correlated with joint pain ***(Lübbeke et al., 2007).*** mRNA expression of leptin and its receptor was higher in RA cartilage (***Simopoulou et al., 2007).***

Leptin has been described to be implicated in RA pathogenesis. However, the results of clinical studies comparing serum or SF leptin concentrations in healthy individuals and RA patients are still ambiguous. Many authors have reported significant elevation

of serum and SF leptin levels in RA patients compared to healthy controls (***Chihara et al., 2020)***, Popa and collaborates reported, in addition, that plasma leptin concentrations were inversely correlated to inflammatory markers in RA patients suggesting that chronic inflammation in RA decreases leptin production (***Popa et al., 1999***).

The role of leptin in RA is not only associated with articular tissues, it might also have a potent effect on cell-mediated immune function (***Fraser et al., 2005***).

2.1.12 Knowledge Gap about the Role of MicroRNAs in RA

- The role of miRNAs in RA still remains to be elucidated. Yet, based on the available data and the overall role of miRNA molecular machinery, it is possible to gain some insight into their participation in RA. Hence, there are some general concepts governing miRNA physiology.

- Their role depends mainly on the target gene. This means that if a gene has an enhancing or suppressive effect on a certain physiological procedure, the down- or up-regulation of the respective miRNA signifies the opposite effect.
- Further on, although it is known that miRNA expression and binding to target-genes are linked to gene negative regulation, the only way to determine miRNA role is through experimental validation; this varies from one pathophysiological condition to another.

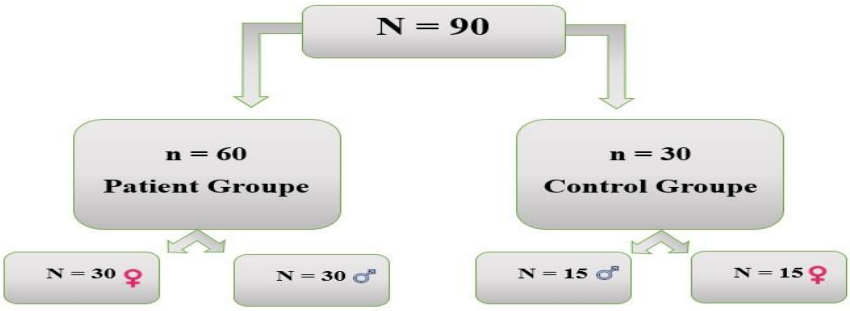
Chapter Three

Methodology

3. Methodology

3.1. Study Design:

The present study included a case-control study for a group of (90) samples: (60) patient samples, and (30) healthy control samples. The study was conducted from October 2022 to September 2023. Patients with Rheumatoid arthritis were selected from Imam Hassan al-Mujtaba Hospital in Karbala. family History, smoking state, job, duration of disease weight and height were taken from each participant. The sociodemographic aspects of the patients were collected through the self-reported technique (student questionnaire) including age, gender, BMI, and any current chronic diseases. They were also exposed to a medical examination for signs and symptoms of rheumatoid arthritis by Specialist doctors based on the World Health Organization (WHO) criteria.



3.2 Instruments:

In this chapter, materials, and instruments were described and listed in Tables (3-1) and (3-2)

Table (3-1): The Tools & instruments used in the study :

NO.	Instruments	Suppliers
1	Centrifuge	Germany
2	Deep freezer	Lebanon
3	Pipette(100-1000µl)	United states
4	Micropipette(10-100 µl)	United states

5	ELISA system	Humen germany
6	Blend Tubes(10ml)	China mheco \china
7	Eppendroff Tubes	China mheco \china
8	Gilson Tips, 100µl -1000µl (blue)	China mheco \china
9	Jell tube	China mheco \china
10	Eppendroff Tubes centrifuge	Germany

Table (3-2): The materials used in the study:

Materials		
1	IL-33 ELISA kit	Bioassay technology laboratory/china
2	human Leptin hormone ELISA kit	Bioassay technology laboratory/china
3	Micro RNA kit	Bioassay technology laboratory/china

3.3.1. Patients Criteria:

All patients were subjected to the full clinical history, clinical examination, and relevant laboratory investigations. The diagnosis of the arthritis clinical conditions was established according to the latest clinical practice guidelines by the WHO. The degree of rheumatoid was identified based on the evaluation of laboratory measurements for the clinical assessment of rheumatoid arthritis.

3.3.2 Control Criteria:

The control group of apparently healthy 30 subjects (15 male and 15 female) were chosen from well-known volunteer participants. Blood samples were drawn from the volunteers, who had no history of rheumatoid arthritis diseases. The percentage of female and male adult individuals were about the same in the patient group. The ages of the participants were also convergent in the whole study group.

Demographic information of the participants was also collected through the self-reported technique (student questionnaire).

3.4 Study variables :

3.4.1 Dependent Variable :

MicroRNA146a, Leptin, IL33

3.4.2 Independent Variable

Age, Gender, smoking state, BMI, and duration of disease

3.5 Approval of the Ethical Committee:

A valid written, signed consent was achieved from the hospital's administration, and valid verbal consent from each patient and control subject before their inclusion in the study. The procedure had been informed before the samples were collected, making sure that they understood the procedure that was to be carried out. The subjects were sentient and they had the right to reject to be included in the study without any detrimental effects. Permission was taken from all subjects of the control group after they were told about the aim and advantages of this study. The protocol of the study was approved by the Ethical Committee of Kerbala College, and the centre of Imam Hassan al-Mujtaba Hospital.

3.6 Measurement and Data Collection:

3.6.1 Data Collection:

A structured questionnaire was specifically designed to obtain information that helps to select individuals according to the selection criteria of the study. Sociodemographic aspects of the subjects (patients and control) were also collected through the self-reported technique (student questionnaire) which included: age,

gender and BMI, smoking state, chronic disease, and duration of disease(for the patient).

3.6.2 Blood Collection and Storage:

Five ml of blood samples were drawn by venipuncture using 5 ml disposable syringes, 4 ml blood was left for (15 min) at room temperature in a gel tube. Serums were separated by centrifuging for 10 minutes at approximately 4000 xg. Serum samples were aliquot into two Eppendorf tups and stored at -20°C to avoid multiple freezing-thawing cycles and used for further measurement. Blood collection tubes were disposable, non-pyrogenic, and non-endotoxin.

3.7 Methods:

Determination of Body Mass Index:

The body mass index (BMI) was calculated by the following equation:

$\boldsymbol{BMI}$=W$\boldsymbol{eight}$ ($\boldsymbol{kg}$)/H$\boldsymbol{eight}$2 (meters)

The body mass index (BMI) is the metric currently in use for defining anthropometric height/weight characteristics in adults and for classifying (categorizing) them into groups as shown in Table (3-3).

Table (3-3) Body mass index.

Weight status	BMI range (kg/m^2)
Underweight	15-19.9
Normal weight	20-24.9
Overweight	25-29.9
Class I obesity	30-34.9
Class II obesity	35-39.9
Class III obesity	≥ 40

3.8 Measurement of IL3 level by using ELISA Technique:

Enzyme-Linked Immunosorbent Assay system (ELISA) was performed using Sandwich-ELISA method to measure the concentrations of serum IL-33

Principle

This ELISA kit uses the Sandwich-ELISA principle. The micro-ELISA plate provided in this kit has been pre-coated with an antibody specific to Human IL-33. Standards or samples are added to the micro-ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for Human IL-33 and Avidin-Horseradish Peroxidase (HRP) conjugate are added successively to each micro plate well and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain Human IL-33, biotinylated detection antibody and Avidin-HRP conjugate will appear blue in color. The enzyme-substrate reaction is terminated by the addition of stop solution and the color turns yellow.

The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The OD value is proportional to the concentration of Human IL-33. You can calculate the concentration of Human IL-33 in the samples by comparing the OD of the samples to the standard curve

Kit reagents:

The ELISA kit applied for the quantitative determination of IL33 concentration in serum, list of reagents was shown in Table (3-4).

Table (3-4): Reagents of determination IL33 concentration.

Components	Quantity (96)	Quantity (48T)
Standard Solution (2400ng / L)	0.5 ml×1	0.5 ml×1
Pre - coated ELISA Plate	12 *8 well strips ×1	12 *4 well strips ×1
Standard Diluent	3 ml×1	3 ml×1
Streptavidin - HRP	6 ml×1	3 ml×1
Stop Solution	6 ml×1	3 ml×1
Substrate Solution A	6 ml×1	3 ml×1
Substrate Solution B	6 ml×1	3 ml×1
Wash Buffer Concentrate (25x)	20 ml×1	20 ml×1
Biotinylated Human IL - 33 Antibody	1 ml×1	1 ml×1
User Instruction	1	1
Plate Sealer	2 Pics	2 Pics
Zipper bag	1 Pics	1 Pics

3.9 Samples and Reagents Preparation

Reagents Preparation:

Stock solutions were prepared based on the procedure of the manufactured kit. All reagents were prepared freshly at room temperature before used. The dilution of standard was shown in Table

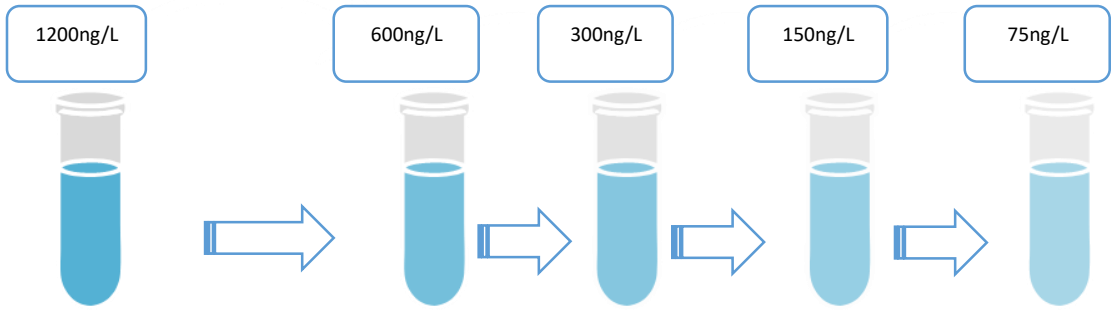
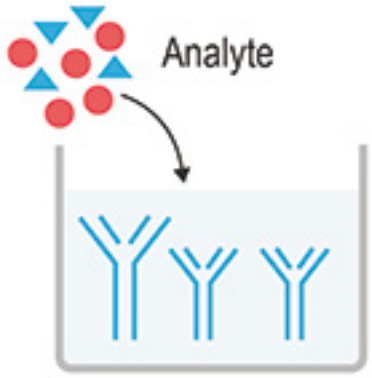


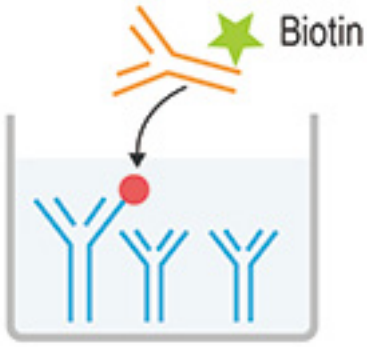
Table (3-5) : Dilution of Standards

1200 ng/L	Standard No.5	120µl Original Standard + 120µl Standard diluents
600 ng/L	Standard No.4	120µl Standard No.1 + 120µl Standard diluents
300 ng/L	Standard No.3	120µl Standard No.2 + 120µl Standard diluent
150 ng/L	Standard No.2	120µl Standard No.3 + 120µl Standard diluent
75 ng/L	Standard No.1	120µl Standard No.4 + 120µl Standard diluent

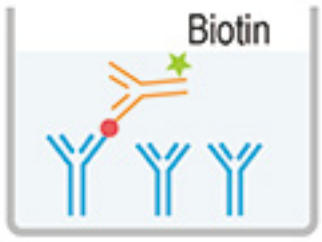
Assay Procedures



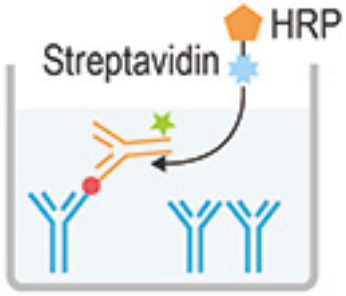
1. A100µL standard or sample were added to the wells and Incubate for 90 min at 37°C



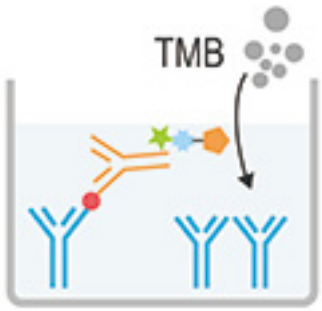
2. Then, A 100µL Biotinylated Detection Ab working solution was added to each well. Incubate for 60 min at 37°C



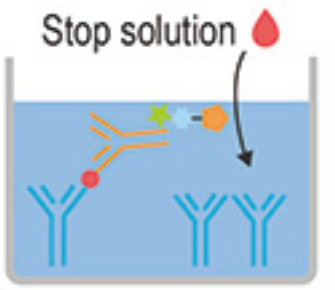
3. The plate was Aspirated and washed for 3 times



4. Then, A100µL HRP conjugate working solution was added. Incubated for 30 min at 37°C. The plate was Aspirated and washed for 5 times



5. A 90µL Substrate Reagent was added . Incubate for 15 min at 37°C



6. A50µL Stop Solution was added

7. The plate was read at 450nm immediately.
Calculation of the results

Calculation of Result

Construct a standard curve by plotting the average OD for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis and draw a best-fit curve through the points on the graph. These calculations can be best performed with computer-based curve-fitting software and the best-fit line can be determined by regression analysis.

3.10 Measurement of serum Human leptin using ELISA Technique:

This competitive enzyme immunoassay kit is for the accurate quantitative detection of human leptin in serum samples

Assay Principle:

Leptin-Ab ELISA kit applies the competitive enzyme immunoassay technique utilizing a Leptin antigen and an Leptin-Ab-HRP conjugate. The assay sample and buffer are incubated together with Leptin-Ab-HRP conjugate in pre-coated plate for one hour. After the incubation period, the wells are decanted and washed five times. The wells are then incubated with a substrate for HRP enzyme. The product of the enzyme-substrate reaction forms a blue colored complex. Finally, a stop

solution is added to stop the reaction, which will then turn the solution yellow. The intensity of color is measured spectrophotometrically at 450nm in a microplate reader. The intensity of the color is inversely proportional to the Leptin-Ab concentration since Leptin-Ab from samples and Leptin-Ab-HRP conjugate compete for the Leptin antigen binding site. Since the number of sites is limited, as more sites are occupied by Leptin-Ab from the sample, fewer sites are left to bind Leptin-Ab-HRP conjugate. A standard curve is plotted relating the intensity of the color (O.D.) to the concentration of standards. The Leptin-Ab concentration in each sample is interpolated from this standard curve.

Reagents :

The ELISA kit applied for the quantitative determination of leptin concentration in serum, list of reagents were shown in **Table (3-6)**

Table 3-6: Reagent Provided

Components	Quantity
Standard Solution (960U/L)	0.5ml x1
Pre-coated ELISA Plate	12 * 8 well strips x1
Standard Diluent	3ml x1
Streptavidin-HRP	6ml x1
Stop Solution	6ml x1
Substrate Solution A	6ml x1
Substrate Solution B	6ml x1
Wash Buffer Concentrate (25x)	20ml x1
Biotinylated human leptin Antibody	1ml x1
User Instruction	1
Plate Sealer	2 pics
Zipper bag	1 pic

Samples and Reagents Preparation

Samples Preparation

- Serum samples were allowed for 10-20 minutes at room temperature.
- Wash Solution - Dilute 10 mL of Wash Solution concentrate (100×) with 990 mL of deionized or distilled water to prepare 1000 mL of Wash Solution (1×).

ASSAY PROCEDURE

- A 100 µL of Standards / Samples were added to the appropriate well in the antibody pre-coated Microtiter Plate, then mixed with 100 µL of PBS (pH 7.0-7.2) in the blank control well.
- After that, 50 µL of Conjugate was added to each well (NOT blank control well). Mix well.
- Then , the plate was covered and incubated for 1 hour at 37°C. The microtiter plate was washed using one of the specified methods indicated below: Automated Washing: Wash plate FIVE times with diluted wash solution (350-400 µL /well/wash) using an auto washer. After washing, dry the plate.
- A 50 µL Substrate A and 50 µL Substrate B were added to each well including blank control well, subsequently. Cover and incubate for 10-15 minutes at 20-25°C.
- A50 µL of Stop Solution was added to each well including blank control well. Mix well.
- Determination the Optical Density (O.D.) at 450 nm was performed using a microplate reader immediately

Calculation of Results

1. The standard curve is used to determine the number of samples.
2. An average of the duplicate readings for each standard and sample were used. All O.D. values are subtracted by the mean value of blank control before result interpretation.
3. Construct a standard curve by plotting the average O.D. for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis, and draw a best fit curve e using graph paper or statistical software to generate a four-parameter logistic curve-fit or linear regression curve. An x-axis for the optical density and a y-axis for the concentration is also a choice. The data may be linearized by plotting the log of the concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis.
4. The concentration of samples corresponding to the mean absorbance from the standard curve was calculated

3.11 Molecular Analyses

3.11.1 MicroRNA analysis

The TransZol Up Plus RNA Kit (Cat#ER501-01, Trans, Beijing, China) was used for total RNA extraction according to the manufacturer's instructions. Total RNA was purified using an RNAClean XP Kit and RNase-Free DNase Set.

Purified total RNA was subjected to quality inspection using a NanoDrop ND-2000 spectrophotometer and Agilent Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA, US).

A: Extraction of microRNAs from the blood

All reagents were brought to room temperature (25ºC) for 30 minutes before each run. To protect the samples from contamination, the workplace was sterilized, disposable gloves (free of powder) were changed at each miRNA extraction step and work was done quickly during all steps. The analysis steps were modified according to our lab conditions to get the required results. Optimization was done for each step to meet a good yield.

1- Lysis done by using 1mL RNAzol RT per (0.5) mL of blood, letting it stand at room temperature for (30) min and centrifuged.

2. The aqueous(upper) phase is transferred to a fresh tube. Centrifuged a second time the aqueous phase is transferred to a clean tube.

3. A volume of 0.4 mL of RNase-free water is added per mL of RNAzol RT used for homogenization. The sample is covered tightly, shacken vigorously for (60) seconds, and allowed to stand for (30) minutes at room temperature.

4. The resulting mixture is centrifuged at 12,000 x*g* for (15) minutes at 4 °C. Centrifugation separates the mixture into a semisolid pellet (containing DNA, proteins, and polysaccharides) and an upper supernatant (containing RNA). The

supernatant is transferred to a new tube, leaving a layer of the supernatant above the DNA/protein pellet.

5. The supernatant is transferred to a fresh tube and a volume of 0.4 mL of 75% ethanol is added to precipitate mRNA.

6. The sample is allowed to stand for 10 minutes at room temperature.

7. Centrifugation is done at 12,000 x*g* for 8 minutes. The mRNA precipitate will form a white pellet on the side and bottom of the tube.

8. The supernatant was transferred to a clean tube, while being sure not to disturb the pellet.

9. A volume of 0.8 ml of 100% isopropanol was added to the supernatant.

10. Sample was allowed to stand for (24 hrs).

11.Centrifugation was done at 12,000 x*g* for 15 minutes. The micro RNA precipitate will form a white pellet on the bottom of the tube.

12.microRNA pellet was washed **twice** with 0.6 mL 75% ethanol (v/v) and 70% isopropanol (v/v), respectively, per 1mL of supernatant used for precipitation. Then centrifugation at 8,000 x*g* for 3minutes at room temperature. Alcohol solution was removed with a micropipette.

13.The RNA pellets were solubilized, **with drying**, in RNase-free water. the samples were Vortexed at room temperature for 5 minutes.

B. Gene Expression Analysis

RNA Measurement kit:

The assay is highly selective for miRNA over other types of RNA and is accurate for initial sample concentrations from 10 pg/µL to 100 ng/µL. The assay is performed at room temperature, and the signal is stable for 3 hours. Common contaminants such as salts, free nucleotides, solvents, detergents, or protein are well tolerated in the assay. The standard and short procedure showing in Figure (3-1).

- The Qubit working solution was prepared by diluting the Qubit miRNA HS Reagent 1:200 in Qubit miRNA HS buffer.
- The volume 190 µL from Qubit working solution has been added to each tube designed to be as a standard, then 10 µl from each provided standard solution has been added into the same tubes, then vortexed.
- The Qubit® working solution of 197 µL has been added to each tube prepared for sample and then 3 µL of the sample has been added individually.
- All composition has been vortexed and incubated at room temperature for 3 minutes.
- Standards tubes have been inserted in Qubit instrument for creating concentration curve.
- Tubes for samples have been added one by one to read the concentration for miRNA in each sample.

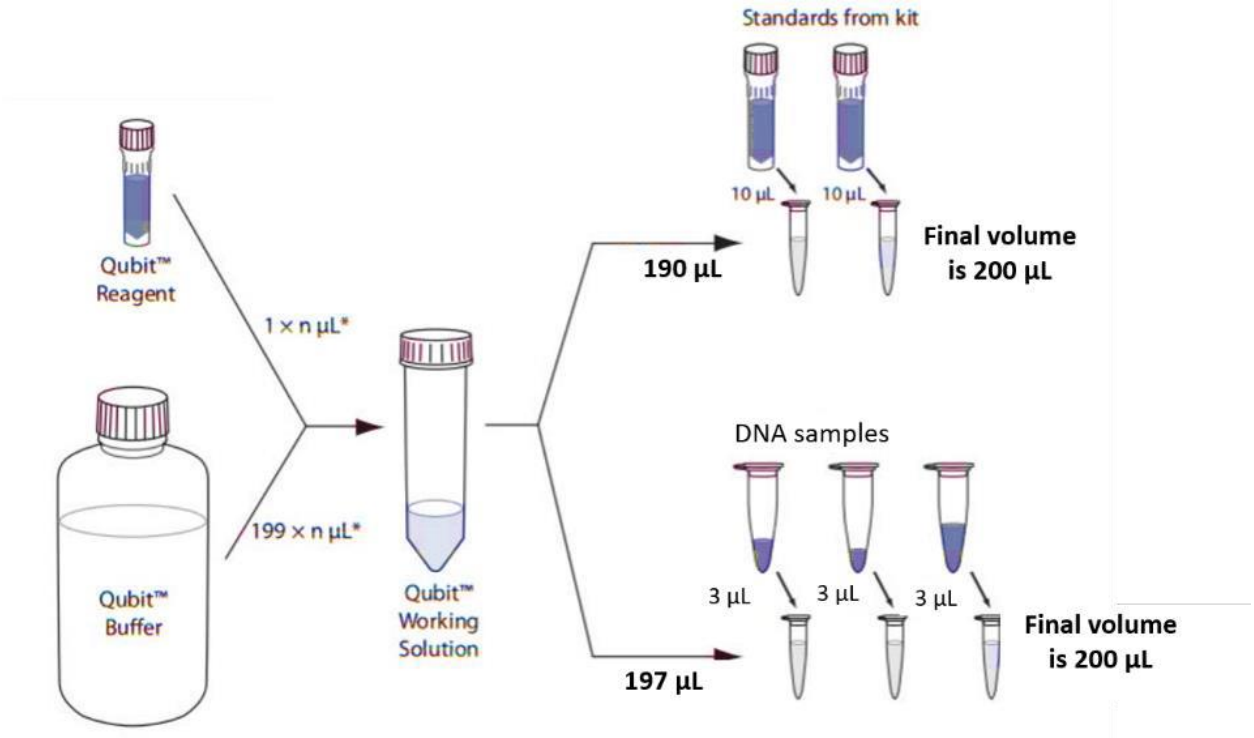


Figure (3.1): Standard procedure for nucleic acid quantification

3.11.2 Real-Time Quantitative PCR (qPCR)

The real time polymerase chain reaction (qPCR) system is based on fluorescent light measurement to determine the amount of complementary DNA (cDNA) of a specific gene. Isolation of total RNA from samples in and Reverse Transcription with the High-Capacity complementary (cDNA) Kit which done according to the kit instruction as the volumes which mentioned in table (3-13). Then submit to Real Time Quantitative PCR (qPCR) reaction.

3.11.3 cDNA Priming

Priming of the cDNA reaction from the RNA template can be done using random primers, oligo-dT, or target-specific primers and the choice of primer can cause marked variation in calculated mRNA copy numbers

3.11.4 RNA Reverse Transcription

To test the expression of PCR target RNA, the method of reverse transcription involves the conversion of RNA to cDNA. All RNA species were converted into cDNA, using oligo-dT primers they were reverse transcribed into cDNA. The oligo-dT primers carry a universal tag sequence on the 5' end to allow amplification of mature miRNA in the step of real-time PCR. Total RNA containing miRNA was used as row material for reverse-transcription reaction. The PCR tube microfuge was used and reverse transcription master was added then gently mixed, the master mix for reverse transcription contains all materials required in cDNA first-strand synthesis except template RNA. Template RNA was added to each tube containing reverse transcription master mix, gently mixed, briefly centrifuged, by using thermal cycler the tubes were incubated for 60 min at 37ºC, then 5 min at95ºC to inactivate reverse transcriptase.

The best way to measure RNA purity is to use a spectrophotometer. This measurement is based on the RNA's absorption of light at wavelengths of 260 and 280 nm. Whereas pure RNA has an absorption ratio of 260/280, ranging between 1.8 and 2.0. That is, the optical density (OD) of RNA at a wavelength of 260 nm should be twice the optical density at a wavelength of 280 nm.

If the absorbance ratio of 260/280 is less than 1.8, the sample contains protein contaminants. If the ratio is higher than 2.0, it means that the sample contains other contaminants, such as organic or mineral impurities.

The ideal ratio of 260/280 absorption for pure RNA is 1.8 to 2.0. That is, the optical density (OD) of RNA at a wavelength of 260 nm should be twice the optical density at a wavelength of 280 nm.

3.11.5 Housekeeping Gene

Quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) is a rapid and sensitive approach to identify mRNA and protein-coding gene expression. However, because of the specially designated reverse transcription and shorter PCR products, very few reference genes have been identified for the quantitative analysis of mRNA expression, and different internal reference genes are needed to normalize the expression of mRNA genes. Therefore, it is particularly important to select the suitable common reference genes for normalization of quantitative PCR of mRNA. Glyceraldehyde-3-phosphate dehydrogenase (gapdh) is one of the most commonly used housekeeping genes used in comparisons of gene expression data.

3.11.6 Detection of miRNA by qPCR

I. Reaction Setup and Thermal Cycling Protocol

The total RNA containing miRNA was the starting material in RT-PCR reaction which was performed in two steps. Due to its short sequence the quantifcation of miRNAs by qRT-PCR requires extending the length of mature miRNAs using stem-loop or adding poly (A)-tails.

II. Two Step RT-PCR

The miRNA gene miRNA146a and U6 (HKG) expression was done by using specific primers as shown in Table (3.7).

Table 3.7: Primer Sequence for miRNA Gene Expression(Designed by NCBI)

Gene	Specific Primers
rnu6 RT-primer	5`-GTTGGCTCTGGTGCAGGGTCCGAGGTATTCGCACCAGAGCCAACAATCAG-3`
rnu6	F 5`- (CTCGCTTCGGCAGCACA) 3` R 5`- (AACGCTTCACGAATTTGCGT) 3`
miR-146a RT-primer	miR-146a RT 5`-(GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTGCACTGGATACGACAACCCA) 3`
miR-146a	F 5` ( GGGTGAGAACTGAATTCCA) 3` R 5` ( CAGTGCGTGTCGTGGAGT ) 3`

3.11.7 Gene Expression Calculation

The result was collected and analyzed by Livak formula

Folding =2-ΔΔCT
ΔΔCT =ΔCT patients - ΔCT control
ΔCT =CT Gene - CT House Keeping gene

3.11.8 Real-Time Quantitative PCR (qPCR) conditions

It was performed for both the cDNA sample from the patient and control at the same run, for each sample there are two PCR tubes one for our target miRNA 146a and a second tube for U6 snRNA which consider a housekeeping gene in this study. The detection of quantity based on the fluorescent power of evergreen. The reaction mix composed from the component with their quantity as mentioned in the table below:

Table 3.8: Real-Time Quantitative PCR (qPCR) materials reaction

Material	Volume
Master mix	10
Forward	1
Reverse	1
cDNA	5
D.W	3
Total	20

Quickly spin for PCR tubes to remove the bubbles and collect the liquid (1 minute at 2000g, then the program for Real-Time PCR was setup with indicated thermocycling protocol as shown in table (3.9):

Table 3.9: PCR protocol

Cycle Step	Temperature	Time	Cycles
Initial Denaturation	95^0C	60 seconds	1
Denaturation Extension	95^0C 60^0C	15 seconds 30 seconds (+plate read)	40-45
Melt Curve	60-95^0C	40 seconds	1

3.12 Statistical Analysis:

Information from the questionnaire and all test results from patients and control samples were entered a data sheet.

The data analysis for this work was generated using the Real Statistics Resource Pack software for Mac (Release 7.2) of the resource pack for Excel 2016. Copy- right (2013 – 2020).

Descriptive statistics was performed on the participants' data of each group. Values were presented as a median for abnormal distribution, and n (%) for categorical variables, respectively. The distribution of the data was checked for normality using the Box plot test.

T- test was used to adjust other risk factors including: age, gender (male, female), BMI. The 95% confidence intervals (95%CI) were also determined for all variables.

Significant differences in continuous variables among the parameters were confirmed through analytical statistical tests. The biomarker were compared to evaluate the relationship between parameters. Results of all hypothesis tests with p-values <0.05 (two-side) were considered to be statistically significant.

Receiver operating characteristics (ROC) curves was also used to test the markers' diagnostic performance in both Rheumatoid arthritis and control groups.

Chapter Four

Results

4. Results

4.1 MicroRNA analysis

4.1.1 Demographic and clinical characteristics

A total of 90 participants were included in this study, 60 samples of Rheumatoid arthritis patients and 30 samples of normal cases as healthy control. The sample size was determined using the formula of calculating equation based on the latest reported prevalence. An interview was conducted to collect the participants history and demographic information

Patients' groups were divided into subgroups based on Age, gender, Duration of disease, and (BMI). The clinical demographic characteristics and laboratory parameters of the study groups were summarized in Table (4.1). The mean level of age in the patients' group was 51.67 years. The age range of participants was (25%) (26 - 40) years old, (36.6%) of the patients were within (41 -55) years, while (38.3%) of the patients were within the age range (more than 55 years).

Also, the analysis of data illustrated that about (42.5 %) of patients were having a duration of disease as (1-6) years, (25%) were having a duration less than one year, and 11.66% of the patients group were having duration (9-12) years, as shown in Figure (4.1)

Table 4.1: Descriptive of the demographic characteristics of the study population (N=90).

Variable	Groups	Patient N=60	Control N=30
Age. Groups	26-40 Years	15	7
	41-55 Years	22	15
	More than 55 Years	23	8
BMI.groups	Normal weight	5	9
	Over weight	15	19
	Obesity	40	2
Gender	Male	30	15
	Female	30	15

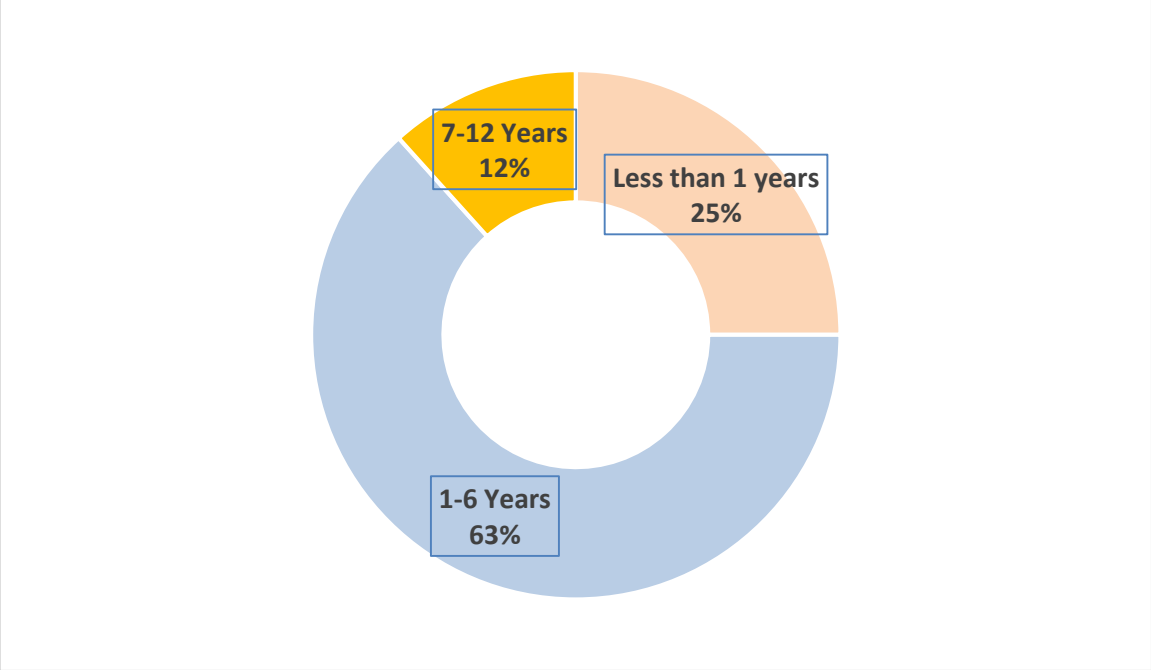


Figure 4.1: Baseline characteristics and Demographic Descriptive of the study population in patients , the number of patients for the duration of disease (n=60)

4.1.2 MicroRNA-146a in Rheumatoid arthritis

Many lines of evidence have suggested that miRNAs can be considered a promising candidate for the next generation of diagnostic biomarkers and therapeutic targets since there is a strong correlation between the status of disease and miRNA expression patterns (***Iorio and Croce., 2012***). Ongoing research on miRNAs has potential implications in the diagnosis and treatment of RA. Their different levels in peripheral blood and synovial fluid between RA patients and healthy population makes them candidates for being used as biomarkers of such disease. Therefore, this study examined the associations of RA with the expression levels of serum miRNA-146a.

4.1.3 Difference between the level of MicroRNA-146a in the Rheumatoid arthritis disease and control groups

Generally, patients with Rheumatoid arthritis disease were shown an increasing range level of the fold change of MicroRNA-146a when compared to the healthy control groups, while the range level of Ct U6 and miRNA-146 a. were decreased compared to healthy control. Results was indicating a significant difference in fold change level among groups, The means were presented in (figure 4.2). The mean levels of Micro146 in patients was (8.49) which was significantly higher than for the Control group (2.07),($p \leq 0.001$), while the mean level of Ct U6 and miRNA-146 a were (17.58) and (36.57) respectively for the patient and (18.79)and (39.09) for control.

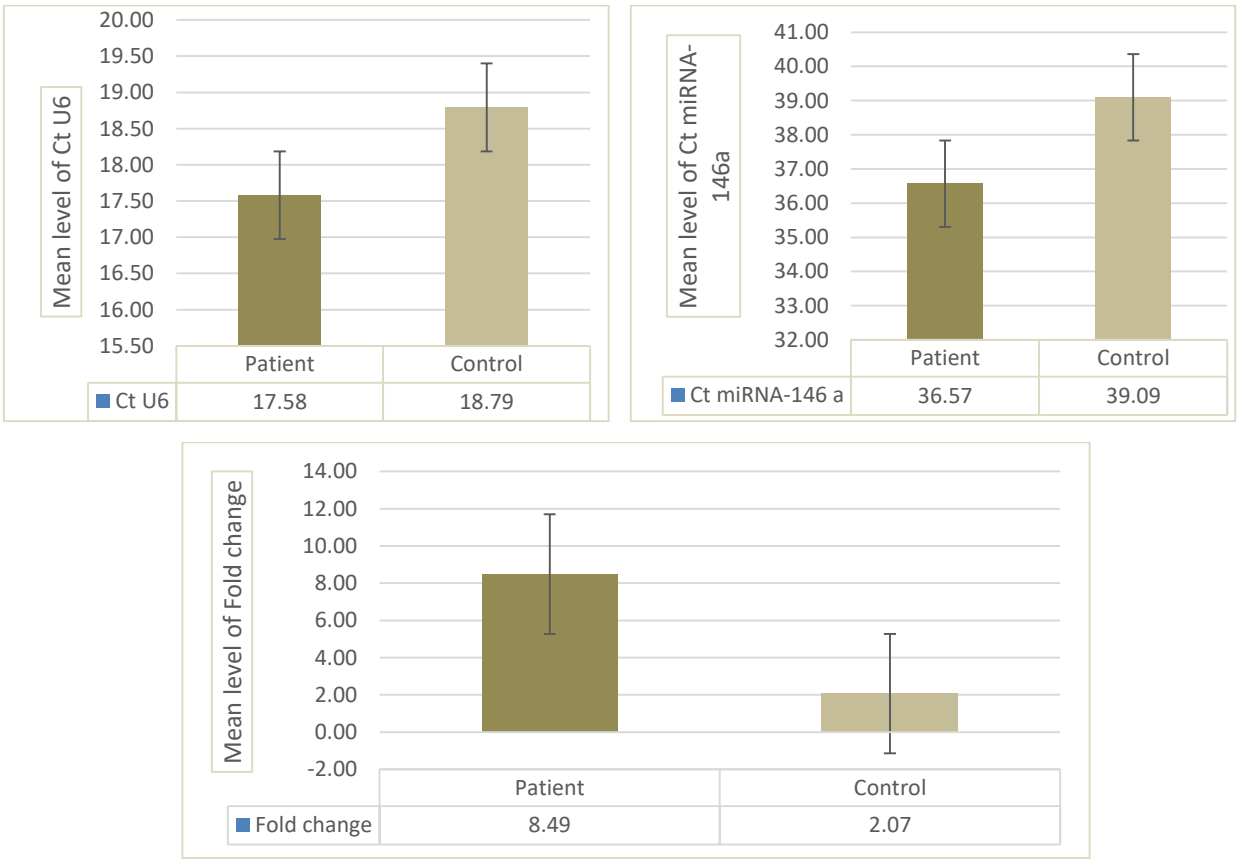


Figure 4.2: Results of the analysis of basic rheumatic arthritis for patients with control groups (T-test was S= significant at $p \leq 0.05$, NS= Non-significant).

4.1.4 Examination of the measured microRNA-146a according to the Age groups

In Figure 4.3: a comparison of serum levels of Ct U6 and miRNA-146 a in different age groups was performed. Both markers were decreased significantly within all age ranges.

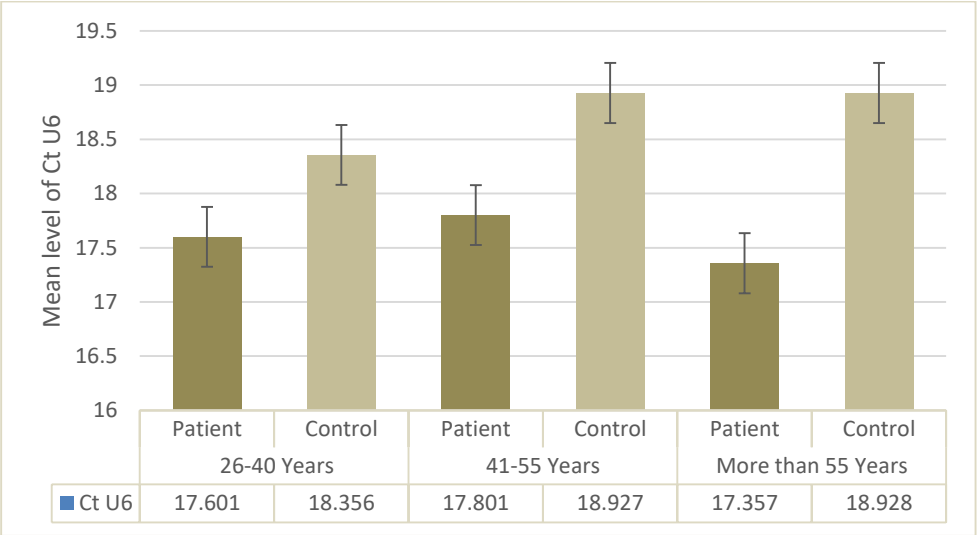


Figure 4.3: The effect of age groups on the Ct U6 according to the patient and control groups (T-test was S= significant at p ≤ 0.05, NS= Non-significant).

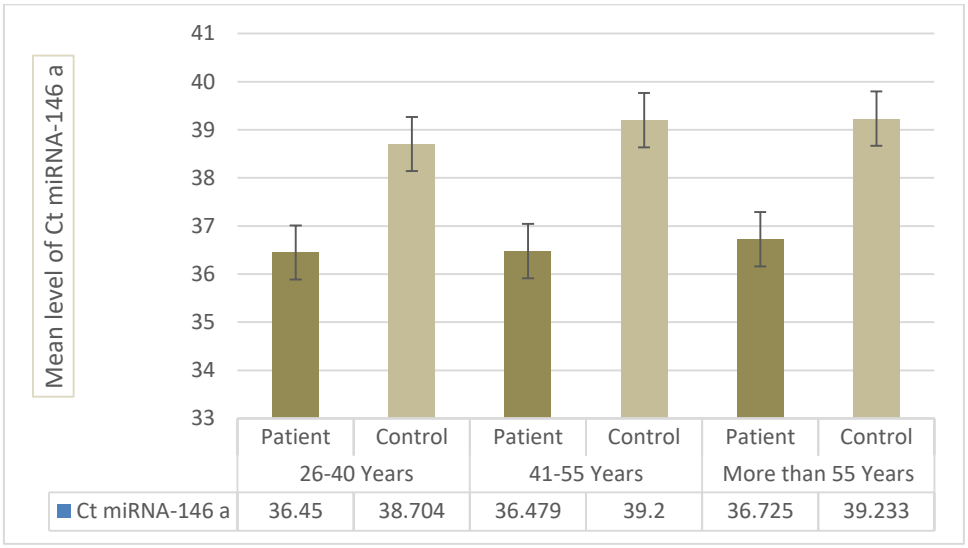


Figure 4.4: The effect of age groups on the miRNA-146 an according to the patient and control groups (T-test was S= significant at p ≤ 0.05, NS= Non-significant).

On the other hand, In Figure (4.5) a comparison level of miRNA-146 a folding change in different age groups were increasingly within the age range (26-40) and (41-55) years ($p<0.05$), but, in the range age more than 55 years was insignificant ($p>0.05$).

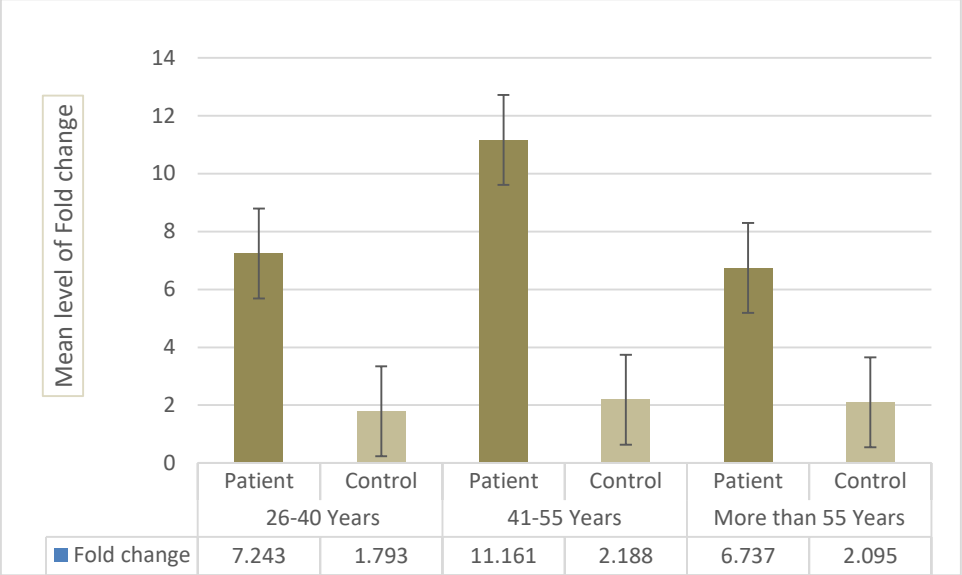


Figure 4.5: The effect of age groups on the fold change according to the patient and control groups .

4.1.5 Examination of the Measured MicroRNA-146a According to the BMI Groups

MicroRNAs have been implicated in the pathogenesis of rheumatoid arthritis (RA), obesity, and altered metabolism. Although RA is associated with both obesity and altered metabolism, the expression of RA-related microRNA in the setting of these comorbidities is unclear (***Andonian et al., 2019***).

Despite of the revolutionary progress in the management of RA inflammation over the past few decades, patients with RA are still at the high risk for sarcopenic obesity-decreased skeletal muscle mass with increased fat mass which contributes to increase in the risks of disability, and other complications (***Biolo et al., 2014***), therefore, this study investigates the microRNA-146a according to the BMI groups. In Figure (4.6) a comparison of serum levels of Ct U6 in different BMI groups was performed. The level of Ct U6 and **microRNA-146a were** decreased within all the BMI groups with highly statistically significant values ($p<0.05$)

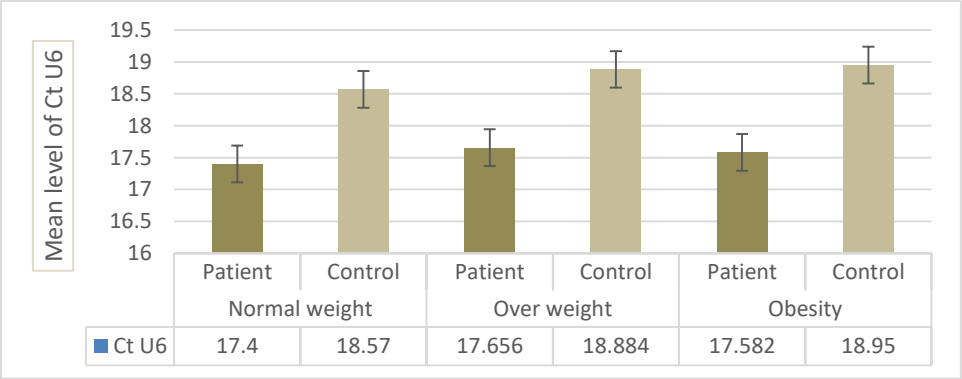


Figure 4.6: Comparison of U6 expression (Ct mean values) according to the BMI of patient and control groups .

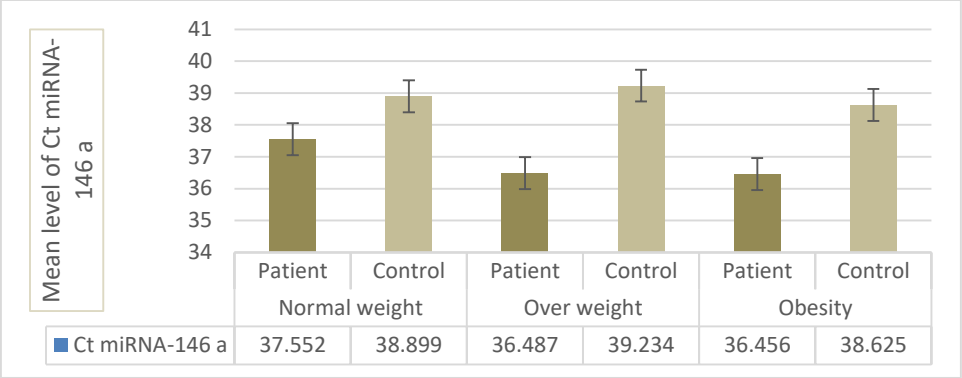


Figure 4.7: Comparison of microRNA-146a (Ct mean values) according to the BMI of patient and control groups (T-test was S= significant at p ≤ 0.05, NS= Non-significant).

In Figure (4.8) a comparison of serum level of miRNA-146a fold change in different BMI groups was also performed. The level of folding change showed a massive increase seen with increasing BMI.

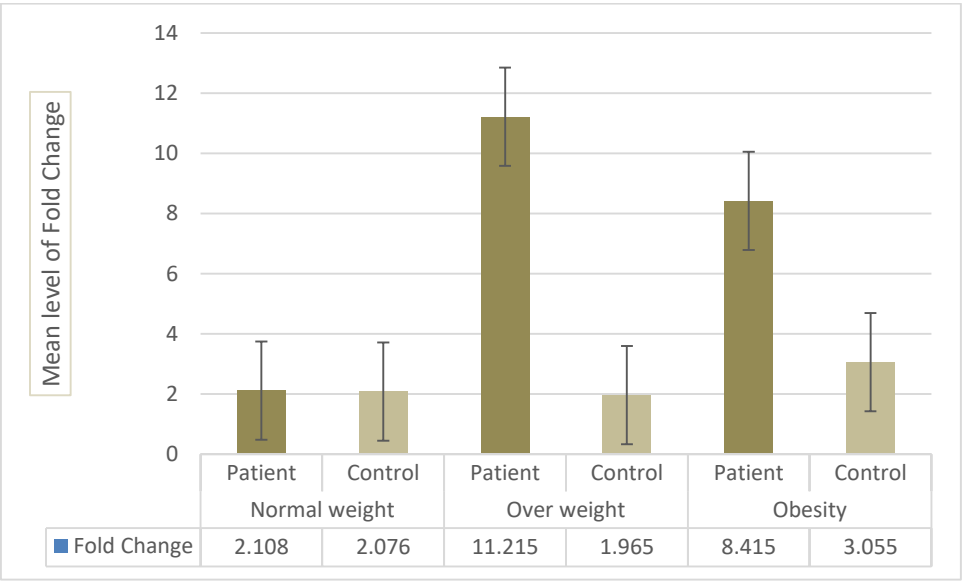


Figure 4.8: The effect of BMI groups on the fold change according to the patient and control groups (T-test was S= significant at p ≤ 0.05, NS= Non-significant).

4.1.6 Examination of the measured microRNA-146a according to the duration of the disease.

In order to understand the role of miRNAs participate in the event and development of many disorders, it was highly worth examining the measured microRNA-146a according to the duration of the disease. Since has been reported the roles of miR-146a in RA, it has become a popular research topic, and levels of expression of these molecules have been investigated in synovial tissues, fibroblasts, and peripheral blood-derived mononuclear cells of RA patients (***Murata et al., 2010***). Nevertheless, there is a lack of reports about the association between the level of these miRNAs in the plasma of RA patients and the activity of the disease (***Bagheri-Hosseinabadi et al., 2021***). In this study, biomarker levels were examined based on the duration of rheumatic arthritis disease. Generally, there were insignificant differences in the mean level of Ct U6, and Ct miRNA-146a. For Ct U6, and Ct miRNA-146a the mean levels were increased slightly in the duration of rheumatic arthritis disease through (7-12 Years) compared to the group who were their duration of less than one year and the group having a duration of rheumatic arthritis disease (1-6 Years), while the level of fold change inversing Ct U6 and Ct miRNA-146a decreasing (7-12 Years) compare to the group who were their duration less than one year and the group having a duration of rheumatic arthritis disease (1-6 Years), but all biomarkers were insignificant p-value > 0.05, as shown in table (4.2)

Table 4.2: Mean difference of Biomarkers based on the duration of rheumatic arthritis

Biomarkers	< 1 years	1-6 Years	7-12 Years	P value
Ct U6	17.51±1.18	17.58±0.90	17.73±0.87	0.892[NS]
Ct miRNA-146a	36.34±1.38	36.55±1.20	37.14±1.05	0.377[NS]
Fold Change	13.18±5.93	6.98±2.40	6.63±3.63	0.340[NS]
NS= Non significant				

4.1.7 Examination of the measured microRNA-146a according to the gender groups

The majority of autoimmune diseases predominate in females. In some diseases such as rheumatoid arthritis (RA), females are three times more affected than men (Carmona, et al.). The human X chromosome is highly enriched in miRNAs as compared to the Y-chromosome. microRNA-146a plays a critical role in inflammation by controlling the differentiation and maturation process of various immune cells, including osteoclast and granulocytic differentiation of myeloid precursors (Li, et al.). However, there are not that many studies exploring differential regulation of microRNA-146a in males versus females in RA cases. Therefore, this study examined the measured microRNA-146a according to the gender groups

Table (4.3) illustrates the mean level of the biochemical in the Patients and control groups according to gender. biomarkers levels were examined based on the gender of rheumatic arthritis disease. The results show that the level of fold change was increased markedly in the patients group in both male and female groups compared to the control, while the Ct U6 and miRNA-146a decreased. all biomarkers were highly statistically significant in both males and females, and p values were <0.05.

Table 4.3: Mean difference of Biomarkers based on gender groups in rheumatic arthritis and healthy control

Biomarker	Male			Female		
	Patients N=30	control N=15	P value	Patients N=30	control N=15	P value
Ct U6	17.71±0.88	18.55±0.44	**<0.001[S]**	17.45±1.03	19.04±0.60	**<0.001[S]**
miRNA-146a	36.70±1.40	39.02±1.16	**<0.001[S]**	36.43±1.05	39.17±1.02	**<0.001[S]**
Fold change	9.51±4.54	1.87±1.45	**<0.025[S]**	7.46±2.99	2.27±1.76	**0.009[S]**
T test was *: significant at p ≤ 0.05 N: number of cases; SD: standard deviation; S: significant						

4.1.8 Correlation

Considering the important role of the measured biomarkers, the Spearman rank test was used to analyze of the correlation between microRNA-146a, U6 expression (Ct mean values), and folding change with IL33 and leptin in rheumatic arthritis cases, as shown in Figure (4.9).

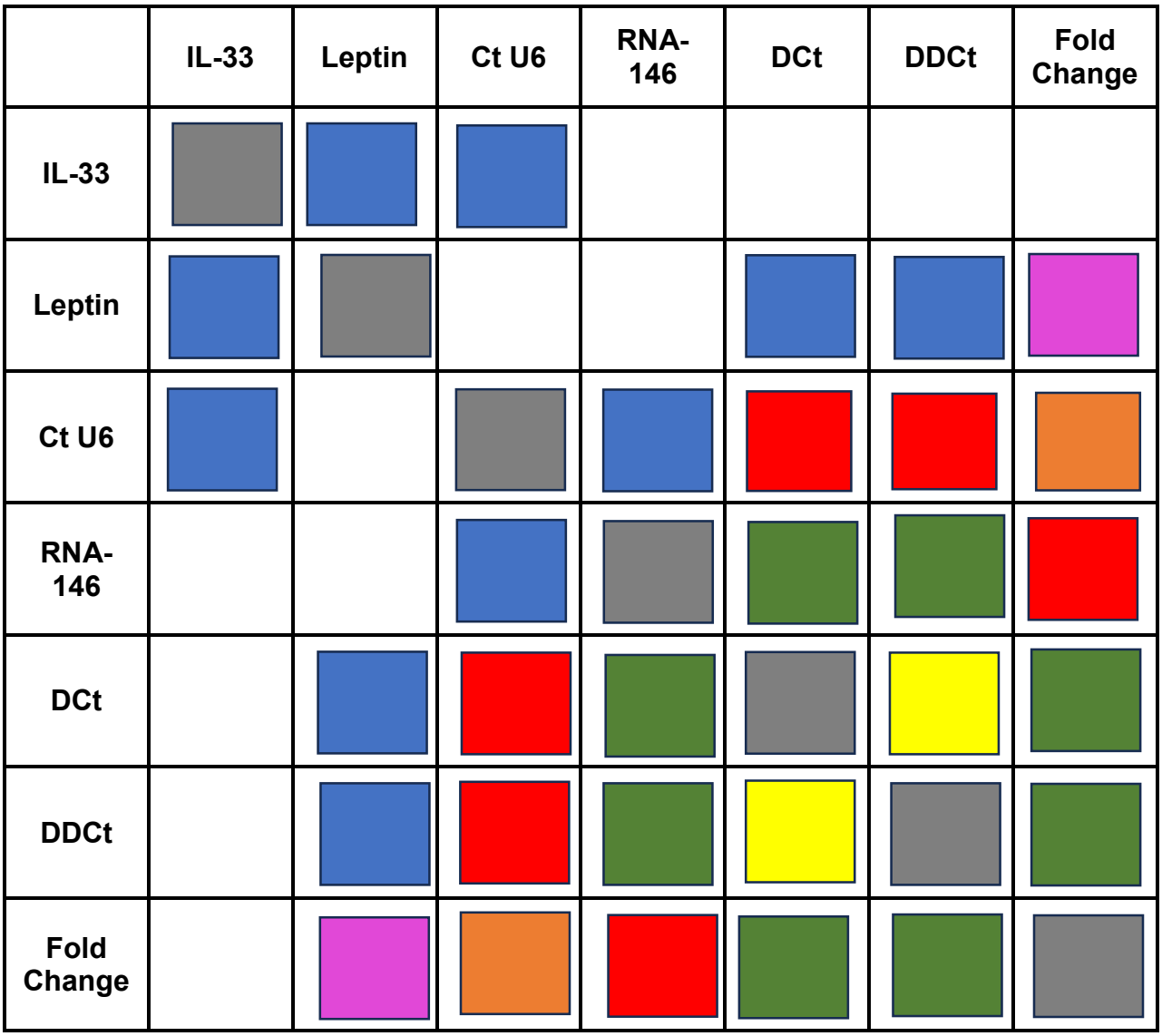


Figure 4.9: Heatmap chart of the Spearman rank test analysis, white boxes Non significant (p>0.05), while coloured boxes reported statistically significant direct and indirect correlations, respectively. The intensity of the colour indicates the following relation: Yellow (r=0.9), green (r=0.8); blue (r=0.3) ; orang(r=0.6), Pink (r= - 0.4, red (r= - 0.7).

4.1.9: Study the association of biomarkers with patients' groups

Multinominal logistic regression was performed to analyze the association between microRNA-146a, U6 expression (Ct mean values), and folding change. It was found that microRNA-146a and folding change was shown highly significant risk factor in rheumatic arthritis disease, miRNA-146 an (OR 1.487; 95% CI: (0.778-2.841) and OR: 3.796; 95% CI: (1.21-5.632)), on the other hand, the U6 is protected factor OR: 0.796; 95% CI: (0.21-1.632), as shown in table (4.4)

Table 4.4: The binary logistic regression of biomarkers in rheumatic arthritis disease (RA)

Variable	OR (Lower-Upper	P value
U6	0.796(0.21-1.632)	**<0.001**
Ct miRNA-146 a	1.487 (0.778-2.841)	**<0.001**
Fold change	1.002 (0.142-1.53)	**<0.001**
p<0.05 considered significantly different- [S]= Significant, [NS]= Non significant, OR= odd ratio		

4.1.10 ROC curve and AUC analysis for the Fold change for rheumatic arthritis disease

ROC curve and AUC analysis for the Fold change for patients compared to the control group were performed. Results of the receiver operating curve (ROC) curve and AUC analysis for the Fold change as a diagnostic parameter showed a fair performance for prediction RA patients, data are presented in Table (4.5).

For Fold change levels: (sensitivity 85 %, specificity 87%) at a level = 3.355 The p-values of the AUC were 0.001 and highly statistically significant. results of the Sensitivity and specificity were confirmed using Youden's J statistics

Table 4.5: Receiver operating characteristic curve showing sensitivity and specificity of Fold change in patients compared to control

Test Result Variable(s)	Fold change
AUP	72.5%
Sensitivity %	85%
Specificity %	87%
Youden index	0.72
Cut-off points	3.355
CI (95%)	0.619-0.830
PPV	88.6%
NPV	47.3%
Accuracy	63.4%
P value	**0.001[S]**

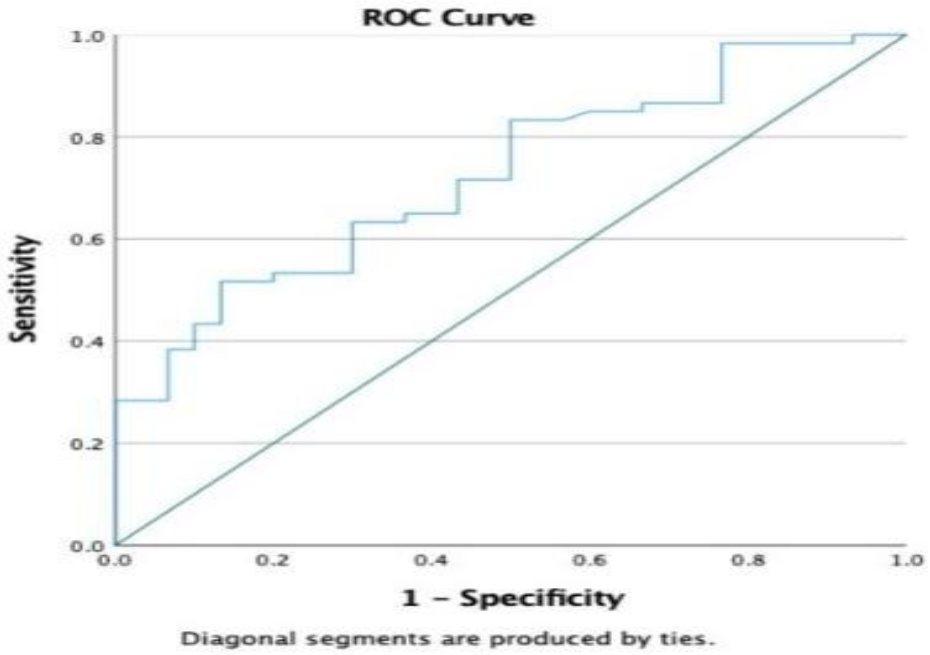


Figure 4.10: Receiver operating characteristics (ROC) curve analysis of folding change levels in Rheumatoid arthritis

4.2 Clinical and Biomarkers

4.2.1 Difference between the level of biological parameters (IL33 and Leptin) in the rheumatic arthritis cases and control group

Generally, patients with rheumatic arthritis disease were shown an increasing range level of IL-33 and Leptin Hormone when compared to the healthy control groups.

Results indicated a significant difference in IL-33 and Leptin Hormone levels among groups, the means, and standard deviations were presented in (Table 4.6). The mean level of IL-33 in patients was (950.80±463.07) which was significantly higher than for the Control group (297.10±44.81), ($p \leq 0.001$) and the mean level of Leptin. The hormone was (7.21±1.54) for the patient and (2.78±0.37) for the control,

Table 4.6: Results of the analysis of basic rhematic arthritis characteristics for disease with control groups.

Biomarkers	Groups	Mean±SD	P value
IL-33	Patient	950.80±463.07	**<0.001 [S]**
	Control	297.10±44.81	
Leptin. Hormone	Patient	7.21±1.54	**0.010 [S]**
	Control	2.78±0.37	
T test was *: significant at $p \leq 0.05$ N: number of cases; SD: standard deviation; S: significant; NS= Non significant			

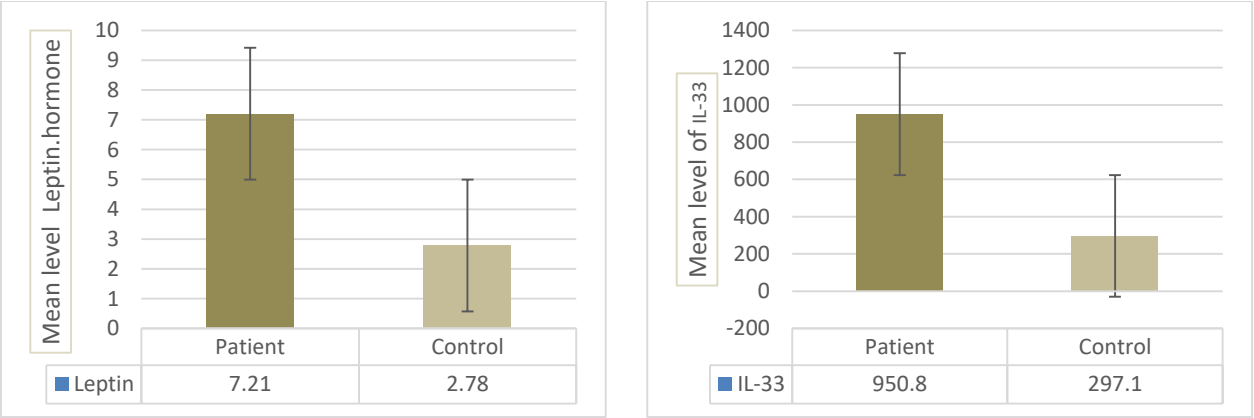


Figure 4.11: Results of the analysis of basic rheumatic arthritis for patients with control groups (T-test was S= significant at $p \leq 0.05$.

4.2.2 Mean Difference of the biological parameters (IL33 and Leptin) based on the duration of disease

Biomarkers levels were examined based on the duration of rheumatic arthritis disease. Generally, there were insignificant differences in the mean level of IL-33 and Leptin Hormone. The mean level of IL-33 was increased slightly with increasing the duration of rheumatic arthritis disease through (7-12 Years) compared to the group who were their duration less than one year and the group having a duration of rheumatic arthritis disease (1-6 Years), the differences were insignificant p-value > 0.05, as shown in Table(4.7) & Figure (4.12)

Table 4.7: Mean difference of biochemical parameters for the duration of rheumatic arthritis disease.

Biomarkers	Groups	Mean±SD	P value
IL-33	Less than 1 years	953.09±240.58	0.967 [NS]
	1-6 Years	881.78±612.96	
	7-12 Years	992.01±153.06	
Leptin. Hormone	Less than 1 years	7.08±0.94	0.460[NS]
	1-6 Years	7.37±1.80	
	7-12 Years	6.61±0.79	
T test was *: significant at p ≤ 0.05 **N: number of cases; SD: standard deviation; S: significant; NS= Non significant**			

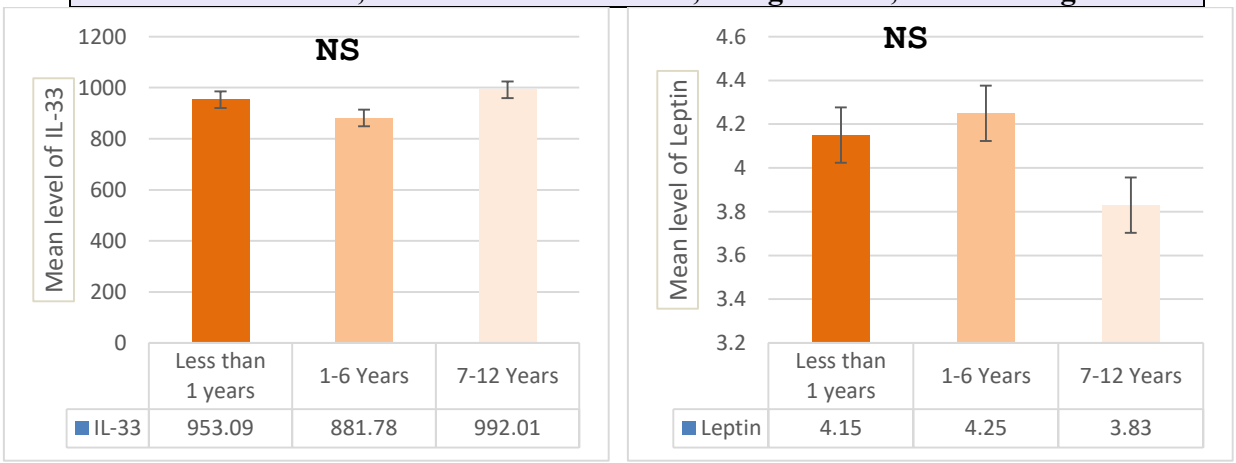


Figure 4.12: Difference between mean levels of biomarkers in rheumatic arthritis disease according to duration of treatment (ANOVA-test was S= significant at p ≤ 0.05, NS= Non-significant).

4.2.3 Examination of the mean differences in the IL-33 and Leptin levels according to the gender groups

Table (4.8) illustrates the mean level of IL-33 and Leptin in the Patients and control groups according to gender. Results showed that the levels of IL-33 and Leptin hormone were increased markedly in the patients group in both males and females compared to the control, p values were <0.001.

Table 4.8: The effect of gender on the biochemical parameters according to the Patients and control groups

Biomarker	Male			Female		
	Patients N=30	control N=15	P value	Patients N=30	control N=15	P value
IL-33	994.1±458.5	401.3±53.5	**<0.001**	830.8±540	352.94± 93	**0.00**
Leptin	6.01±0.43	3.80±0.61	**<0.001**	5.95±0.3	4.54±2.5 9	**0.003**
T test was *: significant at p ≤ 0.05 N: number of cases; SD: standard deviation; S: significant; NS= Non significant						

4.2.4 Examination of the mean differences in the IL-33 and Leptin levels according to the Age groups

In Figures (4.13 and 4.14) a comparison of serum levels of IL-33 and Leptin (pg/ml) in different age groups was performed. The levels of IL-33 and Leptin increased significantly within all the age ranges and were highly statistically significant ($p<0.05$).

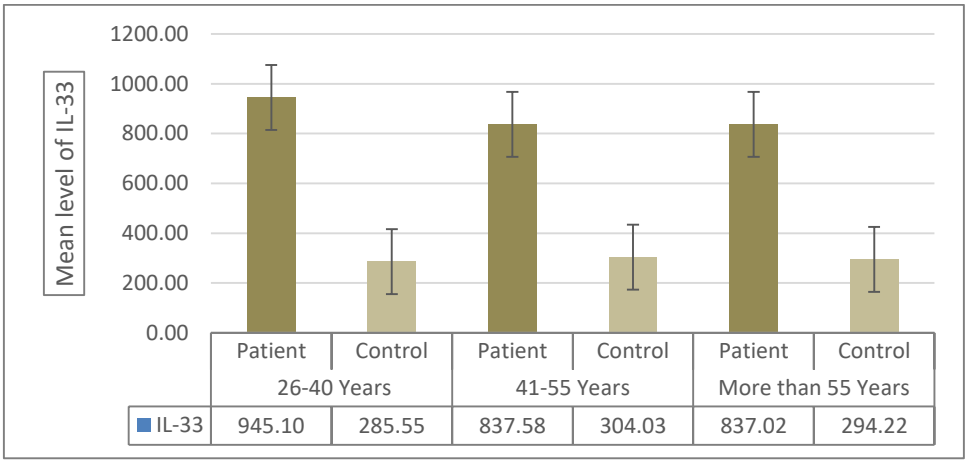


Figure 4.13: The effect of age groups on the IL-33 according to the patient and control groups (T-test was S= significant at $p \leq 0.05$, NS= Non-significant).

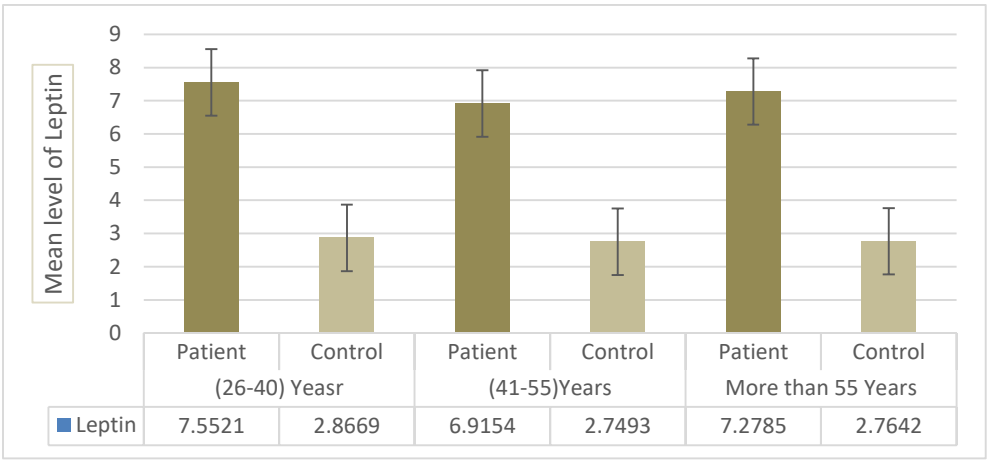


Figure 4.14: The effect of age groups on Leptin according to the patient and control groups (T-test was S= significant at $p \leq 0.05$, NS= Non-significant).

4.2.5 Examination of the mean differences in the IL-33 and Leptin levels according to the BMI groups

In Figures (4.15 and 4.16) a comparison of serum levels of IL-33 and Leptin (pg/ml) in different age groups was performed. Both levels of IL-33 and Leptin were increased within all the BMI ranges and were highly statistically significant ($p<0.05$).

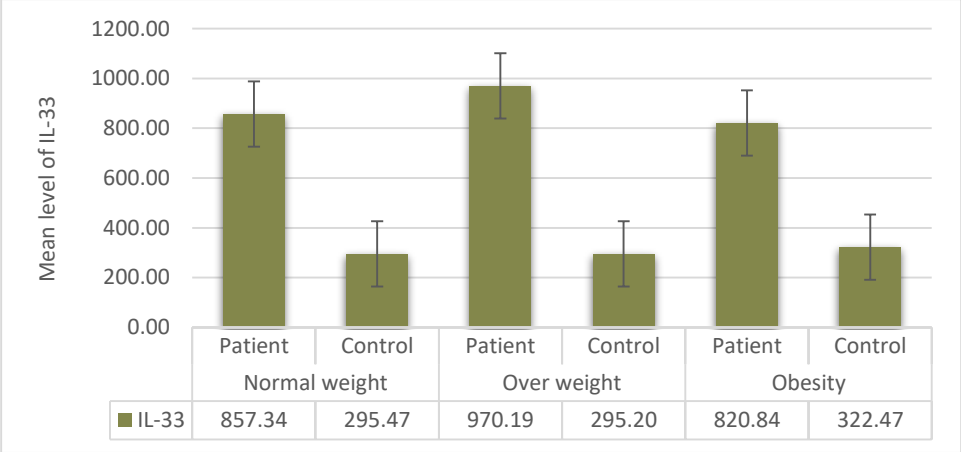


Figure 4.15: The effect of BMI groups on the IL-33 according to the patient and control groups (T-test was S= significant at $p \leq 0.05$, NS= Non-significant)..

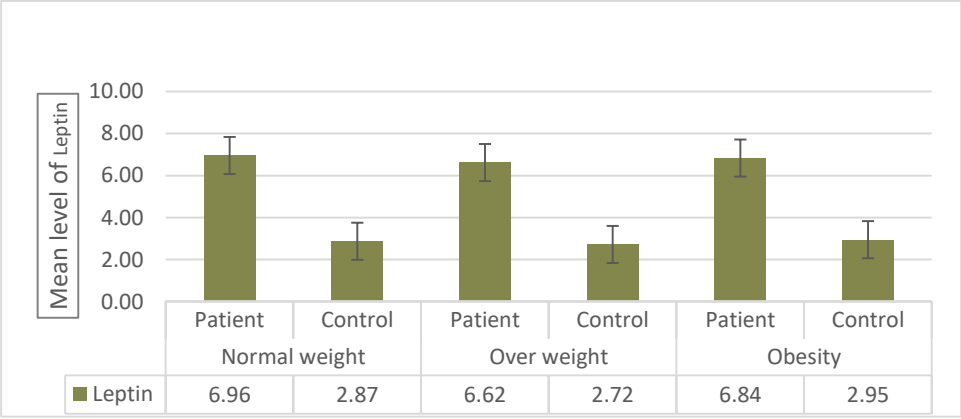


Figure 4. 16: The effect of BMI groups on Leptin according to the patient and control groups (T-test was S= significant at $p \leq 0.05$, NS= Non-significant).

4.2.6 Study the association of IL-33 and Leptin with Rheumatoid arthritis disease

Multinominal logistic regression was performed to analyse the association between IL-33 and Leptin Hormone with rheumatoid arthritis disease. It was found that both biomarkers were shown highly significant differences in such disease and represented a risk factor. Leptin was illustrated to be a three-time risk factor for Rheumatoid arthritis disease than IL33. The odd ratio of IL33 was (OR 1.487; 95% CI: (0.778-2.841) and for leptin hormone was (OR: 3.796; 95% CI: 1.21-5.632), as shown in Table (4.9)

Table 4.9: The binary logistic regression of rheumatic arthritis disease (RA) with levels of biomarkers

Biomarker	OR (Lower-Upper)	P value
Leptin Hormone	3.796(1.21-5.632)	**0.004**
IL-33	1.487 (0.778-2.841)	**0.001**
p<0.05 considered significantly different, [S]= Significant, [NS]= Non significant OR= Odd ratio		

4.2.7 ROC curve and AUC analysis for the IL-33 for rheumatic arthritis disease

ROC curve and AUC analysis for the IL-33 for patients compared to the control group were performed. Results of the receiver operating curve (ROC) curve and AUC analysis for the IL-33 as a diagnostic parameter showed that IL-33 has a good performance for predicting such cases, data are presented in Table (4.10).

For IL-33 levels: (sensitivity 83.3 %, specificity 97%) at a level = 593. The p-values of the AUC were <0.001 and highly statistically significant. Results of the Sensitivity & Specificity were confirmed using Youden's J statistics to the parameters.

Table 4.10: Receiver operating characteristic curve showing sensitivity and specificity of IL-33 in patients compared to control

Test Result Variable(s)	IL-33
AUP	88.5%
Sensitivity %	83.3%
Specificity %	97%
Youden index	0.803
Cut-off points	593.464
CI (95%)	0.811-0.959
PPV	99%
NPV	75%
Accuracy	88.8%
P value	<0.001[S]

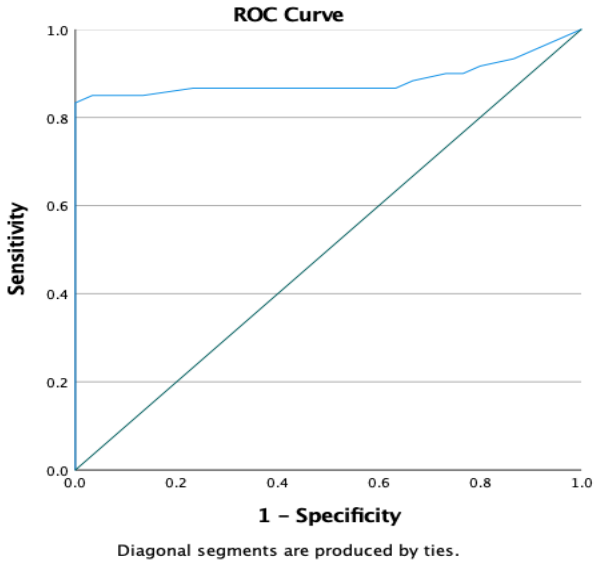


Figure 4.17: Receiver operating characteristics (ROC) curve analysis of IL33 levels in Rheumatoid arthritis cases

4.2.8 ROC curve and AUC analysis for the Leptin for rheumatic arthritis disease

ROC curve and AUC analysis for the IL-33 for patients compared to the control group were performed. Results of the receiver operating curve (ROC) curve and AUC analysis for Leptin as a diagnostic parameter was shown that Leptin has a excellent performance for predicting such cases, data are presented in Table (4.11).

For Leptin levels: (sensitivity 98.3%, specificity 99%) at a level = 5.0387. The p-values of the AUC were <0.001 and highly statistically significant. results of the Sensitivity & Specificity were confirmed using Youden's J statistics to the parameters.

Table 4.11: Receiver operating characteristic curve showing sensitivity and specificity of Leptin in patients compared to control

Test Result Variable(s)	Leptin
AUP	98.3%
Sensitivity %	98.3%
Specificity %	99%
Youden index	0.883
Cut-off points	5.0387
CI (95%)	0.962-1.000
PPV	99%
NPV	75%
Accuracy	88.8%
P value	**<0.001[S]**

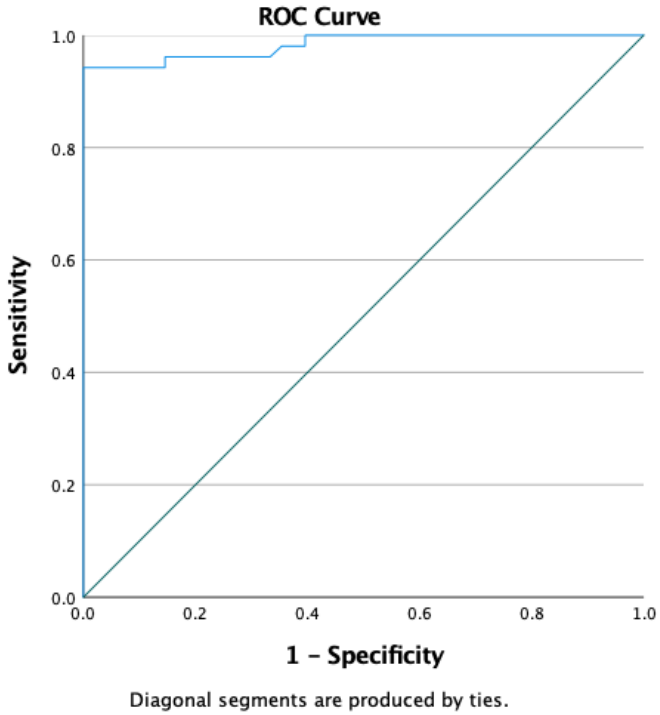


Figure 4.18: Receiver operating characteristics (ROC) curve analysis of Leptin levels in Rheumatoid arthritis cases

Chapter Five

Discussion

5. Discussion

5.1 Difference between the level of MicroRNA-146a in the Rheumatoid arthritis disease and control groups

miRNA-146a (miR-146a) was first described by Taganov (K.D.Taganov., *et al., 2006)*. studies showed that its expression is increased by proinflammatory cytokines and is considered an important modulator of differentiation and function of cells of innate and adaptive immunity (V. Furer, et al., 2010). It has been implicated in the pathogenesis of RA via the regulation of multiple target genes linked to inflammation and apoptosis (A.V. Churov, et al., 2015).

Since its discovery, several reports considered miR-146a an important modulator of differentiation and function of cells of innate and adaptive immunity and associated it with the development and pathogenesis of autoimmune diseases (***Churov et al., 2015)***. In the current study, it was evaluated in RA, Statistically significant differences ($p < 0.001$) were observed between patients and healthy controls as regards miR-146a folding change.

Many studies used the synovial fluid, tissues and fibroblasts as reported by Kriegsmann et al (***Chan et al., 2013***). In all of these studies elevated levels of expression of miR-146a were documented, suggesting its significance as a potential biomarker for RA diagnosis. However, the invasiveness of the synovectomy procedure encouraged researchers to investigate the usefulness of other less invasive samples as indicators for the levels of miRNA-146a expression. Pauley et al. (***Kriegsmann et al., 2016***) and Abou-zeid et al. (***Pauley et al., 2008***) reported upregulated miRNA-146a expression in peripheral blood-derived mononuclear cells (PBMC) from patients with RA than in healthy controls, and in patients suffering from other autoimmune disorders.

It was found that in patients with RA, PBMCs exhibit elevated miRNA expression in a pattern similar to that observed in RA synovial tissue. Then, Ormseth et al. found mR-146a levels of expression elevated in the plasma of patients with RA (**Ormseth *et al*., 2011*)***.

In the current study a whole blood was used, and so did Mookherjee and El-Gabalawy (**Mookherjee *et al*., 2015**) who stated that whole blood samples could accurately reflect miRNA levels in PBMC and would be useful in monitoring the expression of miRNAs as biomarkers.

The current results showed positive correlation between levels of miR-146a and disease activity as demonstrated by the highly significant statistical difference in the folding change of miR-146a among different patients' subgroups ($p < 0.001$), the results were constant with other research who reported the same finding (***Ormseth et al., 2015*** ; ***Mookherjee et al., 2013***).

The mechanisms by which miR-146a affect the developments of RA are varied, one of the most common is the role of miR-146a and TLR4/NF-κB signaling pathway in the proliferation and pro-inflammatory cytokine production of rheumatoid arthritis fibroblast-like synoviocytes (RA-FLSs).

RA-FLSs increased expression of both proinflammatory genes and matrix-destructive enzymes, together with numerous alterations in cell signaling (***Heba et al., 2017***).

A previous study reported that miR-146a could control TLR and cytokine signaling by targeting the TNF receptor–associated factor 6 and IL-1 receptor-associated kinase (***Iwamoto et al., 2018***). Similarly, other results demonstrated that miR-146a overexpression inhibits RA-FLS cell proliferation and pro-inflammatory cytokine production by inhibiting the TLR4/NF-κB signaling pathway. MiR-146a

targets interleukin-1 receptor-associated kinase (IRAK1) and modulates the function of toll-like receptors and related cytokines (***Stanczyk et al., 2008***).

Pauley et al. showed that miR-146a expression controlled the release of some pro-inflammatory cytokines and therefore regulated the extent of stimulation (***Tan et al., 2013***).

TLRs recognize many extracellular molecules associated with pathogens or tissue damage and activate transcription factors that promote the expression of inflammatory factors (***Mitsui et al., 2014***). When TLR4 is bound by its ligand, it activates NF-κB through myeloid differentiation factor-88 (MyD88) dependent and independent pathways (***Li et al., 2012***). The NF-κB family of transcription factors translocate from the cytoplasm into the nucleus upon activation to induce cytokine expression (***Krum et al., 2010***).

These cytokines promote the intracellular expression of NOS mRNA and the release of NO, which promotes inflammation (***Hsu et al., 2013***). Therefore, lower miR-146a expression promotes the release of proinflammatory cytokines, thus exacerbating RA pathology.

Recently, many studies have reported an age-related decline in circulating miR-146a levels (***Maffioletti et al., 2020***). Moreover, adjustment for age did not modify the results. The reason for this discrepancy is unknown; however, the effect of age on miR-146a-5p levels was observed in very old subjects (***Mensà et al., 2019***).

Previously, it has been demonstrated that the miR-146a might be involved in increases the severity of inflammatory arthritis by regulating fibroblast

pathogenicity (***Saferding et al., 2017***). This study also investigated the role of microRNA-146a as a critical factor, which regulates bone loss during aging.

miR-146a has also been implicated in immune dysfunction during aging, as it has been shown to accumulate in macrophages and dendritic cells of aged mice, leading to dysfunction of these cells (***Olivieri et al., 2013***). Myeloproliferation, systemic inflammation, and myelofibrosis are important phenotypes that develop in miR-146a-deficient mice with age (***Magilnick et al., 2017***).

Obesity is closely associated with many dysregulation, and recent reports found decreased miR-146a levels. miR-146a is required for protection from obesity and metabolic disease and loss of miR-146a causes underlying inflammation that acts as a predisposing factor. This is likely caused by pro-inflammatory adipokines expressed during caloric excess that act in concert with inflammatory pathways to signal a need for increased fat storage (***Runtsch et al., 2019***).

MiR-146a is highly expressed within the Stromal Vascular Fraction (SVF) of adipose tissue which is rich in leukocytes, suggesting a role for this miRNA within immune cells. cell types that express miR-146a could play a role, such as B or T lymphocytes or preadipocytes, which are also found in the SVF. miR-146a has been shown to function in each of these cell types in other contexts (***Pratama et al., 2015***).

MiR-146a is well-known for regulating genes that are part of the inflammatory response, which we have shown to be dysregulated during obesity; however, other regulatory functions of miR-146a are not well-studied and could be key to understanding its role in such disease. It was found that miR-146a regulates the

metabolism of activated macrophages, a previously unrecognized function for miR-146a. activated miR-146a macrophages undergo metabolic reprogramming characterized by decreased oxidative phosph orylation. It was also suggested that miR-146a represses Traf6 not only to control inflammatory gene expression, but also to limit the switch from oxidative phosphorylation to glycolytic metabolism during inflammation (***O'Neill et al., 2016***).

5.2 Difference between the level of biological parameters (IL33) in the rheumatic arthritis cases and control group

Generally, patients with rheumatic arthritis disease were shown an increasing range level of IL-33 and Leptin Hormone when compared to the healthy control groups.Results indicated a significant difference in IL-33 and Leptin Hormone levels among groups.

A large number of literature shows that the occurrence and development of RA are also closely related to various cells and cytokines in the body. Most RA-related cytokines are produced by immune cells, which bind to the specific receptors of effector cells through paracrine and autocrine pathways. The effect of cytokines on inflammation can be divided into pro-inflammatory and anti-inflammatory factors. When the impact of pro-inflammatory factors is more significant than anti-inflammatory factors, the condition of RA will be aggravated, resulting in more inflammatory severe reactions and immune disorders (***Jang et al., 2022*** ; ***Kondo et al., 2021***).

In recent years, scholars have confirmed that interleukin-33 (IL-33) regulates immune and inflammatory responses, which is related to the pathogenesis of many diseases. Related reports also point out that IL-33 is significantly correlated

with many cytokines such as rheumatoid factor (RF), which may be a breakthrough in the treatment of RA and become a hot spot of current research(***Önnheim et al., 2022***).

Unlike some cytokines, which have classical secretion patterns, IL-33 is normally localized in the nucleus (***Pichery et al., 2012***). Although the localization of IL-33 in the cytoplasm has been reported in the literature, the results were not obtained under normal conditions. In both physical and pathological inflammatory conditions, the main cellular sources of IL-33 are Endothelial cells, epithelial cells, fibroblasts, and myofibroblasts in humans and mice which were demonstrated to be the main cells expressing IL-33(***Küchler et al., 2008***). Many studies have shown elevated IL-33 levels in both serum and local joint synovial fluid in patients with RA (***Xu et al., 2008)*** IL-33 was also expressed in synovial fibroblasts (***Boissier et al., 2011***).

Inflammatory factors (such as TNF-α) could stimulate synovial fibroblasts to produce IL-33; and IL-33 not only upregulated matrix metalloproteinase-3 (MMP-3), IL-8, and IL-6 but also upregulated B-cell lymphoma-2 (Bcl-2) to inhibit apoptosis and promote proliferation (***Kunisch et al., 2012***). Other studies also revealed that IL-33 was mainly produced in inflamed joints (***Matsuyama et al., 2010***). Hong et al. also reported that in patients with RA, the serum level of IL-33 and sST2 was significantly higher than that of healthy controls. Accordingly, in the synovial fluid, the level of IL-33 was significantly higher than that of osteoarthritis patients (***Heog et al., 2011***). All these results confirmed the fact that IL-33/ST2 signaling played a vital role in the joint inflammation of human RA and the experimental CIA model (***Duan et al., 2013***).

Previous studies have shown that IL-33 is essential in maintaining homeostasis and reducing inflammation by inducing M2 macrophages to polarize and activate immunosuppressive cells. Macrophages can be polarized under the catalysis of different cytokines, forming two cell subsets of classical activated M1 and substituting activated M2, which regulate the immune response. M1 macrophages are the essential regulator of Th1 cell-mediated immune response, while M2 macrophages can control Th2 cell-mediated immune response and reduce inflammation (***Ross et al., 2021***).

Some studies showed that monocytes were extracted from mouse bone marrow and induced into M0 macrophages. M0 macrophages were induced and cultured *in vitro* in the presence of IL-33, which promoted the polarization of macrophages to M2. It is considered that the imbalance of macrophage polarity can lead to the disorder of affected cytokines, which further aggravates the polarity imbalance, which is considered to be the molecular mechanism of RA (***Xia et al., 2020***).

In this study, Serum IL-33 sensitivity in predicting RA cases was estimated, Receiver operating characteristic (ROC) curve for the performance of serum interleukin-33 was performed.

It has been reported that the expression of IL-33, a cytokine that belongs to the IL-1 family, is induced by damage to the epithelial and endothelial cells (***Liew et al., 2016***) and it acts as an alarm signal to maintain homeostasis through binding with its specific receptor known as suppression of tumorigenicity 2 (ST2) (***Chan et al., 2019***). The IL33/ST2 axis plays an important immunoregulatory role and it is implicated in the pathogenesis of many infectious and inflammatory diseases (***De la Fuente et al., 2015***).

Therefore, AUC analysis for IL-33 as a diagnostic parameter showed that IL-33 has a good performance for predicting such cases. This can be explained by its ability to identify subclinical synovial inflammation due to their involved role in the B-cell

mediated pathology which was confirmed by Schmitz et al. (***Schmitz et al., 2005)*** who suggested that IL-33 can enhance the production of autoantibodies by B cells through enhancement of the secretion of IL-5 and IL-13 which are known Th2 cytokines.

5.3 The difference between the level of biological parameters (Leptin) in the rheumatic arthritis cases and control group Rheumatoid arthritis

Rheumatoid arthritis is a severe chronic and progressive autoimmune disorder characterized by synovium inflammation (***Smolen et al., 2018***). (Smolen J.S., et al.) Although the pathological mechanisms involved in RA are different, the onset and progression of both diseases are associated with inflammation, immune mechanisms, and metabolic factors (***Firestein and McInnes., 2017***).

Mechanical loading and inflammatory mediators such as adipose-tissue-derived cytokines (adipokines) have been reported as a link between obesity and RA (***Francisco et al., 2018***). Adipokines including leptin secreted principally by white adipose tissue (WAT) (***Smekal and Vaclavik., 2017***). Through their endocrine, autocrine, or paracrine actions, they are implicated in several physiological and pathological processes and lead to a "low grade inflammatory state"(***Lago et al., 2007***). Indeed, they are demonstrated to be involved in the pathogenesis of rheumatic diseases by the modulation of the inflammatory process in the joint, the imbalance between catabolic and anabolic factors, and the remodeling of bone and cartilage (***Carrión et al., 2019***).

In this study, Leptin levels have greatly increased in the serum of RA patients. Leptin is implicated in both innate and adaptive immune responses. It promotes the synthesis and secretion of pro-inflammatory cytokines. it enhances T-cell proliferation and memory-T-cells differentiation to T-helper (Th1), and inhibits regulatory-T-cell (Treg) proliferation (***La Cava., 2017***).

In RA, Autoantibodies and rheumatoid factor (RF) are the first immune abnormalities detected, followed by joint damage starting in the synovial membrane. Synovium inflammation appears in the early stages of the disease after activation of endothelial cells that express adhesion molecules and chemokines following the infiltration of leukocytes through the synovium, Leptin has been described to be implicated in RA pathogenesis(***Alam et al., 2017***).

Leptin has been described to modulate bone homeostasis by both locally and centrally mediated mechanisms. It inhibits osteoclast differentiation in peripheral blood mononuclear cells (PBMCs) and murine spleen cells in bone culture via the RANKL/RANK/OPG system and thus contributes to the inhibition of bone resorption (***Holloway et al., 2002***).

Increasing levels of leptin were confirmed due to their role in activated macrophage inducing the release of IL-6 and TNF-α. In vitro, the chemotactic activity of macrophage is associated with the induction of leptin. In autoimmune diseases, deregulated immune response of cells is affected by the alteration of metabolic process within these cells(***Francisco et al., 2008***), because leptin binds to its long isoform receptor (Ob-RB) to induce its biological and physiological effect through JAK/STAT signaling pathway. JAK/STAT signal transduction is caused by the involvement of Janus kinase 2 (*JAK2*), activators of transcription (STAT) and transducers found on longer receptor isoform (Ob-Rb) (***La Cava., 2017***).

In addition to leptin, this signaling pathway requires the interaction between complex molecules, including node-like receptor pyrin domain-containing protein 3 (*NLRP3*), and IL-33 (***Crispino and Ciccia., 2021***).

Throughout the roc analysis, leptin level was found to be a reliable surrogate biomarker of RA disease progression. Our results were consistent with other (Giles JT, 2011) that reported that the predictive value of leptin showed a good diagnostic value toward RA.

Since Leptin is a pro-inflammatory factor that stimulates the innate and acquired immune response, and its concentration increases during infection and inflammation, this study analyzed the cut-off point of Leptin in patients with RA. Given that this value has been defined in the kerbala population compared to controls; for this reason, it is worth highlighting the values to identify a more sensitive value for the other disease and population.

5.4Conclusion and Recommendation

5.4.1Conclusion

From all data and correlations of different variables in the present study, it could be concluded that: -

1. Deregulation of miRNAs 146a has a role in the impaired cellular functions in RA pathogenesis, and progressive joint damage.
2. miR-146a could be used as a potential non-invasive sensitive marker for RA diagnosis and screening.
3. It has been demonstrated that RA may be evaluated as a long-term consequence based on a high BMI value, which can also be a useful indicator of the disease's severity.
4. Both IL-33 and leptin were also shown highly significant differences in such disease and represented a risk factor. Leptin was illustrated to be a three-time risk factor for Rheumatoid arthritis disease than IL33.

5.4.2 Recommendation

1. In light of these findings, it was suggested that miR-146a and miR-233 could be used as a non-invasive sensitive marker for the detection and diagnosis of rheumatoid arthritis.

2. In order to confirm the results, we also highly recommended to evaluate this biomarker in a large population study to ascertain its role as a screening test for RA.

5.4.3 Future works

1. Study the role of miRNAs 146 in identifying the subtypes of RA. For example, the levels of miR-7 and miR-214-5p are significantly increased in the serum of patients with RA associated-interstitial lung disease.
2. Further studies should be performed to translate this knowledge for clinical applications and resolve the current inconsistent results among different studies employing different methods or populations. For example, studies of miR-99, miR-143, and miR-197 as landmark miRNAs for predicting the response to anti-TN-α therapy
3. Future development of miRNA-based baseline RA polygenetic risk score models, particularly in conjunction with HLA, is needed.
4. It would be a good idea in future to study the genetic variants in specific miRNAs which can increase or decrease the risk and disease severity of RA in various races.

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Appendices

Appendices

RESEARCHER AHMED J. HASSAN

Arthritis Disease Questionnaire :

Code sample: 1

Patient information:

Name : basim hassan kadhem		Age: 32
Height :1.7	Weight : 80	BMI : 27.68
Gender : Male ☑ Female ☐		Duration of diseases: 3y

Chronic diseases :

Diabetes	no	Hypertension	yes

Arthritis in :

Knee joint		Wrist joint	✓	Ankle joint	
Elbow joint	✓	Shoulder joint		Humerus joint	
Pelvic joint		Another joint			

Required tests :

ESR	40	CBC	Normal
Vit D level		Ca level	
Bone density		CRP	
other:		other:	

Active Smoker no passive smoker no

Biological treatment. Yes ☐ No ✓


Test
Report
Parameter
System
Statistics
Maintain
Item parameters
1-alpha
EGF
IL-6HH
IL-10HH
HBM
GSH H
IgG F
GDF
VEG
GSH F ATIMA
TPO-AB
TGF-B ETA N
TG-AB N
INSU LIN N
NO ta tima
Adro pin m
Gpx m
CD99 m
il-17-m
SOD-1
il-6 zainb
IL6-R ZAIN
TNF-A LPHAZ
liptin bt
il-33 bt
Plate Direction
Row
Column
Start sampleNo
0001
Hole position
E
9
H
12
Setting
Plate current hole position item information
Test parameters
Test template
Setting
Testing
default
Setting
Sample
NC
PC
PC2
CR
QC1
QC2
QC3
QC4
QC5
Blank
Clear
1
2
3
4
5
6
7
8
9
10
11
12
A
B
C
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G
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Test
Stop testing
Clear All
Result
ABS
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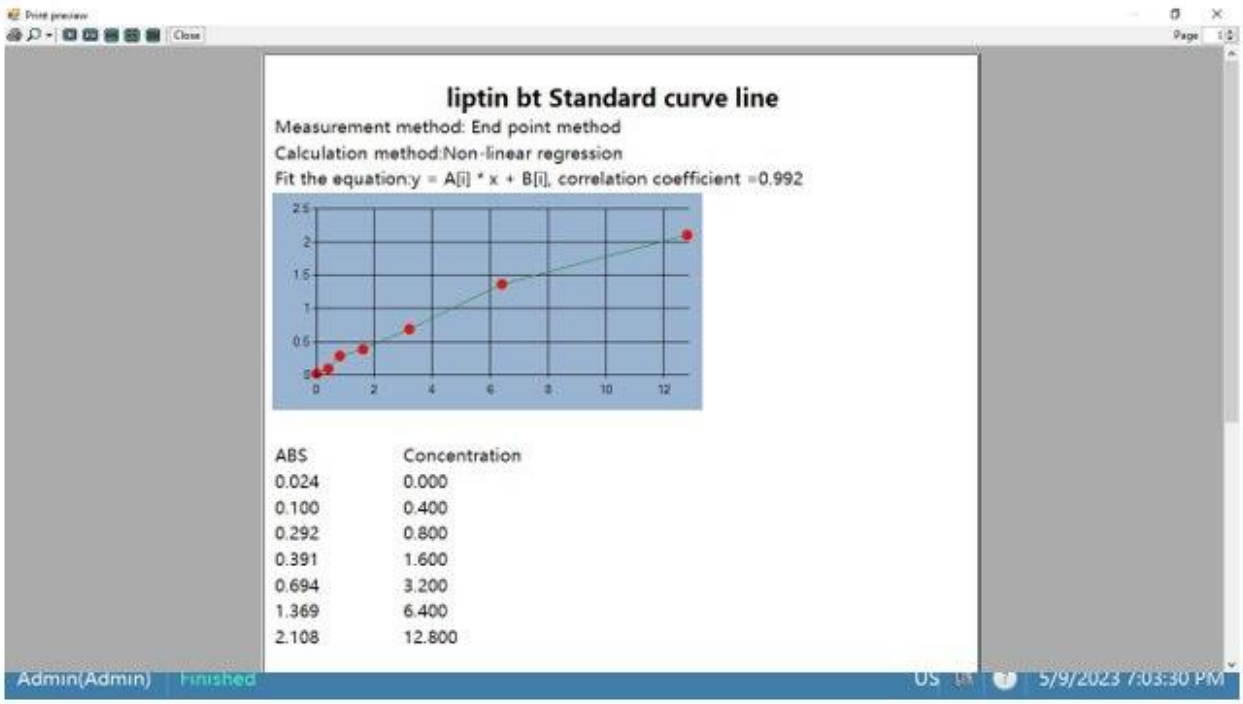
B


liptin bt Standard curve line
Measurement method: End point method
Calculation method:Non-linear regression
Fit the equation:y = A[i] * x + B[i], correlation coefficient =0.992
ABS Concentration
0.024 0.000
0.100 0.400
0.292 0.800
0.391 1.600
0.694 3.200
1.369 6.400
2.108 12.800
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Test
Report
Parameter
System
Statistics
Maintain
Item parameters
t3 HIV HBs Ag HCV TSH
TB IGM CD19 Bcell IL12 ahmed test erythro
indol eamin indol indol eami2 prolactin ferritin
HEPC IL-6 MCP-1 CYP EPO-
EPO HIV1/2 TN-C TN C ASPORIN
Test parameters
Test template Setting Testing default Setting
Sample NC PC PC2 CR QC1 QC2 QC3 QC4
QC5 Blank Clear
Plate Direction Row Column
Start sampleNo 0001
Hole position A 9 -- D 12 Setting
Plate current hole position item information
Test Stop testing Clear All Result ABS Quantitative Qualitative Save Print
Print Preview Export Send
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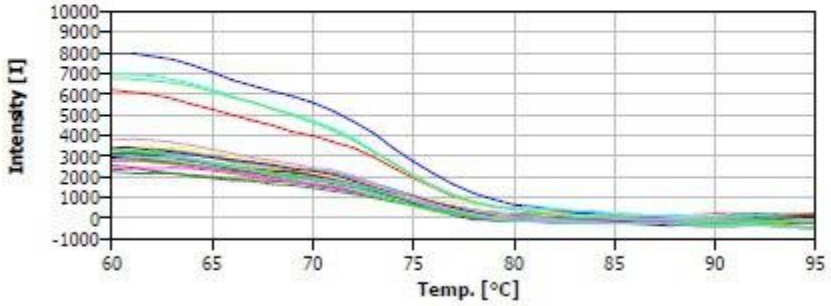
C

Ct

Well		Sample name	Sample type	Dye	Gene	Ct	Mean Ct
A1		11 MiR146	Unknown	FAM		30.09	30.09
A2		15 MiR146	Unknown	FAM		33.23	33.23
A3		16 MiR146	Unknown	FAM		38.35	38.35
A4		17 MiR146	Unknown	FAM		35.3	35.3
A5		19 MiR146	Unknown	FAM		41.28	41.28
A6		20 MiR146	Unknown	FAM		42.36	42.36
A7		21 MiR146	Unknown	FAM		No Ct	
B1		22 MiR146	Unknown	FAM		40.54	40.54
B2		23 MiR146	Unknown	FAM		37.45	37.45
B3		25 MiR146	Unknown	FAM		37.9	37.9
B4		27 MiR146	Unknown	FAM		No Ct	
B5		28 MiR146	Unknown	FAM		40.1	40.1
B6		29 MiR146	Unknown	FAM		39.04	39.04
B7		32 MiR146	Unknown	FAM		38.2	38.2
C1		33 MiR146	Unknown	FAM		30.76	30.76
C2		35 MiR146	Unknown	FAM		37.16	37.16
C3		36 MiR146	Unknown	FAM		38.41	38.41
C4		39 MiR146	Unknown	FAM		37.97	37.97
C5		45 MiR146	Unknown	FAM		38.43	38.43
C6		46 MiR146	Unknown	FAM		38.24	38.24
C7		51 MiR146	Unknown	FAM		No Ct	
D1		56 MiR146	Unknown	FAM		34.18	34.18
D2		60 MiR146	Unknown	FAM		36.13	36.13
D3		64 MiR146	Unknown	FAM		No Ct	
D4		66 MiR146	Unknown	FAM		38.12	38.12
D5		67 MiR146	Unknown	FAM	6	38.14	38.14

Monitoring - MeltingData

All colors



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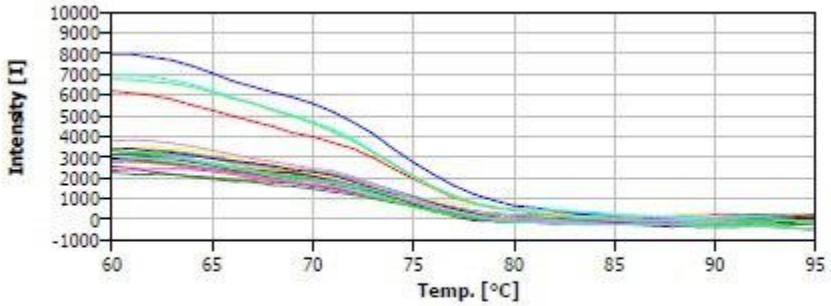

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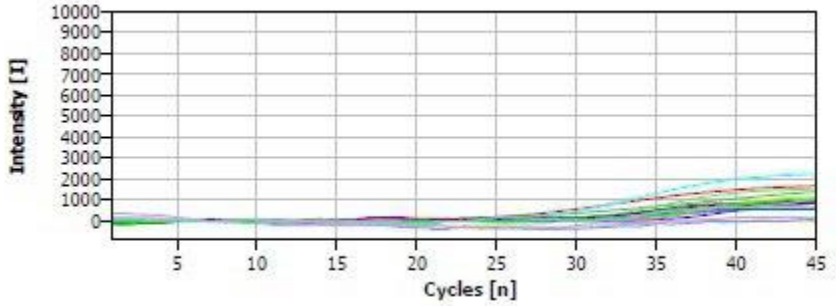

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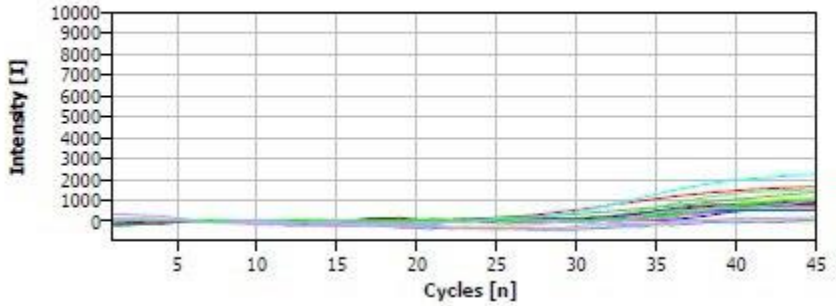
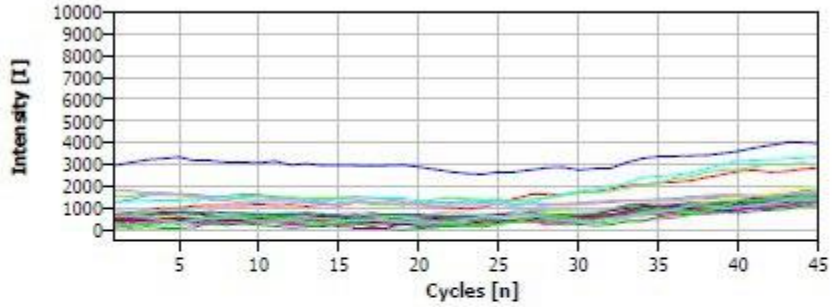


Monitoring - RawData

All colors



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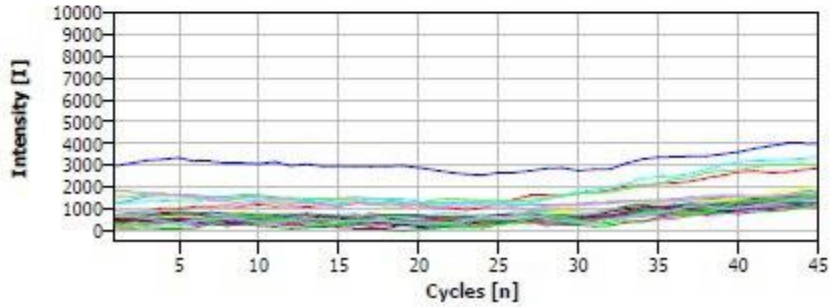
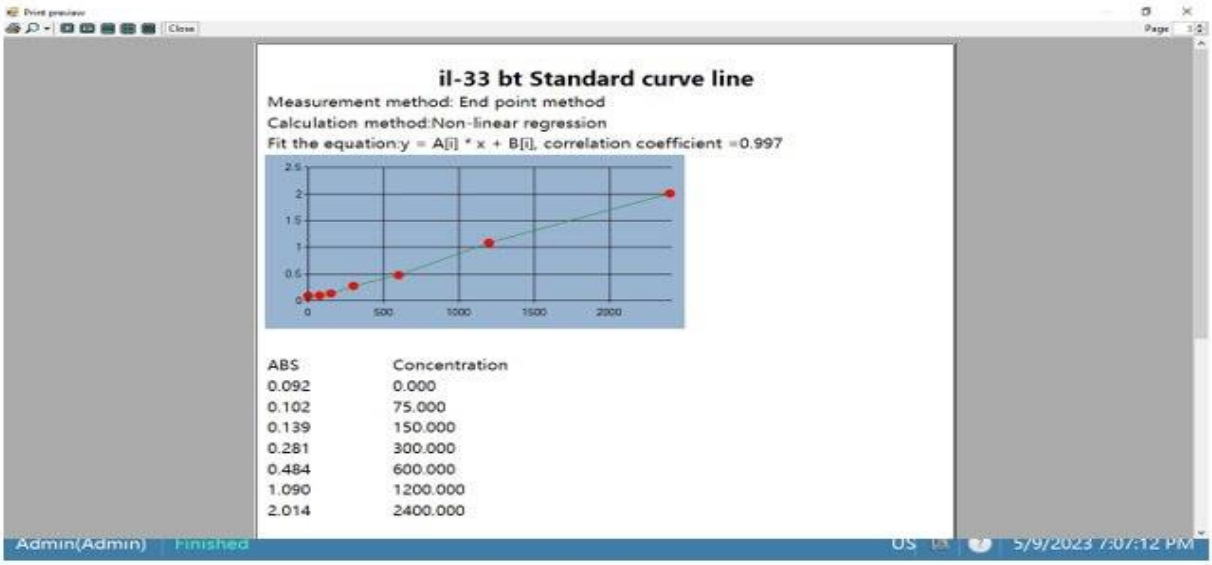


Ct

Well		Sample name	Sample type	Dye	Gene	Ct	Mean Ct
C7		10 MiR146	Unknown	FAM		43.76	43.76
D7		10 U6	Unknown	FAM		17.38	17.38
A8		2 MiR146	Unknown	FAM		38.55	38.55
B8		2 U6	Unknown	FAM		17.63	17.63
C1		3 MiR146	Unknown	FAM		30.31	30.31
D1		3 U6	Unknown	FAM		14.44	14.44
C2		4 MiR146	Unknown	FAM		37.34	37.34
D2		4 U6	Unknown	FAM		17.29	17.29
C3		5 MiR146	Unknown	FAM		36.42	36.42
D3		5 U6	Unknown	FAM		17.29	17.29
C4		6 MiR146	Unknown	FAM		38.48	38.48
D4		6 U6	Unknown	FAM		17.84	17.84
C5		8 MiR146	Unknown	FAM		35.42	35.42
D5		8 U6	Unknown	FAM		16.39	16.39
C6		9 MiR146	Unknown	FAM		37.56	37.56
D6		9 U6	Unknown	FAM		16.25	16.25
A1		C1 MiR146	Unknown	FAM		31.86	31.86
B1		C1 U6	Unknown	FAM		15.86	15.86
A6		C12 MiR146	Unknown	FAM		38.85	38.85
B6		C12 U6	Unknown	FAM		20.18	20.18
A7		C13 MiR146	Unknown	FAM		40.01	40.01
B7		C13 U6	Unknown	FAM		22.93	22.93
A2		C2 MiR146	Unknown	FAM		No Ct	
B2		C2 U6	Unknown	FAM		26.08	26.08
A3		C4 MiR146	Unknown	FAM		38.56	38.56
B3		C4 U6	Unknown	FAM		16.97	16.97
A4		C8 MiR146	Unknown	FAM		40.28	40.28
B4		C8 U6	Unknown	FAM		19.37	19.37
A5		C9 MiR146	Unknown	FAM		37.76	37.76
B5		C9 U6	Unknown	FAM		17.61	17.61

Il-33


Print preview
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il-33 bt Standard curve line
Measurement method: End point method
Calculation method:Non-linear regression
Fit the equation:y = A[i] * x + B[i], correlation coefficient =0.997
2.5
2
1.5
1
0.5
0
0
500
1000
1500
2000
ABS Concentration
0.092 0.000
0.102 75.000
0.139 150.000
0.281 300.000
0.484 600.000
1.090 1200.000
2.014 2400.000
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جمهورية العراق

محافظة كربلاء المقدسة
دائرة صحة كربلاء المقدسة
مركز التدريب والتنمية البشرية
شعبة ادارة المعرفة / وحدة ادارة البحوث

Holy Karbala governorate
Karbala Health Department
General manager's office
Training and Human Development
Center

العدد: ٢٠٥٩
التاريخ / / ٢٠٢٢
٢٢/١١/٢٩

إلى / جامعة كربلاء/كلية العلوم

الموضوع /تسهيل مهمة

تحية طيبة....

كتابكم المرقم ع.٦/٤١٢٤ في ٢٠٢٢/١١/٢٨

نود إعلامكم بأنه لا مانع لدينا من تسهيل مهمة طالب ماجستير (احمد جواد حسن) لإنجاز بحثها الموسوم حول(التحري عن دور miRNA كمؤشر حيوي في المرضى الذين يعانون من التهاب المفاصل الرثوي وهشاشة العظام وارتباطة ببعض المعلمات الفسيولوجية) وباشراف الدكتورة (تماضر مهدي صاحب) على ان لاتتحمل دائرتنا اي نفقات مادية مع الاحترام .

الدكتورة
تقوى خضر عبد الكريم
مدير مركز التدريب والتنمية البشرية
/ /٢٠٢٢

نسخة منه الى
مدينة الامام الحسين (عليه السلام)الطبية /. اجراء اللازم مع الاحترام .
مستشفى الامام الحسن (عليه السلام) اجراء اللازم مع الاحترام

الخلاصة:

أظهرت الدراسة أن مرضى التهاب المفاصل الرثوي لديهم مستويات أعلى بشكل ملحوظ من microRNA-146a (miRNA-146a) مقارنة بمجموعة المقارنة الصحية.

كان متوسط مستوى الارتفاع في المجموعة المصابة (8.49) وهو أعلى بشكل ملحوظ عن مجموعة الاصحاء (2.07) وكانت القيمة المعنوية $P \leq 0.001$.

كما أشارت الدراسة إلى ارتفاع مستوى miRNA-146a بشكل ملحوظ مع زيادة مؤشر كتلة الجسم (BMI) للمرضى , حيث كان متوسط الارتفاع في المرضى الذين يعانون من زيادة الوزن (11.2) بينما كان في الوزن الطبيعي (2) وكانت القيمة المعنوية ($P = 0.05$) بالإضافة إلى ذلك, أظهرت الدراسة أن الذكور المصابين لديهم مستوى ارتفاع أعلى بشكل ملحوظ (9.51) مقارنة بالإناث المصابات (7.46) حيث ان القيمة المعنوية ظهرت ($P = 0.009$) .

وخلصت الدراسة إلى أن مستوى الارتفاع لل microRNA-146a يعتبر من عوامل الخطورة المهمة في الإصابة بالتهاب المفاصل الرثوي .

أظهر تحليل منحنى ((Receiver Operating Characteristic Curve)) (ROC) ومنطقة أسفل المنحنى (AUC)أن مستوى ارتفاع miRNA-146a له أداء جيد في تشخيص مرضى التهاب المفاصل الروماتويدي. علاوة على ذلك, أشارت النتائج إلى اختلاف ملحوظ في مستويات إنترلوكين-33 (IL-33) والليبتين بين المجموعتين .

كان متوسط مستوى إنترلوكين-33 في المرضى (950.80) وهو أعلى بشكل ملحوظ عن المجموعة الضابطة (297.10) والقيمة المعنوية كانت ($P \leq 0.001$) وكان متوسط مستوى الليبتين (7.21) للمرضى و (2.78) لمجموعة الاصحاء .

وأظهرت الدراسة أن كلا العلامين البيولوجيين لهما اختلافات كبيرة في هذا المرض ويمثلان عوامل خطورة. وأشارت النتائج إلى أن الليبتين يعتبر عامل خطورة أعلى بثلاث مرات من إنترلوكين-33 للإصابة بالتهاب المفاصل الروماتويدي. وأظهر تحليل منطقة أسفل المنحنى (AUC) للإنترلوكين-33 كمعلمة تشخيصية , أن إنترلوكين-33 له أداء جيد في توقع الإصابة بالمرض .

في الختام, أشـــارت الدراســـة إلى أن microRNA-146a قد يكون مرشـــحًا جيدًا للعلامات البيولوجية في تشـــخيص المرض وتوقعـه وعلاجـه وتطبيقـاتـه الســـريريـة الأخرى. على الرغم من أن عـدد قليـل من microRNA تشـارك في تكوين التهاب المفاصـل الرثوي , إلا أن الباحثين يحتاجون إلى الاهتمام أكثر بأنواع أخرى من microRNA والتي قد تكون خطوة جيدة لفهم شـبكات تنظيم الجينات المسـاهمة في تكوين المرض بشكل أفضل .

جامعة كربلاء

كلية العلوم

قسم علوم الحياة

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الى مجلس كلية العلوم / جامعة كربلاء

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من قبل

أحمد جواد حسن

بكالوريوس علوم الحياة – جامعة بابل 2006

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