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Evaluation the Role of Serum Levels of anti-Carbamylated Protein, anti-14-3-3η Protein, and Dual Specificity Phosphatase 11 antibodies in Diagnosis and Severity among Patients with Rheumatoid Arthritis

A Thesis
Submitted to the Council of the College of Medicine/ University of Kerbala in Partial Fulfillment of the Requirements for the Degree of Master in Medical Microbiology

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بسم الله الرحمن الرحيم

قَالُوا سُبْحَانَكَ لَا عِلْمَ لَنَا إِلَّا مَا عَلَّمْتَنَا إِنَّكَ

أَنتَ الْعَلِيمُ الْحَكِيمُ

صدق الله العلي العظيم

(البقرة – ٣٢)

Supervisors Certification

We certify that this thesis entitled (**Evaluation the Role of Serum Levels of anti-Carbamylated Protein, anti-14-3-3η Protein, and Dual Specificity Phosphatase 11 antibodies in Diagnosis and Severity among Patients with Rheumatoid Arthritis**) was prepared under my supervision at the college of medicine/university of Kerbala as a partial fulfilment of the requirements for the degree of master in medical microbiology by MSc Student **(Zahraa Qasim Ali)**.

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We, the examining committee, certify that we have read M.Sc. thesis entitled: (**Evaluation the Role of Serum Levels of anti-Carbamylated Protein, anti-14-3-3η Protein, and Dual Specificity Phosphatase 11 antibodies in Diagnosis and Severity among Patients with Rheumatoid Arthritis**).

We have examined the student (**Zahraa Qasim Ali**) in its contents. In our opinion it meets the standards of a thesis for the Degree of Master of Science in Medical Microbiology.

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DEDICATION

To The God who gave us knowledge and faith

To The prophet of the nation and the imams

To Martyrs

To My country Iraq

To Science and Scientists

To All humanity

To My Grandfather and Grandmother

To My mother and father

To My brother and sisters

To My husband

Zahraa **(2023)**

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Summary

Rheumatoid arthritis is an autoimmune disease that mostly affects joints and surrounding tissues (bones, cartilage, and synovial tissues) and symmetrically affects the small joints of the hands and feet. All patients of rheumatoid arthritis must be satisfied six of the ten American College of Rheumatology 2010 criteria, with the recommended laboratory assays being rheumatoid factor and anti-citrullinated peptide antibody.

The study objective is to evaluate the role of carbamylated protein antibody, 14-3-3eta protein antibody, and dual specificity phosphatase 11 antibodies in diagnosis and in assessment the disease severity among rheumatoid arthritis patients both (seropositive and seronegative) patients.

The current study is case-control study conducted in Iraq/Kerbala government/Imam Al-Hassan Al-Mujtaba hospital-rheumatology unit during the period from October 2022 to April 2023, the study included 270 subjects divided into two main groups: 180 rheumatoid arthritis cases and 90 healthy controls, and then cases divided into: 90 seropositive and 90 seronegative according to the presence or absence rheumatoid factor and anti-citrullinated peptide antibody. The mean age and sex of the study groups were matched. After that, all the study participants completed questionnaires, and then venous blood samples were drawn and divided into a sodium citrate tube for the Westergren method erythrocyte sedimentation rate test and a gel tube for serum sample separation. Those were stored at -20 °C in four small Eppendorf tubes for serological assays that included a C-reactive protein test and a rheumatoid factor test by Nephelometry, an anti-citrullinated peptide antibody, a carbamylated protein antibody, the 14-3-3eta protein antibody, and a dual specificity phosphatase 11 antibody that was done by the Enzyme Linked Immune

Sorbent Assay. The statistical analysis was done using statistical package for the social sciences version 26.

The current study results showed highly statistically significant difference at *P*.value less than (0.01) for rheumatoid arthritis patients when compared to healthy control regarding to erythrocyte sedimentation rate, C-reactive protein, rheumatoid factor, anti-citrullinated peptide antibody, and the current study markers. The current study markers anti-carbamylated protein antibody, anti-14-3-3eta and anti-dual specificity phosphatase 11 antibody were statistically significant difference in seronegative group than in seropositive at *P*.value less than (0.05), and in patients with regular treatment than irregulars at *P*.value less than (0.05), when compared with routine tests rheumatoid factor and anti-citrullinated peptide antibody which were highly statistically significant difference in the seropositive group at *P*.value less than (0.01), and in patients with irregular treatment at *P*.value less than (0.01). The current study markers associated significantly with mild to moderate disease activity score28 at *P*.value less than (0.05), when compared with routine tests which associated significantly with severe disease activity score28 at *P*.value less than (0.05). The current study markers have positive statistically significant correlation among them at *P*.value less than (0.05), except anti-dual specificity phosphatase 11 antibodies have negative statistically significant correlation with disease duration and disease activity score28 at *P*.value less than (0.05).

The study suggests that anti-carbamylated protein, anti-14-3-3eta , and anti-dual specificity phosphatase 11 antibodies can be used as diagnostic markers, particularly in seronegative rheumatoid arthritis patients, as they are associated with mild to moderate disease activity and regular treatment compared to routine tests, this making them useful prognostic markers.

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List of Abbreviations

Abbreviations	*Meaning*
+Ve	Positive
Ab	Antibody
ACPA	Anti-Citrullinated Peptide Antibody
ACR	American College of Rheumatology
Ag	Antigen
Anti-14-3-3η	Anti-14-3-3 Eta
Anti-CarP	Anti-Carbamylated Protein Antibody
Anti-CCP	Anti-cyclic Citrullinated Peptide Antibody
Anti-DUSP11	Anti-Dual Specificity Phosphatase 11 Antibody
APC	Antigen presenting cell
APRs	Acute Phase Reactants
AUC	Area Under Curve
CRP	C-Reactive Protein
CS	Cigarette Smoking
CXCL13	Chemokine motif Ligand 13
D.D	Disease Duration
DAS	Disease Activity Score
DC	Dendritic Cell
DMARDs	Disease Modifying Anti-Rheumatic medications
ELISA	Enzyme Linked Immunosorbent Assay
Ems	Extra-articular Manifestations
ESR	Erythrocyte Sedimentation Rate
EULAR	European League Against Rheumatism
FDRs	First-Degree Relatives
FLSs	Fibroblast-Like Synoviocytes
GCs	Glucocorticoids
GM-CSF	Granulocyte-Macrophage Colony Stimulating Factor
GWAS	Genome-Wide Association Studies
hnRNP (antiRA33Ab)	Heterogeneous nuclear ribonucleoprotein
IC	Immune Complex
Ig	Immunoglobulin
IL	Interleukin
INF-ɣ	Interferon gamma

List of Abbreviations

MAPKs	Mitogen-Activated Protein Kinases
MENA	Middle East and North Africa
MHC	Major Histocompatibility Complex
MMP	Matrix Metalloproteinase
NK	Natural killer cell
NOD	Nucleotide-binding Oligomerization Domain
NSAIDs	Non-steroidal Anti-inflammatory Drugs
OD	Optical Density
P.value	Probare value
PAD	Peptidyl Arginine Deiminase
PAMPs	Pathogen-Associated Molecular Patterns
PD-1	Programmed cell Death protein 1
PRRs	Pattern-Recognition Receptors
R	Correlation coefficient
RA	Rheumatoid Arthritis
RANKL	Receptor Activator of NF-kB Ligand
RF	Rheumatoid Factor
ROC	Receiver Operating Characteristic Curve
ROS	Reactive Oxygen Species
SE	Shared Epitope
SPSS	Statistical Package for the Social Sciences
TGF_{β}	Transforming Growth Factor beta
Th	T-helper cell
TLR	Tool-Like Receptor
TNF	Tumor Necrosis Factor
TNF-α	Tumor Necrosis Factor alpha
Treg	T-regulatory cell
-Ve	Negative
WHO	World Health Organization
Y	Year

1.1. Introduction

Rheumatoid arthritis (RA) is a systemic autoimmune disease with synovial joint manifestations that are frequently accompanied by extra-articular manifestations (Baig and DiRenzo, 2023).

The term 'rheumatoid arthritis' derives from the Greek word for inflamed and swollen joints. In 1880, French physician Auguste Jacob Landré-Beauvais was the first person that describes and classifies this debilitating disease (Ding *et al.*, 2023)

Rheumatoid arthritis affects all racial groups and manifests at a variety of ages, the average ages of women and men at diagnosis are approximately 50 and 60 years old. The estimated global prevalence is between 0.5 and 1 percent, women are roughly three times more prone to developing RA than men (Deane *et al.*, 2023).

The causative agents of Rheumatoid arthritis are unknown but believed to involve complex interaction between genetic and environmental factors (Zaccardelli *et al.*, 2019). Consequently, a positive family history multiplies the risk of RA by three to five, indicating a statistically significant difference genetic component to this process, the genetic susceptibility to RA is most evident in the HLA-DR epitope, particularly HLA-DR4, which is known as the "susceptibility epitope." This is present in 70% of RA patients and is associated with disease severity (Siouti and Andreakos, 2019).

The clinical manifestation of the disease is repeated and symmetrical affects the hand, wrist, foot, knee, and other joints. In the early phases redness, swelling, heat, pain, and joint dysfunction. In the later phases, rigidity and deformity of the joints are observed (Fresneda AlarconMcLaren and Wright, 2021). The extra-articular involved the eyes, nerves, epidermis, kidney, lungs, liver, and heart (Ding *et al.*, 2023).

Early diagnosis could prevent joint damage, a large body of evidence indicates that statistically significant difference permanent joint damage can occur within the first two years of disease onset (Cheng *et al.*, 2021) ,consequently, optimal management of RA is crucial during the first three to six months. Thus, reliable biomarkers are required for early disease diagnosis, an accurate prognosis, and enhanced disease management (Mueller *et al.*, 2021).

Rheumatoid arthritis is typically diagnosed according to the 2010 ACR-EULAR (American college of rheumatology-European league against rheumatism) (Ishida *et al.*, 2021).

The routinely test like C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) are used as clinical biomarkers to ascertain the general inflammatory state of RA patients (He *et al.*, 2020).

Multiple antibody have been identified in rheumatoid arthritis based on the antigens to which these antibodies attach, these include anti-citrullinated protein antibodies (ACPA) and rheumatoid factor (RF) and according to their positivity in serum of patients with RA, patients usually divided into seropositive and seronegative (van Delft and Huizinga, 2020).

The most clinically statistically significant difference autoantibodies ACPA target citrullinated peptides, whereas RF targets the Fc region of IgG (Baig and DiRenzo, 2023).

Antibodies to heterogeneous nuclear ribonucleoprotein (hnRNP) also known as anti-RA33 antibodies have been characterized in RA (Cappelli *et al.*, 2022).

Recently, a number of novel candidates have been proposed as potential RA biomarkers and implicated in development of RA, seronegative RA is problematic from both a diagnostic and a pathogenic standpoint (SokolovaSchett and Steffen, 2021).

In 2011, it was found that patients with RA have antibodies against carbamylated protein antigen, or anti-CarP or ACP. Since then, studies have shown how important these antibodies are for prognosis and prediction, as well as how they contribute to the pathophysiology of RA (Mohamed *et al.*, 2019).

Compared to traditional diagnostic indicators, the eta protein antibody (anti-14-3-3η) may be more sensitive and specific in the early detection of RA (Alashkar *et al.*, 2022).

More recent methods, like high-density protein microarrays, have recently found a variety of other potential autoantibody specificities in approximately 35% of ACPA-negative patients, with more than 90% specificity for RA. Anti-DUSP11 was discovered to have the best diagnostic performance, regardless of ACPA status (De Stefano *et al.*, 2021).

The drugs that maintain joint function as conventional synthetic disease-modifying anti-rheumatic medications (DMARDs), biologic DMARDs, and targeted synthetic DMARDs, a novel class of non-biologic DMARDs. Nonsteroidal anti-inflammatory drugs (NSAIDs) and glucocorticoids (GCs) are used to reduce inflammation in RA patients with inadequate symptom control (Radu and Bungau, 2021).

1.2. Aims of The Study

- To evaluate the serum level of Carbamylated Protein Antibody (anti-CarP), the 14-3-3η Protein Antibody (anti-14-3-3η), and Dual Specificity Phosphatase 11 Antibody (anti-DUSP11) in RA patients and healthy peoples.
- To evaluate the serum level of anti-CarP, anti-14-3-3η, anti-DUSP11 in seropositive and seronegative RA patients with the diagnosis and severity of disease.
- These aims achieved by following objectives:
  1. Assess the level of (ESR) by Westergren Method.
  2. Detection of (RF) and (CRP) by Nephelometry.
  3. Calculate disease activity score for the severity of disease by (DAS) calculator.
  4. Measurement of (ACPA, anti-CarP, anti-14-3-3η, and anti-DUSP11) by ELISA test**.**
  5. Analysis the study data by (SPSS).

1.3. Literature Review

1.3.1. Rheumatoid Arthritis (RA)

Rheumatoid arthritis is a chronic inflammatory autoimmune disorder that causes progressive and irreversible joint injuries due to sustained synovitis (Peng *et al.*, 2023).

Clinically, RA manifests as a chronic symmetrical disease that affects minor joints such as the proximal interphalangeal and metacarpophalangeal joints before progressing to larger joints. In general, these patients experience pain and rigidity in multiple joints, resulting in a diminished quality of life, fever, fatigue, and weight loss (Conforti *et al.*, 2021).

Prevalence and incidence measures of RA vary by population and have varied throughout time (Radu and Bungau, 2021).

Rheumatoid arthritis is a heterogeneous disorder caused by an abnormal autoimmune response initiated by the complex interactions of genetic and environmental factors that contribute to RA etiology (Zamanpoor, 2019). There appears to be an essential interplay between components of the adaptive immune system and the innate immune system, abnormalities in the cellular and humoral immune responses contribute to the occurrence of autoantibodies (SchererHäupl and Burmester, 2020).

The overproduction of pro-inflammatory cytokines, such as tumor necrosis factor (TNF) and interleukin-6 (IL-6), results in the proliferation of synovial cells in joints and the consequent formation of pannus, cartilage destruction, and bone erosions (Nattagh-Eshtivani *et al.*, 2021).

On the basis of the presence of autoantibodies, rheumatoid factor (RF) and anti-citrullinated protein antibodies (ACPA), RA can be divided immunologically into two major groups: seropositive and seronegative (Gravallese and Firestein, 2023).

1.3.2. Rheumatoid Arthritis History

Rheumatoid arthritis comes from the Greek word for inflamed and swollen joints. Dr. Alfred Baring Garrod, a UK rheumatologist coined the term "rheumatoid arthritis" in 1859. In 1880, a French physician Auguste Jacob Landré-Beauvais was the first individual to describe and classify this debilitating disease, Landré-Beauvais documented the statistically significant difference symptoms of the disease as "asthenic gout," indicating that the condition was prevalent in females (Ding *et al.*, 2023).

1.3.3. Rheumatoid Arthritis Epidemiology

Epidemiologic variations in the incidence and prevalence of rheumatoid arthritis have been observed based on ethnic and geographic dispersion (Nair *et al.*, 2019). The estimated global prevalence of RA ranges from 0.24 to 1%, although rates differ by region and country (Almoallim *et al.*, 2021).

The epidemiology of RA is poorly understood in the Middle East and North Africa (MENA) region, data on its prevalence and disease activity among Arab populations are rare (Bedaiwi *et al.*, 2019)

A recent global burden study estimated the prevalence of RA in the MENA region to be 0.16 percent, and RA disease severity and management differ geographically within the region (Yip and Navarro-Millán, 2021).

The incidence of RA in Iraq was 1.1% in 2014 and 2.2% in 2019, compared to 1.6% and 2.1% in 2001 and 2011, respectively. Although this variation is not statistically significant difference, it may be attributable to disruptions in the healthcare system and immigration during this time period (Al_Badran *et al.*, 2022).

During the previous decade, it was observed that the prevalence and clinical characteristics of rheumatic diseases varied markedly by region, lifestyle, and social status, indicating that genetic and environmental factors play a substantial role in the onset and progression of rheumatic diseases (Batko *et al.*, 2019).

1.3.4. Rheumatoid Arthritis Risk Factors

Numerous studies on the etiology of RA have been conducted over the past few decades and the evidence suggests that environmental and genetic factors play a statistically significant difference role in causing RA. Indeed, the susceptibility genes HLA-DRB1, TNFRSF14, and PTPN22 are closely associated with RA (Dedmon, 2020).

Environmental factors such as smoking, personal dietary patterns and hygiene, which directly affect the post-transcriptional modification of certain genes or indirectly affect susceptibility genes via epigenetic mechanisms are also crucial in the development of RA (Nemtsova *et al.*, 2019).

The interaction of environmental factors, epigenetics, and susceptibility genes will lead to changes in the relative levels and expression of encoded proteins, which may contribute to autoimmune tolerance disorders (Ding *et al.*, 2023).

1.3.4.1. Genetic and Epigenetic Factors

Several indicators strongly suggest that genetics play a statistically significant difference role in the development of RA. These factors include the overall increased prevalence of RA within families, resulting in an estimated familial risk contribution of 40–50% of seropositive RA with the greatest risks observed in first-degree relatives (FDRs), the risk of RA among first-degree relatives is 1.5 times that of the general population (Rhida and Mahdi, 2022).

Genome-wide association studies (GWAS) and independent replication studies have identified potential genes associated with RA susceptibility, particularly major histocompatibility complex (MHC) genes, which are subdivided into class I (HLA-A, B, and C), class II (HLA-DR, DP, and DQ) and class III sub-regions (Dedmon, 2020). It has been established that HLA-DR, particularly the HLA-DRB1 locus contributes statistically significant difference to the risk of developing RA by encoding MHC class II antigen-presenting molecules that can accommodate a wide variety of peptide ligands (WysockiOlesińska and Paradowska-Gorycka, 2020). Shared epitope (SE) refers to the similar amino acid sequences at positions 70–74 on the HLA-DR chain shared by the majority of RA-associated HLA-DRB1 alleles (Croia *et al.*, 2019).

Genetic heterogeneity does not fully explain the characteristics of RA. Consequently, the study of epigenetic factors and mechanisms associated with the progression of a disease and its response to treatment become increasingly vital (Nemtsova *et al.*, 2019).

Changes in gene expression that are inherited without transforming the DNA sequence determine which genes are active or inactive. Histone modification, DNA methylation, and non-coding RNA mechanisms are the primary mechanisms linked to this process (Karami *et al.*, 2020).

1.3.4.2. Environmental Factors

Numerous ambient factors have been identified as risk factors for the development of RA, with cigarette smoking being the strongest and most consistently identified (Cush, 2022). There is growing evidence that chronic mucosal inflammation such as periodontitis, dysbiosis of the intestine and airway inflammation, is associated with an increased risk of developing RA (Wang *et al.*, 2019).

Epstein-Barr virus and other viruses have been implicated for decades as probable risk factors for RA (Baig and DiRenzo, 2023).

1.3.4.2.1. Smoking

Cigarette smoking is the most known external factor identified as trigger of RA (Croia *et al.*, 2019). Cigarette smoking has been implicated as an environmental risk factor for seropositive RA, possibly by inducing autoimmunity in the pulmonary mucosa and causing the body to emit inflammatory cytokines that contribute to the joint and organ damage associated with RA, up to 35% of the risk of seropositive RA is attributable to cigarette consumption (Ren *et al.*, 2023).

Recent studies have investigated passive cigarette smoking as a possible risk factor for RA in non-smoking patients (PriscoMartin and Sparks, 2020).

Passive smokers had a risk of RA that was 12% greater than that of non-exposed individuals, the risk of developing RA was 34% higher in individuals exposed to passive smoking during childhood compared to those who were not exposed. RA risk may be associated with passive smoking, particularly in childhood exposures (Zhang *et al.*, 2023b).

1.3.4.2.2. Traffic Pollution

Recent research has examined the potential impact of air pollution on the development of autoimmune diseases like rheumatoid arthritis. Evidence suggests that the airway tissues may be capable of transforming airborne particles into antigens and presenting them as an interface between the circulation and the airway. Thus, the particles in ambient air function as autoimmunity precursors and triggers (Sigaux *et al.*, 2019).

The World Health Organization (WHO) currently lists air pollution as one of the most statistically significant difference health concerns. It is well

established that smoking and silica exposure which induce an inflammatory and oxidative stress response can increase the risk of RA (Zhang *et al.*, 2023a), pollutants in the environment are mixture of gases, Ozone (O3), nitrogen dioxide (NO2), carbon monoxide (CO) and sulphur dioxide (SO2) are the pollutants with the strongest evidence of public health risk (Alsaber *et al.*, 2020).

In addition, a previous study revealed that the lung may be the site of early autoimmunity-related damage in RA. Exposure to air pollution disrupts oxidation-reduction homeostasis in the respiratory mucosa and induces pro-inflammatory immune responses in multiple immune cells, suggesting that air pollution may be a risk factor for RA (PriscoMartin and Sparks, 2020).

1.3.4.3. Host Factors

1.3.4.3.1. Sex and Hormones

Sex is the most influential epidemiological factor associated with the onset of RA. Due to its autoimmunity, RA is more prevalent in women than in men approximately three times as often (Intriago *et al.*, 2019). Due to stronger inherent and adaptive immune responses compared to males. Women with diabetes mellitus in Iraq become more susceptible to symptomatic arthritis get older (Rashid, 2023).

Considering the female predominance in the distribution of RA, hormonal and sex-related factors have been studied for long time as disease risk factors (ChancayGuendsechadze and Blanco, 2019).

The hormone imbalance is commonly attributed to estrogens, which are commonly characterized as pro-inflammatory, in contrast to the decreased anti-inflammatory effects of progesterone and androgens in RA patients (Romão and Fonseca, 2021).

For example, androgens, particularly testosterone, have an anti-inflammatory and immunosuppressive function, whereas estrogens depending on their concentration exhibit a dual effect (Benagiano *et al.*, 2019). In fact, high estrogen levels (e.g., per ovulatory or pregnancy levels) promote a shift from a pro-inflammatory T helper 1 (Th1)/Th17 cell immune response to an anti-inflammatory Th2/T-regulatory cell (T-reg) response, whereas low estrogen levels (e.g., luteal or postmenopausal levels) induce the opposite shift. Consequently, menarche, pregnancy, the postpartum period, menopause, and the use of hormone replacement therapies all have an effect on disease activity (Dupuis *et al.*, 2021).

1.3.4.3.2. Vitamin-D Deficiency

Vitamin-D is a steroid hormone and one of the most important immunomodulatory endocrine mediators (SaponaroSaba and Zucchi, 2020), dietary sources of Vitamin-D include oily fish, eggs and dairy products. In human, 7-dehydrocholesterol endures a series of ultraviolet-light-mediated modifications to generate vitamin-D (Harrison *et al.*, 2020).

Vitamin-D is known to exert anti-inflammatory effects on multiple immune cells [macrophages, dendritic cells, lymphocytes, and fibroblast-like synoviocytes (FLSs)] that express the vitamin-D receptor. It regulates the production and release of autoantibodies by B cells (AoKikuta and Ishii, 2021). It inhibits the proliferation and differentiation of activated B-cells by inducing apoptosis. Vitamin-D inhibits T cell proliferation and the production of IL2, interferon gamma (INF-ɣ), and TNF-α (alpha) cytokines (Aslam *et al.*, 2019).

The relationship between the immune system and vitamin-D has been revealed over the past two decades. This information along with the repeated finding that low vitamin-D levels and vitamin-D deficiency were

prevalent among RA patients prompted the investigation of vitamin-D as a potential RA protective factor (Romão and Fonseca, 2021).

A deficiency in vitamin-D negatively impacts bone mass, resulting in rickets in children and adolescents ,osteoporosis and osteomalacia in adults, also it has been linked to the onset or maintenance of other diseases such as cardiovascular diseases, chronic obstructive pulmonary diseases, allergic asthma, type two diabetes, and autoimmune diseases (Sizar *et al.*, 2022).

The active form of vitamin-D is [1,25$(OH)_2$D] that exerts immunologic activities on both innate and adaptive immune system components (Bikle and Christakos, 2020), that inhibits inflammation by inhibiting the expression of Toll-like receptor (TLR) 2 and 4, and the production of inflammatory cytokines, such as interleukin IL-1, IL-6, and TNF-α , which play a crucial pathogenic role in autoimmune diseases (Dupuis *et al.*, 2021).

1.3.5. Rheumatoid Arthritis Immunological Mechanism

An abnormal immune response is mediated by the formation of antigen-antibody aggregates known as "immune complexes" in type III hypersensitivity reactions. They can precipitate in diverse tissues, including the epidermis, joints, blood vessels, and glomeruli, and activate the classical complement pathway (Dispenza, 2019).

At the site of immune complexes, complement activation recruits inflammatory cells (monocytes and neutrophils) that release lysosomal enzymes and free radicals, causing tissue injury. RA is the most prevalent disease associated with a type III hypersensitivity reaction (Usman and Annamaraju, 2021).

The network of innate and adaptive immune systems plays crucial roles in the pathogenesis of RA. Autoimmunity in joints or other organs is the initial symptom of RA, which is a state of continuous cellular

activation. The majority of the disease's clinical manifestations occur following synovial inflammation and joint injury (KondoKuroda and Kobayashi, 2021).

Fibroblast-like synoviocytes (FLS) play a vital function in these pathological conditions. A non-specific inflammatory stage, amplified by T-cell activation in the synovium, a chronic inflammatory stage, and a tissue injury stage mediated by cytokines such as IL-1, IL-6, and TNF-α are reported as the three stages of RA progression (Ding *et al.*, 2023).

Autoantibody production has been linked to severe symptoms such as joint damage and increased mortality (Lucchino *et al.*, 2019). This is probable because autoantibodies against citrullinated peptides (ACPA) generate immune complexes with citrulline-containing antigens. These complexes then adhere to rheumatoid factors (RF), resulting in the activation of the complement system (JangKwon and Lee, 2022).

1.3.5.1. Role of Innate Immune System

The innate immune system is activated by host responses to pathogen-associated molecular patterns (PAMPs) induced by interactions with pattern-recognition receptors (PRRs) on synovial joint immune cells, including neutrophils, macrophages, dendritic cells (DC), natural killer cells (NK), mast cells and eosinophils, PRRs include Toll-like receptors (TLRs) and nucleotide-binding oligomerization domain (NOD)-like receptors (Croia *et al.*, 2019).

Macrophages secrete reactive oxygen intermediates, nitrogen intermediates, matrix-degrading enzymes, inducible nitric oxide synthase, and proinflammatory cytokines such as TNF-α and IL1$_\beta$, which are indicative of an M1 macrophage phenotype (Cutolo *et al.*, 2022).

Neutrophils are the first cells to reach the synovium and the most prevalent leukocytes in inflamed joints, neutrophils bind the immune

complexes on the synovium through their Fc receptors on the neutrophil membrane, triggering their degranulation and reactive oxygen species (ROS) production, this increased ROS production by neutrophils at the site of inflammation causes endothelial dysfunction and tissue injury (EdilovaAkram and Abdul-Sater, 2021).

1.3.5.2. Role of Adaptive Immune System

Autoantibodies and the genetics of RA place adaptive immunity at the center of early pathogenesis. The synovium contains abundance of myeloid cells and plasmacytoid dendritic cells that express cytokines (IL-12, and IL 23), HLA class II molecules, and costimulatory molecules necessary for T-cell activation and antigen presentation (Zhao *et al.*, 2021).

Although RA is conventionally believed to be a disease mediated by type 1 helper T cells, the role of type 17 helper T cells (Th17), a subset that produces IL-17A, 17F, 21, and 22 and TNF-α, has received increasing attention (Giannini *et al.*, 2020).

Macrophage and dendritic cell derived transforming growth factor beta (TGFβ) and interleukin-1β, 6, 21, and 23 stimulate Th17 differentiation and inhibit regulatory T-cell differentiation, thereby shifting T-cell homeostasis towards inflammation (Tu *et al.*, 2021).

Antigen presentation to T cells is another function of joint-infiltrating B cells, which likely contributes to the pathogenesis of RA, B cells and T cells that have been activated typically aggregate in the synovium (CheminGerstner and Malmström, 2019). Some B cells in the synovium differentiate into plasma cells that produce auto-antibodies such as ACPA and RF, whereas others differentiate into effector B cells that produce pro-inflammatory cytokines and express the anti-inflammatory molecule interleukin-6 (Testa *et al.*, 2021).

The receptor activator of NF-kB ligand (RANKL) is essential for the development of osteoclasts involved in joint destruction in RA. It is well

known that RANKL is expressed on fibroblasts, osteoblasts, and T cells, but a portion of B cells also express RANKL (Takeuchi *et al.*, 2019).

Recently identified CD4 T cell subset peripheral helper T cell which is characterized by the expression of programmed cell death protein 1 (PD-1) is an inhibitory receptor expressed as a negative feedback mechanism on activated T cells and production of the chemokine C-X-C motif ligand 13 (CXCL13) (Lowe *et al.*, 2023) , inflamed synovial tissues create chemokine which attracts B cells after antigen-dependent interaction with antigen-presenting cells (APC),activated monocytes and T-cells create this factor, and IL-21 is implicated (SchererHäupl and Burmester, 2020).

The latter might be mediated by Th1-like CD4 T cell subsets that can produce multiple pro-inflammatory cytokines (Jiang *et al.*, 2021), including IFN-ɣ, TNF-α, and Granulocyte-Macrophage Colony Stimulating Factor(GM-CSF), and express cytotoxic molecules such as perforin, granzymes, and granulysin. CD8 T cells within the synovium are capable of producing substantial quantities of IFN-ɣ, However, the role of these lymphocytes in the pathogenesis of RA remains unverified (Yamada, 2022).

1.3.5.3. Role of Complement System

The complement system is a component of innate immunity and functions to recognize foreign antigens and initiate an inflammatory response. There are three distinct but interconnected pathways: classical, lectin, and alternative (GrandNavrazhina and Frew, 2020).

Since the 1950s, when a group of researchers began measuring complement levels in the synovial fluid and plasma of patients with RA and discovered contradictory results, the complement pathway has been investigated in RA (Pabón-Porras *et al.*, 2019). Patients with RA have elevated complement levels in their serum but lesser levels in their synovial

fluid compared to healthy individuals and those with other types of joint disease (de Seny *et al.*, 2020).

In rheumatoid arthritis numerous antibodies react with antigens in the joints and have the potential to create immune complexes (IC) within cartilage and synovial pannus tissue (XieJane-Wit and Pober, 2020). IC activates complement, is opsonized by early complement components, and is subsequently taken up by phagocytes containing complement receptors. In fact, elevated levels of complement activation products and increased C3 and C4 consumption can be detected in the synovial fluids of RA patients (Dijkstra *et al.*, 2019).

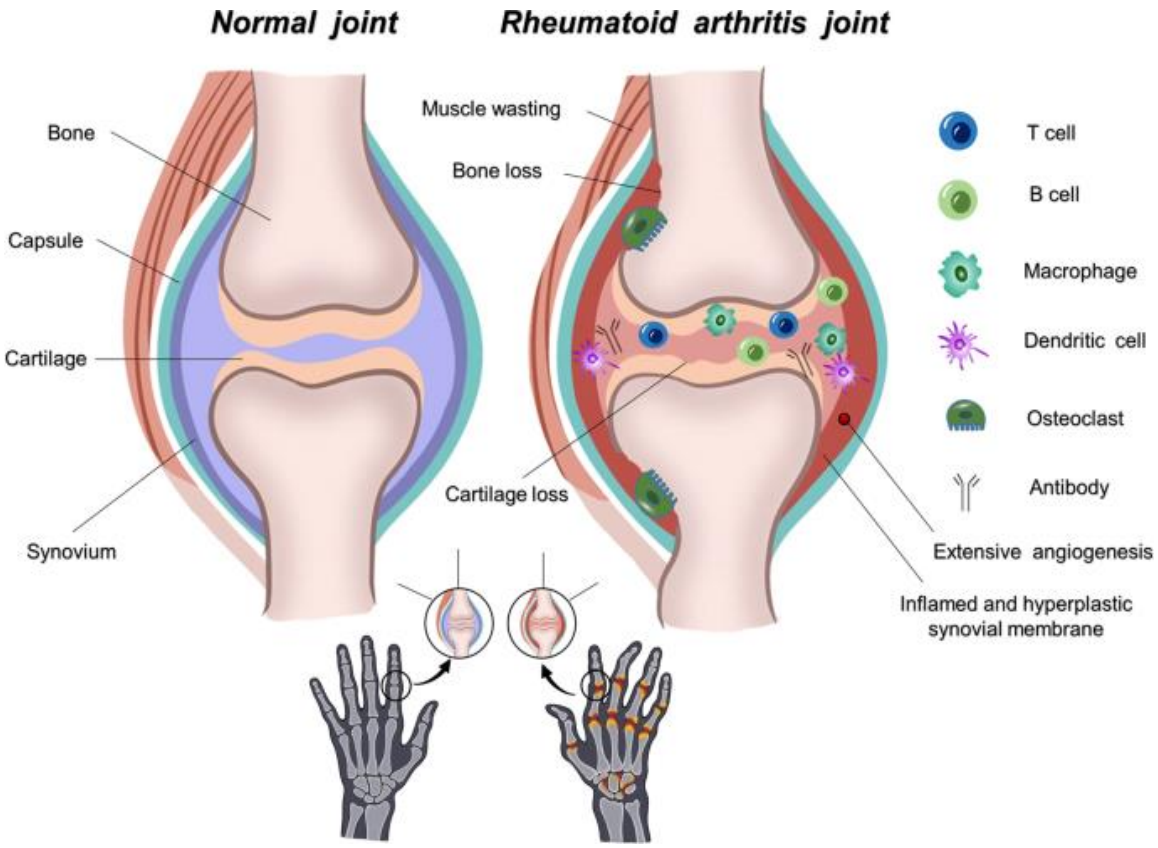
The synovial fluid of RA patients shows deposition of immunoglobulin (Ig), C1q, C3, and C4 that could lead to activation of infiltrated macrophages, mast cells, fibroblasts, and granulocytes by pro-inflammatory cytokines, resulting in their degranulation and release of proteolytic enzymes leading to joint erosion (Fukami *et al.*, 2022), (Banda *et al.*, 2022).

There was statistically significant difference positive correlation between circulating IC and C4/C4b, but not C3/C3b. However, complement activation during RA does not appear to be limited to the classical pathway, as there is evidence that the concentration of Bb fragments generated during the formation of the C3 convertase of the alternative pathway also increases in the synovial fluid of affected patients (Goldberg and Ackerman, 2020). It is important to remember that the alternative pathway acts as an efficient amplification loop for the classical pathway at the stage of C3b production. Consequently, the alternative pathway is also essential for processes that were initiated by the activation of the classical pathway (HarrisonHarris and Thurman, 2023).

1.3.6. Clinical manifestations

Rheumatoid arthritis is an autoimmune, inflammatory disease that begins with minor joints and progresses to larger joints causes function loss in various joints (most frequently the hands, wrists, and knees) (Prasad *et al.*, 2023). The lining of the affected joint becomes inflamed, resulting in tissue injury, chronic pain (IqbalRattu and Shah, 2019).

All of this injury to the joints results in deformities and bone erosion, which are typically extremely painful for the patient. Common RA symptoms include morning rigidity of the affected joints for more than thirty minutes, fatigue, fever, weight loss, swollen, warm joints, and rheumatoid nodules on the skin. The onset of this disease occurs between the ages of 35 and 60 (Bullock *et al.*, 2019).



(Ding *et al.*, 2023)

Even though synovitis is the pathological hallmark of RA, it is likely that many extra-articular manifestations (EMs) and comorbidities occur due to the disease's complex, chronic, inflammatory, and autoimmune features, resulting in increased morbidity and premature mortality (Laria *et al.*, 2022). Chronic inflammation in RA can cause EMs such as vasculitis, as well as cardiovascular (CV), pulmonary, neurological, gastrointestinal, renal, and hematologic diseases (Figus *et al.*, 2021).

1.3.7. Rheumatoid Arthritis Diagnosis

The classification criteria (ACR/EULAR-2010) American college of rheumatology (ACR) and European league against rheumatism (EULAR) are used to diagnose RA. The application of these criteria yields a value between 0 and 10, with a score of 6 being sufficient for diagnosing definite RA (JangKwon and Lee, 2022). ACR/EULAR criteria for 2010 included serologic testing Rheumatoid Factor (RF) and Anti-citrullinated peptide antibody (ACPA) (Ding *et al.*, 2023).

According to previous mention antibodies RA patients were classified into seropositive and seronegative groups (Luan *et al.*, 2021).

It has long been known that seronegative RA is a form of RA in which RF is absent, ACPA is absent, or both are absent during the course of the illness. In distinct ways, seropositive and seronegative RA appears to "behave" differently (Paalanen *et al.*, 2021).

RA is typically diagnosed by a combination of patient symptoms, the results of the doctor's examination, the assessment of risk factors, family history, joint assessment by ultrasound sonography, and laboratory markers such as elevated levels of CRP and ESR in serum and the detection of RA-specific autoantibodies (LinAnzaghe and Schülke, 2020).

Criteria	Score
A. Joint involvement	
1 large joint	0
2-10 large joints	1
1-3 small joints (with or without involvement of large joints)	2
4-10 small joints (with or without involvement of large joints)	3
>10 joints (at least 1 small joint	5
B. Serology (at least 1 test result is needed for classification)	
Negative RF **and** negative ACPA	0
Low-positive RF **or** low-positive ACPA	2
High-positive RF **or** high-positive ACPA	3
C. Acute-Phase Reactants (at least one is needed for classification)	
Normal CRP **and** normal ESR	0
Abnormal CRP **or** abnormal ESR	1
D. Duration of Symptoms	
<6 weeks	0
≥6 weeks	1

(Aletaha *et al.* 2010).

1.3.7.1. Acute Phase Reactants

Acute phase reactants (APRs) are proteins whose serum concentrations increase or diminish by at least 25% during inflammatory states. The effects of cytokines, such as IL6, IL-1 TNF-α, and IFN-ɣ, are primarily responsible for alterations in APR levels (Almoallim and Cheikh, 2021).

The clinical biomarkers to ascertain the general inflammatory state of RA patients like CRP and ESR are routinely used (Shapiro, 2021).

C-Reactive Proteins (CRP) is an acute-phase reactant made up of five 23-kDa subunits from the pentraxin protein family. In the presence of infection, inflammation, or tissue injury, its serum concentration can increase by at least three log steps (Pathak and Agrawal, 2019).

Erythrocyte sedimentation rate (ESR) is a widely used standard laboratory test that measures the rate at which erythrocytes settle in a test

container containing a blood sample from the patient in question. In the presence of inflammatory processes, infections, and autoimmune disorders like RA, as well as pregnancy, anemia, certain kidney diseases, and certain cancers, increased concentrations of fibrinogen in the blood induce red blood cell coagulation (Narang et al., 2020).

During this process, the erythrocytes form bundles known as "rouleaux" that settle more quickly in the test tube due to their increased density. The international committee for standardization in hematology (ICSH) reference procedure for measuring the ESR is based on findings described by Westergren a century ago (Tishkowski and Gupta, 2022).

1.3.7.2. Disease Activity Score (DAS)

The Disease Activity Score DAS/DAS28 is a continuous assessment of the activity of the RA disorder that incorporates data from acute phase response, tender joints, swollen joints, and general health (Van Riel and Renskers, 2016).

A laboratory assessment of acute inflammation, general health, swollen joints, and tender joints is used to calculate DAS28 scores, which range from 0 to 9.4 (Greenmyer *et al.*, 2020).

The Disease Activity Score based on 28 joints (DAS28) has been increasingly used in clinical practice and research studies of RA. Studies have reported calculating DAS28 based on ESR (DAS28-ESR) and CRP (DAS28-CRP) in patients with RA (Tamhane *et al.*, 2013).

1.3.7.3. Rheumatoid Factor Antibody(RF)

Rheumatoid factor (RF) antibodies detecting the Fc-tail of immunoglobulin (IgG) was the first autoantibodies identified in RA and were utilized in the 1987 ACR classification criteria for RA, regardless of its lack of specificity. The test can be considered useful in clinical examinations where a high pre-test probability of RA exists (van Delft and Huizinga, 2020).

In 1948, these antibodies were identified in patients with RA and in 1952, due to their strong association with RA, they became known as RF. RF are autoantibodies that directly bind to the Fc portion of aggregated IgG and are generated locally by B cells in lymphoid follicles and germinal center-like structures that develop in inflamed RA synovium (RochaBaldo and Andrade, 2019).

Multiple studies have shown that the RF response utilizes a wide range of isotypes, including IgM, IgG, and IgA (SokolovaSchett and Steffen, 2021).

Furthermore, RF IgA has been shown to play an important role in RA disease manifestation (Brandl *et al.*, 2021). In addition to RA, RF have been identified in non-rheumatoid conditions such as leprosy, Kala Azar, syphilis, pulmonary tuberculosis, chronic liver disease, and sarcoidosis, as well as in many rheumatologically diseases such as SLE and Sjogren's disease. The frequency of RF IgM increases with age, while RF IgG, surprisingly decreases (Pertsinidou *et al.*, 2021).

1.3.7.4. Anti-Citrullinated Peptide Antibody

Anti-citrullinated peptide antibody (ACPA) is another well-known autoantibody in RA that is also used in diagnostics. Citrullination is a post-

translational modification (PTM) that is recognized by ACPA. Citrullination is the enzymatic transformation of arginine into citrulline by peptidyl arginine deiminase (PAD) (CatrinaKrishnamurthy and Rethi, 2021).

Anti-citrullinated peptide antibody by recognition numerous citrullinated antigens, including α-enolase, fibrinogen, filaggrin, vimentin, and type II collagen (CII), in 50–70% of RA patients. ACPA employs a wide range of isotypes, including IgM, IgG, and IgA (Brevet *et al.*, 2021).

The extensive glycosylation of the V domain of ACPA was recently discovered to be a unique physicochemical characteristic of ACPA. This extensive V-domain glycosylation is absent from IgM ACPA and is predictive of the development of RA (van Delft and Huizinga, 2020).

Some studies have demonstrated that when certain environmental factors change, arginine is converted to citrulline by peptidyl arginine deiminases (PADs), and citrullinated proteins can through antigen-presenting cells (APCs) presented to T cells by specific MHC, produce ACPA and simultaneously generate autoimmune responses to citrullinated self-antigens in RA patients (Ding *et al.*, 2023).

Anti-citrullinated peptide antibody is frequently distinguished by anti-cyclic citrullinated peptide (anti-CCP) antibodies (Okamoto *et al.*, 2022). Four generations of anti-CCP antibody tests have been developed to date, the antigen for the first generation of anti-CCP (antiCCP1) antibody tests was cyclic citrullinated peptides derived from the filaggrin protein using different cyclic peptides (ElabdKhalfalla and Bolad, 2022).

The second generation of anti-CCP (anti-CCP2) antibody tests was developed in 2002. In 2012, the third generation of anti-CCP (anti-CCP3)

antibody tests containing a unique peptide was introduced. Followed in 2013 by the most recent version, anti-CCP3.1, which can detect the combination of IgA and IgG isotypes. In comparison to earlier anti-CCP antibody tests that could only detect a single Ig. This latest generation, which has a higher sensitivity in RF-negative patients, has proven to be statistically significant difference superior (Azalan *et al.*, 2023).

1.3.7.5. Anti Carbamylated protein Antibody

Antibodies against carbamylated protein antigen (anti-CarP or ACP) antibodies were discovered in RA patients in 2011, subsequent research has demonstrated the predictive and prognostic value of this antibody system and play a role in the pathogenesis of RA (Mohamed *et al.*, 2019).

Unlike citrullination, which is an enzyme-mediated conversion of arginine to citrulline, carbamylation is a chemical conversion of lysine residues with cyanate to form homo-citrulline the only distinction between homo-citrulline and citrulline is the addition of one CH2 to its side chain (O'Neil *et al.*, 2020).

Carbamylation occurs when there is abundance of cyanate, which can be caused by an overabundance of urea and enhanced myeloperoxidase activity (inflammation) or by direct consumption (smoking). ACP-Ab existence and role in atherogenesis and renal failure have been brought to light for quite some time (Ricchiuti *et al.*, 2022).

Van Delft and Huizinga demonstrated the presence of anti-CarP antibodies is predictive for the progression to RA in arthralgia patients as well as increased joint destruction over time, particularly in ACPA-negative RA patients, where the anti-CarP antibody response utilizes a wide range of isotypes and IgG subclasses, including IgM, IgG1-4, and

IgA. In addition, anti-CarP antibodies recognize numerous Carbamylated proteins, including both self- and non-self-proteins (Van Delft and Huizinga, 2020).

1.3.7.6. Anti 14-3-3η protein Antibody

The protein eta [14-3-3η proteins] constitutes a family of intracellular chaperonins expressed only in eukaryotic cells. The 14-3-3η family is capable of interacting with over 200 intracellular proteins. Thus, it coordinates a number of biological processes, such as protein trafficking, signaling, and cytoskeletal transport. Seven isoforms that share over 50% amino acid similarities have been isolated [alpha α, beta β, epsilon ε, gamma γ, eta η, zeta ζ, and sigma σ] (Guan *et al.*, 2019).

In 2007, Kilani *et al.* discovered for the first time that serum anti-14-3-3η Ab was substantially associated with two routine biomarkers of RA in arthritic patients. A study by Zeng *et al.* found that serum anti-14-3-3η, a novel, highly specific RA biomarker was statistically significant difference elevated in patients with accelerated RA disease progression and was implicated in the pathogenesis of RA (Zeng *et al.*, 2020).

High levels of anti-14-3-3η were detected in the supernatants of TNF-α stimulated macrophages. These findings suggest that TNF-α promotes 14-3-3η secretion by inducing necroptosis in macrophages, which is a novel mechanism for anti-14-3-3η level elevation in RA synovial fluid (Trimova *et al.*, 2020).

The eta protein antibody (anti-14-3-3η) has been shown to generate inflammatory mediators such as IL-1 and IL-6 and has been linked to the development of joint injury due to its promotion of receptor activator of

nuclear factor-kB ligand (RANKL) and matrix metalloproteinase (MMP) (Abdelnaser Awad *et al.*, 2023).

Alashkar *et al.* suggest the eta protein antibody (anti-14-3-3η) has the potential to be used in the early diagnosis of RA with greater sensitivity and specificity than conventional diagnostic biomarkers. The addition of anti-14-3-3η as a novel biomarker to RF and ACPA is advantageous for early diagnosis of RA and early therapeutic intervention to reduce disease progression and structural damage (Alashkar *et al.*, 2022).

1.3.7.7. Anti-Dual Specificity Phosphatase11 Antibody

Anti-Dual specificity phosphatas11 (DUSP11) also known as phosphatase that interacts with RNA belongs to the atypical DUSP protein tyrosine phosphatase family, only as an RNA phosphatase that modulates the stability of noncoding RNA is DUSP11 known (Yang *et al.*, 2020).

Anti-Dual specificity phosphatase family phosphatases are the main group of protein phosphatases that regulate mitogen-activated protein kinases (MAPKs) activity in mammalian cells (Guler *et al.*, 2022).

Important components of cell signaling pathways are MAPKs. It controls physiological and pathological responses to diverse extracellular stimuli and environmental stresses (Yue and López, 2020). MAPK signaling pathways are involved in gene transcription, mRNA translation, protein stability, protein localization, and enzyme activity. Thereby regulating diverse cellular functions such as cell proliferation, cell differentiation, cell survival, and cell death, MAPK signaling pathways are also associated with several diseases, including inflammation and malignancy (ChenChuang and Tan, 2019).

In combination, DUSP proteins are differentially involved in T cell activation, T cell senescence and exhaustion, and maintaining the homeostasis of effector and T-reg subsets, rendering them highly prospective diagnostic biomarkers and therapeutic targets for a variety of immune-related disease contexts (Zhang *et al.*, 2021).

In general, DUSP appear to function as signal repressors to prevent T cell hyper activation and effector activity and inhibit the immune response. Conversely, sustained T cell activation results in the overexpression of DUSP proteins. The mechanisms by which chronic T cell activation causes DUSP up-regulation are not yet entirely understood (Sun *et al.*, 2021).

Recently, many studies have described a number of novel candidates that have been proposed as potential RA biomarkers, seronegative RA is problematic from both a diagnostic and a pathogenic standpoint. DUSP11 antibodies are among the novel candidate autoantibodies that have been identified in this subset of patients. In one study, these antibodies were detected in 30–40% of both ACPA-positive and ACPA-negative patients (SokolovaSchett and Steffen, 2021).

1.3.8. Treatment

Nonsteroidal anti-inflammatory drugs (NSAIDs), which are primarily used to control pain and inflammation, glucocorticoids (GCs), and disease-modifying anti-rheumatic drugs (DMARDs) comprise the current standard treatment for RA (Abbasi *et al.*, 2019).

Early-diagnosed RA patients may benefit from these therapies to alleviate inflammation and other disease symptoms, as these therapies inhibit inflammatory mediators to treat symptoms and prevent disease progression (Mueller *et al.*, 2021).

Conventional synthetic DMARDs (csDMARDs), biologic DMARDs (bDMARDs), and targeted synthetic DMARDs (tsDMARDs) are subcategories of DMARDs (Sepriano *et al.*, 2020). The principal approved medications, the drugs currently being evaluated in clinical trials, and a number of pre-clinical drugs for the treatment of RA. Methotrexate (MTX) is the most widely used csDMARDs and has been considered a first-line treatment for years (Conigliaro *et al.*, 2019).

The chemical structure of methotrexate (MTX) is comparable to that of the antifolate drug folic acid. This molecule was initially used for cancer chemotherapy, and now it is administered in modest doses for RA treatment (Friedman and Cronstein, 2019).

Nanomedicine has emerged as a novel therapeutic strategy to effectively localize anti-rheumatoid arthritis (RA) medications in inflamed joints (Jeong and Park, 2020).

Among the available nanomedicine approaches, water-soluble polymer-drug conjugates offer a number of benefits, including enhanced pharmacokinetics of the carried drug and simple handling and storage, as the final products can be stored in solid form and prepared for parenteral administration by simple dissolution in a physiological solution (Libánská *et al.*, 2023).

Throughout the past few decades, newer therapy methods have been developed in order to gain deeper understanding of the literature surrounding the actual cause of RA. The therapeutic potential of newer targets, such as granulocyte macrophage colony stimulating factor, dendritic cells and RANKL inhibitors (Shah *et al.*, 2022).

2.1. Subjects

2.1.1. Study Design

Case–Control study design.

2.1.2. Study setting

Iraq/ Kerbala government/ Imam Al Hassan Al-Mujtaba Hospital.

2.1.3. Ethical Approvals

Ethical approvals from the College of Medicine at Kerbala University and the Iraqi Ministry of Health / Kerbala Health Directory were obtained (Appendices).

2.1.4. Study Population

Study subjects included people who attended Imam Al-Hassan Al-Mujtaba Hospital/Rheumatology Unit/Kerbala-Iraq during a period from October 2022 to April 2023. The current study included 270 subjects, and after obtaining acceptance from all of them to participate in the study, according to questionnaires, they were divided into two main groups: 180 rheumatoid arthritis cases and 90 healthy controls, and then cases were divided into 90 seropositive and 90 seronegative RA according to the presence or absence of rheumatoid factor and anti-citrullinated peptide antibody.

2.1.5. Inclusion and Exclusion Criteria

- Rheumatoid arthritis patients fully acquired the American college of rheumatology (ACR)/European league against rheumatism (EULAR) 2010 (ACR/EULAR-2010) RA classification criteria.
- Controls group should be people not have any rheumatological disorder.
- Exclude all other rheumatological disorders or others autoimmune disorders and Infectious diseases.

2.2. Materials

2.2.1. Equipment's:

In the practical aspect of the study, many types of equipment used in the current study are illustrated in table (2-1).

Table (2-1): List of the equipment's used with their companies and origin.

NO.	Equipment	Company	Origin
1.	Gel tube	AFCO	Jordon
2.	Sodium citrate tube for ESR	AFCO	Jordon
3.	Eppendorf tube	AFCO	Jordon
4.	Tourniquet	-	China
5.	Syringes	Medica	AUE
6.	ESR reader	JOKOH	Japan
7.	Automatic pipettes	Dragon	China
8.	Sterile yellow tips	Service bio	China
9.	Disposable gloves	Mumu	Malaysia
10.	Sterile Wooden sticks	ALS	China
11.	Centrifuge	Hettich	Germany
12.	Eppendorf centrifuge	Hettich	Germany
13.	Deep freezing	Kirtsh	Germany
14.	Glass cylinder	-	China
15.	Incubator	Memmert	Germany
16.	Specific protein analyzer	Hipro	China
17.	Microplate washer stat fax®	Bio Front	USA
18.	Microplate reader chromate	Awareness	USA

2.2.2. Laboratory Kits

The kits were used in the current study are illustrated in table (2-2).

Table (2-2): The kits that used in current study

Erythrocytes Sedimentation Rate	
Principle of Test	Westergren Method
Test Number/kit	100 Test
Number of kits	3 kits
Company/Origin	AFCCO/Jordan
C-Reactive Protein Titer	
Principle of Test	Nephelometry
Test Number/kit	25 Test
Number of kits	11 kits
Company/Origin	Hipro Biotechnology/China
Human Rheumatoid Factor Titer	
Principle of Test	Nephelometry
Test Number/kit	25 Test
Number of kits	11 kits
Company/Origin	Hipro Biotechnology/China
Human Anti-Citrullinated Peptide Antibody	
Principle of Test	Sandwich -ELISA
Test Number/kit	96 Test
Number of kits	3 kits
Company/Origin	Sun long-Biotech/China
Human anti-Carbamylated Protein Antibody	
Principle of Test	Direct -ELISA
Test Number/kit	96 Test
Number of kits	3 kits
Company/Origin	Sun long-Biotech/China
Human 14-3-3 Protein Eta Antibody	
Principle of Test	Sandwich ELISA
Test Number/kit	96 Test
Number of kits	3 kits
Company/Origin	Sun long-Biotech/China
Anti-Dual Specificity Phosphatas11Antibody	
Principle of Test	Sandwich ELISA
Test Number/kit	96 Test
Number of kits	3 Kits
Company/Origin	My BioSource/USA

Table (2-3): Materials provided with the ACPA kit

Materials provided with the kit 96 determinations			Storage
1	User manual	1	R.T.
2	Closure plate membrane	2	R.T.
3	Sealed bags	1	R.T.
4	ELISA plate 96 wells	1	2-8℃
5	Standard： 108 U/ml	0.5ml×1 bottle	2-8℃
6	Standard diluent	1.5ml×1 bottle	2-8℃
7	HRP-Conjugate reagent	6ml×1 bottle	2-8℃
8	Sample diluent	6ml×1 bottle	2-8℃
9	Chromogen solution A	6ml×1 bottle	2-8℃
10	Chromogen solution B	6ml×1 bottle	2-8℃
11	Stop solution	6ml×1 bottle	2-8℃
12	Wash solution	20ml (30X)×1bottle	2-8℃

Table (2-4): Materials provided with the Anti-CarP kit

Materials provided with the kit 96 determinations			Storage
1	User manual	1	R.T.
2	Closure plate membrane	2	R.T.
3	Sealed bags	1	R.T.
4	ELISA plate	1	2-8℃
6	Standards:[50,25,10,5,0] ng/l	0.5ml×5 vials	2-8℃
7	HRP-Conjugate reagent	6ml×1 bottle	2-8℃
8	Sample diluent	6ml×1 bottle	2-8℃
9	Chromogen solution A	6ml×1 bottle	2-8℃
10	Chromogen solution B	6ml×1 bottle	2-8℃
11	Stop solution	6ml×1 bottle	2-8℃
12	Wash solution	20ml (30X)×1bottle	2-8℃

Table (2-5): Materials provided with the Anti-14-3-3η kit

Materials provided with the kit 96 determinations			Storage
1	User manual	1	R.T.
2	Closure plate membrane	2	R.T.
3	Sealed bags	1	R.T.
4	ELISA plate	1	2-8℃
5	Standard: 180ng/L	0.5ml×1 bottle	2-8℃
6	Standard diluent	1.5ml×1 bottle	2-8℃
7	HRP-Conjugate reagent	6ml×1 bottle	2-8℃
8	Sample diluent	6ml×1 bottle	2-8℃
9	Chromogen solution A	6ml×1 bottle	2-8℃
10	Chromogen solution B	6ml×1 bottle	2-8℃
11	Stop solution	6ml×1 bottle	2-8℃
12	wash solution	20ml (30X)×1bottle	2-8℃

Table (2-6): Materials provided with the Anti-DUSP11 kit

NO	Materials	Color	96 well kit
1	**ELISA** Plate	---	96 well plate
2	Standards[20,10,5,2.5, 1.25,0.625]ng/ml	S1(Red) S2(Pink) S3(Blue) S4(Green) S5(Yellow) S6(White)	0.5ml×6 vials
3	Sample diluent	Blue	6.0ml×1 bottle
4	HRP-Conjugate reagent	Red	10ml×1 bottle
5	20×Wash solution	White	25ml×1 bottle
6	Stop solution	Yellow	6.0ml×1 bottle
7	Chromogen solution A	Purple	6.0ml×1 bottle
8	Chromogen solution B	Black \ Brown	6.0ml×1 bottle
9	Closure plate Membrane	---	2×pieces
10	Manual	---	1×paper

Case – Control Study Design

Included 270 subjects

Healthy Control = 90

180 Patients with RA

Seronegative = 90

Seropositive = 90

Study Groups sub-classification Regarding to

- Case Group and Control Group
- Serological Test (Seropositive and Seronegative).
- Sex (Males and Females).
- Age and Age Groups (G1, G2, and G3).
- Smoking Factor (Smoker, Non Smoker and –Ve Smoker).
- Family History to RA (Positive and Negative).
- Disease Activity Score (Mild, Moderate and Severe).
- Treatment Intake (Regular and Irregular).

Five milliliter of venous blood from

- Patients with RA
- Healthy Subjects

Routine Assay

- ESR level [Westergren Method].
- CRP titer [Nephelometry].
- RF titer [Nephelometry].
- ACPA titer [ELISA].

Study Markers

- Anti-CarP [ELISA].
- Anti-14-3-3η [ELISA].
- Anti-DUSP11 [ELISA].

2.3. Method

2.3.1. Sample Processing

Five milliliters of venous blood were drawn from patients and controls 1.6 ml were collected in ESR tube, and the remaining in Gel tube, centrifuged for separation of serum after allowing to clot at room temperature. Then, serum sample was divided into in four Eppendorf tubes for each subject and stored at deep freezing (-40 to -80C) until used, ESR level, CRP titer and RF titer immediately measured.

2.3.2. Parameters

2.3.2.1. Erythrocyte Sedimentation Rate

❖ **Procedure of Test**

- The venous blood 1.6 was added to the ESR tube (containing 0.4 ml of sodium citrate anticoagulant). After that, it was mixed well and applied to the Jokoh automated ESR instrument. After one hour, the results were automatically displayed in digital screen and recorded.
- Normal values for the erythrocyte sedimentation rate (ESR), as derived using the Westergren method, are as follows:
  Male: ≤15 mm/hr. Female: ≤ 20 mm/hr. Child: ≤10 mm/hr.

2.3.2.2. C-Reactive Protein Titer Test

❖ **Principle of Test**

- The latex surface is coated with the antibody. Through latex agglutination reactions, the CRP and antibodies in the sample form immune complexes. Immune complexes are responsible for light scattering, which is proportional to the intensity of scattered light and CRP levels. The concentration of CRP is determined by comparing the

turbidity of samples to the standard using a specific protein analyzer to measure the intensity of scattered light.

❖ **Procedure of Test**

1. The kit was restored to room temperature (25 °C) before testing.
2. The capillary of the sample collector aspirated the sample.
3. Then the sample collector is in the cuvette.
4. The test cuvette was inserted into the specific protein analyzer.
5. The result appeared after 90 seconds on a digital screen.
6. The result was recorded in questionnaire sheet.
7. The normal value of CRP Titer is ≤ 10 mg/L

2.3.2.3. Human Rheumatoid Factor Titer Test

❖ **Principle of Test**

➢ On the surface of the latex, the RF units conjugate lgG. Latex agglutination in the liquid phase forms immune complexes from sample RF and IgG. The intensity of light scattering from immune complexes is proportional to RF levels. Protein analyzers determine RF concentration by comparing representative samples' turbidity to the standard concentration.

❖ **Procedure of Test**

1. The kit was kept at room temperature (25 °C) before testing.
2. The capillary of the sample collector aspirated the sample.
3. Then the sample collector is in the cuvette.
4. The test cuvette was inserted into the specific protein analyzer.
5. The result appeared after 130 seconds on a digital screen.

6. The result was recorded in questionnaire sheet.

2.3.2.4. Anti-Citrullinated Peptide Antibody ELISA Test

❖ **Principle of ELISA Test:** Sandwich-ELISA

❖ **Procedure of ACPA Test**

1. Standards were diluted as in the following chart.

S1	72 U/ml	300µl Original Standard + 150µl Standard diluents
S2	48 U/ml	300µl Standard No.1 + 150µl Standard diluents
S3	24 U/ml	150µl Standard No.2 + 150µl Standard diluent
S4	12 U/ml	150µl Standard No.3 + 150µl Standard diluent
S5	6 U/ml	150µl Standard No.4 + 150µl Standard diluent

2. Then pipetted the volume of 50µl from each tube to the standard well.
3. One well in the ELISA plate was left empty as a blank control. In sample wells, 40µl sample dilution buffer and 10µl sample are added. With gentle shaking, the ingredients mixed well. Avoid touching the ELISA well wall.
4. The ELISA plate was incubated for 30 minutes at 37 °C after being sealed with the closure plate membrane.
5. Behind that, the concentrated washing buffer was diluted with distilled water 30 times for 96 tests.
6. The washing procedure was then concluded; the membrane of the closure plate was carefully peeled off, aspirated, and refilled with wash solution. After resting for 30 seconds, discard the wash solution. Five times the washing process was repeated.
7. The HRP-conjugate reagent was added 50µl to each well except the blank control well.

8. Then the plate was incubated for 30 minutes at 37 °C after being sealed with the closure plate membrane.
9. After that, the process of washing was done again, as in number five.
10. Then the colouring step was done by adding chromogen solution A (50µl) and chromogen solution B (50µl) to each well, mixing with gentle shaking, and incubating at 37 °C for 15 minutes. Please avoid light during colouring.
11. The reaction was stopped by adding 50µl of stop solution to each well. The color of the well was changed from blue to yellow.
12. The absorbance was read at 450nm using an ELISA plate reader. The optical density (OD) value of the blank control well is set to zero. Assay was carried out within 15 minutes after adding the stop solution.

2.3.2.5. Human Anti-Carbamylated Protein Antibody ELISA Test

- **Principle of procedure:** Direct- ELISA
- **Procedure of Anti-CarP Test**

1. One well was left as blank well (no sample and HRP was add).
2. Added 50µl of standard (S1, S2, S3, S4, and S5) to the standards wells, respectively the standards concentration were (50, 25, 10, 5, and 0) ng/l respectively. In the sample wells, 40µl sample dilution buffer and 10µl of the sample are added without touching the wall of the well with mixed well with gentle shaking.
3. Incubated for 30 minutes at 37°C after being sealed with a membrane closure plate.
4. Distilled water was used to dilute the concentrated washing buffer 30 times for 96 tests.

5. Aspirated and then refilled with the wash solution after carefully peeling off the closure plate membrane and dumping the wash solution after 30 seconds. The washing procedure was repeated five times.
6. The HRP-conjugate reagent was added 50µl to each well except the blank well.
7. Then incubated for 30 minutes at 37°C after being sealed with a membrane closure plate.
8. The washing procedure that was mentioned in number five was repeated five times.
9. The colouring was done by adding 50µl of chromogen A and 50µl of chromogen B to each well, mixing well with gentle shaking, and keeping at 37 °C for 15 minutes without light.
10. The reaction was terminated by adding 50µl of stop solution to each well, and the color of each well changed from blue to yellow.
11. The optical density (OD) was read at 450nm by using a microtiter plate reader. After adding the stop solution, the pate was measured within 15 minutes.

2.3.2.6. Human 14-3-3η Protein Antibody ELISA Test

❖ **Principle of procedure:** Sandwich-ELISA.

❖ **Procedure of Anti-14-3-3η Test**

1. Standards were diluted as in the following chart.

S1	120ng/L	300µl Original Standard + 150µl Standard diluents
S2	80ng/L	300µl Standard No.1 + 150µl Standard diluents
S3	40ng/L	150µl Standard No.2 + 150µl Standard diluent
S4	20ng/L	150µl Standard No.3 + 150µl Standard diluent
S5	10ng/L	150µl Standard No.4 + 150µl Standard diluent

2. Then, the first well of the ELISA plate was left empty as a blank control. 50µl from each tube of standards was pipetted into the ELISA plate wells as a standard, and 40µl sample dilution buffer and 10µl

sample were added to the sample wells and mixed with gentle shaking. There was no touching of walls.

3. The incubation process was done by incubating for 30 minutes at 37 °C after sealing with a closure plate membrane.
4. The concentrated dilution buffer was diluted with distilled water (30 times for 96 tests).
5. Then the washing process was done after peeling off the closure plate membrane, aspirating, and refilling with the wash solution. Discard the wash solution after resting for 30 seconds. The washing procedure was repeated five times.
6. The HRP-conjugated reagent was added at a volume of 50 µl to each well except the well serving as the blank.
7. The incubation process was repeated as described in Step 3.
8. The washing process was duplicated as described in Step 5.
9. The chromogen solutions A and B were added to each well in a volume of 50µl, respectively, gently shake them to mix and incubate at 37°C for 15 minutes. Light during coloring process was avoided.
10. The termination process was applied by adding 50µl of stop solution to each well to terminate the reaction, and the color in the well was changed from blue to yellow.
11. The absorbance was read at 450nm using an ELISA plate reader. The OD value of the blank control well was set to zero. The assay was carried out within 15 minutes after adding the stop solution.

2.3.2.7. Human DUSP11 Antibody ELISA Test (Sandwich-ELISA)

❖ **Procedure of DUSP11 Ab Test**

1. Before beginning the assay, they allowed the plate, all reagents, and samples to reach room temperature (18°C–25°C).
2. Removed the plate from the foil pouch and the blank, standard, and sample wells were marked.
3. The blank well was left empty, then 50µl were added as standard (S1, S2, S3, S4, S5, and S6) that concentration were (0.625, 1.25, 2.5, 5, 10 and 20) ng/ml respectively.
4. Then 50µl of the samples were added to every sample well.
5. The HRP-conjugate reagent was added 100µl to every well except blank wells. The plate was covered with a closure plate membrane and incubated for 60 minutes at 37 °C.;
6. The ELISA plate wells were washed 4 times.
7. All wells received (50µl) of Chromogen Solution A and B. Mix gently and incubated the plate at 37 °C for 15 minutes with light-protected ELISA plate.
8. Stop Solution was added (50µl) to every well and the color of wells chanced from red to yellow.
9. Optical Density (O.D.) was read at (450nm) using an ELISA reader within 15 minutes after adding stop solution.

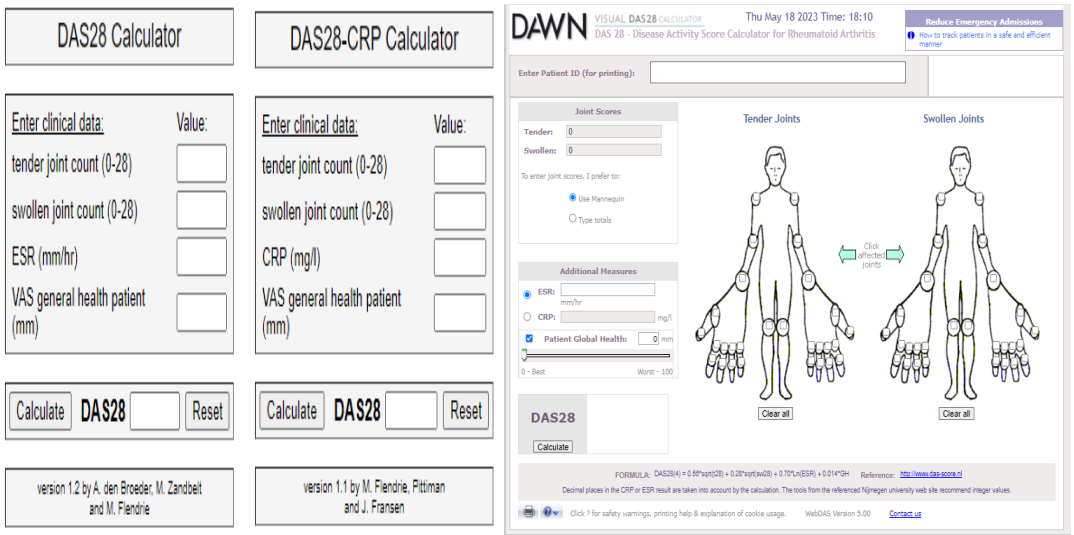
2.3.3. Disease Activity Score (DAS)

The disease activity score (DAS) was calculated by online DAS calculator when used the CRP titer or ESR level.

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

2.4. Programs and Software:

- Microsoft Office version-2021.
- ELISA reader software.
- SPSS version-26 for statistics analysis.
- Pad Prism version-11.
- Online DAS- calculators.



https://www.das-score.nl/das28/DAScalculators/dasculators.html

https://www.4s-dawn.com/DAS28/

2.5. Statistical Analysis:

The SPSS program was used to do statistical analysis of the current study. The Kolmogorov-Smirnov and Shapiro-Wilk tests was applied to all study variables and groups to show the current study data distribution, and according to the statistic test, the statistical analysis will follow either a parametric or a non-parametric statistical test.

Statistically significant difference values are less than 0.05 and highly statistically significant difference values are less than 0.01.

2.6. Standard Curve of Current Study Markers

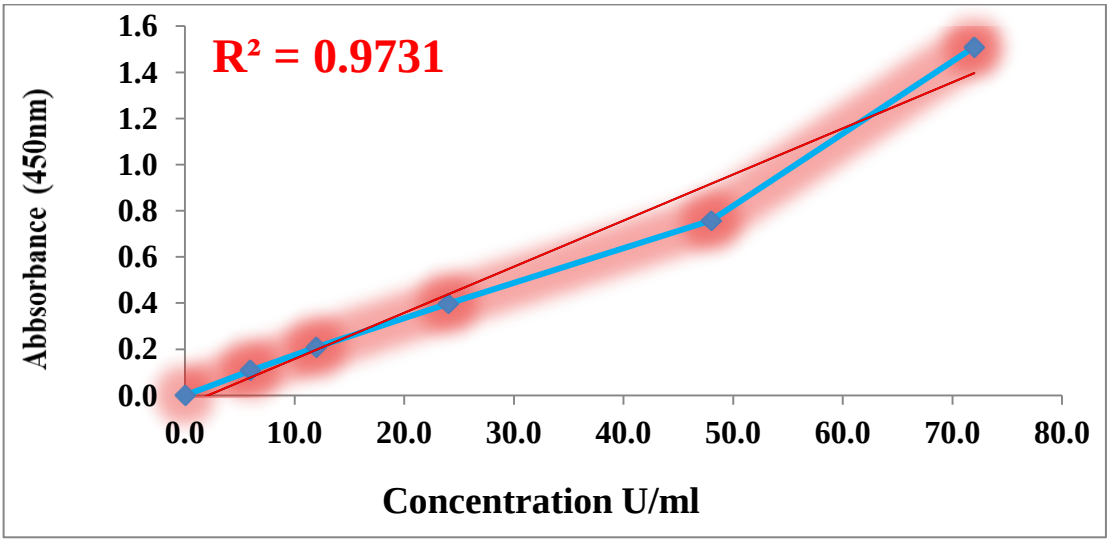


Figure 2-1: the standard curve of ACPA

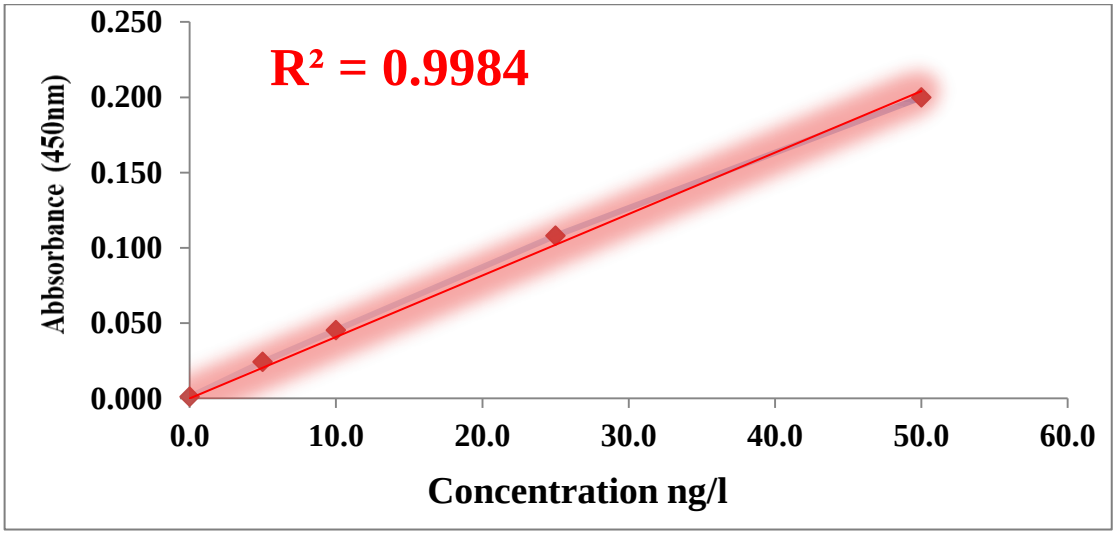


Figure 2-2: the standard curve of Anti-CarP

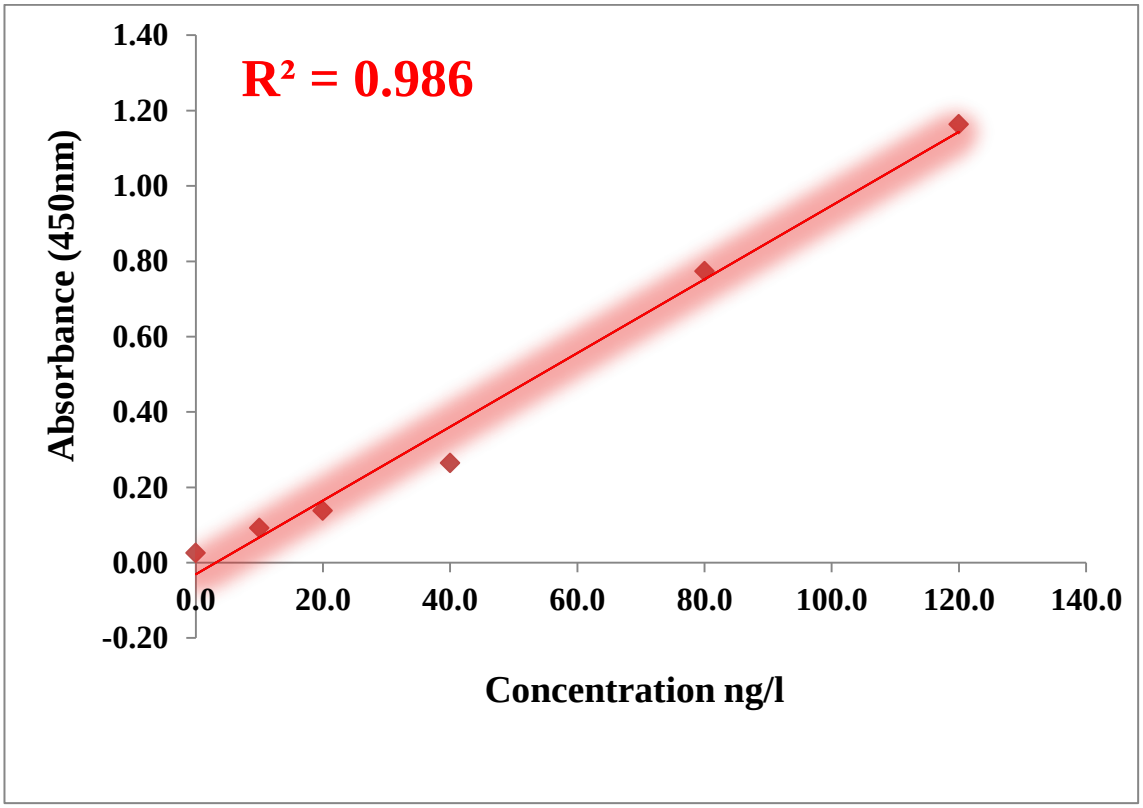


Figure 2-3: the standard curve of Anti-14-3-3η

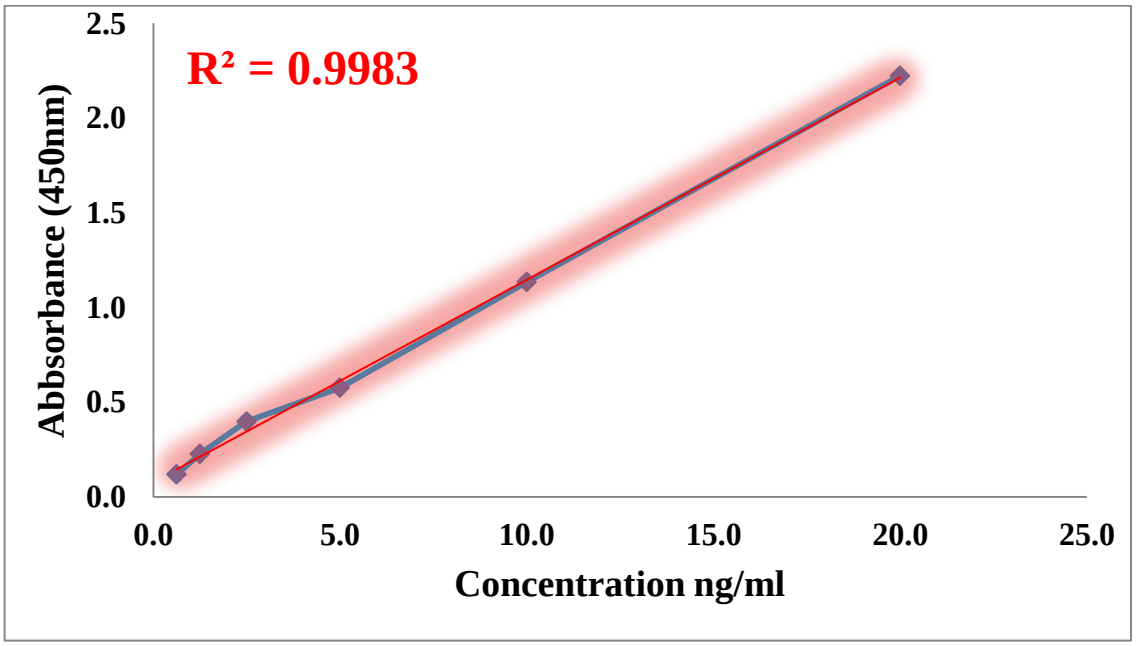


Figure 2-4: the standard curve of Anti-DUSP11

3. Results

3.1. Distribution of sociodemographic characters of study groups RA patients and healthy control

The mean±SE of age of the RA patients and healthy control group are 49.82±86 and 49.00±0.99 respectively at *P*.value (0.568), according to the age variable of RA patients and healthy control in the current study, they are subclassed into three groups: Group 1 (20-39) years of RA patients is 33(18.3%) and healthy control is 9 (10.0%), Group 2 ((40-59) years of RA patients is 113(62.8%) and healthy control is 73 (81.1%), Group3(60-79) years of RA patients is 34(18.9%) and healthy control is 8(8.9%), at *P*.value (0.009). The sex distribution in the current study shows that Males are 20(11.1%) in RA patients group and 12(13.3%) in healthy control group while Females are 160(88.9%) in RA patients group and 78(86.7%) in healthy control group, at *P*.value (0.594). The smoking status divided into three groups: Non Smokers group are 151(83.9%) in RA patients group and 88(97.8%) in healthy control group, Smokers group are 19(10.6%) in RA patients group and 2(2.2%) in healthy control group, Negative Smokers group are 10(5.6%) in RA patients group and 0(0.0%) in healthy control group. Treatment intake divided into: Regulars group are 113(62.8%) in RA patients group and 0(0.0%) in healthy control group, Irregulars group are 67(37.2%) in RA patients group and 0(0.0%) in healthy control group. The RA family history is divided into: Positive RA family history group are 101(56.1%) in RA patients group and 0(0.0%) in healthy control group, Negative RA family history group are 79(43.9%) in RA patients group and 90(100.0%) in healthy control group. All these results show in the table (3-1).

Table (3-1): Distribution of sociodemographic characters of study groups RA patients and healthy control

Variables		RA patients	Healthy Control	*P.* Value
Age **(mean±SE)**		49.82±0.86	49.00±0.99	0.568
Age groups **N (%)**	Group1(20-39)	33(18.3%)	9 (10.0%)	0.009
	Group2(40-59)	113(62.8%)	73 (81.1%)	
	Group3(60-79)	34(18.9%)	8(8.9%)	
Sex **N (%)**	Males	20(11.1%)	12(13.3%)	0.594
	Females	160(88.9%)	78(86.7%)	
Smoking **N (%)**	Non Smokers	151(83.9%)	88(97.8%)	-
	Smokers	19(10.6%)	2(2.2%)	
	Negative Smokers	10(5.6%)	0(0.0%)	
Treatment-Regularity **N (%)**	Regulars	113(62.8%)	0(0.0%)	-
	Irregulars	67(37.2%)	0(0.0%)	
RA Family History **N (%)**	Positive	101(56.1%)	0(0.0%)	-
	Negative	79(43.9%)	90(100.0%)	

*Significant *P*.value < 0.05
Statistical test: Chi-square test

3.2. Distribution of sociodemographic characters of seropositive and seronegative RA patients

The mean±SE of age of the seropositive and seronegative RA patients are 47.73±1 and 51.90±1 respectively at *P*.value (0.182), according to the age variable of seropositive and seronegative RA patients in the current study, they are subclassed into three groups: Group 1: (20-39) years of seropositive are 23(25.6%) and 10(11.1%) of seronegative, Group2 (40-59) years of seropositive are 55(61.1%) and 58(64.4%) of seronegative, Group3(60-79) years of seropositive are 12(13.3%) and

22(24.4%) of seronegative RA patients, at *P*.value (0.225). Sex distribution in the current study including in seropositive group Males are 11 (12.2%) and in seronegative group are 9(10.0%) while Females are 79(87.8%) in seropositive group and 81(90.0%) in seronegative group, at *P*.value (0.635). The smoking status divided into three groups: Non Smokers group are 75(83.3%) in seropositive group and 76(84.4%) in seronegative group, Smokers group are 11(12.2%) in seropositive group and 8(8.9%) in seronegative group, Negative Smokers group are 4(4.4%) in seropositive group and 6(6.7%) in seronegative group, at *P*.value (0.644). Treatment intake divided into: Regulars group are 58(64.4%) in seropositive group and 55(61.1%) in seronegative group while Irregulars group are 32(35.6%) in seropositive group and 35(38.9%) in seronegative group, at *P*.value (0.643). The RA family history is divided into: Positive RA family history group are 51(56.7%) in seropositive group and 50(55.6%) in seronegative group, Negative RA family history group are 39(43.3%) in seropositive group and 40(44.4%) in seronegative group, at *P*.value (0.881). All these results show in the table (3-2).

Table (3-2): Distribution of sociodemographic characters of seropositive and seronegative RA patients

Variables		Sero Positive N=90	Sero Negative N=90	*P*. Value
Age **(mean±SE)**		47.73±1	51.90±1	0.182
Age groups N (%)	Group1(20-39y)	23(25.6%)	10(11.1%)	0.225
	Group2(40-59y)	55(61.1%)	58(64.4%)	
	Group3(60-79y)	12(13.3%)	22(24.4%)	
Sex N(%)	Males	11 (12.2%)	9(10.0%)	0.635
	Females	79(87.8%)	81(90.0%)	
Smoking N (%)	Non Smokers	75(83.3%)	76(84.4%)	0.644
	Smokers	11(12.2%)	8(8.9%)	
	Negative Smokers	4(4.4%)	6(6.7%)	
Treatment - Regularity N (%)	Regulars	58(64.4%)	55(61.1%)	0.643
	Irregulars	32(35.6%)	35(38.9%)	
RA Family History N (%)	Positive	51(56.7%)	50(55.6%)	0.881
	Negative	39(43.3%)	40(44.4%)	

*Significant *P*.value < 0.05
Statistical test: Chi-square test

3.3. Disease Activity Score DAS- CRP and DAS- ESR between Seropositive and Seronegative RA patients group

Disease activity score according CRP between seropositive and seronegative show in the mild group 28(31.1%) and 12(13.3%), in the moderate group 39(43.3%) and 60(66.7%) and in the severe group 23(25.6%) and 18(20.0%) respectively, at *P*.value (0.074).

Disease activity score according ESR between seropositive and seronegative show in the mild group 14(15.6%) and 4(4.4%), in the moderate group 45(50.0%) and 48(53.3%) and in the severe group 31(34.4%) and 38(42.2%) respectively, at *P*.value (0.226).

The frequencies show the highest number in the moderate group in both seropositive and seronegative RA patients. All these results show in the table (3-3).

Table (3-3): disease activity score CRP and ESR among Seropositive and Seronegative RA patients group

Disease Activity Scores		Sero Positive	Sero Negative	*P.* Value
Disease Activity Score-CRP	Mild	28(31.1%)	12(13.3%)	0.074
	Moderate	39(43.3%)	60(66.7%)	
	Severe	23(25.6%)	18(20.0%)	
	Total	90(100%)	90(100%)	
Disease Activity Score-ESR	Mild	14(15.6%)	4(4.4%)	0.226
	Moderate	45(50.0%)	48(53.3%)	
	Severe	31(34.4%)	38(42.2%)	
	Total	90(100%)	90(100%)	

*Significant *P*.value < 0.05
Statistical test: Chi-square test

3.4. Distribution of the Sex

The sex distribution in the current study between the study groups are shown in the following figures: [3-1A] shows that 160 (88.9%) are females and 20 (11.1%) are males among the total RA patients, figure [3-1 B] show that 78(86.7%) are females and 12(13.3%) are males among the healthy control group , figures [3-1 C and D] show the highest frequencies of females sex than males in both seropositive and seronegative 79 (87.8%) and 81 (90%), respectively; while males are 11 (12.22%) and 9 (10%), respectively.

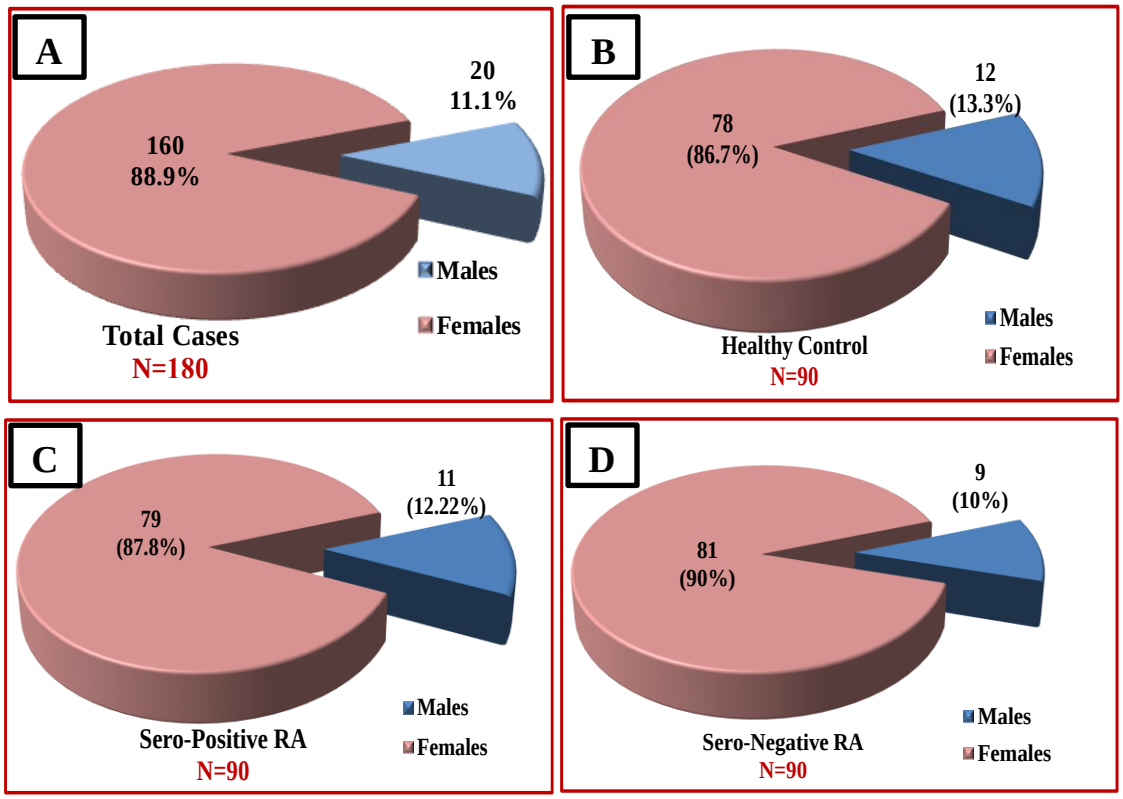


Figure (3-1): Sex Distribution in the study group.

A- Sex Distribution of Total RA Patients.

B- Sex Distribution of Healthy Control.

C- Sex Distribution of Seropositive RA Patients.

D- Sex Distribution of Seronegative RA Patients.

3.5. Comparison of Study Variables between RA Patients and Control Groups:

There are no statistically significant difference between the mean±SE of age of the RA patients which is 49.82±0.86 and the control group which is 49.0±0.99 at the *P*.value 0.508, while the mean±SE of the disease duration 6.30±0.43, ESR 38.73±1.64 , CRP 18.61±2.06, RF 43.55±4.29, ACPA 55.81±4.24, anti-CarP 4.43±0.49, anti-14-3-3η 10.02 ±0.84, and anti-DUSP-11 4.92±0.29at *P*.value 0.000 are statistically significant difference for RA patients group compared to the control group are disease duration 0.00±0.00, ESR 6.83±0.21, CRP 0.95±0.037, RF 5.44±0.23, ACPA 3.97±0.096, anti-CarP 2.29±0.21, anti-14-3-3η 3.94±0.23 and anti-DUSP11 2.25 ±0.14 at *P*.value 0.000,as in the table (3-4).

Table 3-4: Comparison of study variables between RA Patient and Control Groups

Parameters	RA Patients N=180 mean±SE	Control N=90 mean±SE	*P.* Value
Age years	49.82 ±0.86	49.0 ±0.99	0.508^{NS}
D. Duration years	6.30 ±0.43 *	-	0.000**
ESR-level mm/h	38.73 ±1.64 *	6.83 ±0.21	0.000**
C-RP-titer mg/dl	18.61 ±2.06 *	0.95 ±0.037	0.000**
RF-titer U/ml	43.55 ±4.29 *	5.44 ±0.23	0.000**
ACPA U/ml	55.81 ±4.24 *	3.97 ±0.096	0.000**
Anti-CarP ng/l	4.43 ±0.49 *	2.29 ±0.21	0.000**
Anti-14-3-3η ng/l	10.02 ±0.84 *	3.94 ±0.23	0.000**
Anti-DUSP-11 ng/ml	4.92 ±0.29 *	2.25 ±0.14	0.000**

NS: Non-Statistically significant difference
: Highly statistically significant difference ***P.* value < 0.01
Statistical test: Student T Test.

3.6. Comparison of Study Variables between Seropositive and Seronegative RA patients:

Seropositive and seronegative shows no statistically significant difference in the mean±SE of age 47.73±1 and 51.9±1 at P.value 0.182, disease duration 5.94±0.6 and 6.69±0.6 at P.value 0.173, ESR 36.66±2 and 40.81±2 at P.value 0.809, and CRP 17.8±2.62 and 19.42±3.2 at *P*.value 0.986 between the previously mentioned groups respectively, but the mean±SE of RF 75.53±7.08 and 11.56±1.1 at *P*.value 0.000 and ACPA 94.4±6.22 and 17.21±0.43 at *P*.value 0.000 in (seropositive and seronegative) respectively is statistically significant difference for the seropositive group than in seronegative group, while the mean±SE of anti-CarP 4.17±0.92 and 4.68±0.36 at *P*.value 0.000, anti-14-3-3η 7.76±0.67 and 12.28±1.5 at *P*.value 0.000, and anti-DUSP-11 4.86±0.46 and 4.98±0.39 at *P*.value 0.032, in (seropositive and seronegative) respectively is statistically significant difference in seronegative group than in seropositive group, as in the table(3-5).

Table 3-5: Comparison of study variables between Seropositive and Seronegative RA patients

Parameters	Seropositive mean±SE	Seronegative mean±SE	*P.* Value
Age years	47.73 ±1	51.9 ±1	0.182^{NS}
D. Duration years	5.94 ±0.6	6.69 ±0.6	0.173 ^{NS}
ESR-level mm/h	36.66 ±2	40.81 ±2	0.809 ^{NS}
C-RP-titer mg/dl	17.8 ±2.62	19.42 ±3.2	0.986 ^{NS}
RF-titer U/ml	75.53±7.08*	11.56 ±1.1	0.000**
ACPA U/ml	94.4 ±6.22 *	17.21±0.43	0.000**
Anti-CarP ng/l	4.17 ±0.92	4.68±0.36*	0.000**
Anti-14-3-3η ng/l	7.76 ±0.67	12.28±1.5*	0.000**
Anti-DUSP-11 ng/ml	4.86 ±0.46	4.98±0.39*	0.032*

NS: Non-Statistically significant difference
* Statistically significant difference P. value < 0.05 **: Highly statistically significant difference *P*. value <0.01 Statistical test: Student T Test.

3.7. Comparison of Study Variables According to DAS-CRP:

The mean±SE comparison of current study variables according to the disease activity score-28 depending on CRP (DAS28-CRP) shows no statistically significant difference in the age mean±SE among mild 47±2, moderate 51±1, and severe 51±2 groups at *P*.value 0.180. There is a statistically significant difference in the mean±SE of disease duration 8.2±1.2 at *P*.value 0.001, ESR 59±4 at *P*.value 0.001, CRP 48.1±6.87 at *P*.value 0.001, RF 76.35±13.3 at *P*.value 0.001, and ACPA 75.63±10.6 at *P*.value 0.004 in the severe disease activity group than in the mild and moderate activity groups, while the mean±SE of anti-CarP 5.25±1.99 and 4.37±0.4 at *P*.value 0.001, anti-14-3-3η 13.14±3.57 and 9.4±0.43 at *P*.value 0.001, and anti-DUSP-11 5.8±0.75 and 4.99±0.42 at *P*.value 0.05 are statistically significant difference in the mild disease activity group and moderate respectively than in the severe group, as in the table(3-6)

Table 3-6: Comparison of study variables according to Disease Activity Score-CRP.

Parameters	Mild mean±SE	Moderate mean±SE	Severe mean±SE	P. Value
Age years	47 ±2	51 ±1	51 ±2	0.180 NS
D. Duration years	4.2 ±0.5	6.4 ±0.5	8.2 ±1.2 *	0.001**
ESR-level mm/h	27 ±2	35 ±2	59 ±4 *	0.001**
C-RP-titer mg/dl	6.26 ±1.10	11.39 ±1.08	48.1±6.87*	0.001**
RF-titer U/ml	34.91 ±5.87	33.45 ±4.46	76.35±13.3*	0.001**
ACPA U/ml	62 ±9.48	45.10 ±4.82	75.63±10.6*	0.004**
Anti-CarP ng/l	5.25 ±1.99 *	4.37 ± 0.4 *	3.76 ±0.25	0.001**
Anti-14-3-3η ng/l	13.14±3.57*	9.4 ±0.43 *	8.5 ±0.47	0.001**
Anti-DUSP-11 ng/ml	5.8 ±0.75 *	4.99 ±0.42 *	3.88 ±0.33	0.05*

NS: Non-Statistically significant difference
* Statistically significant difference *P*. value < 0.05
**Highly statistically significant difference *P*. value <0.01
Statistical test: ANOVA test

3.8. Comparison of Study Variables between Seropositive and Seronegative RA Patients According to DAS-CRP

Comparison of study variables between seropositive and seronegative according DAS- CRP in the current study about the mean of age show statistically significant difference for seronegative RA in moderate activity group at *P.* value (0.020), and non- significant in mild activity group, about the mean of disease duration there are non- statistically significant difference between seropositive and seronegative RA patients among disease activity group, ESR level show statistically significant difference for seronegative RA in moderate activity group at *P.* value (0.014), CRP-titer show non- statistically significant difference between seropositive and seronegative among activity group, ACPA and RF- titer show highly statistically significant difference for seropositive RA among activity group especially for severe activity group at *P.* value (0.0001), anti-Carp show statistically significant difference for seronegative RA in moderate and severe activity group at *P.* value less than (0.05) while non- significant in mild activity group between seropositive and seronegative, anti-14-3-3η show highly statistically significant difference for seronegative RA among activity group especially in mild activity group at *P.* value less than (0.01), anti-DUSP-11show statistically significant difference for seronegative RA in mild and moderate activity group at *P.* value less than (0.05) while non-significant between seropositive and seronegative in severe activity group. All these results show in the table (3-7).

Table (3-7): Comparison of Study Variables between Seropositive and Seronegative RA Patients According to DAS-CRP

Study parameters /DAS28-CRP		Sero Positive Mean±SE	Sero Negative Mean±SE	*P.* Value
Age years	Mild	47±2	47±4	0.987 NS
	Moderate	48±1	52±1	0.020*
	Severe	49±3	53±3	0.347 NS
D. Duration years	Mild	3.8±0.6	5.0±1.0	0.304 NS
	Moderate	6.2±1.0	6.5±0.6	0.797 NS
	Severe	8.1±1.2	8.3±2.2	0.936 NS
ESR-level mm/h	Mild	26±3	31±4	0.318 NS
	Moderate	31±2	38±2	0.014*
	Severe	60±5	58±7	0.816 NS
CRP-titer mg/dl	Mild	6.16±1.16	6.50±2.58	0.904 NS
	Moderate	10.29±1.19	12.10±1.60	0.365 NS
	Severe	44.72±7.62	52.45±12.45	0.590 NS
RF-titer U/ml	Mild	46.27±7.41	8.39±1.38	0.0001**
	Moderate	66.11±8.82	12.22±1.57	0.0001**
	Severe	127.11±17.58	11.49±1.24	0.0001**
ACPA U/ml	Mild	81.74±11.70	15.92±1.51	0.0001**
	Moderate	87.83±8.51	17.32±0.51	0.0001**
	Severe	120.95±12.31	17.71±0.88	0.0001**
Anti-Carp ng/l	Mild	5.86±2.85	3.84±0.30	0.319 NS
	Moderate	3.51±0.61	4.92±0.52	0.049*
	Severe	3.24±0.21	4.43±0.45	0.008**
Anti-14-3-3η ng/l	Mild	8.27±1.68	24.51±2.83	0.0001**
	Moderate	7.75±0.95	10.46±0.30	0.004**
	Severe	7.18±0.55	10.18±0.62	0.0002**
Anti-DUSP-11 ng/ml	Mild	5.32±0.60	6.78±0.37	0.044*
	Moderate	3.13±0.78	4.90±0.49	0.029*
	Severe	3.72±.49	4.07±0.43	0.664 NS

NS: Non-Statistically significant difference
* Statistically significant difference *P.* value < 0.05
**Highly statistically significant difference *P.* value <0.01
Statistical test: ANOVA test

3.9. Comparison of Study Variables According to DAS-ESR

Comparison of current study variables according to the disease activity score-28 depending on ESR (DAS28-ESR) is no statistically significant difference in the age mean±SE among mild 46±3, moderate 49±1 and severe 52±1 groups at *P*.value 0.204, and there are a statistically significant difference in the mean±SE of disease duration 8.0±0.8 at *P*.value 0.001, ESR 55±3 at *P*.value 0.001, CRP 34.8±4.7 at *P*.value 0.001, RF 57.13±8.8 at *P*.value 0.015, and ACPA 58.42±7.1 at *P*.value 0.018 in the severe disease activity group than in the mild and moderate activity groups, while the mean±SE of anti-CarP 7.80±4.42 at *P*.value 0.025, anti-14-3-3η 15.92±6.94 at *P*.value 0.032, and anti-DUSP-11 7.51±1.41 at *P*.value 0.003, are statistically significant difference in the mild group than in the moderate and severe activity groups, as in the table(3-8)

Table 3-8: Comparison of study variables according to Disease Activity Score-ESR

Parameters	Mild mean±SE	Moderate mean±SE	Severe mean±SE	P. Value
Age years	46±3	49±1	52±1	0.204 NS
D. Duration years	3.0±0.6	5.7±0.5	8.0±0.8 *	0.001**
ESR-level mm/h	22±3	30±1	55±3 *	0.001**
C-RP-titer mg/dl	6.29±1.76	8.97±0.79	34.8 ±4.7 *	0.001**
RF-titer U/ml	36.6±7.94	34.8 ±4.72	57.13±8.8 *	0.015*
ACPA U/ml	83.1±19.4	48.6±4.9	58.42±7.1 *	0.018*
Anti-CarP ng/l	7.80±4.42 *	3.98±0.32	4.15±0.42	0.025*
Anti-14-3-3η ng/l	15.92±6.94 *	9.73±0.87	8.88±0.37	0.032*
Anti-DUSP-11 ng/ml	7.51±1.41 *	4.80±0.39	4.40±0.42	0.003**

NS: Non-Statistically significant difference
* : Statistically significant difference *P*. value < 0.05
**: Highly statistically significant difference *P*. value <0.01
Statistical test: ANOVA test.

3.10. Comparison of Study Variables between Seropositive and Seronegative RA Patients According to DAS-ESR

Comparison of study variables between seropositive and seronegative according DAS- ESR in the current study about the mean of age, D. Duration, ESR-level, and CRP-titer show non-statistically significant difference between seropositive and seronegative among activity group, RF-titer and ACPA show highly statistically significant difference for seropositive RA among activity group especially in severe activity group at *P*.value (0.0001), anti-CarP show statistically significant difference for seronegative RA in moderate and severe activity group at *P*.value less than (0.05) while non- significant in mild activity group, anti-14-3-3η show statistically significant difference for seronegative RA in mild and severe activity group at *P*.value less than (0.05) while non- significant in moderate activity group, anti-DUSP-11 show non- statistically significant difference between seropositive and seronegative RA among activity group. All these results show in the table (3-9).

Table (3-9): Comparison of Study Variables between Seropositive and Seronegative RA Patients According to DAS-ESR

Study parameters /DAS28-ESR		Sero Positive Mean±SE	Sero Negative Mean±SE	*P.* Value
Age years	Mild	48±4	40±7	0.332 NS
	Moderate	47±1	51±2	0.075 NS
	Severe	49±2	54±2	0.078 NS
D. Duration years	Mild	3.0±0.7	3.3±1.1	0.818 NS
	Moderate	5.7±0.8	5.7±0.6	1.000 NS
	Severe	7.6±1	8.3±1.2	0.654 NS
ESR-level mm/h	Mild	20±3	29±8	0.293 NS
	Moderate	29±2	31±2	0.480 NS
	Severe	56±4	55±4	0.859 NS
CRP-titer mg/dl	Mild	5.00±0.85	10.8±7.69	0.454 NS
	Moderate	8.99±1.11	8.96±1.13	0.984 NS
	Severe	36.38±6.23	33.55±6.82	0.758 NS
RF-titer U/ml	Mild	44.40±9.19	9.28±3.00	0.0001**
	Moderate	61.32±8.06	9.96±0.65	0.0001**
	Severe	110.21±14.59	13.83±2.42	0.0001**
ACPA U/ml	Mild	102.84±22.30	13.90±3.73	0.0001**
	Moderate	82.37±7.41	16.93±0.59	0.0001**
	Severe	108.06±10.21	17.92±0.58	0.0001**
Anti-CarP ng/l	Mild	8.73±5.70	4.55±0.74	0.403 NS
	Moderate	3.08±0.53	4.45±0.32	0.019*
	Severe	3.12±0.16	4.99±0.73	0.008**
Anti-14-3-3η ng/l	Mild	9.02±3.32	40.07±14.98	0.043*
	Moderate	7.90±0.85	11.45±1.44	0.106 NS
	Severe	7.00±0.42	10.40±0.45	0.0001**
Anti-DUSP-11 ng/ml	Mild	6.67±1.62	10.44±2.65	0.226 NS
	Moderate	5.09±0.70	4.52±0.39	0.341 NS
	Severe	3.70±0.39	4.97±0.68	0.075 NS

NS: Non-Statistically significant difference
* Statistically significant difference *P.* value < 0.05
**Highly statistically significant difference *P.* value <0.01
Statistical test: ANOVA test

3.11. Comparison of Study Variables According to Treatment Regularity:

In the current study variables comparisons regarding regularity of treatment intake, there is no statistically significant difference in the age mean±SE between regular 50±1 and irregular 50±1 groups at *P*.value 0.902, and there is a statistically significant difference in the mean±SE of disease duration 7.9±0.8 at *P*.value 0.001, ESR 50±3 at *P*.value 0.001, CRP 29.57±4.62 at *P*.value 0.001, RF 63.93±9.35 at *P*.value 0.001, and ACPA 63.56±7.92 at *P*.value 0.001 in the irregular groups than in the regular group, while the mean±SE of anti-CarP 4.72±0.75 at *P*. value 0.047, anti-14-3-3η 10.46±1.29 at *P*.value 0.044, and anti-DUSP-11 5.14±0.40 at *P*.value 0.001, are statistically significant difference in the regular groups than in the irregular groups, as in the table(3-10)

Table 3-10: Comparison of study variables according to Treatment Regularity

Treatment Status	Regulars mean±SE	Irregulars mean±SE	*P.* Value
Age years	50 ±1	50±1	0.902 NS
D. Duration years	5.4 ±0.5	7.9 ±0.8 *	0.001**
ESR mm\h	32 ±2	50 ±3 *	0.001**
C-RP mg\dl	12.12 ±1.55	29.57 ±4.62 *	0.001**
RF U\ml	31.46 ±3.61	63.93 ±9.35 *	0.001**
ACPA U\ml	51.21 ±4.83	63.56 ±7.92 *	0.001**
Anti-CarP ng\l	4.72 ±0.75 *	3.93 ±0.42	0.047*
Anti-14-3-3η ng\l	10.46 ±1.29 *	9.28 ±0.60	0.044*
Anti-DUSP-11 ng\ml	5.14 ±0.40 *	4.54 ±0.44	0.001**

NS: Non-Statistically significant difference
* : Statistically significant difference *P*. Value < 0.05
**: Highly statistically significant difference *P*. Value <0.01
Statistical test: Student T Test

3.12. Comparison of Study Variables between Seropositive and Seronegative RA Patients According to Treatment Regularity

Comparison of study variables between seropositive and seronegative RA according treatment regularity in the current study about the mean of age show non- statistically significant difference between seropositive and seronegative among activity group, D. Duration show statistically significant difference for seropositive RA in irregulars group at *P*.value (0.012), ESR- level and CRP-titer show statistically significant difference for both seropositive and seronegative especially for seropositive in irregulars group of RA patients at *P*.value less than (0.05), RF-titer show highly statistically significant difference for both seropositive and seronegative especially for seropositive in irregulars group of RA patients at *P*.value (0.0001), ACPA show statistically significant difference for seropositive RA in irregulars group at *P*.value (0.024), anti-CarP show non-statistically significant difference between seropositive and seronegative RA between regulars and irregulars treatment intake, anti-14-3-3η show statistically significant difference for seronegative RA in regulars group at *P*.value (0.025), anti-DUSP-11 show statistically significant difference for seronegative RA in regulars group at *P*.value (0.018). All these results show in the table (3-11).

Table (3-11) Comparison of Study Variables between Seropositive and Seronegative RA Patients According to Treatment Regularity

Study parameters / Sero-prevalence		Regulars Mean±SE	Irregulars Mean±SE	*P.* Value
Age years	Sero Positive	49±1	46±2	0.181 NS
	Sero Negative	51±2	54±2	0.290 NS
D. Duration years	Sero Positive	4.8±0.7	7.9±1.0	0.012*
	Sero Negative	5.9±0.6	7.9±1.3	0.164 NS
ESR-level mm/h	Sero Positive	29±2	50±5	0.0013**
	Sero Negative	35±3	50±4	0.0031**
CRP-titer mg/dl	Sero Positive	10.31±1.34	31.38±6.36	0.0007**
	Sero Negative	14.02±2.84	27.91±6.74	0.043*
RF-titer U/ml	Sero Positive	51.79±5.89	118.55±14.1	0.0001**
	Sero Negative	10.02±0.60	13.99±0.71	0.0001**
ACPA U/ml	Sero Positive	83.64±7.16	113.90±11.06	0.024*
	Sero Negative	17.01±0.57	17.53±0.65	0.522 NS
Anti-CarP ng/l	Sero Positive	4.77±1.43	3.08±0.14	0.094 NS
	Sero Negative	4.66±0.33	4.72±0.78	0.943 NS
Anti-14-3-3η ng/l	Sero Positive	7.54±0.83	8.18±1.16	0.704 NS
	Sero Negative	13.55±1.43	10.29±0.38	0.025*
Anti-DUSP-11 ng/ml	Sero Positive	4.97±0.62	4.66±0.64	0.715 NS
	Sero Negative	5.32±0.30	4.13±0.41	0.018*

NS: Non-Statistically significant difference
* Statistically significant difference *P.* value < 0.05
**Highly statistically significant difference *P.* value <0.01
Statistical test: ANOVA test

3.13. The ROC Curve for Study Parameters

The area that is shown in the following figure (3-2) shows the highly sensitivity and specificity of current study markers anti-CarP, anti-14-3-3η , and anti-DUSP-11 with routine acute phase reactants and gold standard antibody markers for RA patient at area 0.964, 0.923, and 0.827 respectively and the *P.*value 0.000 these result display in table (3-9).

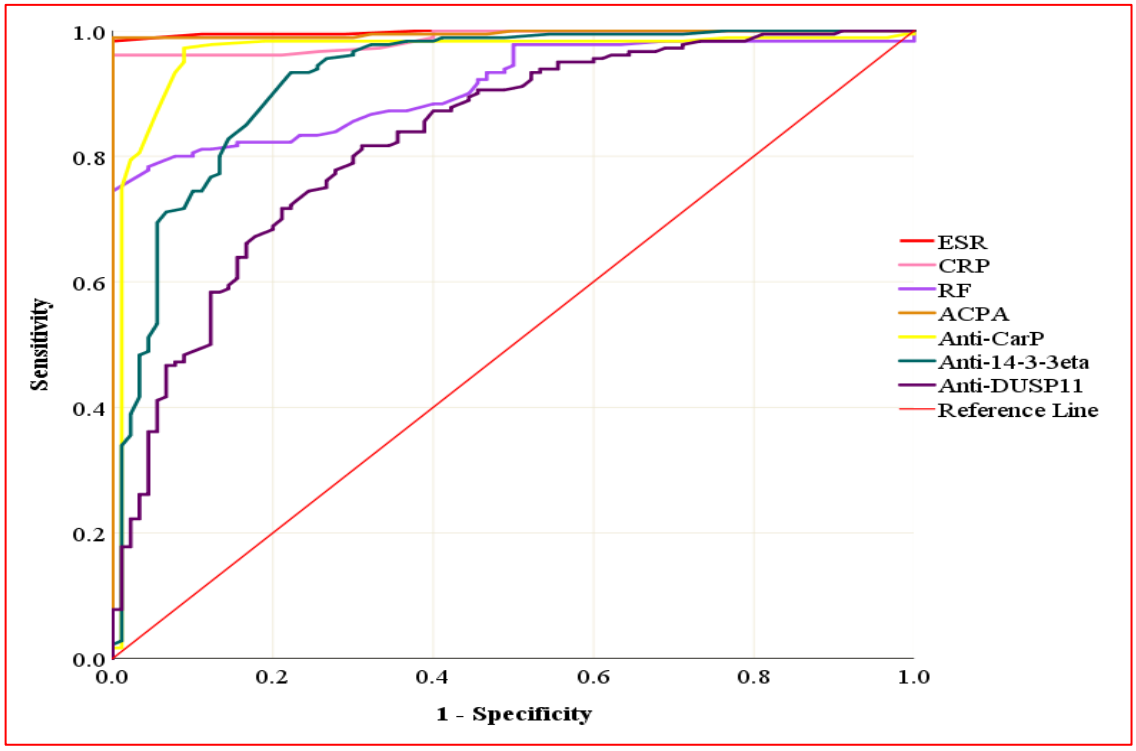


Figure (3-2): ROC Curve among Study Markers.

Table (3-12): The statistical test among study markers regarding ROC Curve.

Routine Test & Study Markers	AUC	SE	*P*. Value
ESR mm/h	0.998	0.002	0.000**
CRP mg/dl	0.987	0.005	0.000**
RF U/ml	0.912	0.017	0.000**
ACPA U/ml	0.996	0.003	0.000**
Anti-CarP ng/l	0.964	0.014	0.000**
Anti-14-3-3η ng/l	0.923	0.019	0.000**
Anti-DUSP11 ng/ml	0.827	0.027	0.000**

ROC curve (receiver operating characteristic curve) mainly used in medical research AUC[(0.9-1)excellent, (0.8-0.89) Good, (0.7-0.79) fair, (0.6-0.69) poor & (0.5-0.59) fail]

3.14. Correlation among Study Parameters in RA patients

According to the age variable in the current study, there is non-statistically significant correlation with ESR, CRP, RF, ACPA, anti-CarP, anti-14-3-3η and anti-DUSP11at the correlation coefficient range (r=0.05-0.1 *P*.value=>0.05), while the disease duration has positive statistically

significant correlation with ESR at *P*.value (0.047) and negative statistically significant correlation with anti-DUSP11 at *P*.value (0.014).

The ESR has positive statistically significant correlation with CRP and RF at *P*.value (0.000) and at *P*.value (0.003) respectively, and negative statistically significant correlation with anti-DUSP11 at *P*.value (0.023).

The DAS28-ESR has negative statistically significant correlation with anti-14-3-3η and anti-DUSP11at *P*.value (0.042) and (0.016) respectively.

The DAS28-CRP has negative statistically significant correlation with anti-DUSP11at *P*.value (0.030).

The RF has positive statistically significant correlation with ACPA at *P*.value (0.000).

The ACPA has negative statistically significant correlation with anti-14-3-3η Abs between them at *P*.value (0.044).

The anti-DUSP11 has positive statistically significant correlation with anti-CarP and 14-3-3 η at *P*.value (0.016) and (0.001) respectively, all the results were shown in the table (3-13).

Table (3-13): Correlation among study parameters in RA patients

Parameters		Age	Disease-Duration	ESR	DAS-ESR	CRP	DAS-CRP	RF	ACPA	Anti-Carp	Anti-14-3-3η
ESR mm/h	r	0.094	0.148*	1							
	P.	0.211	0.047								
CRP mg/dl	r	0.051	0.072	0.673**	0.424**	1					
	P.	0.497	0.336	0.000	0.000						
RF U/ml	r	-0.104	0.130	0.221**	0.162*	0.154*	0.243**	1			
	P.	0.163	0.083	0.003	0.030	0.039	0.001				
ACPA U/ml	r	-0.108	0.091	0.030	-0.048	0.087	0.082	0.616**	1		
	P.	0.150	0.223	0.687	0.523	0.243	0.271	0.000			
Anti-Carp ng/l	r	-0.052	-0.075	-0.083	-0.106	-0.070	-0.075	0.027	-0.109	1	
	P.	0.485	0.320	0.267	0.159	0.353	0.316	0.720	0.144		
Anti-14-3-3η ng/l	r	-0.019	0.012	-0.091	-0.145*	-0.047	-0.139	-0.144	-0.151*	-0.023	1
	P.	0.803	0.868	0.222	0.042	0.531	0.064	0.054	0.044	0.763	
Anti-DUSP11 ng/ml	r	-0.099	-0.184*	-0.170*	-0.180*	-0.097	-0.162*	-0.100	-0.031	0.179*	0.242**
	P.	0.185	0.014	0.023	0.016	0.195	0.030	0.184	0.677	0.016	0.001

- (r): Correlation coefficient
- Sig: *P.* value (*) significant <0.05 and (**) highly significant <0.01

3.15. Correlation among Study Parameters in Seropositive RA patients

According to the age variable in the seropositive RA patients in the current study, there is non-statistically significant correlation with ESR, CRP, RF, ACPA, anti-CarP, anti-14-3-3η and anti-DUSP11 at the correlation coefficient range (r=0.05-0.1 *P*.value=>0.05, while the disease duration has positive statistically significant correlation with ESR, CRP, RF, and ACPA at *P*.value (0.006), (0.000), (0.004), and (0.008) respectively, and negative statistically significant correlation with the anti-DUSP11 at *P*.value (0.029).

The ESR has positive statistically significant correlation with CRP and RF at *P*.value (0.000).

The DAS28-ESR has negative statistically significant correlation with the anti-DUSP11 at *P*.value (0.028).

The CRP has positive statistically significant correlation with RF and ACPA at *P*.value (0.002) and (0.029) respectively.

The DAS28-CRP has positive statistically significant correlation with the RF and ACPA at *P*.value (0.000) and (0.021) respectively

The RF has positive statistically significant correlation with ACPA at *P*.value (0.000).

The anti-CarP have positive statistically significant correlation anti-DUSP11 at *P*.value (0.010).

The anti-14-3-3η has non-statistically significant correlation with anti-DUSP11 at *P*.value (0.098), all the results were shown in the table (3-14).

Table (3- 14) Correlation among Study Parameters in Seropositive RA patients

Parameters		Age	Disease-Duration	ESR	DAS-ESR	CRP	DAS-CRP	RF	ACPA	Anti-Carp	Anti-14-3-3η
ESR mm/h	r	-0.011	0.29**	1							
	Sig	0.919	0.006								
C-RP mg/dl	r	-0.087	0.38**	0.73**	0.51**	1					
	Sig	0.417	0.000	0.000	0.000						
RF U/ml	r	-0.002	0.3**	0.45**	0.369**	0.32**	0.44**	1			
	Sig	0.99	0.004	0.000	0.000	0.002	0.000				
ACPA U/ml	r	0.02	0.28**	0.17	0.09	0.23*	0.24*	0.41**	1		
	Sig	0.83	0.008	0.118	0.427	0.029	0.021	0.000			
Anti-Carp ng/l	r	-0.12	-0.13	-0.15	-0.18	-0.09	-0.12	-0.01	-0.13	1	
	Sig	0.26	0.242	0.151	0.089	0.400	0.276	0.959	0.239		
Anti-14-3-3η ng/l	r	-0.01	-0.09	-0.064	-0.11	-0.05	-0.064	-0.09	0.01	-0.02	1
	Sig	0.94	0.389	0.549	0.321	0.655	0.548	0.407	0.940	0.834	
Anti-DUSP11 ng/ml	r	-0.08	-0.23*	-0.17	-0.23*	-0.14	-0.144	-0.15	-0.037	0.27**	0.18
	Sig	0.48	0.029	0.115	0.028	0.204	0.177	0.166	0.731	0.010	0.098

- (r): Correlation coefficient
- Sig: *P.* value (*) significant <0.05 and (**) highly significant <0.01

3.16. Correlation among Study Parameters in Seronegative RA patients

According to the age and disease duration variables in the seronegative RA patients in the current study, there is non-statistically significant correlation with ESR, CRP, RF, ACPA, anti-CarP, anti-14-3-3η and anti-DUSP11 at the correlation coefficient range (r=0.05-0.1 *P*.value=>0.05).

The ESR has positive statistically significant correlation with CRP and ACPA at *P*.value (0.000) and (0.023) respectively.

The anti-14-3-3η has negative statistically significant correlation with DAS28-ESR, DAS28-CRP and ACPA at *P*.value (0.017), (0.016) and (0.001) respectively.

The anti-CarP have non-statistically significant difference negative correlation with anti-14-3-3η and anti-DUSP11 at *P*.value (0.526) and (0.586) respectively.

The anti-DUSP11 has positive statistically significant correlation with the anti-14-3-3η at *P*.value (0.002), all the results were shown in the table (3-15).

Table (3-15) Correlation among Study Parameters in Seronegative RA patients

Parameters		Age	Disease-Duration	ESR	DAS-ESR	CRP	DAS-CRP	RF	ACPA	Anti-Carp	Anti-14-3-3η
ESR mm/h	r	0.17	0.02	1							
	Sig	0.111	0.980								
C-RP mg/dl	r	-0.03	-0.17	0.64**	0.37**	1					
	Sig	0.755	0.115	0.000	0.000						
RF U/ml	r	-0.03	-0.01	0.13	0.18	0.05	0.07	1			
	Sig	0.755	0.915	0.241	0.089	0.663	0.519				
ACPA U/ml	r	0.10	-0.06	0.24*	0.19	0.02	0.12	0.05	1		
	Sig	0.375	0.601	0.023	0.069	0.842	0.27	0.674			
Anti-Carp ng/l	r	0.08	0.01	0.06	0.07	-0.06	0.03	-0.03	0.12	1	
	Sig	0.480	0.942	0.595	0.514	0.551	0.77	0.788	0.265		
Anti-14-3-3η ng/l	r	-0.09	0.04	-0.15	-0.25*	-0.06	-0.25*	-0.02	-0.35**	-0.068	1
	Sig	0.428	0.730	0.162	0.017	0.581	0.016	0.840	0.001	0.526	
Anti-DUSP11 ng/ml	r	-0.14	-0.14	-0.18	-0.12	-0.07	-0.20	0.01	-0.03	-0.058	0.321**
	Sig	0.195	0.194	0.093	0.273	0.548	0.065	0.944	0.780	0.586	0.002

- (r): Correlation coefficient
- Sig: *P*. value: (*) significant <0.05 and (**) highly significant <0.01

4. Discussions

4.1. Distribution of sociodemographic characters of study groups RA patients and healthy control

The sociodemographic characters that showed in the table (3-1) in the current study that included 270 subjects, classified into two groups (case-control) with the mean±SE of age 49.82±86 and 49.0 ±0.99 respectively and the age rang was from 20-73 years, the study results show the adequate matching in the mean age and range among study groups and these results agree with (Hussein, 2019) the study show the RA patients mean age was 47.82years and control mean age was 46.82years, and agree with (Osman *et al.*, 2021) that show mean age was 48.5 years ,also agree with (Kolarz *et al.*, 2021) that mean age was (52.1) years and the age ranged (18-70) years.

Regarding to the age groups in the current study table (3-1) the group two has highest group numbers that age ranged from (40-59) years represent 62.8% as 113 subjects, These frequencies agree with (Abbood, 2019) in Iraq, that showed the patients age group range from (40-59 years) was 112 subject that represented (62.2%) of the study group. The highest age range from (40-59) years may be due to the disease progression with age and hormonal effect especially in Females. In fact, the low estrogen levels postmenopausal encourage the proinflammatory effects so induce RA.

The sex distribution in the current study are showed in the table (3-1) that appear the females sex in the RA patients represent 160(88.9%) and this results agree with (Oweis *et al.*, 2020) that showed females sex represented 252 (88.4%), also agree with (Iannone *et al.*, 2017) in Italy that showed the female 77(80.68%) of total RA patients, and agree with (Osman *et al.*, 2021) that show females were 79 (92.9%), and males were 6 (7.1%) in the case group. The sex distribution of females more than males may due to females have stronger immune system and hormonal imbalance

is commonly attributed to estrogen make them more susceptible to autoimmune diseases more than males, also females with diabetes mellitus in Iraq become more susceptible to symptomatic arthritis get older, and disagree with (Nilsson *et al.*, 2021) in the Sweden that showed the female sex were represented (68%) of RA patients this disagreement may due to the sample selection, inclusion and exclusion criteria.

The family history of RA patients in the current study is positive family history in 101(56.1%) of total RA patient, that means predisposed to RA and this result may benefits in early diagnosis for RA and adopting healthy life style to prevent disease progression, this result disagree with (Flyeh, 2019) this study showed only (6%) of the RA patients in the study had positive family history, these discrepancy due to variation about previously unknown between the current study population when compared with previous period and study population.

4.2. Comparison of study variables between RA Patients and Control Groups

The results in the table (3-4) show, the Anti-CarP is statistically significant difference at *P.* value less than (0.01) for RA patients group. These results agree with the following studies (Elsayed *et al.*, 2019) in Egypt, (Mohamed *et al.*, 2020) in Egypt, (Sidiras *et al.*, 2021) in Belgium and (Kolarz *et al.*, 2021) in Poland, all of these studies demonstrated statistically significant differences in anti-CarP levels between RA patients and healthy controls, that is may due to Carbamylation which occurs when there is abundance of cyanate, which can be caused by an overabundance of urea and enhanced myeloperoxidase activity (inflammation) or by direct consumption (smoking).

The anti-14-3-3η is statistically significant difference at *P.* value less than (0.01). This finding agrees with (LiuLiao and Shi, 2019) in China and

(El-Sherif *et al.*, 2019) in Egypt, these studies showed that the 14-3-3η Abs were high level in early RA than control, that due to the anti-14-3-3η has greater sensitivity and specificity than conventional diagnostic biomarkers, and agree with (Bonifacio *et al.*, 2019) in Italy, that showed the 14-3-3η Abs, a new proinflammatory mediator implicated in RA than controls.

The anti-DUSP-11 is statistically significant difference with *P.* values less than (0.01). This result agrees with (Li *et al.*, 2022) in China, these studies showed that DUSP11 Abs had specificity for RA patients when compared with healthy control.

4.3. Comparison of study variables between Seropositive and Seronegative

Regarding to the results in the current study that were shown in the table (3-5) that reveal the age, disease duration, ESR, and CRP have non-statistically significant difference at *P.*value (0.182), (0.173), (0.809) and (0.986) respectively, between seropositive and seronegative RA. This result agrees with (Oweis *et al.*, 2020) in Jordan, and agrees with (Liu *et al.*, 2021) in China.

The results in table (3-5) that show the RF and ACPA are statistically significant difference at *P.*value less than (0.01) for seropositive than seronegative. So these results agree with (Reed *et al.*, 2020) in Sweden this study revealed that RA patients were classified as seropositive or seronegative, depending on the presence or absence of ACPA and RF, and agree with (Kronzer *et al.*, 2021) in Sweden that showed the ACPA and RF were specific for seropositive RA patient,.

The study markers anti-CarP, anti-14-3-3η, and anti-DUSP-11 in the table (3-5) are statistically significant difference at *P*.value (0.000), (0.000) and (0.032) respectively in seronegative RA patients compared to seropositive RA patients.

The anti-CarP antibodies result agrees with the following studies (Sidiras *et al.*, 2021) in Belgium, that showed the anti-CarP were associated with seronegative RA, and (Lamacchia *et al.*, 2021) in Switzerland, this study showed the significant difference at *P*.value 0.02 for seronegative when compared with seropositive patients, also (Wang et al., 2023) in China, that said the presence of anti-CarP antibodies may aid in the early detection of RA, and (Markovic *et al.*, 2023) in Italy, that revealed that anti-CarP antibodies were detected in a substantial proportion of RA patients in about 35% of seronegative patient and also agree with (Ucci *et al.*, 2023) in UK they indicated that citrullinated and carbamylated protein expression in the extracellular micro-vesicles of rheumatoid arthritis patients.

The anti-14-3-3η antibody result agrees with the following studies (Salman *et al.*, 2019) in Turkey, that showed the importance of anti-14-3-3η Abs in the seronegative RA patients at *P*. value 0.001, and (Zhang *et al.*, 2020) in China, that revealed the values of anti-CarP and anti-14-3-3η Abs in the boosting of RA diagnosis in compensation with gold standard markers, also agree with (Wu *et al.*, 2022) in China, that determined that the addition of anti-14-3-3η Abs can provide incremental benefits for the diagnosis of RA, and also agree with (Chawla and Jain, 2023) in India, they indicated that anti-14-3-3η Abs levels are substantially elevated in RA patients and may be used as an additional diagnostic test for RA.

The anti-DUSP11 result agrees with (Lu *et al.*, 2021) in China, this study showed that DUSP11-Abs significant for seronegative RA patients, and agree with (SokolovaSchett and Steffen, 2021) in Germany, that indicated the presence of anti-pentraxin 3 and anti-dual specificity phosphatase 11 (DUSP11) antibodies in about 30–40% of both ACPA-positive and ACPA-negative RA patients, also agree with (Li *et al.*, 2022) in China , this study revealed that DUSP11-Abs significant for diagnosis of seronegative RA cases, and also agree with (Romão and Fonseca, 2022) in Portugal, that revealed that anti-DUSP11 present in serum of RA patients as 32%.

4.4. Comparison of study variables According to DAS28-CRP

Regarding to the findings in the table (3-6) the results were compared the mean±SE of the current study variables on the bases of DAS28-CRP, therefore the result of mean±SE age among mild, moderate and severe groups have non-statistically significant difference at *P*.value (0.180) while the mean±SE of disease duration, acute phase reactants (ESR and CRP), RF and ACPA have statistically significant difference at *P*.value less than (0.01) among the DAS28-CRP groups especially for severe than moderate and mild groups. These results agree with (Hussein, 2019) in Iraq, that documented the statistically significant difference for severe DAS28-CRP in RA patients than other two groups, and agree with (Abbood, 2019) in Iraq, that demonstrated the highly significant value for the mean of ESR and CRP for severe groups than mild and moderate DAS28-CRP. This may be due to delayed diagnosis and progression of the disease.

The current study markers anti-CarP Abs, anti-14-3-3η Abs, and anti-DUSP11Abs are associated with the mild and moderate groups at statistically significant difference *P.* value (0.001), (0.001) and (0.05)

respectively, which were shown clearly in table (3-6). These results agree with (Wang *et al.*, 2023) in China, they demonstrated that anti-CarP Ab the associated with mild DAS28 and may use as novel marker in compensation with other markers, but disagree with the following studies (De Stefano *et al.*, 2021) Italy, that said that anti-DUSP11 Abs associated with worst outcomes high DAS28 patients, and (Alashkar *et al.*, 2022) they documented the anti-14-3-3η had positive correlation with disease activity, and also disagree with (Abd Elsamea *et al.*, 2023) in Egypt, they show 14-3-3η protein levels were significantly high level in RA and significantly correlated with inflammation. These discrepancies are due to the dose and type of treatment intake that affected the level of acute phase reactant and patients behaviors and pain relive that finally affected the disease activity score.

4.5. Comparison of study variables between seropositive and seronegative RA patients According to DAS28-CRP

Regarding to the findings in the table (3-7) the results in the current study were compared the mean±SE of the current study variables between seropositive and seronegative RA patients on the bases of DAS28-CRP therefore the result of RF and ACPA at *P.* value (0.0001) agree with (Carbonell-Bobadilla *et al.*, 2022). These results indicate the routine tests ACPA and RF associated with severity of disease, RA progression, joint destruction and bone erosion in seropositive RA patients.

The results of study markers (anti-CarP, anti-14-3-3η, and anti-DUSP11) Abs is unique in this field with regard to the markers used in the study that separated seropositive and seronegative RA patients according DAS28-CRP. These results indicate the study markers can be aid in the diagnosis of RA patients especially seronegative RA to prevent disease progression.

4.6. Comparison of study variables according to DAS28-ESR

The results were shown in the table (3-8) in the current study revealed the non-statistically significant difference at *P*.value (0.204) of the mean±SE age among mild, moderate and severe activity group regarding DAS28-ESR. This result agrees with (Sparks *et al.*, 2019) in USA, while the disease duration ,ESR, CRP, RF and ACPA show statistically differences with sever disease at *P*.value (0.001), (0.001), (0.001), (0.015) and (0.018) respectively, that progression with time. This result agrees with (Madan *et al.*, 2019) in India that demonstrated the association of disease severity with elevated level of ESR, CRP, RF and ACPA, and agree with (Vadell *et al.*, 2020) in Sweden that showed the severity increased with disease duration, the ESR, CRP, RF and ACPA are significantly difference among DAS28 groups especially with severe group and also agree with (El debsy *et al.*, 2021) in Egypt that documented the highly association among ESR, CRP, RF and anti-CCP with worst radiological damage and high DAS28 score.

Regarding to DAS28-ESR groups the study marker anti CarP Abs, anti-14-3-3η Abs, and anti-DUSP11 Abs results showed statistically significant difference at *P.* values (0.025, 0.032, and 0.003) respectively for mild DAS28-ESR RA patients than other groups. These results agree with (El Hawary *et al.*, 2022) in Egypt they documented that anti-CarP Abs were a marker of disease activity in RA patients, but disagree with (Truchetet *et al.*, 2017) in France that demonstrated the high level of anti-CarP Abs may help to identify patients at risk of erosive progress of RA and (Zhang *et al.*, 2020) in China they documented that anti-CarP Abs were a potential marker of disease activity and bone erosion in RA

The result of anti-14-3-3η agrees with (El-Sherif *et al.*, 2019) that documented the use of this marker for preventing disease progression, especially before RF and ACPA become positive and agree with (Dammona *et al.*, 2020) in Egypt, they concluded that anti-14-3-3η associated with non-erosive damaging outcome in RA and disagrees with (Raft *et al.*, 2022) in Denmark they demonstrated that 14-3-3η Abs associated with severity of disease, the anti-DUSP11 result agrees with (Qian *et al.*, 2022) in China they showed that anti-DUSP11 inverse correlation with tender and swollen joints weakly with DAS28 and disagrees with (De Stefano *et al.*, 2021) in Italy they said the anti-DUSP11was high in the sever DAS28-ESR patients. These disparities in the results may be due to differences in the dose and kind of medication used, as well as population or personal variation, and pain alleviation, which influenced the level of ESR thus affecting the disease activity score.

4.7. Comparison of study variables between seropositive and seronegative RA patients According to DAS28-ESR

Regarding to the findings in the table (3-9) the results in the current study were compared the mean±SE of the current study variables between seropositive and seronegative RA patients on the bases of DAS28-ESR therefore the results of RF and ACPA at *P*. value (0.0001) agree with (SargınKöse and Şentürk, 2019) and (Liang *et al.*, 2022). These results indicate the routine tests ACPA and RF associated with severity of disease, RA development, joint destruction and bone erosion in seropositive RA patients.

The results of study markers (anti-CarP, anti-14-3-3η, and anti-DUSP11) Abs is unique in this field with regard to the markers used in the study that separated seropositive and seronegative RA patients according DAS28-ESR. These results indicate the study markers can be aid in the

diagnosis of RA patients especially seronegative RA to prevent disease progression and bone erosion.

4.8. Comparison of study variables According to Treatment Regularity

According to the results that were shown in the table (3-10) in the current study reveals the comparisons between regular and irregular groups with study variables; the results of the mean±SE age variable show non-statistically significant difference at *P*.value (0.902) between regular and irregular treatment intake while the mean±SE of disease duration, ESR, CRP, RF and ACPA statistically significant difference with irregular group at *P*.value (0.001). This result agrees with (Ghaseminasab-Parizi, 2022) in Iran they documented the regular specific treatment received RA patients were significant low DAS28 and laboratory test.

The current study results about the current study markers anti-CarP, anti-14-3-3η, and anti-DUSP11 have statistically significant difference with the regular group at *P*.value (0.047), (0.044) and (0.001) respectively of RA patients, these results agree with (Zeng *et al.*, 2020) in China the study documented the high level of anti-CarP, anti-14-3-3η with regular treatment intake and can be used as prognostic markers for RA patients follow up.

4.9. Comparison of study variables between seropositive and seronegative RA patients According to Treatment Regularity

According to the results that were shown in the table (3-11) in the current study reveals the comparisons between seropositive and seronegative RA patients according treatment regularity with study variables, therefore the results of RF and ACPA at *P*. value less than (0.05)

agree with (Ridha *et al.*, 2022) and (Curtis *et al.*, 2023). These results indicate the routine tests RF and ACPA associated with disease severity and bad prognostic markers for RA monitoring in seropositive RA patients.

The results of study markers (anti-CarP, anti-14-3-3η, and anti-DUSP11) Abs is unique in this field with regard to the markers used in the study that separated seropositive and seronegative RA patients according treatment regularity. These results indicate the study markers associated with disease monitoring and can be used as good prognostic markers for RA patients especially for seronegative RA.

4.10. The ROC Curve among Study Parameters

The results that were shown in figure (3-2) and table (3-12) in the current study show that statistically significant associated of the sensitivity and specificity of the study markers anti CarP, anti-14-3-3η, and anti-DUSP11with ESR, CRP, RF and ACPA. These results agree with (Othman *et al.*, 2017) in Holland that documented the sensitivity and specificity of anti-CarP at *P*.value= 0.005, and agree with (Zhu *et al.*, 2019) in China they documented the sensitivity and specificity area under the ROC curve (AUC) were 0.76 at *P.* value< 0.000, also agree with (Mohamed *et al.*, 2020) in Egypt they showed the ROC curves appeared that anti-CCP had highest AUC than both anti-CarP and RF.

The anti-14-3-3η results agree with (Poornima, 2018) in India who demonstrated the ROC curve about anti-14-3-3η significant sensitivity and specificity at AUC 0.948 and *P.* value< 0.000.

The anti-DUSP11 results are agree with (Lu *et al.*, 2021) in China they demonstrated the ROC curve an indicator combining sensitivity and specificity, which demonstrated the intrinsic effectiveness of diagnostic tests, the AUC RA was 0.869, 0.875, and 0.899 for CRP, RF and ACPA

with anti-DUSP11 respectively, these results indicate the study markers more sensitive and specific for RA diagnosis.

4.11. Correlation among Study Parameters in RA patients

The result of correlation among the current study variables in the table (3-13) shows the highly correlation between ESR and CRP at correlation coefficient 0.673 and among the ESR and CRP with RF are low to medium correlation and with ACPA highly correlation. These results agree with the following studies (Khojah *et al.*, 2016) in Saudi Arabia they documented the positive correlation of ESR and CRP with RF and anti-CCP, and agree with (Hamadi, 2023) in Iraq that documented the positive correlation among ESR, CRP, RF and ACPA, while disagreed with (HassanAbdullah and Zakair, 2022) in Iraq that said were no significant correlation between DAS28 and CRP. This discrepancy may due to population and sample variation and differences in sampling exclusion and inclusion criteria of patient's selection.

The anti-CarP antibody has negative correlation with DAS-28 ESR and DAS-28 CRP. These results agree with (Kumar *et al.*, 2021) in Italy that and (Wu *et al.*, 2021) in Taiwan. These results indicate the anti-CarP antibody has the potential to be an effective clinical response predictor.

The anti-14-3-3η antibody has negative correlation with ESR, CRP, RF and ACPA. These results agree with (Dammona *et al.*, 2020) in Egypt about ESR, CRP the study documented the serum 14-3-3η protein was negative correlated with ESR, CRP while this study disagrees with current study result about RF and ACPA that showed the serum RF (*P.*= 0.048) and ACPA (*P.*= 0.003). So, this discrepancy may be due to different in sample size and include patients sample that take moderate and severe DAS-28 cases only.

The anti-DUSP11 antibody has negative statistically significant correlation with DAS28-CRP, DAS28-ESR and disease duration. These results agree with (Li *et al.*, 2021) in China these results indicate the anti-DUSP11 antibody has the potential to be an effective clinical response predictor and early diagnostic marker for RA.

4.12. Correlation among Study Parameters in seropositive and seronegative RA patients

The results of correlation among the current study variables for seropositive RA patients in the table (3-14) show the age variable non-statistically significant correlation with ESR, CRP, RF and ACPA at (r=0.05-0.1 *P*.value=>0.05. So, these results agree with (TakanashiTakeuchi and Kaneko, 2023) in Japan, while the disease duration have positive statistically significant correlation with ESR, CRP, RF and ACPA at *P*.value less than (0.01) these results agree with (Rivellese *et al.*, 2020) in UK

DAS28-ESR and DAS28-CRP have positive statistically significant correlation with RF and ACPA at *P*.value less than (0.01 and 0.05). These results agree with (Seri *et al.*, 2021) in Sudan.

RF has highly positive statistically significant correlation with ACPA at *P*.value (0.000). These results agree with (Kolarz *et al.*, 2021) in Poland.

With regard to the results of the current study markers anti-CarP Abs, anti 14-3-3η Abs, and anti-DUSP11 Abs for seropositive and seronegative RA patients which are shown in the tables (3-14) and (3-15). This current study is unique in this field with regard to the markers used in the study that separated seropositive and seronegative RA patients according the correlation among study parameters. The anti-14-3-3η has negative significant correlation with DAS28-ESR, DAS28-CRP and ACPA at

P.value (0.017), (0.016) and (0.001) respectively. These results indicate the anti-14-3-3η can be used to diagnosis and prognosis of RA patients especially seronegative RA to prevent disease progression, cartilage destruction and bone erosion.

5. Conclusions and Recommendations

5.1. Conclusions

The current study concludes the following:

1- Anti-CarP, anti-14-3-3η, and anti-DUSP11 can be aid in the diagnosis of RA patients (especially seronegative patients).

2- Use of anti-CarP, anti-14-3-3η, and anti-DUSP11 markers in integrated with gold standard antibodies (RF and ACPA) in the diagnosis of RA patients.

3- The anti-CarP, anti-14-3-3η, and anti-DUSP11 markers show high sensitivity and specificity at AUC 0.964, 0.923 and 0.827 respectively.

4- Anti-CarP, anti-14-3-3η, and anti-DUSP11 associated with mild and regular treated RA patients, so can be used as prognostic markers.

5.2. Recommendations:

The current study is recommending the following:

1- Further studies with larger sample size to evaluate the serum level of anti-CarP, anti 14-3-3η, and anti-DUSP11in RA patients.

2- Further studies are required to establish the potential relevance of anti-CarP, anti-14-3-3η , and anti-DUSP11 markers in RA etiology to clarify their utility in disease activity, treatment monitoring, and understanding disease progression especially in seronegative RA patients

3- Further identification of anti-CarP, anti-14-3-3η, and anti-DUSP11 markers associated with genetic variants in RA patients using personalized medicine, which is critical for disease course prognosis and treatment plan selection.

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7. Appendices

7.1. Systematic Ethical Approvals

جمهورية العراق

وزارة التعليم العالي والبحث العلمي
جامعة كربلاء
كلية الطب
معاون العميد لشؤون العلمية
شعبة الدراسات العليا

العدد: د ك / 326
التاريخ: 26 / / 2023

الى/دائرة صحة كربلاء المقدسة/مستشفى الامام الحسن المجتبى(ع) التعليمي
شعبة المختبر
م/ تسهيل مهمة

جامعة كربلاء كلية الطب
الصادر

تحية طيبة:

يرجى تفضلكم بتسهيل مهمة طالبة الدراسات العليا/ماجستير/احياء مجهرية طبية (زهراء قاسم علي) في مشروع بحث رسالة الماجستير الموسومة :

Evaluation The Role of Serum Level of Carbamylated protein Antibody,The 14-3-3η protein Antibody, and Dual Specificity Phosphatase 11 Antibody in The Diagnosis and Severity of The Rheumatoid Arthritis Patients.

لغرض جمع عينات البحث، شاكرين تعاونكم معنا خدمة للحركة العلمية في بلدنا العزيز

... مع التقدير ...

أ.م.د.علي عبد الرضا أبو طحين
معاون العميد للشؤون العلمية
2023/ / 26

الاستاذ المساعد الدكتور
بشير عقيل العلي
معاون العميد للشؤون الادارية

**نسخة منه:
- مكتب السيد العميد المحترم للتفضل بالاطلاع مع التقدير.
- مكتب معاون العميد للشؤون العلمية المحترم للتفضل بالاطلاع مع التقدير.
- فرع الاحياء المجهرية.. للتفضل بالاطلاع مع التقدير.
- شعبة الدراسات العليا/الحفظ.
- الصادرة.

جمهورية العراق

وزارة التعليم العالي والبحث العلمي
جامعـــــة كربـــــلاء
كلية الطب
معاون العميد للشؤون العلمية
شعبة الدراسات العليا

العـدد: د/ 3071 / 16

التاريخ: 2022/ 11 / 9

أمر اداري

م/اقرار مشروع بحوث طلبة الدراسات عليا/ماجستير/احياء مجهرية طبية

إشارة الى ما جاء في محضر مجلس الكلية بالجلسة الثانية المفتوحة المنعقدة بتاريخ (2022/10/13) والمصادق عليها من قبل رئاسة جامعة كربلاء /أمانة مجلس الجامعة بكتابهم المرقم (ج/1498 في 2022/11/2)، واستناداً للصلاحيات المخولة لنا تقرر:

- اعتماد خطط ومشاريع بحوث طلبة الدراسات العليا/ماجستير/احياء مجهرية طبية واسماء السادة التدريسيين المشرفين على خطط مشاريع البحوث حسب الجدول ادناه واعتباراً من تاريخ كتاب مصادقة أمانة مجلس الجامعة على محضر مجلس الكلية.

ت	اسم الطالب	عنوان البحث	اسم المشرف
1	فاطمة عبد الحسين كاظم	Evaluation of Immune-Related Genes (Integrin-alpha M Chain (ITGAM),Tumour Necrosis Factor (TNF) Alpha Induced Protein 3 (TNFAIP3))and Cytokine Markers (Interferon-alpha(IFN-α),Toll-like Receptor-7(TLR-7)for Progression of Systemic Lupus Erythematosus in Iraqi Patients.	أ.د. ستار جبار راهي احياء مجهرية -مناعة جامعة كربلاء-كلية الطب
			أ.د. رياض ضيهود الزبيدي الطب الباطني والغدد الصم جامعة كربلاء-كلية الطب
2	زهراء قاسم علي	Evaluation The Role of Serum Level of Carbamylated protein Antibody,The 14-3-3η protein Antibody, and Dual Specificity Phosphatase 11 Antibody in The Diagnosis and Severity of The Rheumatoid Arthritis Patients.	أ.د. الاء سعد حنفوش بورد مناعة جامعة كربلاء-كلية الطب
			أ.د. ضمياء مكي حمزة احياء مجهرية-طفيليات جامعة كربلاء-كلية الطب
3	مروة محمد علي	Immunological role of interleukin-37,38 and 17A in patients with bacterial infection of diabetic foot ulcers.	أ.م.د .سوسن محمد جبار احياء مجهرية-مناعة جامعة كربلاء-كلية الطب
			م.د. مي محمد علي احياء مجهرية -بكتريولوجي جامعة كربلاء-كلية الطب

أ.م.د. علي عبد الرضا أبو طحين
معاون العميد للشؤون العلمية
2022/ 11/ 9

*نسخة منه:
- مكتب السيد العميد المحترم للتفضل بالاطلاع مع التقدير.
- مكتب معاون العميد للشؤون العلمية المحترم للتفضل بالاطلاع. مع التقدير.
- فرع الاحياء المجهرية. للتفضل بالاطلاع لتبليغ السادة المعنيين.
- الحسابات...للتفضل بالاطلاع ...واتخاذ ما يلزم.
- شعبة الدراسات العليا/اضابير الطلبة.
- الصادرة.

7.2. Current Study Questionnaire

1

NO =

Questionnaire For Rheumatoid Arthritis Patients

I'm researcher from university of Karbala/ faculty of medicine / medical microbiology department: I do research on RA patients do you accept to participate in this research.

Yes: ☐ **NO:** ☐

Seropositive RA Patients

Name:		**Gander ♀ or ♂:**		
Age in years :				
ACR /EULAR Classification		**Typical RA**	**Atypical RA**	
Disease Duration, years:				
Family History:				
Other Autoimmune Disorders:			**1st :**	**2nd :**
Smoking Status:		**Non Smoker**	**Smoker**	**-Smoker**
Treatment	**Biological**	**Regular**	**Irregular**	
	MTX (non-Bio DMARD)	**Regular**	**Irregular**	
Number of Swelling Joints (NSJ)				
Number of Tender Joints (NTJ)				
Pain Assessment (0 - 100 mm)				
Patient Global Assessment (PGA)				
Anti-CCP Antibody titer (ACPA)				
Anti-Rheumatoid Factor titer (RF)				
CRP titer mg/dl				
ESR mm/h				
DAS28-CRP				
DAS28-ESR				
Anti-CarP				
Anti-14-3-3				
Anti-DUSP11				

2

NO =

Questionnaire For Rheumatoid Arthritis Patients

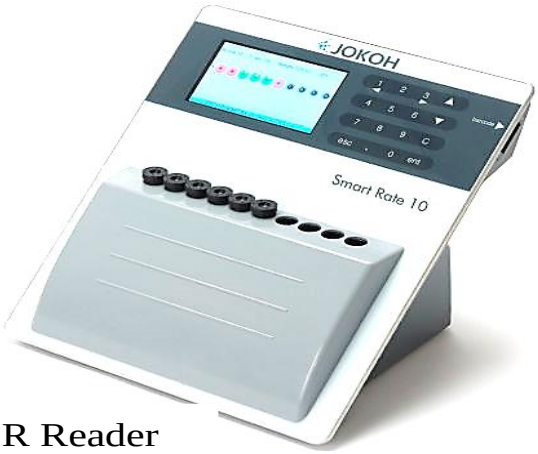
I'm researcher from university of Karbala/ faculty of medicine / medical microbiology department: I do research on RA patients do you accept to participate in this research.

Yes: ☐ **NO:** ☐

Seronegative RA Patients

Name:		**Gander ♀ or ♂:**		
Age in years :				
ACR /EULAR Classification		**Typical RA**	**Atypical RA**	
Disease Duration, years:				
Family History:				
Other Autoimmune Disorders:			**1st :**	**2nd :**
Smoking Status:		**Non Smoker**	**Smoker**	**-Smoker**
Treatment	**Biological**	**Regular**	**Irregular**	
	MTX (non-Bio DMARD)	**Regular**	**Irregular**	
Extra articular manifestation (EAM)				
Elbow Involvements				
Number of Swelling Joints (NSJ)				
Number of Tender Joints (NTJ)				
Pain Assessment (0 - 100 mm)				
Patient Global Assessment (PGA)				
Anti-CCP Antibody titer (ACPA)				
Anti-Rheumatoid Factor titer (RF)				
CRP titer mg/dl				
ESR mm/h				
DAS28-CRP				
DAS28-ESR				
Anti-CarP				
Anti-14-3-3				
Anti-DUSP11				

7.3. ESR Tube and ESR Reader for ESR Test



ESR Reader

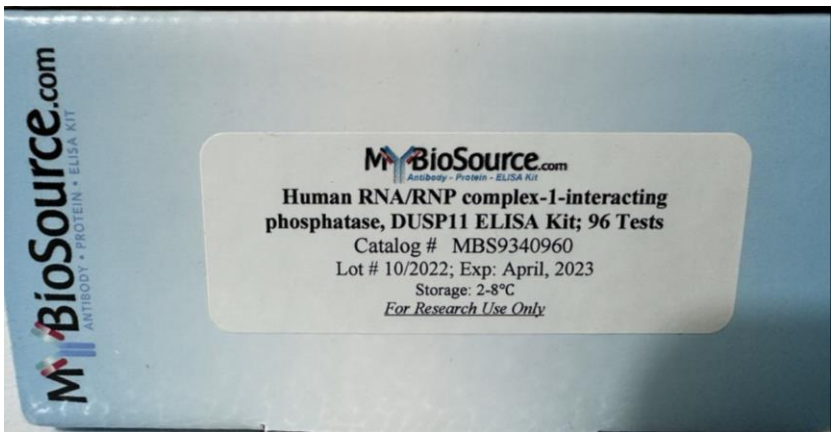
ESR Tube

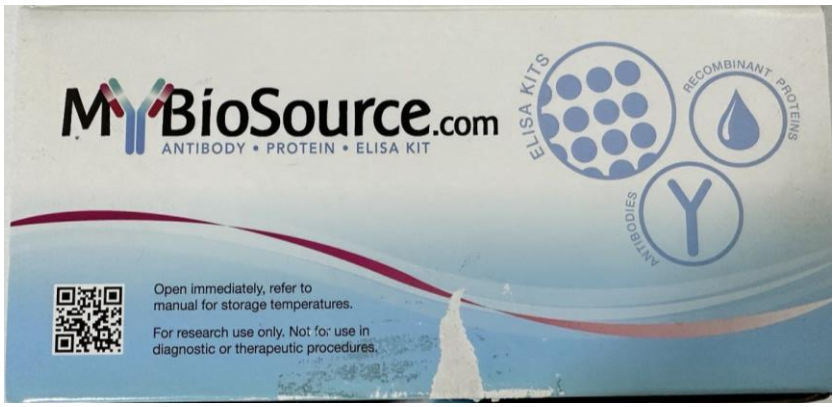
7.4. Specific Protein Analyzer and Gel Tube for CRP Test

Gel Tube

Specific Protein Analyzer

7.5. Anti-DUSP-11 Kits





7.6. Anti-14-3-3η Kits

7.7. Anti-CarP Kits

الخلاصة:

التهاب المفاصل الرثوي هو أحد أمراض المناعة الذاتية التي تؤثر في الغالب على المفاصل والأنسجة المحيطة بها (العظام والغضاريف والأنسجة الزليلية) وتؤثر بشكل متناظر على المفاصل الصغيرة في اليدين والقدمين. يجب أن يستوفي جميع مرضى التهاب المفاصل الرثوي ستة من معايير الكلية الأمريكية لأمراض الروماتيزم العشرة مع فحوصات المختبر الموصى بها وهي العامل الرثوي والأجسام المضادة الببتيدية المضادة للسيترولين.

هدف الدراسة هو تقييم مستويات المصل للأجسام المضادة للبروتين المكربن، ١٤-٣-٣أيتا والفوسفات ثنائي الخصوصية الحادي عشر في مرضى التهاب المفاصل الرثوي سواء إيجابيي المصل أو سلبيي المصل، والأشخاص الأصحاء لتحديد شدة المرض وانتظام العلاج.

دراسة الحالات والشواهد تمت في العراق / محافظة كربلاء / مستشفى الإمام الحسن المجتبى - وحدة أمراض الروماتيزم من أكتوبر ٢٠٢٢ إلى أبريل ٢٠٢٣ حيث قسمت ٢٧٠ شخصًا إلى مجموعتين رئيسيتين: ١٨٠ حالة مرضية التهاب المفاصل الرثوي و ٩٠ فرد سليم، ثم قسمت حالات التهاب المفاصل الرثوي إلى مجموعتين فرعيتين: ٩٠ إيجابي مصلي و ٩٠ سلبي مصلي بالإعتماد على وجود أو غياب العامل الرثوي والأجسام المضادة للببتيد المضاد للسيترولين، تمت مطابقة متوسط العمر والجنس لمجموعات الدراسة. بعد ذلك، أكمل جميع المشاركين في الدراسة الاستبيانات، ثم تم سحب عينات الدم الوريدي وتقسيمها إلى أنبوبين، أنبوب سترات الصوديوم الخاص بطريقة ويسترجرين لاختبار معدل ترسيب كرات الدم الحمراء وأنبوب هلامي لفصل عينة المصل التي تم تخزينها عند (-٢٠) درجة مئوية في أربعة أنابيب إيبندورف صغيرة لإجراء فحوصات مصلية شملت اختبار البروتين المتفاعل سي واختبار العامل الرثوي بواسطة جهاز قياس الكدر ،أما الجسم المضاد للببتيد المضاد للسيترولين، الجسم المضاد للبروتين المكربن، ١٤-٣-٣أيتا والفوسفات ثنائي الخصوصية الحادي عشر الذي تم إجراؤه بواسطة مقايسة المادة الماصة المناعية المرتبطة بالإنزيم. تم إجراء التحليل الإحصائي باستخدام برنامج الحزمة الإحصائية للعلوم الإجتماعية الإصدار ٢٦.

أظهرت نتائج الدراسة الحالية فروقات معنوية ذات أهمية إحصائية عالية عند القيمة الإحتمالية أقل من (٠,٠١) لمرضى التهاب المفاصل الرثوي عند مقارنتها بالأشخاص الأصحاء، وقد أشارت إختبارات الدراسة الحالية إلى وجود معنوية احصائية للأجسام المضادة للبروتين المكربن، ١٤-٣-٣أيتا والفوسفات ثنائي الخصوصية الحادي عشر في المجموعة السلبية المصلية

عنها في المجموعة الإيجابية المصلية عند القيمة الإحتمالية أقل من (٠,٠٥)، وفي الحالات العلاجية المنتظمة عنها في غير المنتظمة عند القيمة الإحتمالية أقل من (٠,٠٥)، عند مقارنتها مع الإختبارات القياسية وهي العامل الرثوي والأجسام المضادة للببتيد المضاد للسيترولين حيث كانت ذات معنوية إحصائية عالية في المجموعة إيجابية المصل عند القيمة الإحتمالية أقل من (٠,٠١)، وفي الحالات العلاجية غير المنتظمة عند القيمة الأحتمالية أقل من (٠,٠١)، أظهرت إختبارات الدراسة الحالية إرتباط بنشاط المرض الخفيف إلى المتوسط عند القيمة الإحتمالية أقل من (٠,٠١) أو مساويا للقيمة الإحتمالية (٠,٠٥)، عند مقارنتها مع الاختبارات القياسية المرتبطة بدرجة نشاط المرض الشديد عند القيمة الإحتمالية أقل من (٠,٠٥)، وكذلك أظهرت إختبارات الدراسة الحالية ارتباط معنوي موجب فيما بينها عند القيمة الإحتمالية أقل من (٠,٠٥)، ولكن كان للأجسام المضادة للفوسفات ثنائي الخصوصية الحادي عشر علاقة سلبية معنوية مع مدة المرض ودرجة نشاط المرض عند القيمة الإحتمالية أقل من (٠,٠٥).

تشير الدراسة الحالية إلى أنه يمكن استخدام الأجسام المضادة للبروتين المكربن، ١٤-٣-٣أيتا والفوسفات ثنائي الخصوصية الحادي عشركإختبارات تشخيصية خاصة في مرضى التهاب المفاصل الرثوي السلبي المصل، حيث أنها مرتبطة بنشاط مرضي خفيف إلى متوسط و بالحالات العلاجية المنتظمة مقارنة بالاختبارات القياسية، مما يجعلها علامات إنذار مفيدة.

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة كربلاء
كلية الطب
فرع الأحياء المجهرية

تقييم دور التركيز المصلي لمستويات الأجسام المضادة للبروتين المكربن و١٤-٣-٣أيتا والفوسفات ثنائي الخصوصية الحادي عشر في تشخيص ومتابعة شدة المرض لدى مرضى التهاب المفاصل الرثوي

رسالة

مقدمة الى مجلس كلية الطب / جامعة كربلاء وهي جزء من متطلبات نيل درجة الماجستير في الأحياء المجهرية الطبية

من قبل الطالبة

زهراء قاسم علي العنزي

بكالوريوس علوم حياة ٢٠١٣

بإشراف

الأستاذ الدكتور
الآء سعد العتابي
بورد مناعة سريرية

الأستاذ الدكتور
ضمياء مكي حمزة
دكتوراه أحياء مجهرية

٢٠٢٣ ميلادي

١٤٤٥ هجري