تقييم بعض علامات أمراض الدم و الالتهابات لدى مرضى التهاب المفاصل الرثوي

رسالة

مقدمة الى مجلس كلية العلوم للبنات جامعة بابل وهو من متطلبات نيل شهادة الماجستير في علوم الحياة

من قبل

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١٤٤٤هـ   ٢٠٢٢ م

Republic of Iraq

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Evaluation of some Hematological and Inflammatory Markers in Rheumatoid Arthritis Patients

A Thesis

Submitted to the Council of College of Science for Women, University of Babylon as a Partial Fulfillment of the Requirements for the Degree of Master of Science /Biology.

BY

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Biology. Sc. / Microbiology/ College of Science, University of Babylon 2018

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2022AD   **1444AH**

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

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Dedication

To My Dear Parents

To My Dear Sisters

To My Dear Brothers

To My Dear Friends

And to all people whom I love, I dedicate this work

Maysaa

Acknowledgment

Summary

Rheumatoid arthritis a class of systemic autoimmune disturbances and manifest with progression inflammation of several Joint in the body. The present results were conducted to evaluate some hematological parameters and some biomarkers in patients affected with RA of both gender (men and women).

The individuals who had involved in the present Study were 80 subjects of patients and healthy individual, they were classified according to gender into two Subclasses, and the first one involved 40 males that were divided into 20 Males Patients with RA and 20 Males healthy control. The second class had also 40 females, 20 of them affected with RA and 20 healthy females as a control group.

All ages of subject were ranged between 40-79 years old and then according to their age, they were classified into four age groups (40-49, 50-59 60-69, 70-79 Years old).

The demographical data that obtained from the present study indicated that the Percentage ratio of women with RA was 60% higher than Men with RA who had recorded 40%. The distribution of patients according to their age groups the percentage ratios were the following: 40-49 first group 30%, 50-59 Second group 30% ,60-69 Third group 25%, 70-79 fourth group 15% respectively).

In addition, the percentage ratios of RA Patients according to body Mass index (BMI), it was that the found patients who had BMI equal to <18kg/m² %20 less than 25-29.9 30 % less than those patients who had BMI ranged between 30-39.9 18kg/m² 45%).

The changes of hematological Parameters had been exhibited a significant (P<0.01) increase in the total count of white blood cells (WBCs), granulocytes%, blood platelets and Erythrocyte sedimentation rate (ESR) in all RA patient's groups when compared with those healthy one. Inversely the levels of lymphocytes, red blood Cells (RBCs) and hemoglobin was significantly (P<0.05) lower in all group of patients with RA in matching with their healthy counterparts.

The correlation Coefficients occurring among studied hematological parameters recorded the following data, it was found a significant (p≤ 0.001) positive correlation between ESR and Platelet in RA patients. Also, a significant (p≤0.005) positive correlation occurring between ESR and WBCs. the same results were found between ESR and granulocytes recorded a significant (p≤0.007) Positive Correlation in RA Patient. Inversely, a significant (p≤0.006) negative correlation between ESR and lymphocytes in RA patients

The observations recorded around studied biomarkers including rheumatoid factor (RF), C-reactive Protein (CRP), fibrinogen, tumor necrosis factor alpha (TNF), and granulocytes-monocytes Colony stimulating factor (GM-CSF) were showed a significant (P<0.05) elevation in all levels, of these biomarkers of all patient's groups compared to healthy subjects.

These studied biomarkers appear closely associated with BMI; therefore, their levels were significantly P<0.05 high in RA Patients with who had BMI equal to <18kg/m² %20 less than 25-29.9 30 % less than those patients who had BMI ranged between 30-39.9 18kg/m² 45%.

The correlation analysis of present study showed that ESR had a significant positive correlation with studied parameters TNF-α, GM-CSF, platelets, WBCs, Granulocytes, Fibrinogen, RF, CRP while it had a significant negative correlation with lymphocytes, also positive correlation between GM-CSF with platelets, WBC, granulocytes, fibrinogen, TNR-α, CRP and RF while it had a significant negative correlation with lymphocytes, also positive correlation between TNF-α with platelets, WBC, GM-CSF, granulocytes, fibrinogen, CRP and RF while it had a significant negative correlation with lymphocytes.

From the present study it can be concluded that RA is associated with development of anemia, this is because of systemic inflammation and oxidative stress, as well as increased inflammatory biomarkers.

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List of Abbreviations

Abbreviations	Meanings
ACPA	Anti- cyclic citrullinated peptide antibodies
BMD	Bone mineral density
BRC	Bone remodeling compartments
CBC	Complete blood count
CTGF	Connective tissue growth factor
CRP	C-reactive protein
CD	Crohn,s disease
CTLA4	Cytotoxic T-cell lymphocyte antigen4
DCs	Dendritic cells
DMP-1	Dentin matrix protein -1
DMARDs	Disease – modifying ant rheumatic drug
DIC	Disseminated intravascular coagulation
eNOS	Endothelial nitric oxide synthase
EBV	Epstein Bar Virus
EBNA-1	Epstein Bar Virus nuclear antigen
ESR	Erythrocyte sedimentation rate
FCγR	FC gamma receptor
FIB	Fibrinogen
GC	Glucocorticosteroids
GM-CSF	Granulocyte monocyte- colony stimulating factor
HLA	Human leukocyte antigen
HIF-α	Hypoxia – induced factor –α
ITAM	Immunoreceptor Tyrosine – based activated motif
IBD	Inflammation bowel disease
IGF-1	Insulin – like growth factor
ICAM-1	Intercellular adhesion molecule -1

IFN	Interferon
IL17	Interleukin 17
IL	Interleukine
IL1	Interleukine 1
IL10	Interleukine 10
IL6	Interleukine 6
LDL-C	Low –density lipoprotein cholesterol
LPA	Lysophasphatidic acid
LPC	Lysophosphatidylcholine
MSCs	Mesenchymal stem cells
MTX	Methotrexate
MCP-1	Monocyte chemoattractant protein -1
M-CSF	Monocytes – colony stimulating factor
mCRP	Monomeric CRP
MAP	Mycobacterium avium para
MDSCs	Myeloid –derived Suppressor cells
NLR	Neutrophil-to-lymphocyte ratio
NO	Nitric oxide
NIS	Noninfections nveitis
NOD/SCID	Nonobese diabetic /severe combined immunodeficent
OPG	Osteoprotegerin
PTH	Para thyroid hormone
PRRs	Pattern recognition receptors
pCRP	Pentameric CRP
PAD	Peptidylarginine deimminases
PRG4	Proteoglycan
Ps	Psoriasis
PsA	Psoriatic arthritis
ROS	Reactive oxygen species
RANKL	Receptor activator of nuclear factor $-_k$B ligand
RBCs	Red blood cells

RF	Rheumatic factor
RA	Rheumatoid arthritis
SOFA	Sepsis – related organ dysfunction score
TRAP	Tartrate - resistant acid phosphatase
Th	T-helper
TACE	TNF-α coverting enzyme
TLRs	Toll –like receptors
TMTNF-α	Transmembrane TNF-α
TREMz	Triggering receptor expressed in myeloid cells z
TNF-α	Tumor necrosis factor alpha
VCAM-1	Vascular cellular adhesion molecule-1
VEGF	Vascular endothelia cell growth factor
WBCs	White blood cells

Chapter one
Introduction

1.1 Introduction:

Rheumatoid arthritis (RA) is a chronic systemic disease in which immunologically mediated inflammation of synovia-lined joints can result in marked disruption of joint structure and function. Over the past two decades, significant advances in basic science research have elucidated the biology of this inflammatory process, including the identification of some of the cytokines that drive chronic synovial inflammation (e.g., TNF-α, IL-1 and IL-6). This has resulted in an explosion of targeted biologic therapies for RA that have proved significantly more effective than previously available treatments in improving disease activity, preventing joint destruction and preserving physical function. Using these new therapeutic agents, remission of disease activity is now a realistic possibility (Salomon-Escoto *et al*.,2011).

Rheumatoid arthritis can be diagnosed at the time patients present with clinically detectable inflammatory arthritis (swollen joints). It is known that immunological alterations are present long before that Choy., (2012). For instance, magnetic resonance imaging (MRI)-detected subclinical inflammation is present weeks to months before arthritis becomes clinically apparent in patients presenting with clinically suspect arthralgia (CSA) and has been shown to be predictive for RA development nonetheless, a long-standing question is where inflammation starts in the joint, or in what order the different tissues of the joints (synovium, tenosynovium, and bone) become inflamed during the development of RA(Van Steenbergen *et al*., 2016).

Classification criteria for Rheumatoid Arthritis were established by the American College of Rheumatology and European League against Rheumatism 2010 (ACR/EULAR) provide a better diagnosis for early stages of the disease and confirm the increased levels of autoantibodies and erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) as an indication of immune activation progression of inflammation. RA may also lead to complications intraarticular cartilage injury, joint stiffness, cardiovascular and respiratory diseases. (Urman *et al.*,2018).

The core pathological features of RA are abnormal. the proliferation of the synovial cell, invasion of inflammatory cell, formation of rheumatoid vasospasm, cartilage degradation, the bone, and then the deterioration of the joint (Feldmann and Maini, 2008).

Symptoms of stiffness and discomfort, along with general malaise and weakness, often occur in many types of arthritis. Diagnosis is often dependent on laboratory testing, clinical evaluations, and X-rays. Classification criteria include anamnestic information, inflammation and tenderness examination of the joints, and autoantibodies blood tests (Anti-cyclic citrullinated peptide antibodies ACPA) and IgM healthy people (Doran *et al.*, 2002).

Sultan *et al.*, (2019) Identified a significant correlation between the use of antibiotics and the development of rheumatoid arthritis. Is attributed to the effect of antibiotics on the microbial group that is naturally present in the human body, or to the induction of the disease that emerged as a result of the microbes themselves that the antibiotics were used against it, or the two causes are compatible.

1.2 The Aims of the study:

The aim of the present study was to predict a rheumatoid arthritis disease by using hematological and biomarkers parameters and highlights interactions among these parameters.

The following Parameters were tested to carry out the aim of this study:

1. Demographic Parameters such us gender, age, BMI, and blood group.
2. Hematological Parameters which included Complete blood count.
3. Study some biomarkers including GM-CSF, CRP, RF, fibrinogen, and TNF-α.

Parameters which are studied:

1. Blood group
2. Erythrocyte Sedimentation Rate (ESR)
3. Complete blood count (CBC)
4. C-reactive proteins (CRP)
5. Rheumatic factor (RF)
6. Fibrinogen concentration
7. Tumor necrosis factor-alpha (TNF alpha)
8. Granulocyte monocyte-colony stimulating factor (GM-CSF)

Chapter two
Literature Review

2. Literature Review:

2.1 Rheumatoid Arthritis:

Rheumatoid arthritis (RA) is a systemic autoimmune disease that preferably affects small joints. Rheumatoid arthritis can also cause many extra-articular manifestations such as pericarditis, pulmonary fibrosis, and peripheral neuropathy, etc. most often, the diagnosis is made when patients with pain and swelling in the peripheral joints, as well as joint stiffness in the morning, seek medical help. Pain due to RA is typically worse in the morning and evening and improves during the day. Awakening in the night because of pain in the joints has also been described as a common symptom of RA, as in most inflammatory rheumatic diseases. Rheumatoid arthritis is a "multicausal" disease that most likely results from a combination of genetic predisposition and various environmental and lifestyle factors. Articular and systemic manifestations can lead to poor long-term outcomes such as disability and death (England and Mikuls, 2020).

Methotrexate (MTX) is a drug which has been used since the early eighties of the last century in the treatment of RA and now it is the first line of treatment for this disease, this drug has several mechanisms supposed to reduce rheumatoid arthritis, the adenosine signaling mechanism is may be the best broadly accepted elucidation for the methotrexate mechanism against RA (Friedman and Cronstein, 2019).

Conducted a meta-analysis study on rheumatoid arthritis patients showing that there was no risk of developing inflammatory diseases when patients received MTX treatment. conclusion through their study that patients with rheumatoid arthritis are more susceptible to viral infections than others, but this risk increases when patients stop treatment with anti-arthritis drugs (Favalli *et al.*, 2020).

2.1.2 Pathogenesis of RA:

In parallel to the gut microbiota, several viral and bacterial infections seem to be responsible for the development of autoimmune diseases, including RA.

Characterizing the infection events in a longitudinal cohort of first-degree relatives of patients with RA, evaluating their associations with the development of the disease (Arleevskaya *et al*., 2018).

Interestingly, they observed that an annual increase of respiratory tract infections was found at the pre-clinical stage of RA, probably due to alteration in the immune system, resulting in susceptibility to infection. The authors also hypothesized that the infectious events can predispose to the development of RA. In particular, the role of Epstein Barr virus (EBV) in the pathogenesis of RA has been demonstrated, although some aspects need to be better defined (Kuusela *et al.*, 2018).

Investigated the prevalence of asymptomatic activation of EBV in RA patients and the correlation of serum EBV DNA with disease activity. Interestingly, they observed that active RA was associated with a lytic EBV infection which may play an active role in the pathogenesis of RA (Coulter and Cant, 2018).

Subsequently, to better characterize, the influence of EBV infection in the development of RA, evaluated the EBV gene in the synovial tissue of RA patients by using nested PCR for the amplification of EBV nuclear antigen-1 (EBNA-1). Interestingly, they observed that EBV infection can contribute to the onset of RA and chronic inflammation in synovial tissue and that the frequency of EBNA-1 gene variants was low and not significantly different between RA and control groups, suggesting that EBNA-1 gene variants are not a risk factor for RA (Masuoka *et al*., 2018).

2.1.3 Classification Requirements and Symptoms RA:

Classification criteria for RA were first suggested, in 1956, and subsequently adopted and published by the American Rheumatism Association (ARA) in 1958 (Sokolove and Strand, 2010).

In 1987, the American Rheumatism Association (ARA), put a new criteria next set of criteria were initially assembled by a committee of the American College of Rheumatology (ACR and developed by a panel of leaders in the field of rheumatic disease (Arnett *et al*., 1988). ACR / EULAR Rheumatoid Arthritis Classification Criteria were introduced (Aletaha *et al*., 2010).

The new criteria were not for diagnostic level, but rather were taxonomic criteria for identifying chronic disease with the possibility of its development (Ravi *et al*., 2012).

These new classification criteria exceeded the 1987 "old" ACR standards and were adapted to early diagnosis of RA. The American College of Rheumatology (ACR) and the European Rheumatology Association (EULAR) jointly published "new" classification criteria. The most common signs and symptoms associated with RA are terminal joint pain, limbs, swelling and stiffness in the morning. Joint swelling may start in only a few of them, but it is generally symmetrical in its fixed form, most often occurring in the upper limbs of most patients. The 2010 standards were better than the 1987 criteria because they revealed many patients with Rheumatoid Arthritis at an early stage. The results of the 2010 criteria were acceptable (Britsemmer *et al*., 2011).

2.2. Causes and the risk factors of RA:

The RA has no certain or specific cause. It is known that there is a link between genetic and natural factors. The key mechanism includes the immune defenses of the body that invade the joints and as a result, the joint becomes a swollen and thick knot. Likewise, it also affects the underlying bone cartilage .There are several hereditary and environmental factors associated with RA, the majority of these are familial associations and first-degree relative's status in particular, but it is not yet known if these correlations are related to genetic or environmental causes, or both, or even potential improved occurrence of families due to disease recognition (Frisell *et al.*, 2016).

Some significant and stable considerations include the presence of shared epitope -containing alleles, female age, Tobacco smoke consumption and certain nutritional causes, such as omega-3 fatty acid intake. Oral contraceptives and some nutritional ingredients may be for protection(Symmons, 2002).

The average age and female factor of the patient are serious factors for the development of the disease and bad results. The risk factor contains some indications for primary and secondary prevention. However, treatment is the greatest determinant of disease prevention and the largest determinant of its results (Klein and Morgan, 2020).

2.2.1 Gender

Some researchers have shown that the disease was more common in women. According to (Kvien *et al*., 2006), the incidence of RA in women was 4-5 times higher than that in men in people under 50 years of age, and the ratio of women to men in people over 60-70 years old was about 1: 2. Men and women had different therapeutic effects (Albrecht,

2014), found that there were gender differences in the prevalence of RA complications. Women are more likely to develop depression, fibromyalgia, and hypothyroidism than men, while men are more common in cardiovascular disease and diabetes. studies have shown that women are more affected by the disease (Forslind *et al*., 2007).

There were significant differences in clinical manifestations and therapeutic effects among different genders of RA. However, it is not clear why RA has these gender differences (Lesuis *et al*., 2012).

2.2.2 Age:

The onset of this disease usually appears between the ages of 30 to 60 and the disease is characterized by exhaustion, pain and physical disability, which have a higher prevalence in severe disease progression and longer disease duration (Uhlig *et al*., 2014).

2.2.3 Smoking:

Smoking is a common environmental risk factor that contributes to double the risk of developing RA. Smoking is one of the major environmental exposures associated with the risk of disease, and it represents about one in every six new cases of RA, with recent results emphasizing the central importance of selected interactions between gene smoking's in developing RA. Moreover, smoking and HLA-DRB1 alleles synergistically increase one's risk of having anti–citrullinated Protein antibody (Wysocki *et al*., 2020).

2.2.4 Hereditary:

The genetics studies of families and monozygotic and dizygotic twins support the existence of a genetic variable in RA. In an attempt to assess the magnitude of this genetic variable, quantitative methods used to evaluate the outcomes of two of the largest twin studies (one from Finland and one from the United Kingdom) were used to estimate the degree to

which RA can be explained by genetic variation. The study showed that approximately 60 percent of the RA susceptibility risk was due to heritability (MacGregor *et al.*, 2000).

Researcher mentioned their variable variant of the host being the main site for histocompatibility. Human leukocyte antigen (HLA) with the histological compound present on chromosome 6A has a strong correlation with RA and high-risk factors for infection. Another genetic risk factor associated with RA joint inflammation is the TNF-α gene which made reference to the important role of TNF-α markers in RA (Tuokko *et al.*, 2001).

2.3 Inflammation of Bone:

When a bone tissue is injured, the blood vessels inside the bone defect disrupt. The blood enters the bone defect area, forming a hematoma. Fibrin in the hematoma coagulates to form a provisional matrix, which contains components from the blood, bone tissue, and surrounding tissues. Inside the defect, macrophages and their recruited immune cells (granulocytes, lymphocytes, monocytes, etc.) mediate inflammatory reactions, playing the most crucial role in this stage they secrete a range of cytokines spatiotemporally to trigger inflammation. During this process, the inflammatory cytokines mainly play a role in recruiting and regulating cells involved in the healing process they also

- Recruit other inflammatory cells through a positive feedback loop to amplify the inflammatory response, clean up bone-tissue fragments, and fight infection.
- Recruit endothelial cells to participate in the formation of new blood vessels and invasion.

- Recruit and promote fibroblast proliferation, promote fibrosis of the fibrin clot, and improve stability.

- Recruit multipotent MSCs and regulate their proliferation and differentiation (Lin *et al.*, 2017).

In the early stage of inflammation, various components of the hematoma participate in the healing regulation. Platelets in the hematoma degranulation and release PDGF and TGF-β1. PDGF promotes the proliferation of MSCs, fibroblasts, osteoblasts, and endothelial cells, and TGF-β1 recruits MSCs. Stimulated by the ischemic and hypoxic environment of the hematoma, as well as the macrophage colony stimulating factor (M-CSF) and RANKL secreted by the osteoblast cell line, macrophages polarize to M1 type (Huang *et al.*, 2017).

M1 type macrophages can secrete a variety of pro-inflammatory cytokines, including TNF-α, IL-1, IL-6, etc., recruiting inflammatory and repair-related cells (e.g., fibroblasts, MSCs, and osteoprogenitor cells), and release angiogenic factors to promote angiogenesis(Michalski and McCauley, 2017).

In addition, the hypoxic environment can induce the up-regulation of the transcription factor hypoxia-induced factor-α (HIFα) in osteoblasts and endothelial cells, which can indirectly promote the release of antigenic factors, including angiopoietin-1 and vascular endothelial growth factor (VEGF), to enhance the revascularization process. When new blood vessels invade, various cellular components respond to chemokines and accompany the blood vessels to enter the bone defect area. Subsequently, the fibrin matrix transforms into granulation tissue. With revascularization and cell recruitment, the hypoxic–ischemic extracellular

environment is relieved. Macrophages transform from M1-type polarization to M2 type (Bahney *et al.*, 2019) .

2.4.1. Bone Cells:

Bone cells make up about 10% of total bone volume. There are four types of cells:

1. **Osteoprogenitor** (Stem) Cells: Osteoprogenitor cells retain the ability to re-differentiate into osteoblasts. They reside in the bone canals, endosteum, periosteum, and marrow they may regulate the influx and efflux of mineral ions into and out of the bone extracellular matrix. They also are responsible for the formation of bone remodeling compartments (BRC) with a specialized microenvironment (Dallas *et al.*, 2013).

2. **Osteoblasts** - Bone Forming Cells: They are tightly packed on the surface of the bone. They synthesize and secrete bone matrix (osteoid). They also regulate bone mineralization by secreting alkaline phosphatase (a marker for bone formation) and a set of proteins known as dentin matrix protein (DMP-1) and bone sialoprotein, which act as nucleates for mineralization.

3. Osteocalcin and osteonectin are calcium and phosphate binding proteins secreted by osteoblasts, which regulate the deposition of mineral by regulating the number of hydroxyapatite crystals. Osteoblasts ultimately have one of two fates: (1) remain quiescent osteoblasts lining cells or (2) become osteocytes. Osteoblasts regulate osteoclastogenesis (osteoclast formation) and osteocyte formation. Vitamin D

and parathyroid hormone (PTH) stimulate osteoblasts to secrete macrophage CSF (M-CSF) and to express Receptor activator or nuclear factor kappa -B ligand (RANKL), which are important for osteoclastogenesis (Caetano-Lopes *et al.*, 2007).

4. **Osteocytes** - Mechanosensing Cells: These account for 90% of all bone cells. They are derived from osteoblasts. They reside within the bone network known as the lacuna canalicular system. They do not normally express alkaline phosphatase but do express osteocalcin and other bone matrix proteins. They maintain a connection with each other and bone surfaces via their cytoplasmic processes. Osteocytes are linked metabolically and electrically through gap junctions. Their primary function is mechanosensation. Osteocytes detect mechanical loading through physical deformation of bone matrix and fluid flow shear stress resulting from the flow of canalicular fluid through the lacuna canalicular network. Osteocytes act as orchestrators of bone remodeling and as a result, are also considered endocrine cells. They secrete FGF23 to regulate serum phosphate levels. FGF23 decreases renal and intestinal sodium and phosphate co-transporter expression and subsequently increases renal phosphate excretion by both kidneys.(Aarden *et al.*, 1994).

5. **Osteoclasts** - Bone Resorbing Cells: These are multinucleated cells that originated from mononuclear monocyte-macrophage cells. RANKL and macrophage CSF (M-CSF) are two cytokines that are critical for osteoclast

formation. They are important for osteoclast precursors to proliferate and differentiate into mature osteoclasts. Osteoprotegerin (OPG) is a membrane-bound secreted protein that binds RANKL to inhibit its action at the RANK receptor and subsequently inhibit osteoclastogenesis. Bone resorption depends on osteoclast secretion of hydrogen ions, tartrate-resistant acid phosphatase (TRAP), and cathepsin K enzymes. Hydrogen ions acidify the resorption compartment beneath osteoclasts to dissolve the mineral component of the bone matrix, whereas cathepsin K and tartrate-resistant acid phosphatase (TRAP) digest the proteinaceous matrix, which is mostly composed of type I collagen. PTH stimulates osteoclast activity while calcitonin inhibits it (Boyce *et al*., 2009).

2.5. Increased Bone Resorption in RA:

Osteoclasts Mediate Bone Destruction in RA Osteoclasts are the only cell type capable of bone resorption. To resorb the bone, osteoclasts secrete protons, which acidify the extracellular compartment beneath their ruffled border in order to solubilize calcium phosphate, and proteases, such as cathepsin-K, which degrade the exposed organic matrix of bone (Boling., 2004).

Significant evidence has accumulated demonstrating the crucial role of osteoclasts in arthritic bone degradation. Indeed, multinucleated cells exhibiting an osteoclastic phenotype, which includes tartrate-resistant acid phosphatase, cathepsin-K, and the calcitonin receptor, have been identified at sites of bone erosion at the pannus-bone interface both in RA patients (Bromley and Woolley, 1984) and animal models of arthritis .

2.5.1 Osteoclast Differentiation in RA:

Osteoclasts originate from the fusion of mononucleated cells belonging to the myeloid hematopoietic lineage in the presence of macrophage colony-stimulating factor (M-CSF) and receptor activator of nuclear factor-κB ligand (RANKL).

Osteoclasts were thought to arise only from common bone marrow myeloid progenitors and peripheral monocytes under inflammatory conditions until it was shown in vitro that conventional human and murine immature dendritic cells were also able to transdifferentiate into osteoclasts(Speziani *et al*., 2007) .

2.5.2 Triggers for Bone Resorption in RA:

2.5.2.1 Innate Immune Mechanisms:

Accumulating evidence suggests a role for innate immune mechanisms in enhanced osteoclastogenesis and bone loss in RA. Osteoclasts express innate immune receptors such as immunoreceptor tyrosine-based activation motif (ITAM)-associated receptors and Toll-like receptors (TLRs) As other immune cells, osteoclasts require co-stimulatory signals for their activation in addition to the signaling pathway induced by RANKL. ITAM-harboring proteins, DNAX-activating protein of 12 kDa (DAP12), and Fc gamma receptor (FcRγ), associate with surface receptors, triggering receptor expressed in myeloid cells 2 (TREM2) and osteoclast-associated receptor (OSCAR), respectively. However, the ligands activating the receptors in osteoclast progenitors are not known. DAP12 and FcRγ co-stimulatory signals are required for osteoclastogenesis through the activation of spleen tyrosine kinase (SYK) and phospholipase C-gamma 2 (PLC-γ2) downstream signaling, regulating the calcium-calcineurin-NFATc1 pathway (Koga *et al*., 2004).

The ITAM signaling pathway is activated in RA. In fact, it has been shown that OSCAR was expressed by osteoclasts at the sites of bone erosions in the joints of RA patients and the percentage of cells expressing OSCAR, FcRγ, DAP12, and TREM2 was significantly higher in RA synovium compared to healthy synovium. These two pathways, RANK and ITAM, cooperatively stimulate the activation of NFATc1 Takayanagi's group has shown that the activation of tyrosine kinase Btk by RANK and BLNK (B-cell linker, phosphorylated adapter by Syk) by receptors with the ITAM domain allows the formation of the Btk/BLNK complex this complex induces the phosphorylation of PLC-γ resulting in a calcium-dependent activation of NFATc1 and thus stimulates osteoclastogenesis. The inhibition of Btk is a promising strategy for the prevention of bone loss in osteoclast-associated bone disorders such as RA (Shinohara *et al.*, 2014).

2.5.2.2 Pro-Inflammatory Cytokines:

The correlation between bone loss and clinical disease activity, as well as the protection from bone erosion progression by a tight therapeutic control of synovitis, supporting the concept that chronic inflammation is the major mechanism involved in bone loss in RA (Tunyogi- Csapo *et al.*, 2008).

Pro-inflammatory cytokines, including TNF, IL-6, and IL-1, play an important role in perpetuating inflammatory and destructive processes in RA. TNF is a key pro-inflammatory cytokine among them as its overexpression is sufficient to induce arthritis in mice (hTNFtg) (Keffer *et al.*, 1991).

TNF stimulates osteoclastogenesis both indirectly and directly. Firstly, TNF upregulates RANK and c-fms (M-CSF receptor) expression

in osteoclast precursors and RANKL expression in synovial fibroblasts in conjunction with IL-6 (Tunyogi-Csapo *et al*., 2008).

Secondly, TNF directly stimulates the differentiation of osteoclasts from mononuclear precursors in synovial tissues in synergy with RANKL (Nozawa *et al*., 2013).

TNF also expands the pool of osteoclast precursors and induces the production of other pro-inflammatory cytokines.   TNF also stimulates the connective tissue growth factor (CTGF) production by synovial fibroblasts in patients with RA (Nozawa *et al*.,2013) CTGF, also known as CCN family member 2 (CCN2), is a bioactive cytokine believed to be a downstream mediator of transforming growth factor (TGF)-β. CTGF promotes osteoclastogenesis and aberrant osteoclastogenesis observed in collagen-induced arthritis (CIA) mice is reduced by neutralizing anti-CTGF monoclonal antibody IL-6 is produced by macrophages and synovial fibroblasts. IL-6 promotes osteoclastogenesis by upregulating RANKL expression in synoviocytes and is also an important factor for Th17 differentiation (Jimi *et al*., 1999).   Similarly, to TNF, IL-1 is produced by monocytes/macrophages and promotes osteoclast differentiation synergistically with TNF. In addition, IL-1 directly increases the ability of osteoclasts to resorb as a consequence, treatments targeting pro-inflammatory cytokines have been shown to retard or arrest the occurrence of bone erosion and improve skeletal remodeling in addition, other cytokines such as IL-15, IL-33, IL-34 promote osteoclastogenis and may represent a new biologic therapeutic target for RA (van Schaardenburg *et al*., 2011).

2.5.3.3 Autoimmunity:

Due to the fact that chronic inflammation is considered as the primary trigger for bone damage, the discovery of systemic bone loss and

bone erosion in healthy ACPA-positive individuals suggests that bone loss and bone resorption precede the clinical onset of inflammation and may be caused not only by inflammation, absent during the pre-clinical phases of RA, but also by autoantibodies such as ACPAs, present long before arthritis .In accordance with this hypothesis of a direct pathogenic role of ACPAs in bone loss, the injection of ACPAs isolated from RA patients into immunodeficient mice induces osteopenia and enhanced osteoclast number (Lu *et al*., 2010).

Direct and indirect mechanisms of ACPA-mediated bone loss have been suggested. Due to the fact that ACPAs mainly belong to the IgG subtype, they are recognized by FcγR in the form of immune complexes with their citrullinated antigens .It was originally proposed that ACPA-containing immune complexes bind to FcγR on immune cells such as monocytes/macrophages resulting in the release of TNF, which in turn stimulates osteoclastogenesis (Krishnamurthy *et al*., 2016).

In addition, as osteoclast precursors belong to the myeloid cell lineage, they also express the FcγR and can therefore bind immune complexes. Crosslinking of these FcγRs directly promotes osteoclastogenesis. Accordingly, conditional knockout mice lacking FcγR IV in osteoclasts are partly protected from bone loss in an arthritis model. Studies have shown that ACPAs bind to citrullinated proteins such as vimentin on the surface of osteoclast precursors and directly stimulate their differentiation into mature osteoclasts , but some have been retracted or corrected (Lacki *et al.*, 1996)

In addition, osteoclast precursors highly express enzymes performing protein citrullination (peptidylarginine deiminases, PAD) and that citrullination of vimentin in osteoclast precursors is increased in RA (Croce *et al*., 2007).

Remarkably, ACPA levels increase in the pre-clinical phase of RA, but ACPA glycosylation profile changes towards a pro-inflammatory profile only within the 3 months prior to the clinical onset of RA. This shift is regulated by Th17 cells in an IL-22- and IL-21-dependent manner (Arkema *et al*., 2013).

2.5.4.4 Inhibition of Osteoformation in RA:

Bone loss in RA is the result of exacerbated osteoclast mediated bone resorption and blunted bone formation. In marked contrast with the physiological situation, bone resorption in the high inflammatory context is not compensated by bone formation. Treatments using anti-RANKL, anti-TNF, or anti-IL-6 receptor aiming at decreasing the high level of these pro-osteoclastogenic cytokines, do not reverse bone formation inhibition in RA patients under therapy (Wehmeyer *et al*., 2016). Furthermore, the current intermittent parathyroid hormone (PTH) anabolic treatment combined with TNF blockers fail to reduce the erosion volume in patients with established RA but with the controlled disease activity, reflecting a total uncoupling between bone resorption and bone formation, which is still not fully understood. By contrast to humans, the treatment of hTNFtg mice with a combined therapy consisting of anti-TNF together with intermittent PTH led to the regression of local bone erosion and bone repair, demonstrating new bone formation Bone development and bone formation rely on Wingless (Wnt) signaling, which is required in osteoblastic lineage differentiation and function. The Wnt pathway controls the different steps of bone forming osteoblast differentiation from the mesenchymal stem cells (MSCs) commitment to the osteoblast progenitor, the amplification of pre-osteoblasts, final osteoblast differentiation, and apoptosis (Kedlaya *et al*., 2013).

To prevent bone over-growth, Wnt pro-osteogenic functions are antagonized by a set of physiological factors interfering with the canonical Wnt receptors frizzle and LPR5 or LPR6. In RA, activated synoviocytes produce high levels of RANKL and TNF which in turn induce, in an autocrine loop, over expression of Wnt antagonists namely Dickkopf proteins DKK-1, -2, and sclerostin (Marenzana *et al*., 2013) leading to bone formation inhibition .

The circulating DKK-1 level is increased in RA patients' sera and correlates with the inflammation and erosion grade, as such it could serve as a biomarker of disease activity. The DKK-1 level is also elevated in arthritic models such as CIA and hTNFtg mice. Therefore, targeting Wnt antagonists to improve bone formation and repair, may offer an alternative to combined therapy. However, the surprisingly enough treatment with anti-DKK1 do not improve bone formation except in hTNFtg mice (Chan *et al*., 2018).

Sclerostin is also an attractive therapeutic target for bone loss pathologies. Sclerostin-neutralizing antibodies have a strong bone-building effect in mice, rats, monkeys, and human (Chen *et al*., 2018).

At bone erosion sites, inflammation leads to an osteoimmune focus of multiple activated cell types producing a locally high level of RANKL and TNF, including inflammatory synoviocytes, Th17 cells, and B-cells. B-cells are enriched at the subchondral and endosteal bone marrow areas in direct contact with the bone surface and osteoblasts. RA B-cells produce high levels of TNF and CCL3 (chemokine ligand3) that directly inhibit bone marrow MSCs osteogenic differentiation through NF-κb and Erk activation. At the inflamed joint, the local source of cytokines which are both pro-osteoclastogenic and anti-osteogenic, team up to achieve bone destruction and bone formation inhibition rendering anabolic treatment

inefficacious in the absence of a pro-osteogenic cytokine as IL-22 in the RA disease (Segal *et al*., 2001) .

2.6. Synovial Fluid:

Synovial fluid, also called synovia is a viscous, non-Newtonian fluid found in the cavities of synovial joints. With its egg white–like consistency (West and Sterling, 2015) the principal role of synovial fluid is to reduce friction between the articular cartilage of synovial joints during movement (Petty *et al*.,2016) Synovial fluid is a small component of the transcellular fluid component of extracellular fluid.

2.6.1 Structure of synovial fluid:

The inner membrane of synovial joints is called the synovial membrane and secretes synovial fluid into the joint cavity (Bay-Jensen *et al*.,2016) Synovial fluid is an ultra-filtrate from plasma, and contains proteins derived from the blood plasma and proteins that are produced by cells within the joint tissues (Bennike *et al*.,2014).

The fluid contains hyaluronic secreted by fibroblast-like cells in the synovial membrane, lubricant (proteoglycan 4; PRG4) secreted by the surface chondrocytes of the articular cartilage and interstitial fluid filtered from the blood plasma (Jay and Waller,2014).

This fluid forms a thin layer (roughly 50 μm) at the surface of cartilage and also seeps into micro cavities and irregularities in the articular cartilage surface, filling all empty space (Edwards *et al*., 2000).   The fluid in articular cartilage effectively serves as a synovial fluid reserve. During movement, the synovial fluid held in the cartilage is squeezed out mechanically to maintain a layer of fluid on the cartilage surface (so-called weeping lubrication). The functions of the synovial fluid include:

• reduction of friction — synovial fluid lubricates the articulating joints (McCracken *et al*.,2000).

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• shock absorption — as a dilatant fluid, that possesses rheopectic properties (Christorpher *et al*.,2014), becoming more viscous under applied pressure; the synovial fluid in diarthrotic joints becomes thick the moment shear is applied in order to protect the joint and subsequently, thins to normal viscosity instantaneously to resume its lubricating function between.

• nutrient and waste transportation — the fluid supplies oxygen and nutrients and removes carbon dioxide and metabolic wastes from the chondrocytes in the surrounding cartilage.

• molecular sieving - pressure within the joint forces hyaluronic in the fluid against the synovial membrane forming a barrier against cells migrating into, or fluid migrating out of, the joint space. This function is dependent on the molecular weight of the hyaluronic (Sabaratnam *et al*., 2005).

2.7 CRP (C-reactive protein):

C-reactive protein (CRP) Called for its ability to precipitate polysaccharide molecules from Streptococcus pneumonia, it was first identified in acute stage protein and is a systemic sign that responds well to inflammation, tissue damage, and inflammatory disease control. The level of CRP in the blood is a sensitive but non-specific sign of inflammation that quickly responds to changes in the activity of underlying inflammatory diseases, making its measurement an important tool for detecting and controlling inflammatory diseases (Ries *et al*., 2019).

2.7.1 Roles of CRP in RA:

In general, CRP plays an important role in host defense mechanisms against infectious agents and in the inflammatory response. CRP binding to immunoglobulin Fc gamma receptors (FcγR) promotes the

production of proinflammatory cytokines leading to an amplification loop of inflammation. It is produced predominantly by hepatocytes in response to stimulation by IL-6, (Choy and Rose-John, 2017) but CRP has also been reported to be expressed by smooth muscle cells, macrophages, endothelial cells, lymphocytes, and adipocytes. A significant correlation has been seen between serum CRP levels and tissue inflammation scores from knee synovium biopsy samples in patients with RA.

Analyses of serum and synovial fluid CRP in patients with RA have shown that CRP levels closely correlate with IL-6 levels (Wang *et al.*, 2013).

2.8 Rheumatoid factor (RF):

Rheumatoid factor (RF) which was already used in the 1987 ACR criteria. RF has been shown to predate the onset of RA. The antibody test for RF has historically been used as a diagnostic marker for RA. However, although RF is present in approximately 80 % per cent of patients with RA, its use as a diagnostic marker for RA is limited due to both sensitivity and specific city issues. RF may also be present in both healthy individuals and individuals with other autoimmune disease (Nishimura *et al.*, 2007).

2.9 Complete Blood Count:

The CBC is the most common and easy-to-perform laboratory test, which provides a wealth of information on individual health status. The appropriate interpretation of this test is pivotal for the early detection of several clinical conditions, which should be further investigated by laboratory and clinical analysis (Opriessnig *et al.*, 2007).

The CBC parameters can be grouped into three categories: (i) white blood cells (WBC), (ii) red blood cells (RBC), (iii) platelets. In this

section, we describe the parameters included in a basic CBC, which can be provided by any hematological analyzer.

2.9.1 White Blood Cells:

White blood cells, also known as leukocytes, are a heterogeneous population including lymphocytes, monocytes, and granulocytes, consisting of neutrophils, eosinophils, and basophils. It can be expressed as a percentage or as an absolute value. The WBC absolute value has clinical significance and is more informative than the relative one (percentage) because it indicates the medullary response to inflammatory stimuli. The relative value is helpful for evaluating which WBC population is mainly involved in the inflammatory process, allowing an etiological diagnosis (Adalsteinsson *et al*., 2012).

Commonly, the increase of total WBC count is indicative of inflammation and infection. However, it can be altered in several clinical conditions, such as hemopathy, and in inflammatory non-infective disorders, such as rheumatoid arthritis, lupus, and malignancy (Pyo *et al*., 2017).

2.9.2 Lymphocytes:

A lymphocyte is a type of white blood cell (leukocyte) in the immune system of most vertebrates (Janeway *et al*., 2001) Lymphocytes include natural killer cells (which function in cell-mediated, cytotoxic innate immunity), T cells (for cell-mediated, cytotoxic adaptive immunity), and B cells (for humoral, antibody-driven adaptive immunity) (Cohn *et al*., 2014).

They are the main type of cell found in lymph, which prompted the name"lymphocyte" Lymphocytes make up between 18% and 42% of circulating white blood cells (Omman *et al*., 2020).

Lymphocytes are key components of the adaptive immune response.　They make up approximately 20–40% of the total leukocyte count.A hallmark of sepsis is the simultaneous presence of pro-inflammatory and immunosuppressive alterations. The latter are characterized by the early massive depletion of lymphocytes due to apoptosis. Studies on mice and humans revealed that sepsis-induced apoptosis is driven either by extrinsic or intrinsic pathways (Hotchkiss *et al.*, 1997).

An increase in lymphocyte concentration is usually a sign of a viral infection (in some rare case, leukemias are found through an abnormally raised lymphocyte count in an otherwise normal person) (Guilbert *et al.*, 2010).

A high lymphocyte count with a low neutrophil count might be caused by lymphoma. Pertussis toxin (PTx) of Bordetella pertussis, formerly known as lymphocytosis-promoting factor, causes a decrease in the entry of lymphocytes into lymph nodes, which can lead to a condition known as lymphocytosis, with a complete lymphocyte count of over 4000 per ul in adults or over 8000 per ul in children. This is unique in that many bacterial infections illustrate neutrophil-predominance instead (Guilbert *et al.*, 2010).

A low normal to low absolute lymphocyte concentration is associated with increased rates of infection after surgery or trauma (Clumeck *et al.*, 2010).

One basis for low T cell lymphocytes occurs when the human immunodeficiency virus (HIV) infects and destroys T cells (specifically, the CD4* subgroup of T lymphocytes, which become helper T cells

Without the key defense that these T cells provide, the body becomes susceptible to opportunistic infections that otherwise would not affect

healthy people. The extent of HIV progression is typically determined by measuring the percentage of CD4* T cells in the patient's blood - HIV ultimately progresses to acquired immune deficiency syndrome (AIDS).

The effects of other viruses or lymphocyte disorders can also often be estimated by counting the numbers of lymphocytes present in the (Wahed *et al*., 2020).

2.9.3 Monocytes:

Monocytes represent the first line of defense against invading pathogens. They are activated by pattern recognition receptors (PRRs) and sepsis-associated hypoxia. Monocytes control both the innate and adaptive immune responses to pathogens by different mechanisms, including phagocytosis; the release of reactive oxygen species, cytokines, and chemokines; the recruiting of neutrophils; antigen presentation; and the activation of lymphocytes (Radzyukevich *et al*., 2021).

Overall, monocytes can be classified into three different sub-population based on the different expression of a co-receptor to lipopolysaccharide (LPS), CD14, and CD16 receptor: classical (CD14+++CD16−), intermediate (CD14++ CD16+), and non-classical (CD14+ CD16++), which present different morphological, functional, and phenotypical characteristics. Under physiological conditions, classical monocytes are the most represented sub-population, accounting for around 85% of total circulating monocytes; the intermediate ones account for around 5%; and the non-classical ones account for the remaining 10% (da Mota *et al*., 2018).

2.9.4 Neutrophils:

Neutrophils represent the most prevalent leukocytes and the most abundant innate cell population in systemic circulation, making up

about 40% to 70% of the total leukocyte count. They are a key component of the innate immune system and act as sentinels to eliminate invading pathogens (Chung *et al.*, 2019).

When infection occurs, neutrophils rapidly migrate to the site of infection and eliminate the invading pathogen by several mechanisms, including phagocytosis and oxidative bursts, neutrophils extracellular traps to execute microbial killing (Witter *et al.*, 2016).

Under physiological conditions, neutrophils undergo apoptosis to maintain their homeostasis (Gao *et al.*, 2021).

Noteworthy, the increase of neutrophils, also known as neutrophilia, can occur in response to a stressor, including physical and emotional stress, as well as smoking. Also, chronic disorders, such as inflammatory bowel disease, rheumatic disease, and hepatitis, as well as congenital disorders, such as Down syndrome, are characterized by baseline neutrophilia (Belok *et al.*, 2021).

2.9.5 Red Blood Cells:

Red blood cells, also termed erythrocytes, are the most abundant circulating cells and are produced within bone marrow through a complex and multi-step process, known as erythropoiesis, which begins with the differentiation of multipotent hematopoietic stem cells into erythroid-committed precursors. The final step leads to the production and release in the bloodstream of reticulocytes, which complete the maturation process into erythrocytes.

Under physiological conditions, RBCs have a characteristic biconcave disc shape, a lifespan of 120 days, and are metabolized by macrophages in the spleen and liver (Anderson *et al.*, 2018) .

The best-known function of RBC is the transport and exchange of O2 and CO2 between the lungs and other tissues. However, they also

have a pivotal role in cellular blood immunity, representing the main circulating bactericidal cells.

2.9.6 Platelets:

Platelets are the smallest elements in the bloodstream. They are anucleate cells produced mainly in the bone marrow by the fragmentation of the megakaryocyte extrusions into the vasculature. Beyond their well-known role in hemostasis, they contribute to the innate immune response to infection and inflammation. Platelets function as sentinels for the rapid detection of microbial invasion and orchestrate a complex intravascular immune defense response that protects against bacterial dissemination (McDonald and Dunbar, 2019).

Activated platelets also release microbicide molecules and chemokines that facilitate pathogen elimination, signal immune cells, and contribute to inflammation. Finally, platelets promote a pro-inflammatory phenotype of neutrophil (Ma and Kubes, 2008), as well as monocyte, differentiation into macrophages.

2.10 Fibrinogen:

Fibrinogen, coagulation factor I, is a 340 kDa glycoprotein that plays an important role in many physiological and biochemical processes. The name "fibrinogen" was used for the first time in 1847 by Rudolf Virchow, while in 1872, Alexander Schmidt indicated that the conversion of fibrinogen to fibrin is an enzymatic process. Physiologically, almost all fibrinogen is found in plasma, and its concentration is 1.5–3.5 g/L (normal concentration ranges may vary slightly among different laboratories), with a half-life (T1/2) of 3–5 days. The plasma concentration of fibrinogen largely depends on the factors regulating its synthesis and genetic predisposition (Murdaca *et al*., 2013).

Fibrinogen is expressed primarily in hepatocytes and is regulated transcriptionally and post-transcriptionally. The biosynthesis of fibrinogen in the liver is primarily constitutive. Fibrinogen synthesis is regulated by acute-phase proteins, mainly by IL-6 (derived by monocytes, macrophages, and vascular endothelial cells), which induces its synthesis in the liver, while IL-1β and tumor necrosis factor-alpha (TNF-α) suppress its synthesis (Neerman *et al*, .2018 and Kattula *et al*., 2017).

Thus, fibrinogen is an acute phase protein because its biosynthesis is increased during inflammation (in the acute phase of inflammation, plasma fibrinogen concentrations can exceed 7 g/L). The production of fibrinogen is also increased by glucocorticosteroids (GC) (Neerman-Arbez and Casini, 2018).

2.10.1 Inflammation - driven coagulation activity and coagulation – driven inflammation:

Inflammation as a regulator of coagulation and fibrinolytic system activity is well recognized. Acute inflammation is known to shift the hemostatic balance toward a prothrombotic and antifibrinolytic state in which there is an increase in circulating levels of several key procoagulant and antifibrinolytic mediators. Activation of the coagulation and fibrinolytic systems following acute inflammatory events can lead to potentially devastating consumptive coagulopathy and disseminated intravascular coagulation5-7 ; however, a reciprocal pathway also exists whereby hemostatic factors affect inflammatory processes (Esmon, 2000).

The potency of fibrinogen as an inflammatory mediator is linked to an ability to influence multiple aspects of leukocyte biology through direct and indirect mechanisms Fibrinogen can both facilitate leukocyte transmigration out of the vasculature and induce leukocyte effector functions by serving as a local, spatially defined cue within damaged

tissue. Fibrinogen can exert such wide-ranging effects by functioning as a ligand for a host of cell surface receptors including VE-cadherin, ICAM-1, $\alpha IIb\beta3$, $\alpha5\beta1$, $\alpha V\beta3$, $\alpha M\beta2$, and $\alpha X\beta2$ expressed by cell types including leukocytes, endothelial cells, platelets, fibroblasts, and smooth muscle cells (Gear and Camerini, 2003).

2.11 Tumor necrosis factor alpha (TNF-α):

Tumor necrosis factor alpha (TNF-α) is a cytokine that has pleiotropic effects on various cell types. It has been identified as a major regulator of inflammatory responses and is known to be involved in the pathogenesis of some inflammatory and autoimmune diseases (Bradley, 2008).

Structurally, TNF-α is a homotrimer protein consisting of 157 amino acids, mainly generated by activated macrophages, T-lymphocytes, and natural killer cells. It is functionally known to trigger a series of various inflammatory molecules, including other cytokines and chemokines. TNF-α exists in a soluble and transmembrane form. The transmembrane TNF-α (tmTNF-α) is the initially synthesized precursor form and is required to be processed by TNF-α-converting enzyme (TACE), a membrane-bound disintegrin metalloproteinase, to be released as the soluble TNF-α (sTNF-α) (Jiang *et al*., 2017).

Osteoclasts in RA induce synovial hyperplasia and angiogenesis. In PsA, activated dendritic cells (DCs) and macrophages secrete TNF-α and IL-23 excessively. IL-23 induces T cells to differentiate in Th17 cells, which secretes IL-17. IL-17 and TNF-α in the blood activate keratinocytes, resulting in psoriasis (PS). TNF-α also activates osteoclasts, leading to synovial fibroblast, which results in joint erosion.

Physiologically, TNF-α is a crucial component for a normal immune response. TNF-α can activate the immune system to regulate; however, the

inappropriate or excessive production of TNF-α can be harmful and may lead to disease. (RA), inflammatory bowel disease (IBD), psoriatic arthritis (PsA), psoriasis (PS) (Raychaudhuri , 2009) , and noninfectious uveitis (NIU) are induced by the abnormal secretion of TNF-α; thus, TNF-α can be classified as a key factor in the pathological development (Lis *et al.*, 2014).

Due to the involvement of TNF-α in the pathogenesis of autoimmune diseases, TNF-α inhibitors have been successfully developed and applied in the clinical treatment of autoimmune diseases such as Crohn's disease (CD) and RA. Therapeutic drugs act as antagonists by blocking the interaction of TNF-α with TNFR1/2 or, in some cases, as agonists by stimulating reverse signaling, causing the apoptosis of TNF-α producing immune cells Several TNF-α inhibitors have been approved for clinical use: etanercept, infliximab, adalimumab, golimumab, and certolizumab. The impact of TNF-α signaling on each type of autoimmune disease will be introduced in this review, as well as the evaluation of current TNF-α inhibitors utilized as therapeutic drugs against autoimmune diseases (Gong *et al.*, 2013).

2.12 Granulocyte-macrophage colony-stimulating factor (GM-CSF):

Granulocyte-macrophage colony-stimulating factor (GM-CSF) has demonstrated significant adjuvant effect when included in DNA vaccines for many infectious diseases (Encke *et al.*, 2006).

For example, the co-administration of plasmid-encoded GM-CSF with an HIV-1 DNA vaccine improved both the magnitude and quality of vaccine-induced T-cell responses, particularly by increasing proliferating CD4+ T cells which simultaneously produce interferon-γ, tumor necrosis factor-α, and interleukin-2 (Reali *et al.*, 2005).

In a murine model of the Ebola virus, the inclusion of interleukin-12 or GM-CSF improved cell-mediated immunity. However, the GM-CSF adjuvant plasmid did not improve neutralizing antibody titers in this model. The combination of GM-CSF with self-amplifying mRNA constructs encoding the influenza virus nucleoprotein significantly improved the magnitude of antigen-specific CD8+ T cell responses and increased recruitment of antigen-presenting cells at the vaccination site. These results imply a local release of GM-CSF. Indeed, the delivery system of GM-CSF seems to have an impact on the initiation of immune response.(Reali *et al*., 2005) observed an increase in the immune response in mice following the local administration of GM-CSF. In contrast, systemic administration of GM-CSF results in immune suppression through the production of myeloid-derived suppressor cells (MDSCs).

To allow sustained local delivery of GM-CSF as an adjuvant in an anti-cancer vaccine, we developed an encapsulated cell technology in which genetically modified cells are loaded into a biocompatible, semipermeable capsule which can secrete human or murine GM-CSF. This medical device is co-implanted in the subcutaneous tissue at the site of antigen injection. This delivery system allows GM-CSF administration which induces recruitment and early maturation of dendritic cells, a critical step for vaccine efficacy (Serafini *et al*., 2004).

Chapter Three

Materials and methods

3. Materials and methods:

3.1. Materials: -

3.1.1. Subjects of the Study:

The present work was performed in many locations including hospitals (Marjan teaching hospital and Imam Al–sadiq hospital) and location including Babylon university/college of science for women and private laboratories. The present study was initiated at a beginning of November 2021 to April 2022, the total number of individuals was eighty (80) of men and women of these twenty (20) men were complained from Rheumatoid arthritis and twenty (20) men were apparently healthy were selected as a control group.

The remaining individuals (40) women, of them, twenty (20) women were affected with Rheumatoid arthritis and twenty (20) women also they were selected as a control group.

All persons of study, had ages ranged between 40-79 years old. The subjects (patients and healthy control) of the present study were classified according to their ages in to four categories (40-49, 50-59, 60-69, 70-79 years old). Excluded criteria (diabetes mullites, osteoporosis, thyrotoxicosis, malignant diseases and pregnancy).

All patients were admitted to hospital and health care centers to check up their own healthy and received therapeutic options. Concerning control subjects, they were selected from public health centers, workers in hospitals, and person who have normal medical history of both sexes.

3.1.2 Design of study:

Total number 80 patients and control

40 men (patients and healthy)

40 women (patients and healthy)

20 rheumatoid arthritis men

20 healthy men (control)

20 healthy women (control)

20 rheumatoid arthritis

Group 1 (40-49) years old

Group 2 (50-59) years old

Group 3 (60-69) years old

Group 4(70-79) years old

Group 1 (40-49) years old

Group 2 (50-59) years old

Group 3 (60-69) years old

Group 4 (70-79) years old

- ✓ Blood group
- ✓ Body mass index
- ✓ Complete blood count (CBC)
- ✓ Tumor necrosis factor alpha (TNT alpha)
- ✓ C-reactive protein (CRP)
- ✓ Fibrinogen concentration
- ✓ Granulocyte monocyte – colony stimulating factor (GM-CSF)
- ✓ Rheumatoid factor (RF)
- ✓ Erythrocyte sedimentation Rate (ESR)

3.1.3. Instruments and apparatus: -

In the following table, the instruments and their origins that used the present study: -

Table (3-1)

Apparatus	Source	Company
Plate Reader for ELISA	USA	Awareness technolog
HumaClot Junior	Germany	Human
Micropipette Different size	Germany	Slamed
Plain tube (10)	Jordan	CMA
Disposable Syringes	Hungary	Dia Gon
Refrigerator	Japan	Royal
Centrifuge	Germany	Heattich
Gloves	Jordan	CMA
Eppendorf tubes	Jordan	CMA
Deep Freeze	Germany	Concord
EDTA Tube (3ml)	USA	Afco
Gel Tube (6ml)	USA	Afco
Sodium Citrate Tube(5ml)	USA	Afco
Pipette tips (yellow)	USA	Afco
Pipette tips (blue)	USA	Afco
Slides	China	Sail Brand
Getein 1100	USA	Biotech
Ichroma AII	Korea	Biotech
ESR tube	USA	Afco

3.1.4. Chemicals: -

Table (3-2) explains the chemicals that were used in the present study: -

Chemical	Source	Company
Tumor necrosis factor alpha (TNF alpha) kit	IRAN	Karmania Pars Gene
Granulocyte monocyte –colony stimulating factor (GM-CSF) kit	USA	Thermo Fisher
Fibrinogen kit	Germany	Hemostat
Rheumatoid factor (RF) kit	Korea	Biotech
C-reactive proteins kit	USA	Biotech

3.2. Methods- :

3.2.1. Collection of Blood Samples: -

Samples of blood were collected from several locations of hospitals and health center cares of Babylon governorate (Marjan medical city and Imam Al–sadiq hospital). Antecubital vein of left arm was selected. The area of collection was firstly warmed by massage to improve blood circulation and protrusion of the vein. A tourniquet was applied around the skin directly about 7 cm above the collection site. The skin was exactly sterilized and cleared with ethyl

alcohol (70%) and then was permitted the skin to completely dry to avoid hemolysis of blood. Needles were selected with 23 gauges and blood samples volumes were approximately 5 milliliters from each Rheumatoid arthritis patients and healthy control subjects.

Collecting about five milliliters of venous blood from each patient. The blood was divided into three parts: one part (approximately two milliliters) was collected into EDTA. And the second part of the blood were placed in gel tube for thirty minutes and the third part of the blood were placed in Sodium Citrate tube for serum and plasma and placed in centrifugation at 3000 rpm for 15 min; after that the serum collected and transferred to Eppendorf tubes and kept in the freezer (-20c°) until it was used for. For future analyses and each tube was given a serial number to refer to each subject (patient and control).

3.2.2 Determination of BMI:

To calculate body mass index, use the following formula

BMI= weight (kg) /height^2 (m)^2

The weight status was classified into five groups according to the values of BMI as shown in table (3-3).

Table (3-3): The weight status groups according to the value of BMI (European society of human reproduction and embryology, 2009)

Weight status	Values of BMI (kg/m2)
Underweight	<18
Normal	18-24.9
Overweight	25-29.9
Obesity	30-39.9

Morbid obesity	≥40

3.3 Hematological Tests:

3.3.1 Erythrocyte Sedimentation Rate (ESR):

The erythrocyte sedimentation rate was determined using the Westergren method according to the following steps: The Westergren technique needs to collect 2 ml of blood extracted from the vein in blood- preserving tubes containing sodium citrate, then kept at a known temperature of 4 cº, then the samples taken are calculated and placed 100 ml at a time of one hour and then we calculate the distance from the upper surface to the first Point.

3.3.2 Complete Blood Count (CBC) assay:

Whole blood sample in EDTA tube was used immediately to get complete blood count using automated 3-part hematology autoanalyzer, samples were swirled several times to mix the sample and then processed in the autoanalyzer to get result within 60 seconds, result printed out and recorded.

3.4 Biomarkers tests:

3.4.1 Measurement of Tumor necrosis factor alpha Levels:

- **Procedure of TNF -α:**

1. About 50 µl /well has been added of standard number 4 to Al, standard number 3 to B1, standard number 2 to C1, and standard number 1to DI. Then, 50 µl of pre-diluted samples were added to other wells and incubated the plate one hour in the room temperature and on the shaker (180 RPM).

2. The wells were Aspirated and washed for three times with 250 µl /well IX wash buffer. The allowing time for soaking (-1 minute) during each wash step increased the effectiveness of the washes. Blot plate to remove any residual buffer

3. About 50 µl /cell of biotin-conjugated anti-TNF-1 antibody were added to all wells

4. The plate has been sealed and incubated at room temperature for one hour.

5. The wells were washed as described in the second step.

6. About 50 µl /well of HRP-Avidin were added to all wells.

7. The plate has been sealed and incubated at room temperature for thirty minutes.

8. The wells were Washed five times as described in the second step.

9. About 50 µl /well of substrate were added to all wells and incubated for fifteen minutes on the shaker (180 RPM).

10. About 25 µl /well of stopping were added to all wells.

11. Read plates at 450 nm. If wavelength subtract on is available, subtract the values of 570 nm from those of 450 nm and analyze data.

3.4.2 Measurement of Granulocyte-macrophage colony-stimulating factor:

• Principles of the test:

An anti-human GM-CSF coating antibody is adsorbed onto microwells Coating Antibody Human GM-CSF present in the sample or standard binds to antibodies adsorbed to the microwells. An HRP-

conjugated anti-human GM-CSF antibody is added and binds to human GM-CSF captured by the first antibody following incubation unbound HRP-conjugated anti-human GM-CSF is removed during a wash step, and substrate solution reactive with HRP is added to the wells.

Second incubation

A colored product is formed in proportion to the amount of human GM-CSF present in the sample or standard. The reaction is terminated by addition of acid and absorbance is measured at 450 nm. A standard curve is prepared from 7 human GM-CSF standard dilutions and human GM-CSF concentration determined.

- **Procedure:**
1. The determination of number of micro well strips required.
2. The micro well strips were washed twice with Wash Buffer.
3. About 50 µl /well has been added of standard dilution on the micro well plate: Added 100 µL Assay Buffer (1x), in duplicated, to all standard wells. Pipette 100 µL prepared standard into the first wells and created standard dilutions by transferred 100 µL from well to well. Discard 100 µL from the last wells. Alternatively external standard dilution in tubes Pipette 100 µL of these standard dilutions in the micro well strips.
4. About 50 µL of Assay Buffer (1x) were added to sample wells.
5. About 50 µL of sample in duplicate were added to designated sample wells.
6. HRP-Conjugate has been Prepared.

7. About 50 μL of HRP-Conjugate were added to all wells.

8. The microwell strips has been covered and incubated three hours at room temperature (18°C to 25°C).

9. The microwell strips has been emptied and washed three times with wash buffer.

10. About 100 μL of TMB Substrate Solution were added to all wells.

11. The microwell strips has been incubated for about ten minutes at room temperature (18°C to 25°C).

12. About 100 μL of stop solution were added to all wells.

13. The absorbance in wells has been read and measure color intensity at 450 nm.

3.4.3 Measurement of C-reactive proteins:

- **Principle of Method:**

Test uses an anti-human CRP monoclonal antibody conjugated with CRP monoclonal antibody coated on fluorescence latex and another anti-human the test line. After the sample has been applied to the test strip, the fluorescentce latex-labelled binds with the CRP in anti-human CP monoclonal antibody sample and antigen-antibody complex. This complex moves to the test forms a marked card antigen-antibody complex is captured on the test line by the detection zone by capillary action. Then marked anti-human CRP monoclonal antibody. The fluorescence intensity of the test line increases in proportion to the amount of CRP in sample then insert test card into Getein1100 Immunofluorescenci Quantitative Analyzer/Getein1600 Immunofluorescenc Quantitative Analyzer (hereinafter referred to as

Getein1130 and Getein1600), the concentration of CRP in sample will S measured and displayed on the screen. The value will 02 stored in Getein1100/Getein1600 and available for downloading the result can be easily transmitted to the laboratory or hospital information system.

- **Procedure:**

1. Specimens has been collected according to user manual.

2. Test card, sample and reagent should must be room temperature before the test.

3. SD card has been confirmed lot No. in accordance with test kit lot No... Perform "SD card" calibration when necessary.

4. The test card has been removed from the sealed pouch immediately before use.

5. The test card has been Putted on a clean table, horizontally placed.

6. About 10 ul of sample were used sample transfer pipette, deliver into one tube of sample diluent, were mixed gently and thoroughly. About then drop 100 pl of sample were mixture into the sample port on the test card.

7. Reaction time: three minutes. The test card was inserted into Getein1100 and pressed "ENT (for Android Getein 1100) after reaction time is elapsed. The result has been showed on the screen and printed automatically.

3.4.4. Measurement of Fibrinogen Concertation:

- **Principle of Method:**

HEMOSTAT FIBRINOGEN is based on the most commonly used method first described by Clauss.1 Thrombin in optimized quantity is added to a prediluted plasma sample the measured clotting time is inversely proportional to the fibrinogen concentration in the specimen.

- **Procedure:**

The steps of Fibrinogen measurement were followed the instruction of kit as showed in the following table:

Table: the steps of Fibrinogen measurement

Bred [RGT] to room temperature before use and pre-warm test tubes.	
Pipette diluted sample/ controls/ (CAL) into pre-warmed test tubes	100 μl
Incubate 3 min. at 37°C	
Add [RGT] (room temperature)	50 μl
Start timer with addition of reagent. Record time required for clot formation	

3.4.5. Measurement of Rheumatoid factor:

- **Principle:**

The test uses a sandwich immunodetection method; the detector antibody in buffer binds to antigen in sample, forming antigen-antibody complexes, and migrates onto nitrocellulose matrix to be captured by the other immobilized-antibody on test strip. The more antigen in sample forms the more antigen-antibody complex and leads

to stronger intensity of fluorescence signal on detector antibody, which is processed by instrument for ichromaTM tests to show RF IgM concentration in sample.

- **Procedure:**

1. The top of the detection buffer tube was Marked a puncture on the top by inserting an empty sample collector.

2. About 10 μL of sample were drawed (Human whole blood) was used a sample collector. If the sample is not whole blood, transfer 5 μL (Human serum / plasma / control) with a pipette to detection buffer tube.

3. (If necessary, wipe out the excess blood outside of the capillary) on the sample collector with paper towel).

4. The sample collector and the tube have been assembled into one.

5. About ten times the sample were Shaked or more until the sample out of the sample collector by inversion. The mixture of buffer and the sample has to be used within 30 seconds.

6. The cap off the top of assembled tube has been removed. Discard two drops of sample mixture onto the paper towel before loading. Load only two drops of the mixture onto the sample well of the cartridge.

7. The sample-loaded test cartridge has been leaved at room temperature for five minutes Scan the sample-loaded cartridge immediately when the incubation time is over. If not, it will cause inexact test result.

8. The sample-loaded cartridge has been scanned, is inserted it into the cartridge holder of the instrument for ichroma TM tests. Ensure proper orientation of the cartridge before pushing it all the way inside the cartridge holder. An arrow has been marked on the cartridge especially for this purpose.

9. 'Select' button has been Pressed on the instrument for ichromaTM tests to start the scanning process.

10. The test result was read on the display screen of the instrument for ichroma TM tests.

3.5. Statistical Analysis:

Results of the present study were illustrated as means ± standard error (SE). The values were statistically analyzed by using SPSS 23 version. Program and analysis of variance were explained. The lowest significant differences (LSD) among studied groups were ($p < 0.05$). Also, Pearson correlation coefficients were calculated to check the relationship between the studied parameters. Receiver operating characteristic (ROC) curves were plotted to find out which of the study parameters are sensitive to RA patients (Daniel, 1999).

Chapter Four
Results

4. Results:

4.1. Demographical Study:

4.1.1. Distribution of patients according to gender.

From present finding it was noticed that women suffer from RA more than of men. An average of 60% of women and 40% for men. As explained in Figure 4.1.
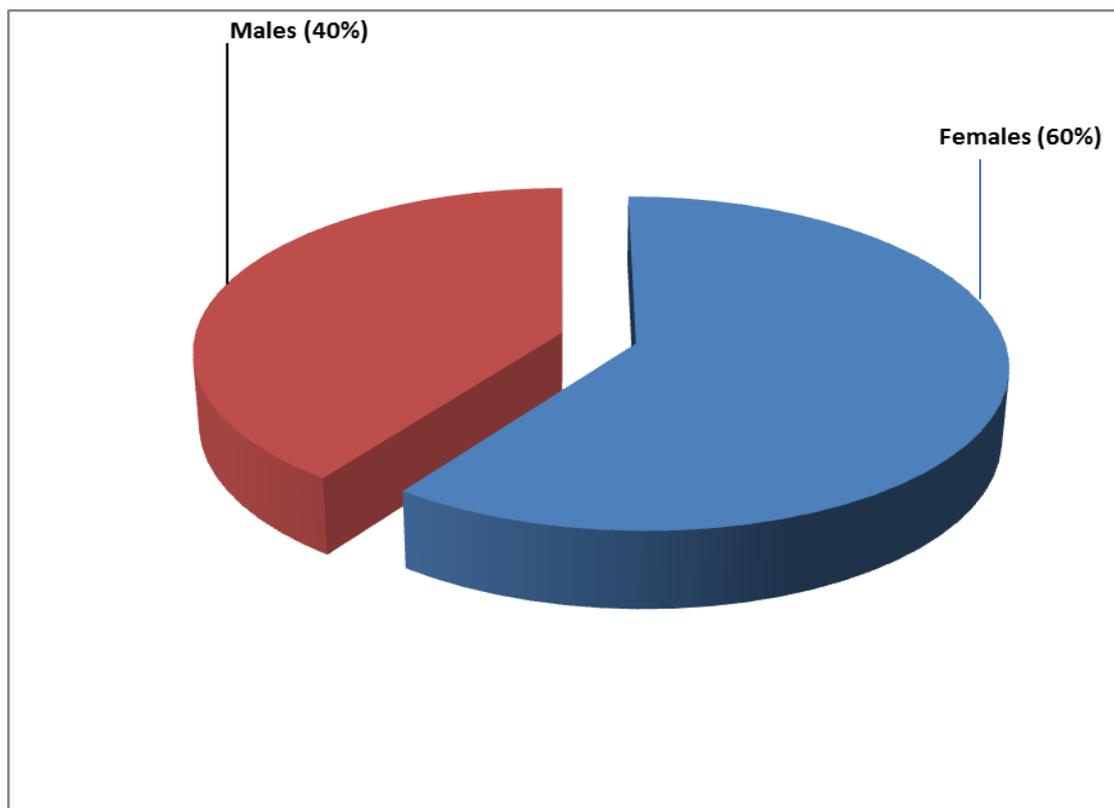
Figure (4.1): Explain the percentage ratio (%) of males and females' patient with RA.

4.1.2 Distribution of patients according to age groups:

The present study, observed the rate of rheumatoid arthritis patients according to age groups as follows:

- ✓ Patients age from 40 to 49 years were 30% of patients.
- ✓ Patients age from 50 to 59 year were 30% of patients.
- ✓ Patients age from 60 to 69 years or more were 25% of patients.
- ✓ Patients age from 70 to 79 years or more were 15% of patients.

As explained in Figure 4.2.
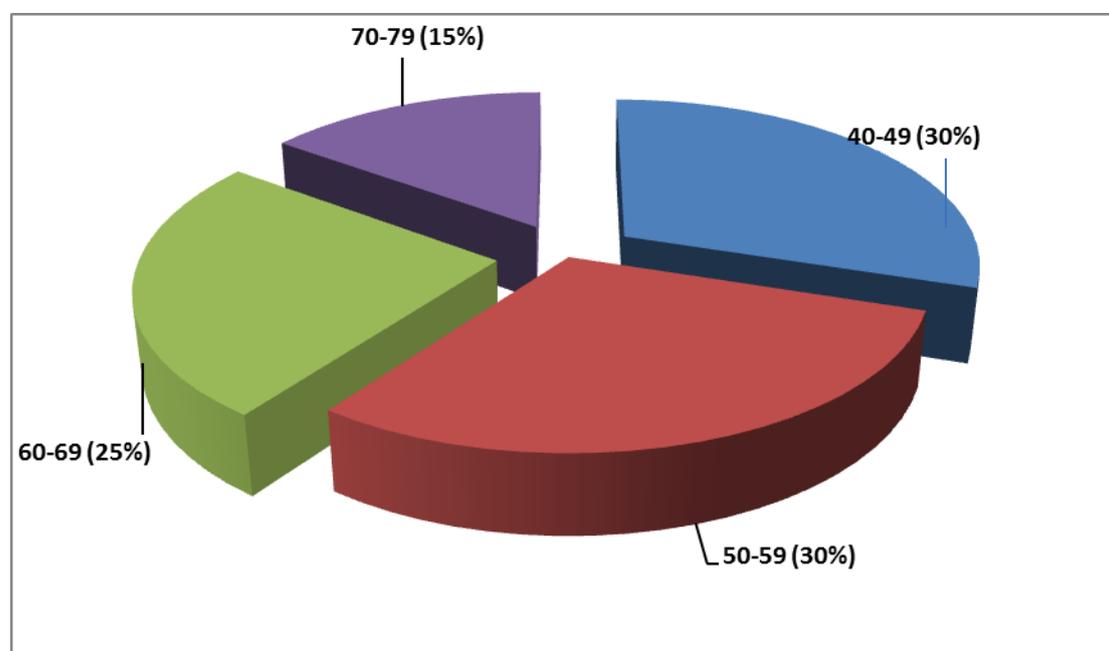
Figure (4.2): Represents the percentage ratio (%) of age groups of patients with RA.

4.1.3 Distribution of patients according to BMI:

From this study, it had been observed that people with high body mass affected more than people who have normal body mass, with an average of 70% for people who have excessive body mass and 30 % for people with normal body mass. This was showed in Figure 4.3
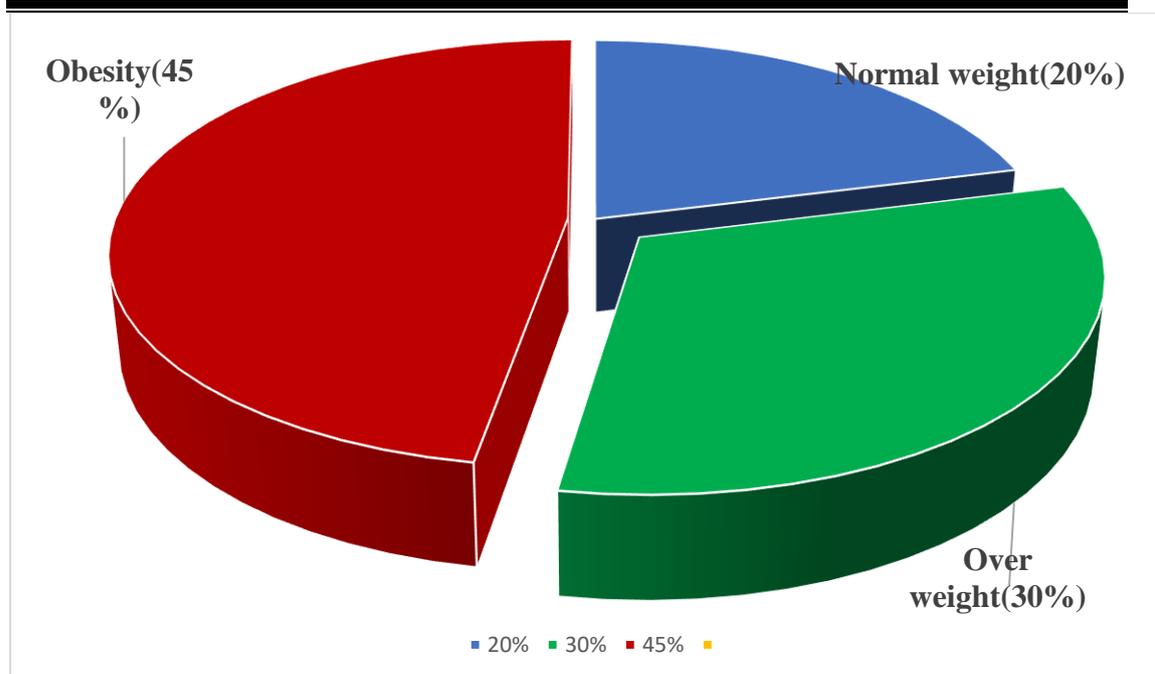
Figure (4.3): Shows the percentage ratio (%) of body mass index for patients with RA.

4.2. Results of hematological parameters:

4.2.1. Blood parameters in RA patients and healthy people:

Results that were shown in table 4-1 explained a significant P<0.01 heightening in the levels of total white- blood cells, Granulocyte, lymphocyte, hemoglobin and erythrocyte sedimentation rate in matching with those healthy control groups, whereas in the levels of RBCs and platelets indicated a significant p<0.05 different in matching with those healthy control groups.

Table (4-1): Shows the results of hematological parameters (total white- blood cells, Lymphocytes, Granulocytes, hemoglobin, RBCs, platelets and ESR) of patients affected with RA and healthy subjects.

Groups	Patient (n=40)	Control (n=40)	Pvalue≤0.05
Parameters	Mean ±S. E		
WBC (cell/mm^3)	8.51±0.16	5.98±0.17	**0.0002****
Granulocyte (%)	80.68±5.55	69.04±4.71	**0.0001****
Lymphocyte (%)	16.99±1.55	28.82±2.78	**0.0003****
Hb (g/dL)	9.35±1.29	12.56±1.21	**0.004****
RBC (cell/mm^3)	3.99±0.60	4.67±0.91	**0.042***
PLT (cell/mm^3)	275.25±17.40	239.48±15.60	**0.034***
ESR (mm/hr)	29.60±2.85	12.59±2.73	**0.0001****

-All values are mean ±SE

- Results with two strikes are significantly different at P≤0.01

-Results with one a strike is significantly different at P≤0.05

4.2.2 Illustrate the biochemical markers in RA Patients and Healthy subjects:

The results that were shown in table 4-2 explained a significant p<0.01 heightening in the levels of TNF-α, fibrinogen, RF, CRP and GM-CSF in matching with those healthy control groups.

Table (4-2): Illustrate the biochemical markers (TNF, fibrinogen, Rf, CRP and Gm-CSF) of patients affected with RA and healthy control.

Groups Parameters	Patient (n=40)	Control (n=40)	Pvalue≤0.05
	Mean ±S. E		
TNF (pg/ml)	20.39±1.58	5.10±1.48	0.0001**
Fibrinogen (mg/dl)	393.25±13.29	212.65±14.01	0.0004**
Rf (IU/l)	25.11±2.47	11.23±1.42	0.0001**
CRP (IU/l)	30.26±2.79	8.59±2.68	0.0001**
Gm-CSF (pg/ml)	340.93±13.69	114.38±11.05	0.0001**

-All values are mean ±SE

-Results with two strikes are significantly different at P≤0.01.

4.2.3 Blood parameters in RA patients and healthy people according to gender:

Data that were illustrated in Table (4-3) showed a significant P<0.05 increase in levels of WBCs, granulocytes, platelets, and ESR. and record a significant P<0.05 decrease in levels of Lymphocyte and hemoglobin but there was insignificant P>0.05 decrease in levels of RBCs in male patient, while in female the results indicated a significant P<0.05 increase in levels of WBCs, Granulocytes, platelets and ESR and showed a significant P<0.05 decrease in levels of Lymphocyte and hemoglobin but there was insignificant P>0.05 decrease in levels of RBCs. when compared with healthy control group in levels of WBCs, Granulocytes, Lymphocyte, hemoglobin, RBCs, platelets, and ESR in males healthy control group, and the levels of WBCs, Granulocytes, Lymphocyte, hemoglobin, RBCs, platelets, ESR for females healthy control group.

Table (4-3): Shows the results of hematological parameters (WBCs, Lymphocytes, Granulocytes, Hb, RBCs, PLT and ESR) of patients and control affected with RA according to gender.

Groups	Patient		Control		LSD (0.05)
	Male	Female	Male	Female	(gender*group)
Parameters	Mean ±S. E				
WBCs (cell/mm³)	8.50±1.5	8.53±1.3	5.88±0.7	6.08±2.1	**0.581***
Granulocytes (%)	79.98±12.1	81.38±8.4	69.70±7.9	68.37±5.4	**2.227***
Lymphocyte (%)	17.53±2.1	16.46±3.3	28.36±2.4	29.28±6.3	**2.276***
Hb (g/dL)	7.82±1.7	10.89±2.1	12.99±1.1	12.14±1.6	**2.629***
RBCs (cell/mm³)	4.10±0.9	3.89±0.9	4.80±0.8	4.54±0.3	**1.308**
PLT (cell/mm³)	273.80±33.2	276.70±12.6	237.05±16.4	241.90±18.8	**22.197***
ESR (mm/hr)	28.91±4.7	30.30±2.9	12.08±9.6	13.09±1.3	**2.666***

-All values are mean ±SE

4.2.4. The results of biochemical markers (TNF-α, RF, CRP and GM-CSF) of patients affected with RA and control group according to gender:

Table (4-4) illustrates the results recorded a significant $p<0.05$ increase in the levels of TNF-α, fibrinogen, RF, CRP and GM-CSF in males patients when compared with control group in the levels of TNF-α , fibrinogen, RF , CRP and GM-CSF in males , while in females patient the values pointed out a significant $P<0.05$ elevation in the levels of TNF-α , fibrinogen, RF, CRP and GM-CSF when compared with control group in the levels of TNF-α , fibrinogen, RF, CRP and GM-CSF in females.

Table (1-4): Illustrates the biochemical markers (TNF-α, fibrinogen, RF, CRP and GM-CSF) of patients affected with RA and control groups according to gender

Groups	Patient		Control		LSD (0.05)
	Males	Females	Males	Females	(gender*group)
Parameters	Mean ±S. E				
TNF (pg/ml)	19.06±2.3	21.73 ±6.3	6.02±0.9	4.17±0.6	**2.708***
Fibrinogen (mg/dl)	395.25±35.4	391.25±12.5	211.95±14.2	213.35±12.7	**12.397***
Rf (IU/l)	24.30±8.2	25.93±3.4	11.07±0.3	11.38±1.3	**1.495***
CRP (IU/l)	29.38±5.3	31.15±4.1	8.64±1.1	8.55±0.7	**2.489***
Gm-CSF (pg/ml)	339.00±17.8	342.85 ±25.3	113.45±10.3	115.30±15.4	**9.156***

-All values are mean ±SE

4.2.5. Hematological parameters according to gender and age groups of patients affected with RA and healthy subjects of both sexes:

Results which were illustrated in the following table (4-5) they explained the effects of age periods on some hematological parameters (total white blood cells, granulocytes%, lymphocytes %, hemoglobin, platelets, and erythrocyte sedimentation rate (ESR mm/hr) of both patients and control, some of these results significantly different at $p<0.05$ when compared among different age groups according to LSD values. **Table (4-5): Shows the results of hematological parameters (WBCs, Lymphocytes, Granulocytes, and hemoglobin, red blood cells, platelets and erythrocyte sedimentation rate) of patients affected with RA and healthy groups according to gender and age.**

Groups	Age (year)	Patient		Control		LSD (0.05)
		Male	**Female**	**Male**	**Female**	
Parameters		**Mean ±S. E**				
WBC (m/mm³)	**40-49**	8.52±0.6	7.83±1.2	5.24±0.6	5.12±0.5	**1.105**
	50-59	8.15±1.2	8.49±0.9	5.84±1.2	5.75±0.5	
	60-69	8.26±1.7	8.82±0.8	6.00±0.3	6.24±1.2	
	70-79	9.08±1.8	8.97±1.3	6.43±0.1	7.22±0.6	
Granulocytes (%)	**40-49**	77.80±22.1	80.32±12.4	67.60±12.4	65.78±12.3	**4.056**
	50-59	78.18±13.4	79.18±9.2	66.88±7.8	65.64±6.7	
	60-69	81.12±7.8	82.94±11.1	71.82±12.4	70.98±8.2	
	70-79	82.82±11.11	83.06±8.6	72.50±8.8	71.08±9.1	
Lymphocytes (%)	**40-49**	19.88±2.3	17.70±2.2	30.64±2.6	32.18±6.3	**4.055**
	50-59	19.16±2.2	18.72±1.7	31.12±3.1	32.34±9.2	
	60-69	16.48±1.7	14.86±1.2	26.16±2.2	27.10±4.7	
	70-79	14.60±0.9	14.56±1.5	25.50±1.9	25.48±5.5	
Hb (g/dL)	**40-49**	7.62±1.1	11.20±0.9	13.53±2.4	12.59±1.2	**1.198**
	50-59	7.57±0.4	10.70±0.6	13.35±1.4	11.98±1.1	
	60-69	7.31±0.6	11.32±0.5	13.47±0.9	12.46±0.9	
	70-79	8.77±0.5	10.36±0.2	11.62±0.8	11.51±1.3	
RBCs (m/mm³)	**40-49**	4.12±0.3	3.91±0.2	5.35±0.3	4.98±0.2	**0.601**
	50-59	4.21±1.1	3.91±0.1	4.93±0.2	4.66±0.3	
	60-69	4.11±1.0	4.05±0.3	4.52±0.2	4.27±0.3	
	70-79	3.96±0.9	3.69±0.4	4.38±0.1	4.25±0.7	
PLT (m/mm³)	**40-49**	226.00±22.6	229.20±12.5	259.40±22.1	264.60±22.3	**35.223**
	50-59	256.40±14.6	258.20±22.3	250.00±14.2	257.80±35.1	
	60-69	301.40±17.6	303.60±33.1	225.80±25.2	229.80±17.9	
	70-79	311.40±33.1	315.80±31.1	213.00±11.7	215.40±14.6	
ESR (mm/hr)	**40-49**	24.82±11.4	26.50±2.6	9.00±2.1	8.86±1.2	
	50-59	25.80±3.3	26.18±2.2	9.66±1.3	11.92±0.9	**4.049**
	60-69	30.26±2.6	32.16±1.9	14.42±1.1	15.30±1.1	
	70-79	34.76±2.9	36.34±1.7	15.24±0.8	16.28±1.0	

-All values are mean ±SE

4.2.6. The results of biochemical markers according to gender and age groups of patients affected with RA and healthy control of both sexes:

All the results had been recorded in the table (4-6) were a significantly (P<0.05) increased in levels of (tumor necrosis factor, fibrinogen, rheumatoid factor, C-reactive protein and granulocyte monocyte-colony Stimulating factor) as the values are shown in the table, in all age groups (40-49,60-69,70-79) in male and female with rheumatic arthritis when compared with healthy groups.

Table (4-6): Illustrate the biochemical markers (tumor necrosis factor, fibrinogen, rheumatoid factor, C-reactive protein and granulocyte monocyte-colony Stimulating factor) of patients affected with RA and healthy groups according to gender and age.

Groups Parameters	Age (year)	Male		Female		LSD (0.05)
		Patient	Control	Patient	Control	
		Mean ±S. E				
TNF (pg/ml)	40-49	16.30±2.1	2.50±0.3	18.90±2.1	1.78±0.06	2.364*
	50-59	16.38±1.2	4.32±0.5	21.66±1.6	2.82±0.1	
	60-69	20.90±1.1	8.26±0.2	21.88±3.3	4.52±0.03	
	70-79	22.64±0.9	9.00±1.2	24.46±3.7	7.56±0.9	
Fibrinogen(mg/dl)	40-49	384.80±22.5	193.20±17.8	385.00±35.2	194.20±15.6	19.738*
	50-59	394.20±17.5	201.40±20.1	395.40±17.8	202.80±20.5	
	60-69	397.60±20.1	205.80±15.5	398.80±16.9	207.20±12.6	
	70-79	404.40±33.2	247.40±9.6	385.80±23.1	249.20±22.7	
Rf (IU/l)	40-49	20.84±1.9	7.84±1.1	23.92±2.6	6.94±0.9	1.855*
	50-59	22.26±2.1	11.12±0.7	25.78±1.3	12.26±1.2	
	60-69	26.80±3.3	12.12±1.1	26.14±2.4	12.96±1.4	
	70-79	27.30±4.1	13.20±1.6	27.86±3.3	13.36±1.1	
CRP (IU/l)	40-49	25.92±2.3	6.12±1.2	26.36±2.3	5.14±0.3	4.037*
	50-59	26.44±3.3	7.26±0.8	29.22±1.7	7.36±0.6	
	60-69	30.40±1.9	10.14±0.5	32.22±3.6	10.36±0.1	
	70-79	34.74±4.4	11.04±1.3	36.80±2.7	11.32±2.1	
Gm-CSF (pg/ml)	40-49	313.20±17.8	108.40±17.4	314.00±23.2	111.00±7.8	9.036*
	50-59	333.20±6.9	114.20±11.6	331.60±15.6	115.40±10.1	
	60-69	344.20±18.9	114.40±9.8	349.20±12.6	116.80±9.9	
	70-79	365.40±36.2	116.80±13.9	376.60±9.8	118.00±7.4	

-All values are mean ±SE

4.2.7. The results of biochemical markers (RF, GM-CSF, TNF-α, fibrinogen, and CRP) of patients affected with rheumatic arthritis according to BMI:

The results that were shown in the table (4-7) indicated a significant p<0.01 elevation in the level of rheumatoid factor, granulocyte monocyte-colony Stimulating factor, tumor necrosis factor, and fibrinogen, C-reactive protein in patients when compared with control groups in the level of rheumatoid factor, granulocyte monocyte-colony Stimulating factor, tumor necrosis factor, fibrinogen, and C-reactive.

Table (4-7): Biochemical marker according to BMI (kg/m^2) of patients with rheumatic arthritis and control groups

BMI groups	BMI (Kg/m^2)		P≤0.05
	Patient (25-33)	**Control (19-23)**	
Parameters	**Mean±S.E**		
Rf (IU/I)	25.84±1.1	11.44±1.2	≤0.0001**
Gm-CSF (pg/ml)	358.40±11.9	121.80±1.9	≤0.0001**
TNF-α (pg/ml)	22.74±1.6	7.08±1.4	≤0.0001**
Fibrinogen(mg/ml)	401.80±10.8	215.00±12.8	≤0.0001**
CRP (IU/I)	32.94±3.1	10.42±2.3	≤0.0001**

All values are mean ±SE

Results with two strikes are significantly different at P≤0.01

4.3. Correlations among studied parameters

4.3.1 Results of correlation among studied parameters of patients affected with RA of both sexes:

The following figures are show the correlation that had been occurred among all studied parameters (hematological and biochemical).

4.3.2. The results that had been explained in the following figure indicated a significant positive correlation (r=0.62, sig=0.0001) between ESR and TNF- α of patients affected with rheumatic arthritis of both sexes.

Figure (4-5): Correlation coefficient between ESR and TNF - α.

4.3.3. Values of ESR and GM-CSF were a significant positive correlated (r=0.75, sig=0.0001) of patients affected with rheumatic arthritis of both sexes.

Figure (4-6): Correlation coefficient between ESR and GM-CSF.

4.3.4. There was significantly positive correlated (r=0. 52, sig=0.001) between ESR and PLT of patients affected with rheumatic arthritis of both sexes.

Figure (4-7): Correlation coefficient between ESR and PLT.

4.3.5. It was found significantly positive correlated (r=0.43, sig=0.005) between ESR and WBCs of patients affected with rheumatic arthritis of both sexes

Figure (4-8): Correlation coefficient between ESR and WBCs.

4.3.6. It was demonstrated a negative correlation (r=0.43) at level p<0.05 between ESR and lymphocytes of patients affected with rheumatic arthritis of both sexes.

Figure (4-9): Correlation coefficient between ESR and lymphocytes.

4.3.7. Data in figure (4-10) indicated significantly positive correlated (r=0.42, sig=0.007) between ESR and granulocytes of patients affected with rheumatic arthritis of both sexes

Figure (4-10): Correlation coefficient between ESR and granulocytes.

4.3.8. Results which were explained in figure (4-11) pointed out a significant positive correlation (r=0.43, sig=0.005) between ESR and fibrinogen of patients affected with rheumatic arthritis of both sexes.

Figure (4-11): Correlation coefficient between ESR and fibrinogen.

4.3.9. It had been found, there was significantly positive correlated (r=0.66, sig=0.0001) between ESR and RF of patients affected with rheumatic arthritis of both sexes.
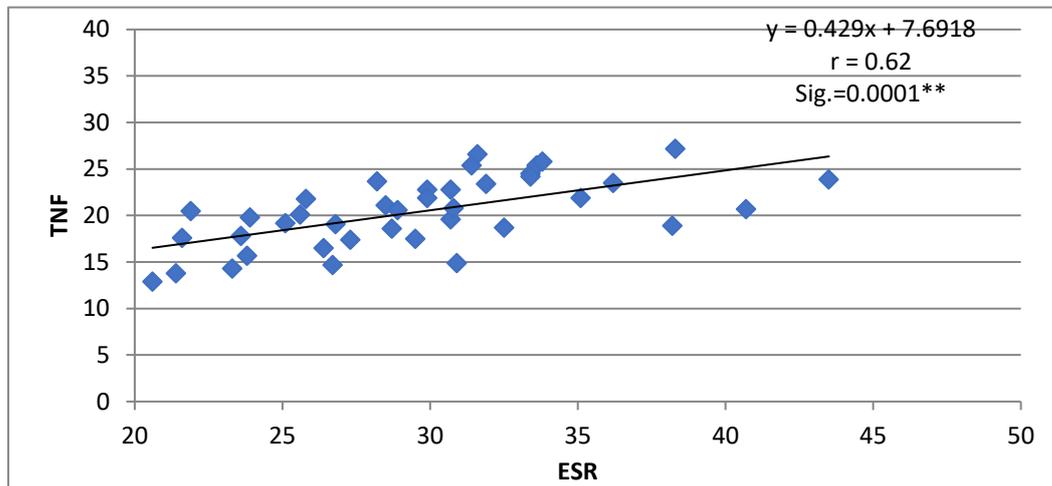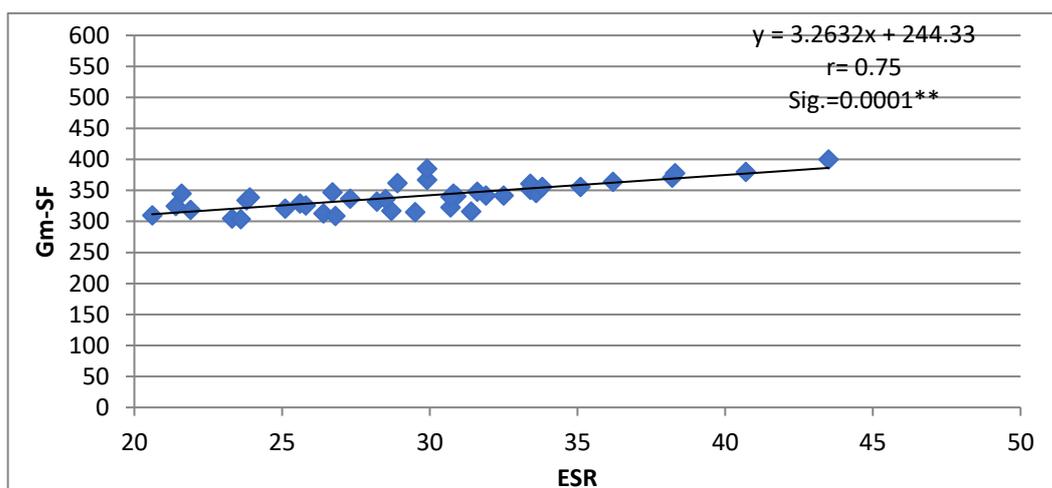

Figure (4-12): Correlation coefficient between ESR and RF.

4.3.10. There was significantly positive correlated (r=0.74, sig=0.0001) between ESR and CRP of patients affected with rheumatic arthritis of both sexes

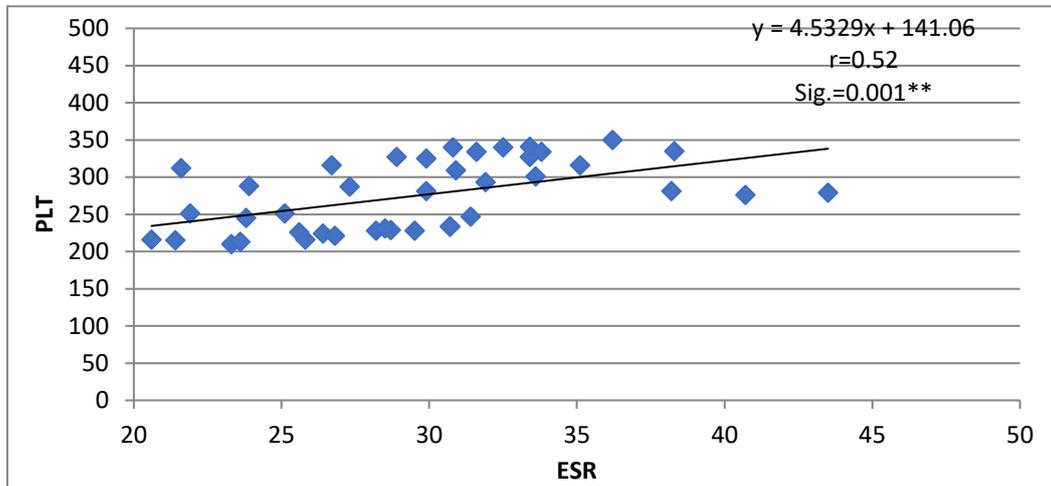Figure (4-13): Correlation coefficient between ESR and CRP.

4.3.11. The results that had been explained in the following figure they were indicated a significant positive correlation (r=0.68, sig=0.0001) between Gm-CSF and PLT patients affected with rheumatic arthritis of both sexes.

Figure (4-14): Correlation coefficient between Gm-CSF and PLT.

4.3.12. Results which were explained in figure (4-15) pointed out a significant positive correlation (r=0.34, sig=0.027) between Gm-CSF and WBCs of patients affected with rheumatic arthritis of both sexes.
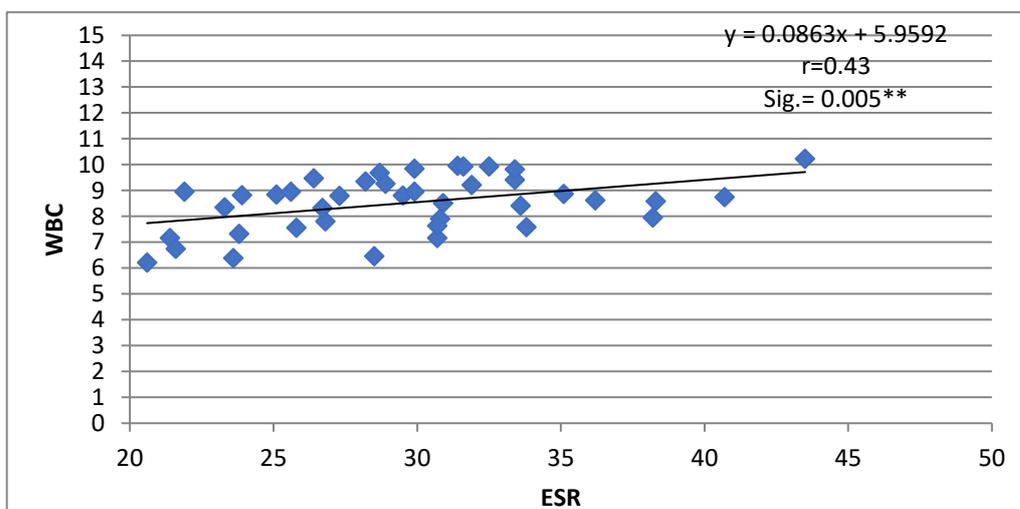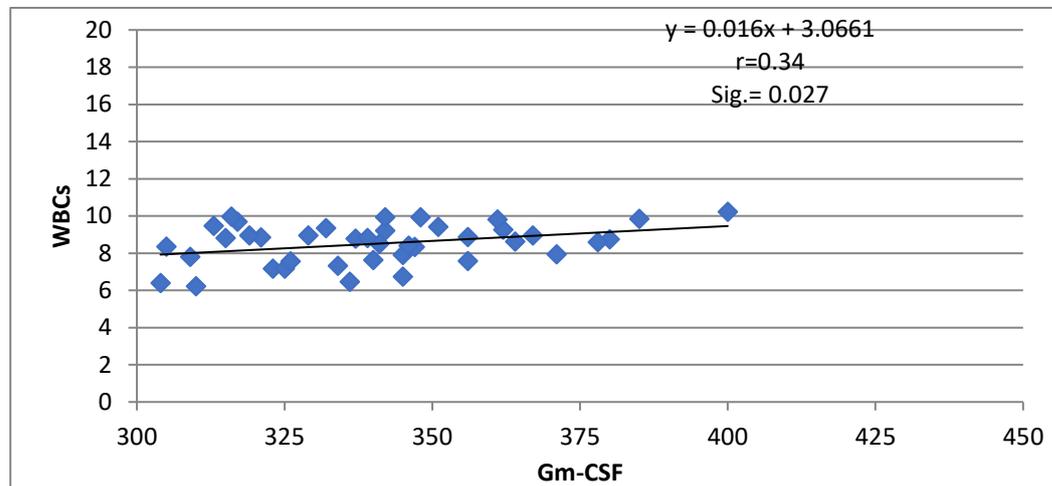

Figure (4-15): Correlation coefficient between Gm-CSF and WBCs.

4.3.13. Results which were illustrated in figure (4-16) confirmed a negative correlation (r=0.41) at levels p<0.007 between Gm-CSF and lymphocytes of patients affected with rheumatic arthritis of both sexes.
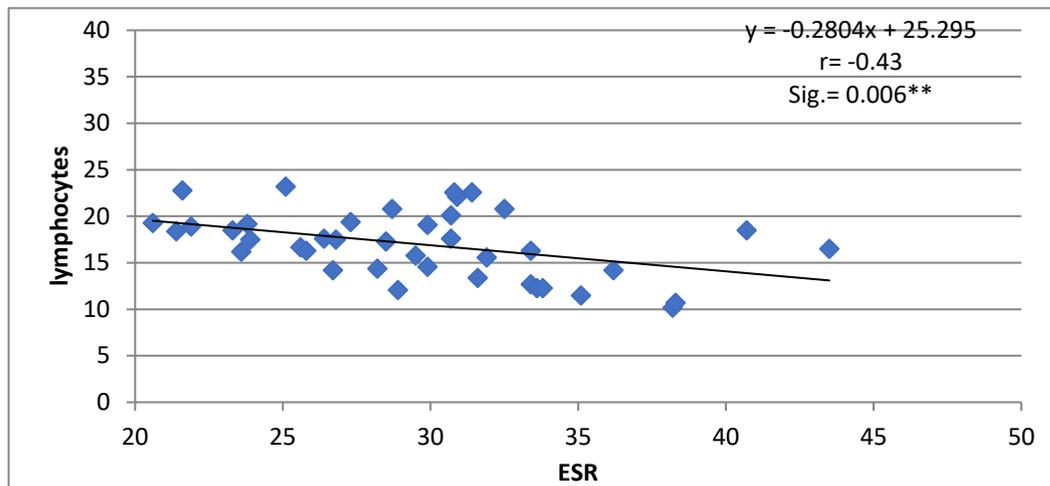

Figure (4-16): Correlation coefficient between Gm-CSF and lymphocytes.

4.3.14. Results that were presented in figure (4-17), it demonstrated a positive correlation (r=0.39) at p<0.012 between Gm-CSF and granulocyte of patients affected with rheumatic arthritis of both sexes.
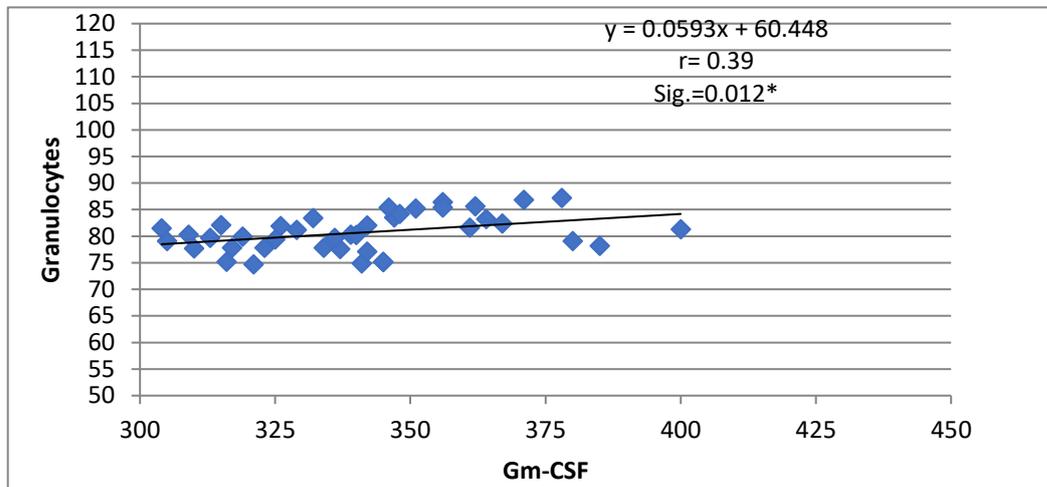
Figure (4-17): Correlation coefficient between Gm-CSF and granulocytes.

4.3.15. It had been found that, there was significantly positive correlated (r=0.34, sig=0.0030) between Gm-CSF and Fibrinogen of patients affected with rheumatic arthritis of both sexes.
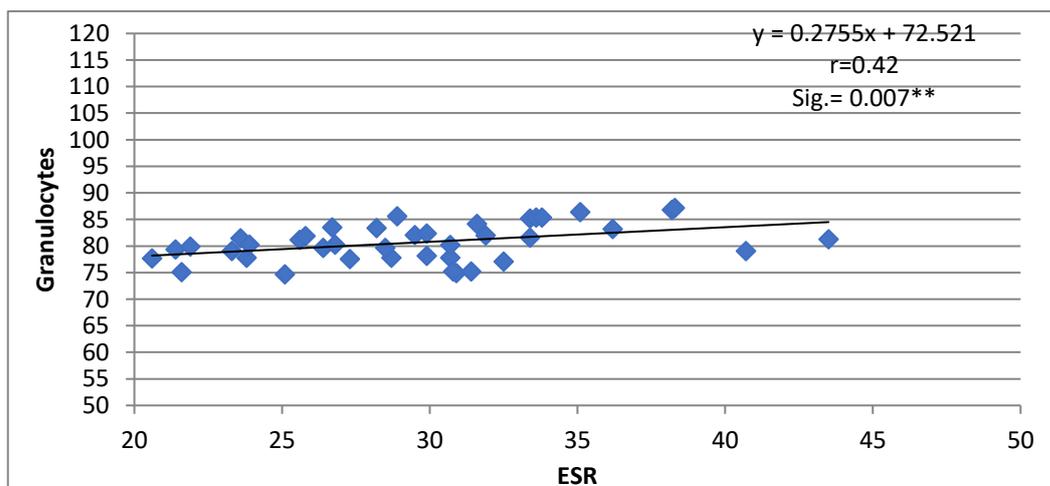

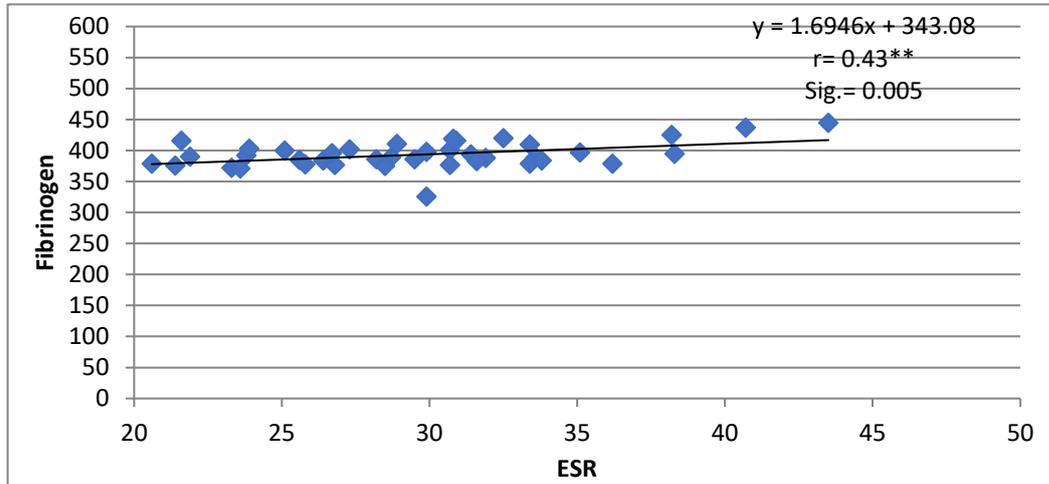Figure (4-18): Correlation coefficient between Gm-CSF and Fibrinogen.

4.3.16. There was significantly positive correlated (r=0.58, sig=0.0001) between Gm-CSF and RF of patients affected with rheumatic arthritis of both sexes.

Figure (4-19): Correlation coefficient between Gm-CSF and RF.

4.3.17. Values of Gm-CSF and CRP were a significant positive correlated (r=0.75, sig=0.0001) of patients affected with rheumatic arthritis of both sexes.

Figure (4-20): Correlation coefficient between Gm-CSF and CRP.

4.3.18. From the observations present in figure (4-21), it was apparently found that there was a positive correlation (r=0.50) at levels p<0.001 between TNF -$\alpha$and Gm-CSF of patients affected with rheumatic arthritis of both sexes.

Figure (4-21): Correlation coefficient between TNF-α and Gm-CSF.

4.3.19. Findings illustrated in the following figure (4-22) pointe out a positive correlation (r=0.46) between TNF-α and PLT at levels p<0.003 of patients affected with rheumatic arthritis of both sexes.
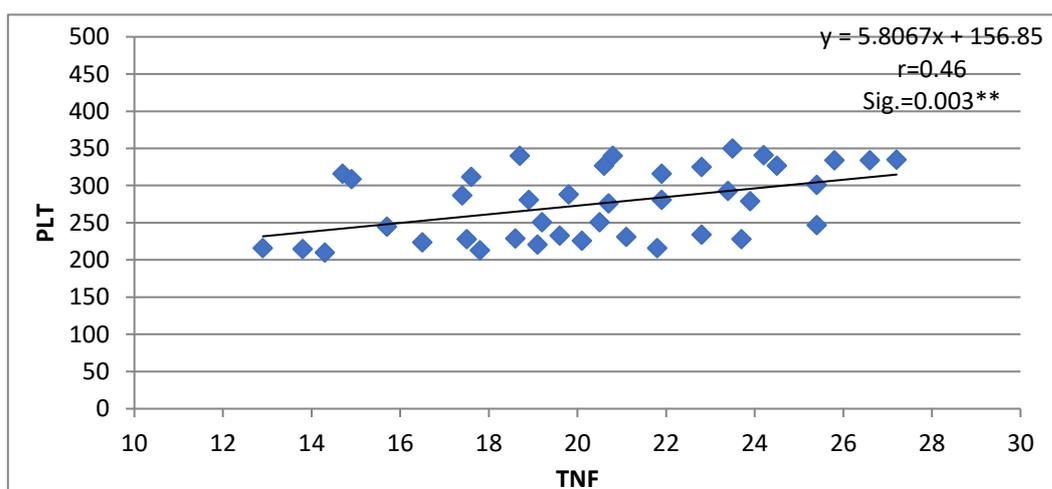
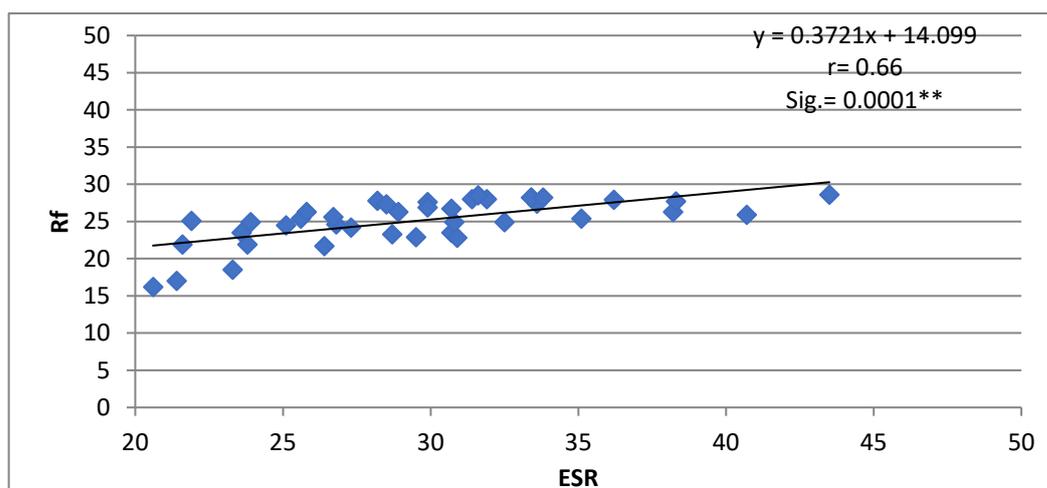Figure (4-22): Correlation coefficient between TNF-α and PLT.

4.3.20. There was significantly positive correlated (r=0.42, sig=0.006) between TNF-α and WBCs of patients affected with rheumatic arthritis of both sexes.
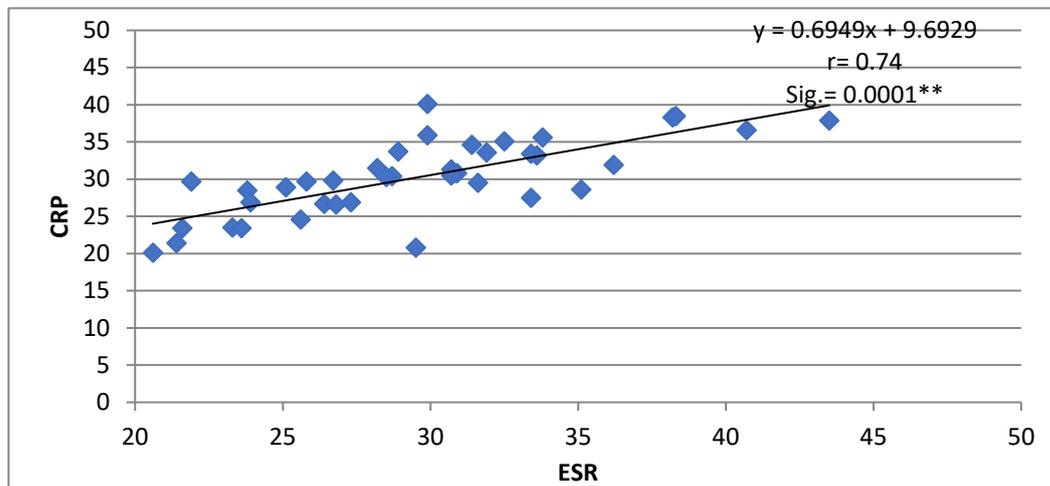

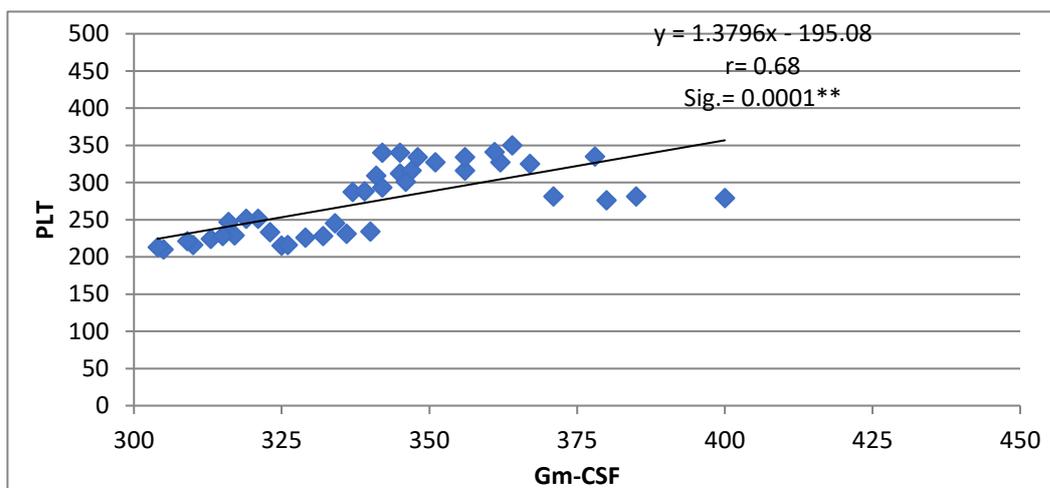$$y = 0.1227x + 6.0129$$
$$r=0.42$$
$$Sig.=0.006**$$
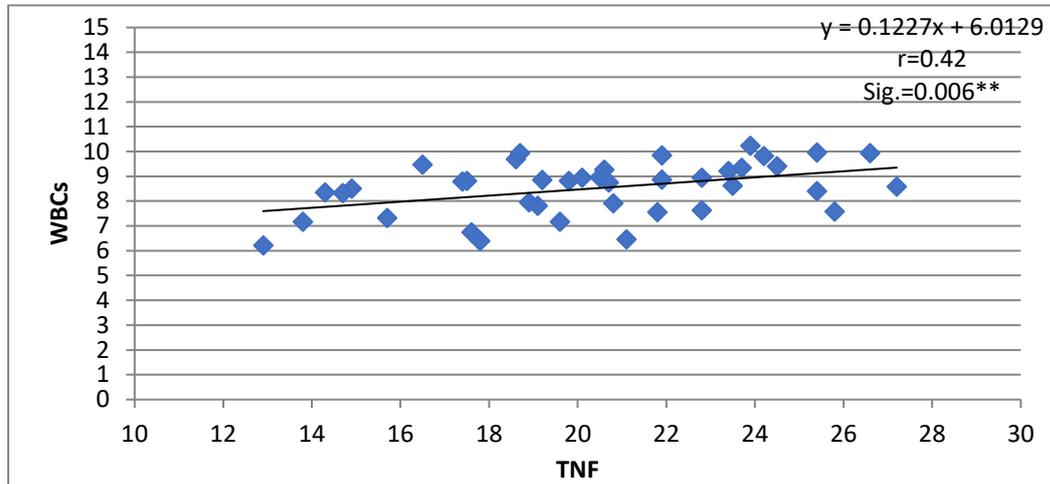
Figure (4-23): Correlation coefficient between TNF-α and WBCs.

4.3.21. Results which were explained in figure (4-24) pointed out a significant negative correlation (r=0.45, sig=0.003) between TNF and lymphocytes of patients affected with rheumatic arthritis of both sexes.

$$y = -0.4281x + 25.724$$
$$r= -0.45$$
$$Sig.=0.003**$$
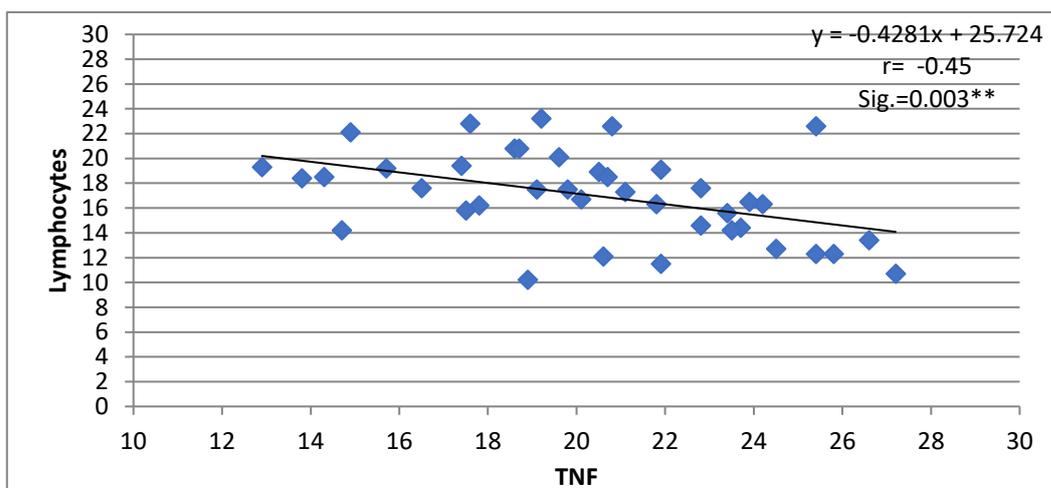
Figure (4-24): Correlation coefficient between TNF-α and lymphocytes.

4.3.22. It had been found, there was significantly positive correlated (r=0.48, sig=0.002) between TNF-α and granulocytes of patients affected with rheumatic arthritis of both sexes.
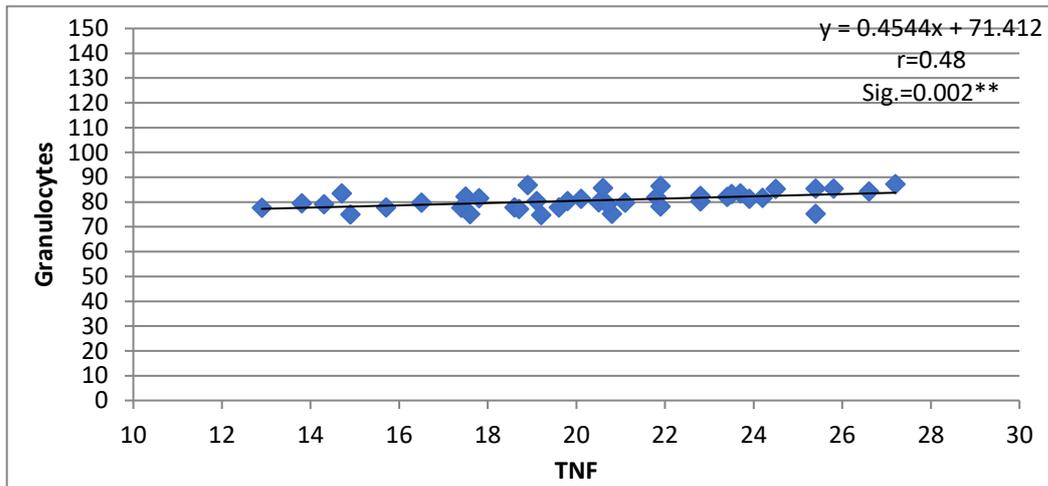
Figure (4-25): Correlation coefficient between TNF-α and granulocytes.

4.3.23. The results that had been explained in the following figure they were indicated a significant positive correlation (r=0.87, sig=0.0001) between of TNF-α and RF patients affected with rheumatic arthritis of both sexes.
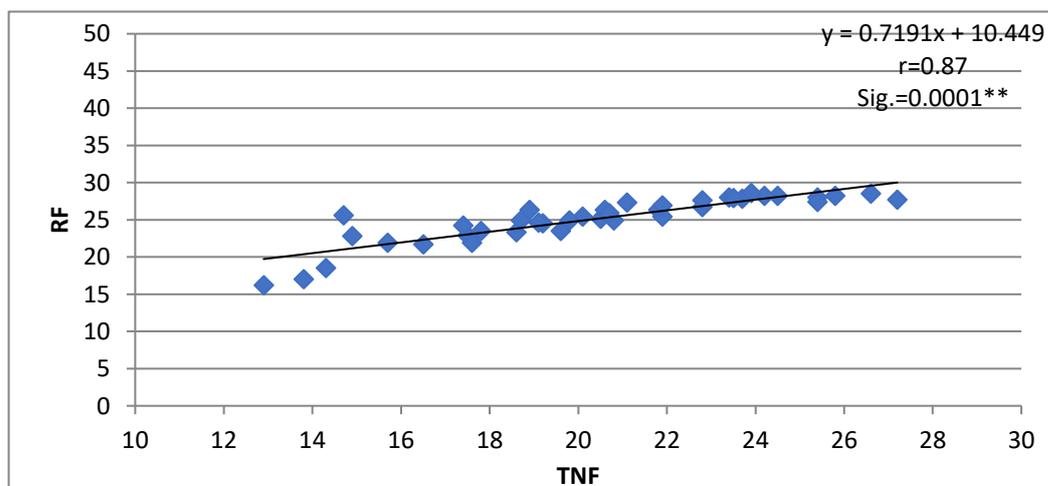
Figure (4-26): Correlation coefficient between TNF-α and RF.

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4.3.24. There was significantly positive correlated (r=0. 62, sig=0.0001) between TNF-α and CRP of patients affected with rheumatic arthritis of both sexes.
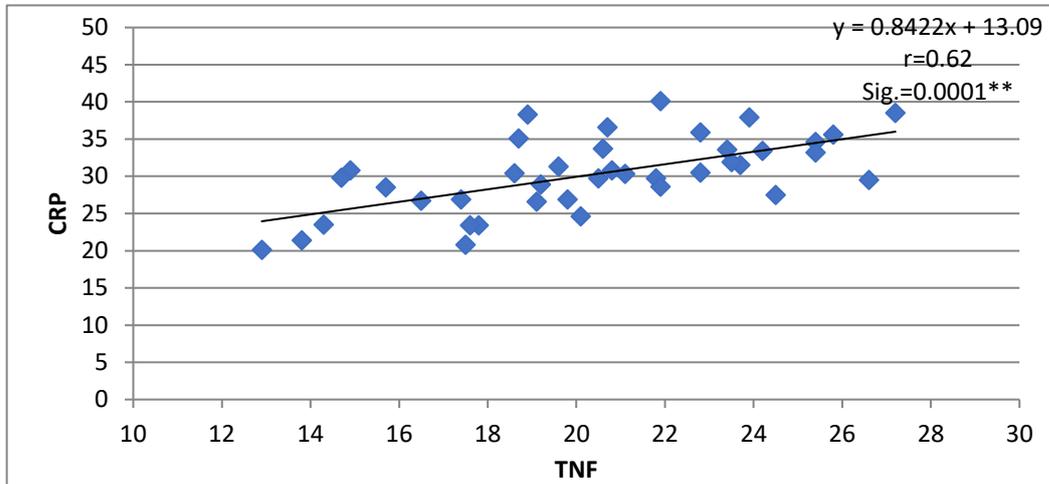
Figure (4-27): Correlation coefficient between TNF-α and CRP.

4.3.25. Data in figure (4-28) indicated significantly positive correlated (r=0.49, sig=0.001) between   RBCs and fibrinogen of patients affected with rheumatic arthritis of both sexes.
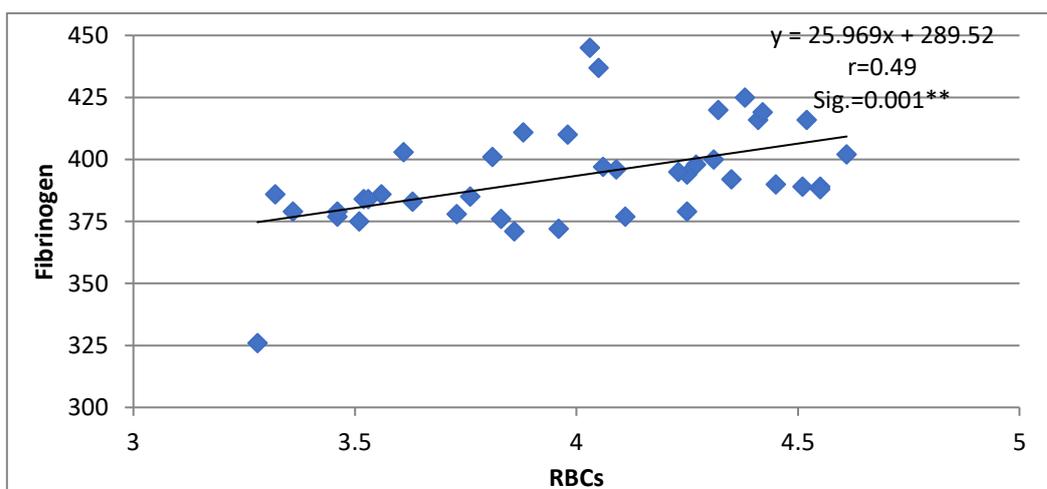
Figure (4-28): Correlation coefficient between RBCs and fibrinogen.

4.3.26. There was significantly positive correlated (r=0.35, sig=0.025) between WBCs and PLT of patients affected with rheumatic arthritis of both sexes.
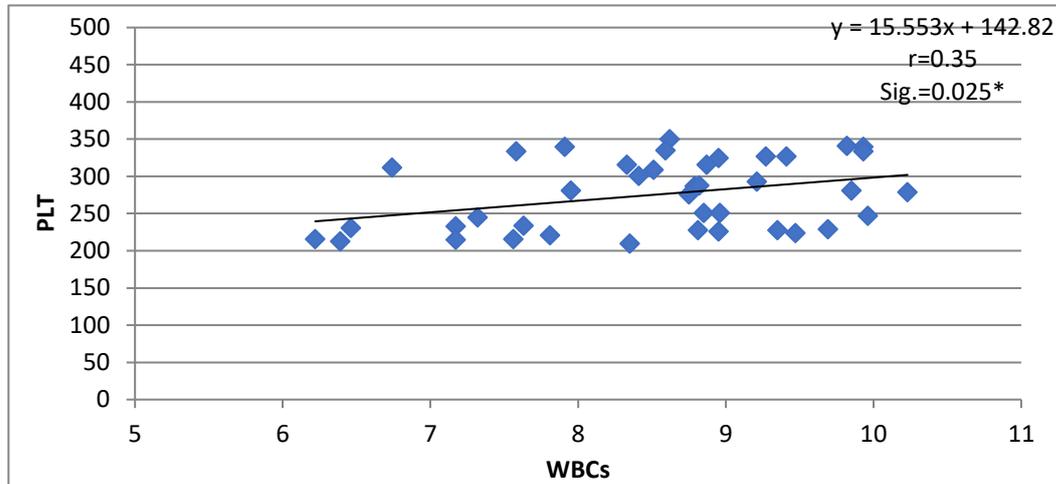
Figure (4-29): Correlation coefficient between WBCs and PLT.

4.3.27. It was demonstrated a negative correlation (r=0.32) at level p<0.044between lymphocytes and PLT of patients affected with rheumatic arthritis of both sexes.
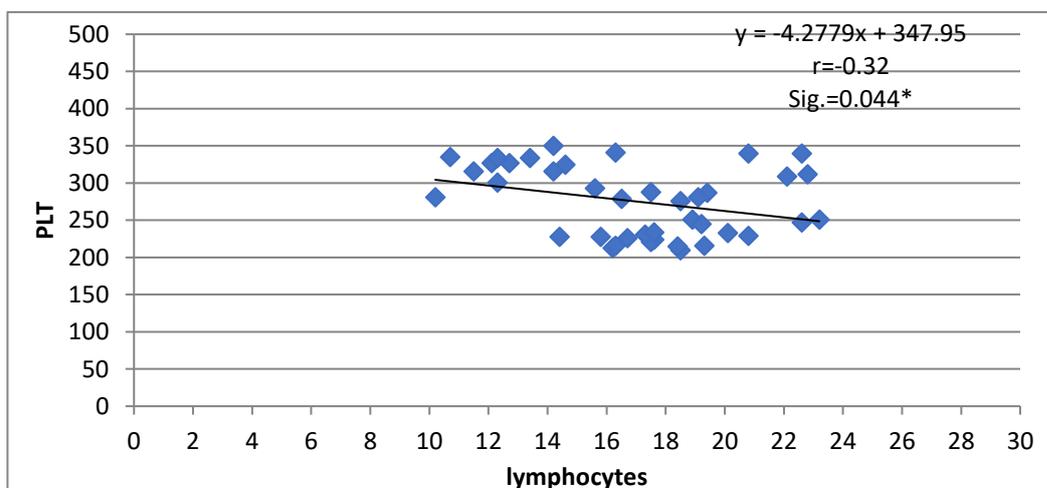
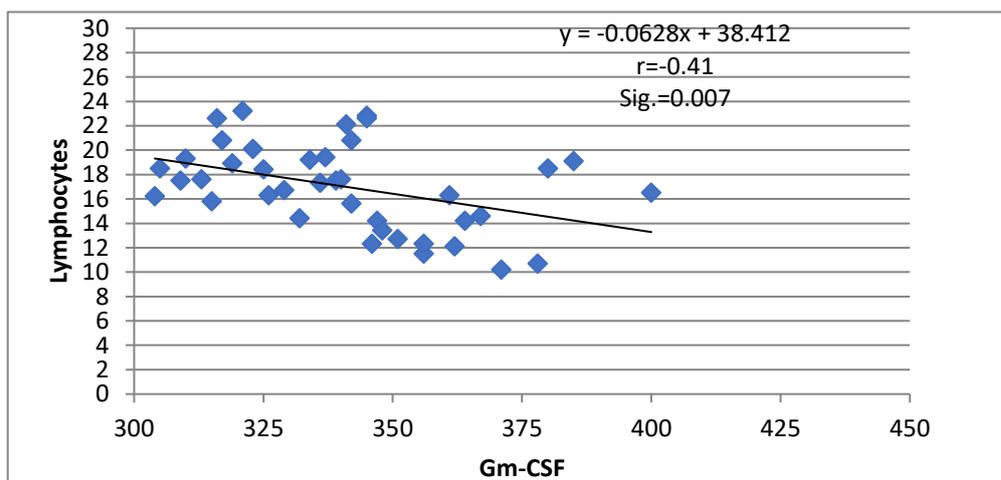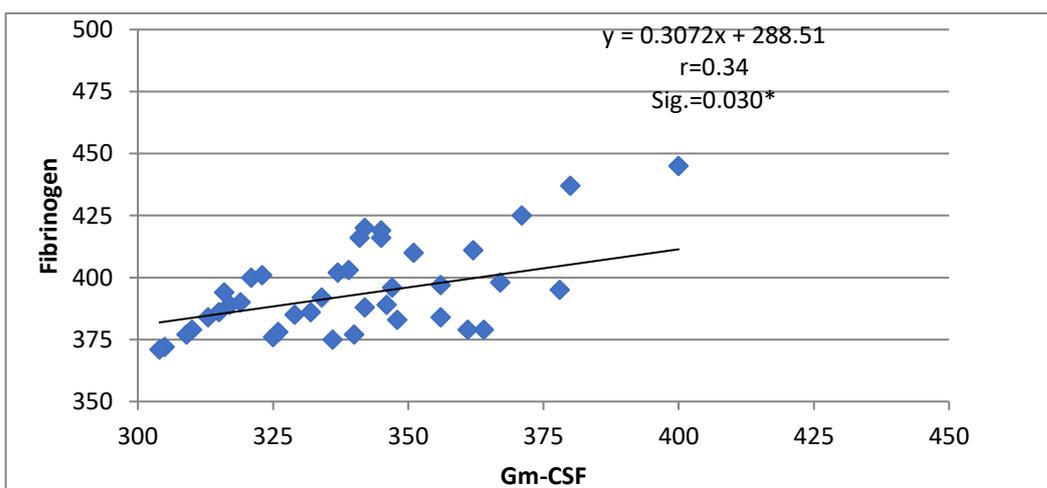Figure (4-30): Correlation coefficient between lymphocytes and PLT.

4.3.28. It had been found, there was significantly positive correlated (r=0.045, sig=0.003) between WBCs and CRP of patients affected with rheumatic arthritis of both sexes.
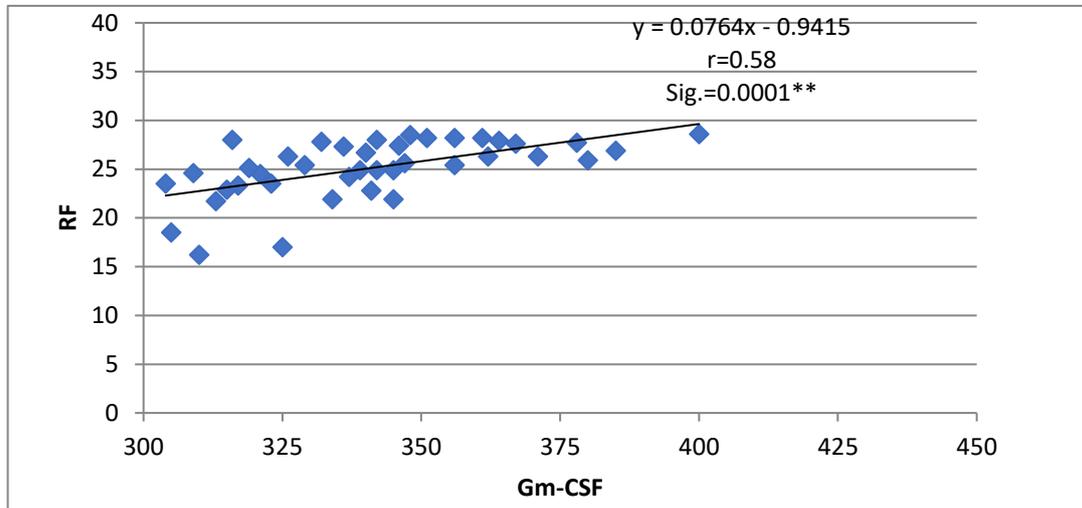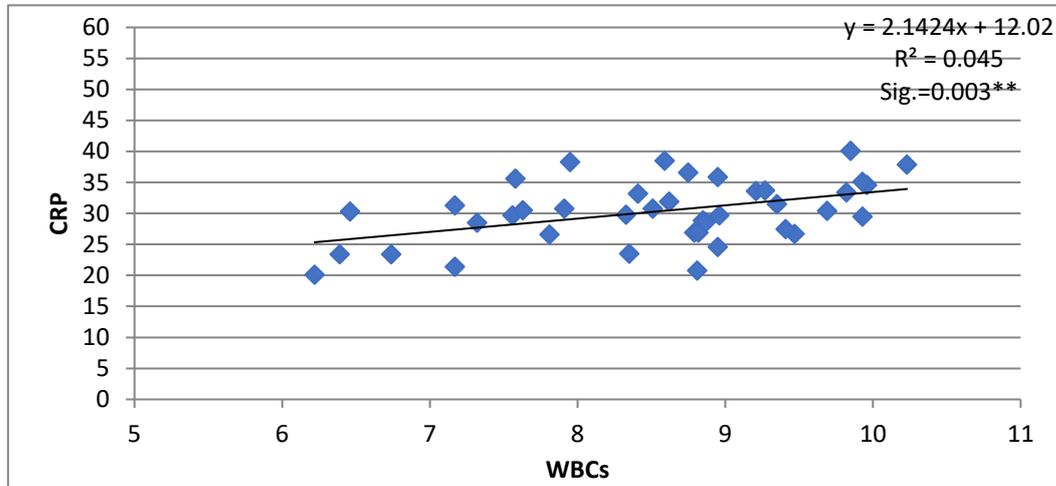

Figure (4-31): Correlation coefficient between WBCs and CRP.

4.3.29. From the observations present in figure (4-32), it was apparently found that there was a positive correlation (r=0.35) at levels p<0.024 between Fibrinogen   and PLA of patients affected with rheumatic arthritis of both sexes.
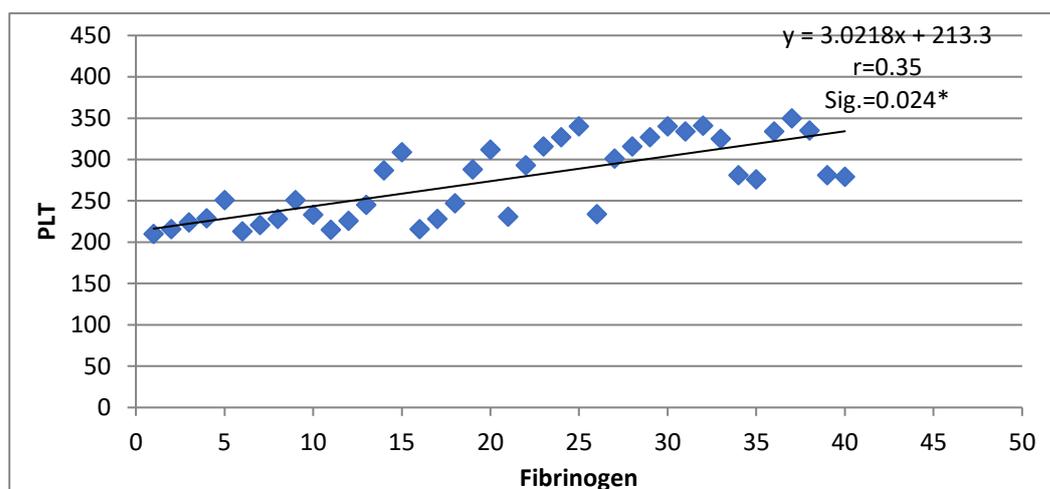
Figure (4-32): Correlation coefficient between Fibrinogen and WBCs.

4.3.30. Findings illustrated in the following figure (4-33) pointe out a positive correlation (r=522) between RF and PLT at levels p<0.001 of patients affected with rheumatic arthritis of both sexes.
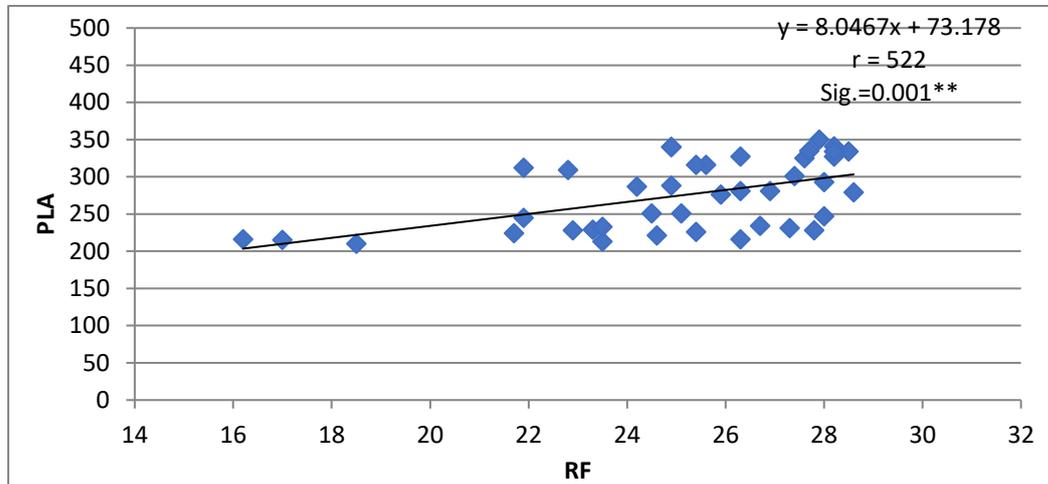
Figure (4- 33): Correlation coefficient between RF and PLT.

4.3.31. Values of CRP and PLT were a significant positive correlated (r=0.52, sig=0.001) of patients affected with rheumatic arthritis of both sexes.
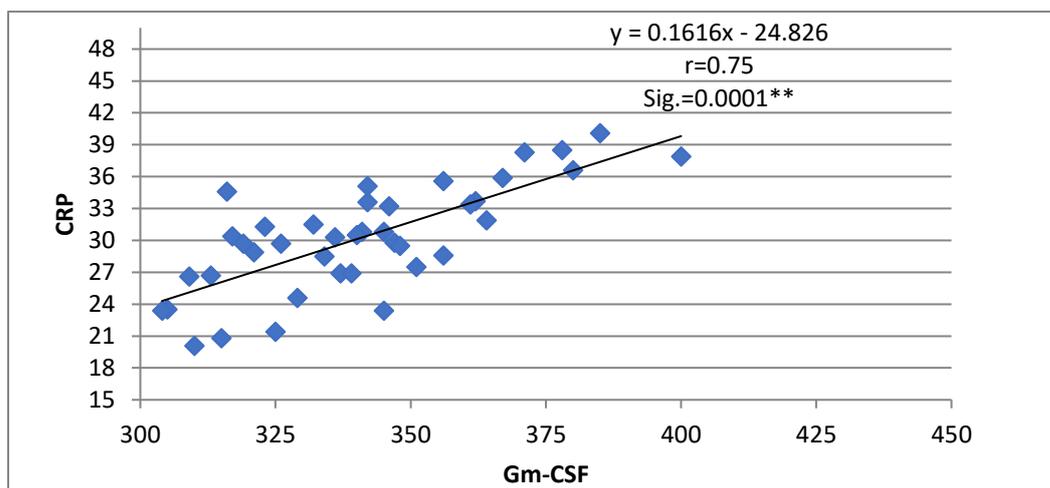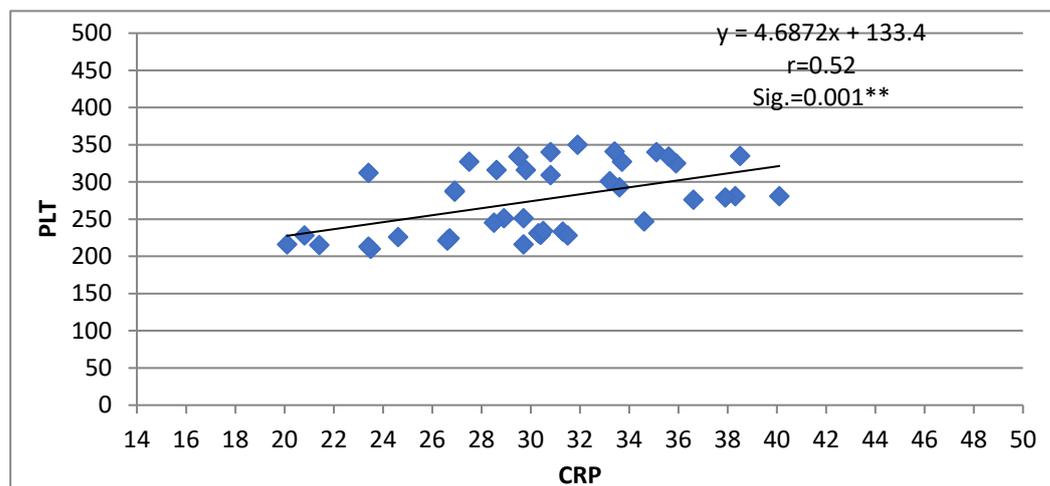

Figure (4-34): Correlation coefficient between CRP and PLT.

4.3.32. From the observations present in figure (4-36), it was apparently found that there was a positive correlation (r=0.48) at levels p<0.001 between WBCs and Rf of patients affected with rheumatic arthritis of both sexes.
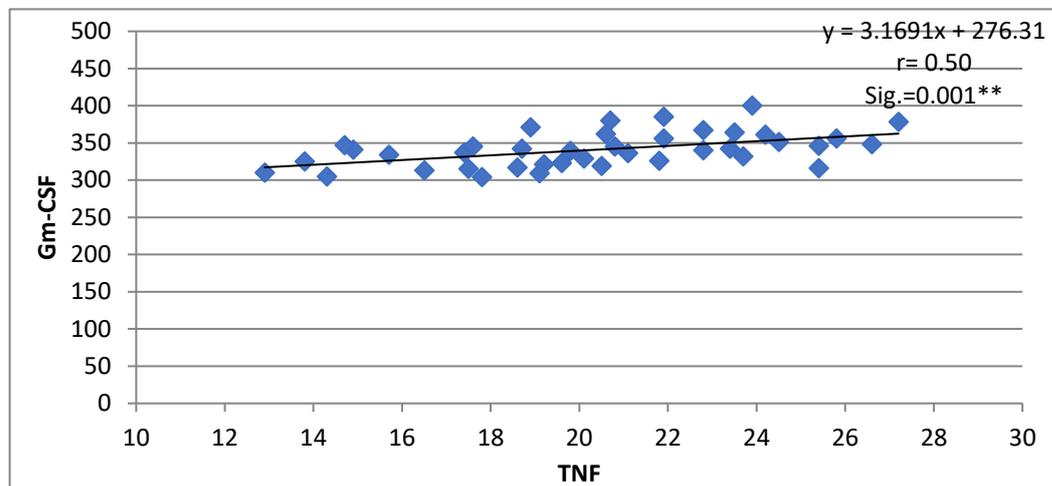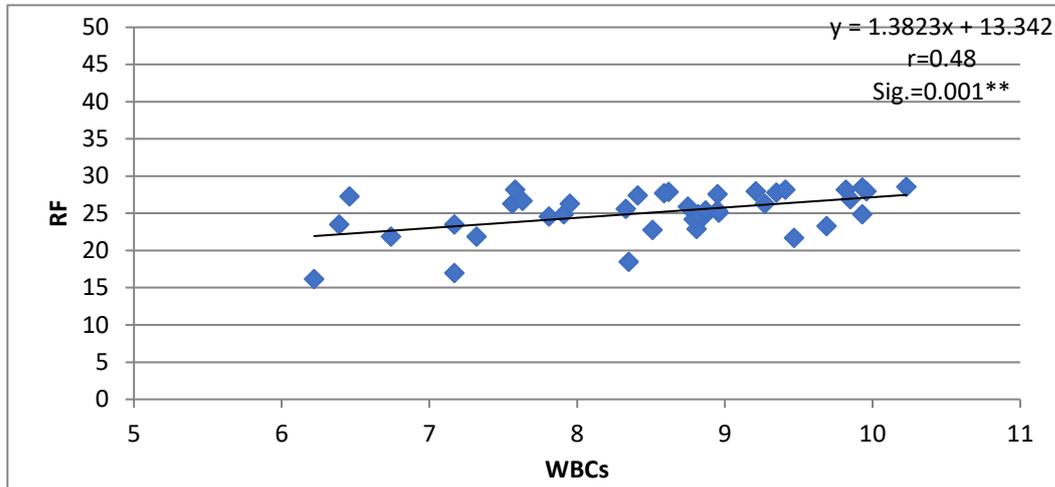

Figure (4-35): Correlation coefficient between WBCs and Rf.

4.3.33. It had been found, there was significantly positive correlated (r=0.72, sig=0.0001) between CRP and Rf of patients affected with rheumatic arthritis of both sexes.
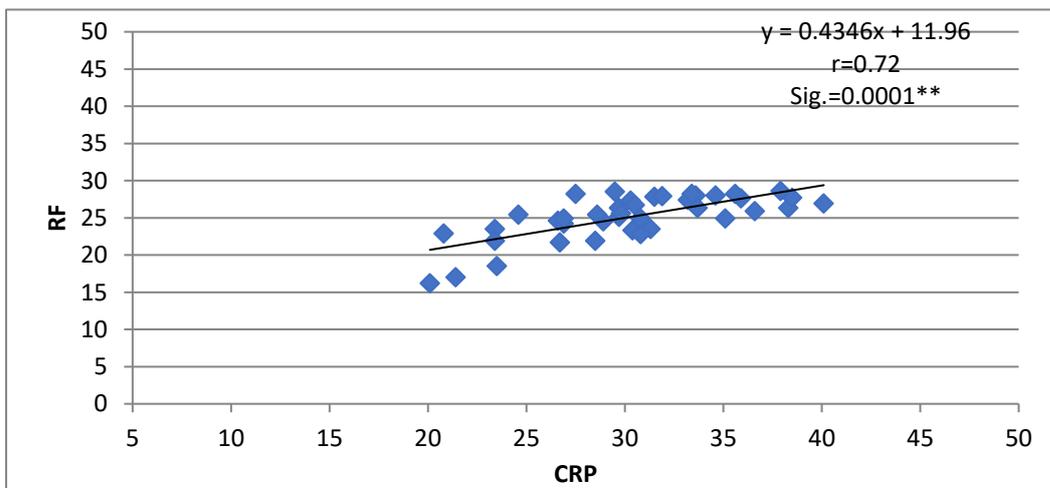
Figure (4-36): Correlation coefficient between CRP and Rf.

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4.3.34. It had been found, there was significantly negative correlated (r=0.99, sig=0.0001) between lymphocytes and granulocyte of patients affected with rheumatic arthritis of both sexes.
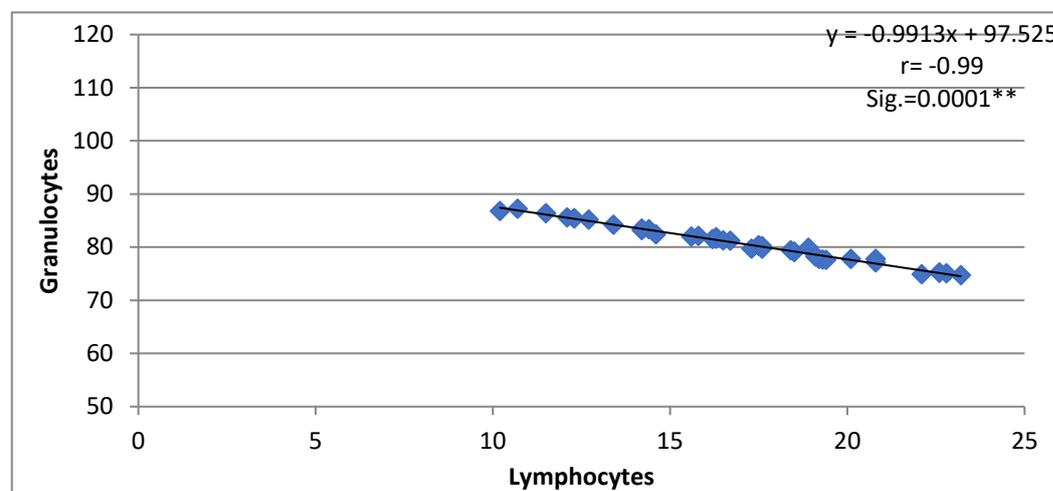
Figure (4-37): Correlation coefficient between lymphocytes   and granulocyte.

4.4. The results of operating characteristic curves of RA:

The ROC analysis yielded a cut off value of TNF-α, GM-CSF, fibrinogen, RF and CRP for prediction of the disease activity by parameters for RA disease.

ROC Table (4-8): the best cut off, sensitivity and specificity for prediction of the disease activity by parameters.

Parameter	Sensitivity	Specificity	AUC	Cut off	95% confidence	p-value
TNF-α	0.825	0.975	0.865	10.600	0.770-0.960	≤0.0001
Gm-CSF	0.875	0.700	0.914	118.50	0.846-0.982	≤0.0001
Fibrinogen	0.800	0.450	0.775	205.00	0.666-0.883	≤0.0001
RF	0.825	0.675	0.864	12.700	0.779-0.950	≤0.0001
CRP	0.800	0.475	0.728	7.850	0.612-0.844	≤0.0001
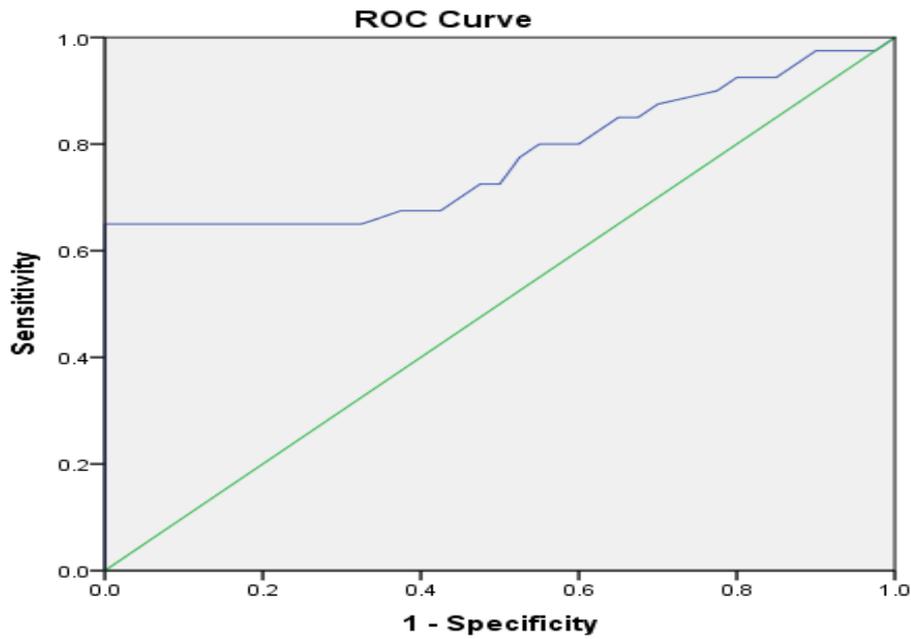
Figure (4-38): ROC curve for prediction of the disease activity by Fibrinogen
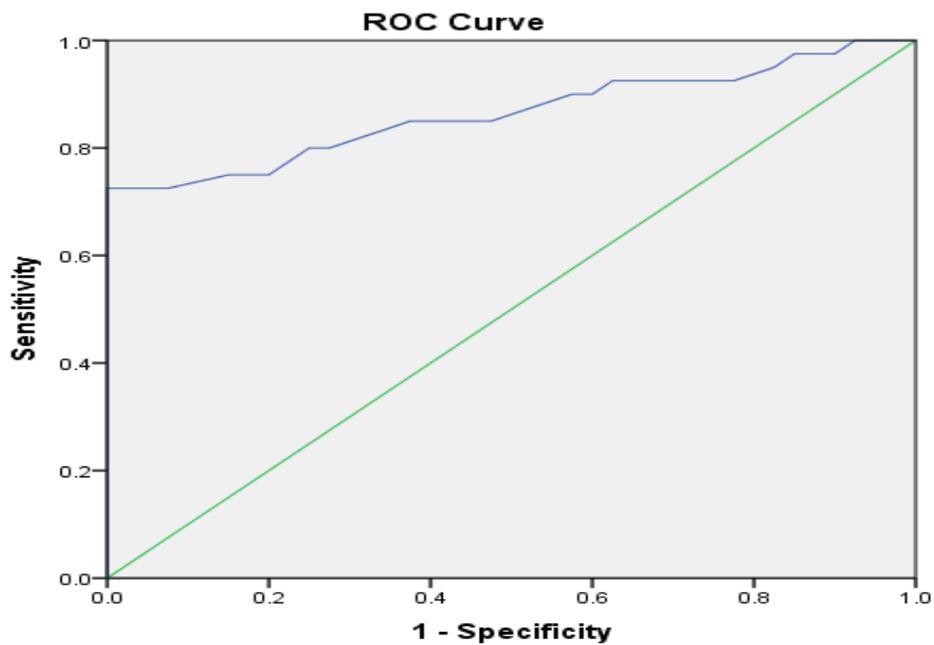
Figure (4-39): ROC curve for prediction of the disease activity by RF
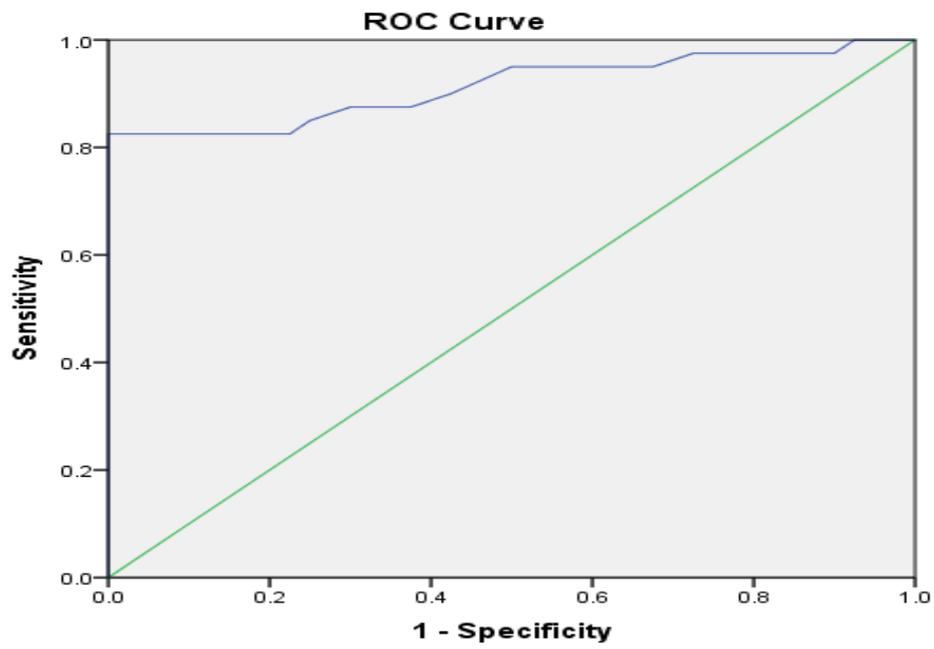
Figure (4-40): ROC curve for prediction of the disease activity by Gm-CSF
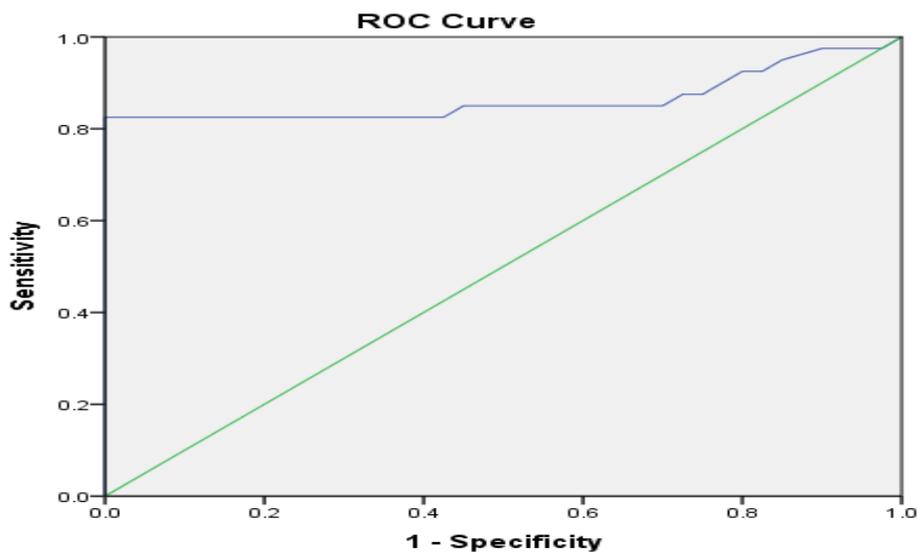
Figure (4-41): ROC curve for prediction of the disease activity by TNF-α
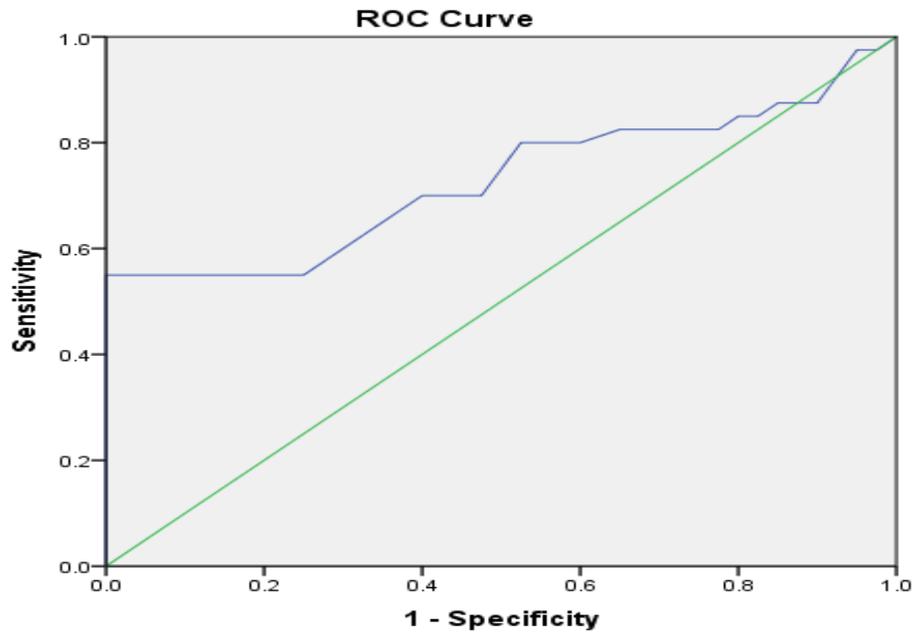
Figure (4-42): ROC curve for prediction of the disease activity by CRP.

Chapter Five

Discussion

5. Discussion:

5.1 The Effect of Gender on the Number of People with RA:

Figure (1) that was yield from the present study indicates that women affected with RA constituted 60% whereas men comprised 40%

Age of women is also enhancing the development of disease RA, since, it is found that women under 50 years old have 4 to 5 times but this ratio is decreased above 60 years old *(Kvien et al.,2006).*

The present described results of this study are supported by ideas of author who confirm the effects of sex in incidence of RA according to epidemiological reports that finally concluded that women affected with RA three folds than of men (Kvien *et al.,*2006).

Concerning the relationship of sex hormones and the development of RA and other autoimmune disease, the growing evidence in this issue indicates that estrogen hormone has impact role in the inflammatory processes, it acts to drop inflammatory mechanisms and at the same time it evokes and triggers production of immunoglobulin (Kristensen *et al.,* 2010).

The experimental study showed that estrogen evokes production excessive levels of interferon-alpha in cells rather than of testosterone (Laffont *et al*., 2014*).*

From pervious observations that were conducted to explain how estrogen affects inflammatory mediators, the experimental study was involved monocyte and macrophages that are derived from blood human and treated with 17 beta estradiol, the final result reported profuse production of pro-inflammatory mediators at low level doses such as IL-1 , IL-6 , TNF-α that in turn up regulates inflammatory processes, but on

other hand , a higher concentration of 17-beta estradiol leads to decrease those inflammatory mediators (Shivers *et al.,*2015).

There are two concepts involved that gender and sex of humans represented important acknowledge, the former concept about sex related to sexual chromosomes (X and Y), Reproductive systems, and effective specific hormones, in regard to word of gender, it used to recognize non-physiological characteristics of sex individual that concern with males and females that have specify to socio-cultural basis of life (Klein and Flanagan, 2016).

These observations were matched with previous studies that indicated gender differences play important roles in incidence and development of RA and those findings were consistent in their conclusions that confirm the incidence and severity of RA is higher in women than of men as well as the outcomes of this disease is poor in affected females *(*Vanvollen hove, 2009; El-Ghany *et al.,* 2019*)*.

Recent study conducted by Maranini *et al*, (2022)*,* that reported sex affected incidence and progressive of different autoimmune disease in particular RA at the same time influence treatment programs.

5.2. The Effect of Age on RA incidence:

The old age is associated with different chronic diseases of both men and women, previous figure (2) established that RA affects human over 65 years old, and also the sex plays pivotal role in the incidence of this disease, it has been found that women with RA worse step by step compared to men and have poor prognosis but the destructions of joins are similar to that of males (Tengstrand *et al.,* 2004)*.*

Another previous study had also recognized that prevalence and incidence of RA is higher among elderly people (Tutuncu and Kavanaugh, 2007).

Sex hormones were suggested to have implication or interactions in incidence of RA and its pathogenesis, women are affected with RA at nearly menopause (< 40 years old), on the other hand, testosterone, concentration tend to decrease slowly at 50 years old, (Sokka *et al.,* 2009).

It is previously proposed that expanding of life expectancy of individual is related with increased incidence of RA, many suggestions were designed that established the accumulated damage of DNA, oxidative stress, and physiological disturbances are implicated in development of RA (Boots *et al.,* 2013).

Some of previous studies established that old patients with RA have high joint damage, but other studies showed no differences in damage of that young and old human (Pease *et al*., 1999; Innala *et al*., 2014).

In the study of mangnus *et al.* (2015), it was published that the severity of RA is associated with age and the observations of this study was supported through increase concentration of powerful inflammatory marker (CRP) in patient with RA.

The yield data confirm that patient's men become improved more than women all age groups that treated with anti-inflammatory drugs, and there for the incidence and severity of RA is age and sex dependent (Andersson *et al.,* 2021).

Another recent study is designed to investigate whether there are differences in radiological characteristics of ageing individual with RA and compare with young individual, the study determined inflammatory mediators such as CRP, Ferritin, and Fibrinogen develop systemic inflammatory process than of elderly individual (Ke *et al.,*2021).

5.3 The Effect of BMI on subjects with RA:

It is well described that obese human have increase inflammatory markers without clinical signs or symptoms, these inflammatory markers increase risk factor in developing of inflammatory disease in particular RA (Panagiotakes *et al.,*2005).

Adiponectin is adipose biomarkers that secreted from adipocytes and act in different part of the body, The levels of these markers is corrected positively with BMI and they are implicated with incidence and development of RA (Chen *et al.,* 2013).

Excess adipose tissue is source in secretion of pro-inflammatory and inflammatory mediators and chemokines such us TNF-α, IL, IL6, IL8, IL10, TGF-β, haptoglobin and serum amyloid (Touyz, 2005; Versini *et al.,* 2014).

It is well known that role of obesity in RA is strong among women rather than men, The implication of obesity in incidence of chronic diseases return to excessive systemic inflammatory processes, this concept is consistent with physiological issue that reveals adipose tissue is an active tissue because of its ability to secret several cytokines called adipokines which contribute in many immunological processes that are negative or positive within the body (Mirpourian *et al.,*2014).

The previous findings are consistent with the present study especially, the epidemiological studies explained there is a positive relationship and risk of RA incidence, and this relevant is stronger in men and women who have high BMI (Feng *et al.,* 2016).

Abnormal accumulation of adipose tissue in patients who have heavy weights is associated with activation of inflammatory and autoimmune processes.

Recent study explored that the activity of RA is associated linearly with gender and BMI, the women is more acceptable to disease than of men, Since, the high level of BMI is significant related with increase pro-inflammatory state and this relation appear clearly with increased levels of C-reaction protein in obese human (Igbal *et al.,* 2020).

It is not surprising that obesity is physiological problem when become over 30 kg/m^3, it is associated with several chronic disease such as autoimmune, diabetes mellitus, and arthritis (Giles *et al.,* 2021).

Data obtained from the present study figure (3), which established by Elalouf *et al.,* (2021) described the association and effects of obesity on tumor necrosis Factor α in patients who have RA.

5.4 Rheumatoid factor:

The results of current study that showed in tables for the gender (4), age groups (6), and BMI (7) indicated a significant (P≤ 0.05) increase in the value of RF in RA patients, also and the result of the ROC / AUC =0.675 showed that RF could concerned as risk factor for RA development. Moreover, the results of correlations analysis showed a significant (P≤ 0.05) positive correlation between RF and platelets, CRP and RF, ESR and RF.

During of pre-clinical stage (the disease dose not onset) some auto-antibodies, pro-inflammatory cytokines, and adipokines can be developed gradually in patients that have susceptible to auto-immune disease including RA and this is related with BMI (obese) having high titer of auto-antibodies and pro-inflammatory cytokines, of those auto-antibodies is RF (Deane *et al*.,2010).

Rheumatoid factor is a group of antibodies that act against the FC structure of immune globulin, this factor represents the principal test in diagnosis of RA patients it is presented in autoimmune disease of other tissue of the body (Ingegnoli *et al.,* 2013).

Previous study has also been highlights on the association between RA and BMI and this study concluded increase RA incidence and titer of RF in those obese patients (Qin *et al*., 2015).

Another study was conducted to show how obesity affect RA and the relation with incidence of RA, and then concluded that obesity induces of RA, and increase levels of RF (Gharbia *et al*., 2018).

It is well documented that RF is not found only in patient complained from RA but it had been diagnosed in wide types of autoimmune diseases, it also found in minimum levels in young and elderly individual (Jandu *et al*.,2020).

Women appear more susceptible for autoimmune diseases in particular RA, it is well found that incidence of RA is affected by sex differences, that is, women have more affected than men especially women that have high BMI (Turesson *et al*., 2016). Recent study indicated the role of sexual hormone in development of autoimmune diseases and pro-inflammatory cytokines, it is well established that

estrogen evokes immune system in contrast to that of testosterone (Harding and Heaton, 2022).

5.5 The relationship of WBCs with RA:

The results of current study that showed in tables for the gender (3) and age groups (5) indicated a significant (P≤ 0.05) increase in the value of WBCs in RA patients, the results of correlations analysis showed a significant positive correlation between WBCs and platelets, WBCs and GM-CSF, WBCs and CRP, WBCs and TNF-α, WBCs and RF.

The present data of this study are consistent with recent study which established by Stepniak *et al*. (2020), which confirmed that levels of WBCs are significantly higher in patient with RA, as well as, this study found that neutrophil – to – lymphocyte and platelet to lymphocyte ratios were significantly higher in those patients with RA as well as lymphocyte to monocytes ratios have been recorded significant increase, these findings are also associated with elevation of ESR and CRP.

More ever, evidence proved the strong relationship between RA and neutrophil to lymphocyte ratio and platelet to lymphocyte ratio (Koiwa *et al*., 2016).

Most if not all inflammation especially those chronic inflammations are accompanied with increase production of several cytokines, antibodies, and growth factors, all these factors can affect hematopoietic centers (Fawzy *et al*., 2017).

From physiological point view, it is well known that the components of immune system include lymphocytes, monocytes, neutrophils, and platelets, these components have a significant function during chronic inflammation especially RA (Zhang *et al*.,2018).

Both ESR and CRP are considering the common inflammatory markers in acute and chronic disease, but those parameters do not give the localization of disease especially in RA (Stepniak *et al*., 2020).

Following study has also been that evaluated of CRP, ESR, leukocyte, and leukocyte esterase in blood of those patients with RA (Zhang *et al*., 2020).

There is clear evidence refers to the increase development and progressive of chronic disease especially RA is associated with large accumulation and stimulation of leukocyte (Manning *et al*., 2021).

Choe and Kim (2022) assess the hematological parameters in RA patients, the blood parameters are affected with systemic inflammation and autoimmune inflammation their observations indicate that CRP, ESR, neutrophil to lymphocyte, platelet to lymphocyte, and neutrophil to hemoglobin were markedly increased in those patients with RA.

5.6. The relationship between platelets, RBCs, and HB with RA:

The results of current study that showed in tables for the gender (3), and age groups (5) indicated different significant in value of platelets, RBCs, and HB and the study showed a significant positive correlation between GM-CSF and platelets, TNF-α and platelets, WBCs and platelets, lymphocytes and platelets, RF and platelets, CRP and platelets, ESR and platelets, RBCs and fibrinogen.

The inflammatory process occurring in RA can affect erythropoiesis process, it was found that two reasons implicated in incidence of anemia associated with RA, the first one involved that gene encoding erythropoietin may be suppressed by actions of pro-and

inflammatory mediator including TNFα, IL-1, IL-6, and CRP (Ferrucci *et al*., 2005)

As well as, the pro-inflammatory mediators especially interleukin-6 exerts suppression effects on erythropoiesis process in bone marrow causing anemia affecting patients with RA (Nikolaisen *et al*., 2008).

Levels of RBCs are influenced in patient with RA because of increased levels of pro-and inflammatory proteins as well as oxidative stress (Staron *et al*., 2012).

This appears through p-selection and other adhesion molecules located on platelets, these molecules involved in interactions occurring among platelets and leukocytes including neutrophils, monocytes, and T-cells (Koupenova *et al*., 2018).

the second, the pro inflammatory mediators can counteract the stimulatory effects of erythropoietin on progenitor cells in bone marrow (Grigorakaki *et al*., 2011).

It is suggested that platelets act as a pathway to deliver high number of inflammatory cytokines, growth factor, and chemokines to maintain and sustain autoimmune inflammatory (Habets *et al*., 2013).

It is documented that platelets number tend to be increased in RA patient with high activity of diseases compared to those moderate level of RA (Talukdar *et al*., 2017).

It has been demonstrated that platelets exert pivotal roles in inflammatory process occurring within the body, since they responsible for regulation and activation of white blood cells by releasing various inflammatory cytokines (Bakogiannis *et al*., 2019).

It is well established that blood cells, in particular, RBCs, and platelets have essential roles in acute and chronic diseases especially RA, the finding which indicate that patient with RA have high number of blood platelets and this is associated with significant lowering in the levels of HB and RBCs therefore the facts concluded from recent published study explained that these above-mentioned parameters can be employed to differential active RA and inactive RA (Xu *et al*., 2022).

5.7 The role of CRP on RA patients:

The results of current study that showed in tables for the gender (4), age groups (6), and BMI (7) indicated a significant ($P \leq 0.05$) increase in the value of CRP in RA patients, also and the result of the ROC / AUC =0.675. The results of correlations analysis significant positive correlation between CRP and platelets, CRP and RF, ESR and CRP.

There are several inflammatory cytokines take part in most of joint inflammation, it is well understanding that increased levels of pro-inflammatory cytokines are associated with damage of joint because of stimulation of proliferation of tissue within joint (Fox,2000).

Moreover, increase levels of pro-inflammatory markers such as IL-1β, TNF-α, and IL-6, They are together act to induce hepatic synthesis of CRP as well as from the extra hepatic tissue including adipose tissues, monocytes, lymphocytes, neurons, and smooth muscle cells of vasculature (Calabro *et al.,* 2005).

Previous study demonstrated and described that CRP is not only markers for severity of disease but also it determines the prognosis of disease (Emery et al., 2007).

In fact, the systemic inflammation of the body is resulted from pro-inflammatory pathway mediator, of these pro-inflammatory cytokines including TNF-α, IL-1β, and IL-6 (MacInne and Schett, 2011).

Concerning the relationship and effects of age and obesity with BMI, it is well noted that age and obesity evoke production profound amount of CRP, the peak of CRP is demonstrated after age 50 years old and subsequent elevated with advanced ages (Kawamoto, 2013).

Study of Kim *et al.*, (2015), had also determined the levels of CRP in synovial fluids and and serum, and this study concluded increase levels of CRP in both serum and synovial fluids and its levels were correlated positively with IL-6 levels, so that CRP may be play a key role in damage of bone and joint structures by proliferation and activation of osteoclasts by recruit more progenitors' cells of osteoclasts.

Another study confirmed a positive relationship between levels of CRP and BMI (Qin et al., 2017).

The data obtained from the present study were consistent with previous study which indicated that CRP represents regulator of inflammatory conditions in those patients complained from RA and its levels is accompanied with incidence and comorbidities RA and its levels remain elevated in RA (Pope and Choy, 2021).

Furthermore, the following study established by Dessie *et al*. (2021), to evaluate the levels of CRP in RA patients and this study confirmed that patients with RA having higher levels of CRP as well as its values associated with severity of disease and high grade of systemic inflammation.

Following inflammatory reactions, IL-6 acts to stimulate production acute phase proteins in particular of general inflammation in patients with RA (Dessie *et al.*, 2021).

5.8 Fibrinogen in rheumatoid arthritis:

The results of current study that showed in tables for the gender (4), age groups (6), and BMI (7) indicated a significant (P≤ 0.05) increase in the value of CRP in RA patients, also and the results of ROC / AUC =0.775, while the study showed a significant positive correlation between ESR and fibrinogen, RBCs and fibrinogen.

The previous study confirmed that FIB is one of the acute phase proteins likes CRP, and its levels are elevated during inflammation, tissue injury, and thrombosis, it is not surprising that this clotting factor represent bridge among these diseases that are commonly occurring in RA (Symmons, 2002).

Fibrinogen is clotting factor (FIB) of coagulation cascade in addition, it has ability to form link between other mechanism such as thrombosis, proliferation, and inflammatory processes, therefore, it is well documented that levels of plasma fibrinogen are significantly higher in those patients complained from RA compared to those healthy subjects (Sharma *et al.,*2018).

As long as RA is autoimmune disease associated with systemic inflammation, there is no doubt that inflammatory mediators can be overshot at a higher level, of these mediator fibrinogen, TNF-α CRP and IL-6 (Rooney *et al.*, 2011).

Previous document confirmed increased hepatic production of FIB during acute phase response to inflammation, it appears clear that RA

which associated with acute and chronic inflammatory processes enhance elevation of synthesis and production of FIB (Yidirim *et al.*, 2004).

A recent study investigated and assessed whether there is a relationship between RA and coagulation markers such as fibrinogen, D-dimer, and fibrin degradation product, the authors of this study recognized that patient with RA have significant higher levels of all coagulation markers and ROC analysis of these markers indicate that AUC of D-dimer marker recorded significant elevation than other coagulation markers (Xue *et al.*, 2021).

In fact, and depending on pathological mechanisms, it obviously demonstrated that coagulation components co-operate with immune system to perform several functions (Arneyh, 2019).

Most if not all, different immune pathogenic inflammatory issues have ability to amplification of coagulation factor in clotting pathways and enhance thrombosis, the powerful activity of fibrinogen in pro-inflammatory signaling pathway, its upstream inflammatory responses and this process is achieved by interactions of fibrinogen with ligand receptors that are localized on immune cells (Sokolove *et al.,* 2011; Beinsberer *et al.*, 2014).

It is well documented that pro-inflammatory mediators can upstream activation of clotting factors associated with drop activities of anticoagulant markers (Wang, 2018).

FIB factor represents a key factor in blood clotting pathway and can be deposited in joint of patient with RA (So, 2003).

5.9 Tumor necrosis factor in RA:

The results of current study that showed in tables for the gender (4), age groups (6), and BMI (7) indicated an increase significant in value of RF, also and the results of the ROC / AUC =0.865. The results of correlations analysis a significant positive correlation between TNF-α and GM-CSF, TNF-α and WBCs, TNF-α and lymphocytes, TNF-α and granulocytes, TNF-α and RF.

TNF-α is implicated in induction of other inflammatory mediators such as GM-CSF, IL-6, IL-1, and IL-8, the stimulatory effects of TNF-α to those inflammatory markers appears by autocrine and paracrine fashion (Bulter *et al*., 1995).

The pathophysiological changes occurring in sites of RA are involved infiltration of inflammatory cells and increased vascular exudation, among recruited cells in inflamed area, are CD4 + T-cells, these cells responsible for secretion several inflammatory cytokines such as TNF-α, IL-1, and IL-6, the TNF-α is powerful inflammatory cytokines in auto immune responses of human with RA (Deon *et al*., 2001).

Substantial evidence and documents refer to that TNF-α is synthesized and secreted by several cells such as T, B, Macrophage, Monocytes, and FIB, and this pro-inflammatory marker considers the essential cytokines that induces inflammation of patients with RA (Choy and Panayi, 2001).

Vasanthi *et al*. (2007) who confirmed that there is a genetic susceptivity to have RA and environmental interactions, and explained a genetic complex mechanisms and environmental factors can individual affected with auto immune diseases especially RA.

On the basis of pathophysiology, the TNF (pro-inflammatory cytokines) exerts substantial effects during systemic inflammatory processes and those occurring in RA, to explain these effects, the experimental studies involved using anti-TNF inhibitors lead to prevent and arrest systemic and loss of bone texture (also called bone mineral density) and improvement fracture healing (Manara and Sinigalia, 2015).

Another study described the relationship between T-cell and TNF-α especially T-regulator and T-effector cells in those patients with RA, and the substantial efforts were carried out to inhibit and prevent progression of RA by application of several therapies, of those, inhibitors of TNF-α therapies since TNF-α inhibitors can affect T-regulatory cells and then controlling RA (Farrugia and Baron,2016).

Another previous study is aimed to explain the role of TNF-α in insulin resistance of patients with RA and then this study concluded that TNF-α is positively correlated with insulin resistance in patients complained from RA as well as, it has significant roles in development of atherosclerosis (Hussein *et al*., 2017).

With age, the homeostatic mechanisms become disturbed and fluctuated, the inflammatory responses become elevated with old age and there are gender differences, pro-inflammatory and inflammatory mediators are significantly higher in old age, but their levels are much higher than of men (Mattos *et al*., 2019).

Study of Dey *et al*., (2020) concluded that increased BMI is associated with increased incidence of RA and attenuate to responses to inhibitory drugs to TNF-α.

Further recent study concluded that there is an interference between TNF-α inhibitors and BMI, that is, administration of TNF-α inhibitors can increase BMI (Patsalos *et al*., 2020).

Generally, it is previously known that TNF-α had been diagnosed as a factor that has ability to cause necrosis of tumor mass, as well as this factor is described to has additional interactions in pathological conditions of that autoimmune diseases, it is binded with specific two receptors that can triggers signal transduction cascades within the cell and this signal leads to different cellular functions including differentiation, development, and survival, however, excessive stimulation of TNF-α signaling pathways can be associated with chronic inflammatory diseases that eventually causing autoimmune diseases in particular RA (Jang *et al*., 2021).

5.10 GM-CSF in rheumatoid arthritis:

The results of current study that showed in tables for the gender (4), age groups (6), and BMI (7) indicated an increase significant in value of GM-CSF and the result of the ROC / AUC =0.914. The results of correlations analysis significant positive correlation between GM-CSF and WBCs, GM-CSF and lymphocytes, GM-CSF and granulocytes, GM-CSF and RF.

From physiological point view the GM-CSF functions as a hemopioetic growth factor necessary to proliferation and development of myeloid progenitor cells within bone marrow as well as acts as inflammatory mediator, and its down regulation is accompanied with profound results on initiation and development of several inflammatory processes such as arthritis, and the inhibition of GM-CSF can lead to positive effects of RA patients (Feetwood *et al*., 2007; Hamilton, 2008).

GM-CSF is well documented to has role in development and progression of inflammatory processes which associated with RA and many suggestions indicate that blocked the GM-CSF can improve the RA status, in this contex, it clearly recognized that loss of GM-CSF level leads to decrease of synovitis and matrix metalloproteinase that mediates neopitope expression (Cook *et al*.,2012).

The present data were supported with clear emerging evidence suggested that GM-CSF has pro-inflammatory functions especially in development and progression of autoimmune diseases (Codarri *et al*., 2011).

Another study of shiomi *et al., (*2016), also proved that GM-CSF has pro-inflammatory effects to the pathogenesis of T-helper 17 cells and development of autoimmune that mediated by T-helper 17.

In addition, there is a relation between GM-CSF contributes with inflammatory processes and those of RA, it is well found that there is profound amount of GM-CSF in serum and synovial fluids of patients with RA (Burmester *et al*., 2017).

There is a fact established that the expression of GM-CSF on peripheral lymphocytes in patients affected with RA and this expression is modulated by using drug affecting RA through determination of CRP and also established that expression of GM-CSF in RA did not correlate to duration of illness and administration of anti-TNF alpha caused drop of GM-CSF in both B and T effector cells (Makris *et al*., 2018).

The differentiation and development of hematopoietic cells that called myeloid is a mechanism that depends on special cytokines called granulocyte _ monocyte colony stimulating factor that abbreviated GMCSF, this factors also to act as pro-inflammatory mediators and exerts

in pathogenetic mechanism of RA, these invitro studies established that GM-CSF has ability to activate macrophages, in particular, in locations of chronic inflammations, this is markedly diagnosed increase of GM-CSF concentration within affected synovial fluids of individuals with RA in matching with those healthy individuals, furthermore, the specify of GM-SCF in perception of intensive pain had also been recognized therefor, administration of antibodies that block GM-CSF should be used (Crotti *et al.*, 2019).

It is well found that evidence confirms GM-CSF receptors are associated with severity of several inflammatory diseases especially those autoimmune diseases of those rheumatoid arthritis had been recognized (Lee *et al*., 2020).

5.11. The correlation analysis among studied parameters of patients affected with RA:

The present study finding showed that ESR had a significant positive correlation with TNF-α, GM-CSF, PLT, WBCs, granulocytes, fibrinogen, RF, CRP, while it had a significant negative correlation with lymphocytes. The positive correlation between ESR and TNF-α previous study found that ESR is significantly and positively correlated with markers of inflammatory immune activation. These correlations, and the prognostic results for ESR, support the hypothesis that inflammatory cytokines (particularly TNF receptors) (Deswal *et al*.,1999).

The positive correlation between ESR and platelet previous studies found ESR evaluation, however, is recommended for chronic inflammatory conditions, including bone-associated inflammatory disease. Increased concentration of fibrinogen, clotting factor and alpha globulins during the pathologic states leads to variation in ESR (Milovanovic *et al*.,2004).

These markers have been reported to indicate the severity of diseases like rheumatoid arthritis, polymyalgia rheumatica, temporal arteritis and systemic lupus erythematosus (Litao *et al*.,2014).

Thrombocytosis (increased platelet count) is reported in children during chronic inflammation, infection, iron-deficiency anaemia, tissue injury and malignancies (Chiarello *et al*., 2011).

At the site of inflammation, platelet release mediators such as interferon γ, IL-2 and chemokine ligands elevate the inflammatory process (Stokes *et al*.,2012).

The positive correlation between ESR and CRP though the explanation of these changes in CBC and ESR of patients is not known but literature review suggests that increased ESR may be due to chronic inflammatory response with polyclonal increase in immunoglobulins (Hamza and Bashir,2016).

The positive correlation between ESR and fibrinogen the relationship between the erythrocyte sedimentation rate (ESR) and plasma proteins was studied within homogenous clinical material and in in vitro models. In acute phase reactions, fibrinogen was the likely cause of the ESR-elevation, but there were significant associations between the ESR and the concentrations of α1-antitrypsin, C3, haptoglobin and albumin. In chronic diseases, the ESR-elevation was probably caused by fibrinogen, mono- or polyclonal increase of IgG, IgA, IgM alone or in combinations. In multiple myeloma of the IgG and IgA subtypes, significant correlations were found between the ESR and the monoclonal proteins or between the ESR and the percentage of plasma cells in bone marrow (Ingebrigt ana Hans, 2011).

The positive correlation between ESR and RA the quantitative usefulness of ESR versus CRP has been evaluated in many studies with no clear consensus (Wolfe *et al*.,1994). ESR and/or CRP are part of the American College of Rheumatology (ACR) core data set for measuring disease activity in RA and have been used in clinical trials as the main laboratory marker of disease activity in RA (Felson *et al*.,1995).

Laboratory tests such as the erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) have been an integral part of the clincians repertoire for many years, used as markers of inflammation, although there is still no clear consensus on when to use one, the other, or both. CRP has recently become the more preferred serological marker for evaluating acute dis- ease activity (Skogh *et al*.,2003) .

In addition, disease activity score (DAS) and its derivatives use ESR or CRP as part of their score and as such they have found increased use and discussion regarding their role in disease activity assessment. Because of t`he way these indices are calculated, ESR and CRP may play a disproportionately significant role in the overall score (Gardiner *et al*.,2005).

The results of GM-CSF correlation analysis showed a significant positive correlation with PLT, WBC, granulocytes, fibrinogen, CRP, RF and TNF, while it had a significant negative correlation with lymphocytes.

The positive correlation between GM-CSF and CBC Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a hematopoietic growth factor known to promote the proliferation and differentiation of precursors of granulocytes and monocytes. GM-CSF at standard doses (125-500 micrograms/m2) alleviates neutropenia secondary to cytotoxic

chemotherapy, myelodysplastic syndromes, and aplastic anemia, but has minimal effect on anemia or thrombocytopenia (Vesole *et al*.,1994).

Granulocyte macrophage colony-stimulating factor (GM-CSF) is one of the early upstream mediators and orchestra- tors of this hyperinflammatory immune response. Increasing levels of circulating GM-CSF have been associated with progression and increasing severity of the disease (Thawaites *et al*.,2021).

The positive correlation between GM-CSF and CRP Like GM-CSF, CRP levels directly correlate disease severity.1 Increases in CRP are driven by elevations of IL-6 during the hyperinflammatory response following infection (Weinhold *et al*.,1997).

Data finding positive correlation between GM-CSF and RF Early studies measuring cytokines in synovial fluid and blood from patients with RA showed increased GM-CSF levels, as well as increased expression of GM-CSFR, in inflamed synovial tissue Administration of GM-CSF to RA patients led to disease flares a genome-wide association study revealed that mutations in CSF2 (Hazenberg *et al*., 1989).

The gene that encodes (GM-CSF) contribute to genetic susceptibility in RA (Based in part on the priming of blood monocytes with GM-CSF, it was recently suggested that GM-CSF neutralization be considered as a potential therapeutic approach for the treatment of ankylosing spondylitis (Shi *et al*.,2020).

The contribution of GM-CSF to the pathogenesis of experimental inflammatory arthritis is well documented in the literature. GM-CSF-deficient mice fail to develop arthritis and associated pain in several inflammatory arthritis models, including collagen-induced arthritis (CIA),

antigen-induced arthritis (AIA), zymosan-induced arthritis (ZIA) and K/BxN serum-transfer arthritis (STA) (Lee *et al*.,2019).

The positive correlation between GM-CSF and TNF-α in vitro studies, mainly with monocyte(s)/macrophages, have shown that GM-CSF can upregulate TNF expression, at least at the mRNA level, while inversely TNF can induce GM-CSF formation and secretion in a number of tissue cell populations, such as fibroblasts, endothelial cells, and chondrocytes (Leizer *et al*.,1990).

In line with the latter type of studies, TNF neutralization of ex vivo RA synovial cell cultures led to reduced GM-CSF levels these findings gave rise to the concepts that TNF and GM-CSF may, in fact, be operating within a cytokine hierarchy (or a cytokine network, the latter concept perhaps helping to explain the chronicity of certain inflammatory lesions (Hamilton *et al*.,2002).

The results of TNF-α correlation analysis showed a significant positive correlation with PLT, WBC, granulocytes, fibrinogen, CRP, RF and GM-CSF, while it had a significant negative correlation with lymphocytes.

The positive correlation between TNF-α and platelet the proinflammatory cytokine tumor necrosis factor-α (TNF-α) is up-regulated in patients and seems to be involved in the pathophysiology of this disease (Aukrust *et al*.,1999). Because previous in vitro studies have demonstrated that TNF-α may promote platelet aggregation, we investigated whether circulating levels of TNF-α could have a role in eliciting platelet aggregation in patients with HF (Soslau *et al*.,1997).

The correlation between TNF-α, WBC and Lymphocytes however, these discovered that some TNFα inhibitors studied in the present study,

such as ADA, may keep the total WBC count significantly above the level in healthy donors. In addition, we detected a higher level of the total lymphocyte counts in GOL and ADA groups.

This might be related to the fact that TNFα has a bi-functional role in the growth of hematopoietic stem and progenitor cells and its inhibition is associated with the onset of lymphoproliferative disorders and pathological abnormalities interestingly, while the therapeutically mediated decrease of the lymphocyte counts is generally correlated to RA activity (Autrel *et al*.,2014).

The positive correlation between TNF-α and ESR serum TNF-α was found to be significantly associated with DAS28-ESR in early RA patients. Patients with a low disease activity score had a significantly lower concentration of serum TNF-α than in the subgroups with moderate and high disease activity. Similar to our findings were the observations made by other studies conducted on newly diagnosed RA patients (Tetta *et al*.,1990).

These findings show that TNF-α is expressed earlier in the pathologic events in DMARD naïve RA patients and can therefore be an important marker for the evaluation of disease activity previously in a study patient with high disease activity scores were reported to have significantly high concentrations of TNF-α in blood and synovial fluid as well however, in our study, synovial fluid samples were not taken (Petrovic-Rackov,2006).

The positive correlation between TNF-α and CRP increases slightly with age, pregnancy, various kinds of mild inflammation and viral infections (10–40 mg/L). Higher levels are observed in severe

bacterial infections. In contrast to CRP, TNF-α is an early indicator of inflammation (Yousef *et al.*,2010).

Arising from both infectious and non-infectious causes. TNF-α plays an important role in pathophysiology of many inflammatory disorders, whether due to infection or due to non-infectious causes.

Therefore, interrogated the diagnostic and prognostic value of both CRP and TNF-α in enhancing the clinical distinction between 1) critically ill patients with and without a clinically defined SIRS, and 2) with and without infection (Schrag *et al.*,2012).

The positive correlation between RBC and Fibrinogen Two potential RBC receptors have been implicated in fibrinogen binding. Fibrinogen–RBC interactions can be inhibited by the integrin-blocking molecule eptifibatide and are not supported by RBCs lacking β3 isolated from patients with Glanzmann thrombasthenia (Carvalho *et al.*,2010), Implicating β3 or a β3-like molecule on the RBC surface. However, that study did not rule out the possibility that RBC-bound platelets mediate this interaction. Fibrinogen–RBC interactions can also be blocked with an antibody against the integrin-associated protein CD47, As CD47 was originally identified for its interaction with αvβ3, αIIbβ3, and α2β1 integrins, it is possible that the RBC binding site comprises a complex with both of these molecules. It is interesting to speculate that interactions between one or both of these receptors and fibrinogen contribute to RBC incorporation in venous thrombi or that blocking these interactions may reduce blood viscosity, and consequently VT risk (De Oliveira *et al.*,2012).

The positive correlation between RF and CRP, higher CRP levels are associated with greater RA disease activity based on the core components of the 28-joint Disease Activity Score (DAS28) (Cylwik *et al*.,2010).

Individual aspects of disease activity, such as swollen joint count, and patient-reported measures, including functional status (Health Assessment Questionnaire score), morning stiffness fatigue, and pain, have also been associated with CRP (Madsen *et al*.,2016).

Indeed, CRP levels are widely used for monitoring systemic inflammation and disease activity in RA. CRP level is a component of several composite disease activity measures: DAS28-CRP, SDAI, and American College of Rheumatology (ACR) and European League Against Rheumatism (EULAR) definitions of remission (England *et al*.,2019).

Yet, the usefulness of CRP testing as a routine measure of RA disease activity is not universal due to the substantial proportion of treated patients who experience flares in their RA but still have normal CRP levels. In fact, as RA clinical trials often specify elevated CRP (for example ≥6 mg/L) as an eligibility criterion, patients with active RA but without elevated inflammatory markers are commonly excluded (Genovese *et al*., 2015).

The present study finding positive correlation between fibrinogen and RF, FIB is an important glycoprotein present in human blood plasma and it is involved in many physiological processes such as wound healing, tissue regeneration and regulation of inflammatory responses moreover, FIB has been shown as an important determinant of inflammatory arthritis through its effects on proinflammatory pathways

such as NF-κB signaling it has been demonstrated that the multiple regions of the FIB molecule could bind to CD11b/CD18 integrin receptor expressed on cells associated with the innate immune system such as circulating monocytes, tissue-specific macrophages, and so on (Ugarova *et al*.,2001).

Emerging evidence from in vitro studies and animal models implicated FIB in the pathogenesis of RA. For example, an in vitro study showed that treatment of synovial fibroblasts with FIB was followed by IL-8 secretion and upregulation of ICAM-1 expression, resulting in increased adhesiveness of lymphocytes (Lishko *et al*.,2004)

Another study using collagen-induced arthritis model revealed that mice lacking FIB or missing only CD11b/CD18 integrin receptor-binding domain had fewer arthritic joints compared with the control mice (Liu *et al*.,2000)

A recent study showed that circulating levels of FIB are elevated in RA and correlated with markers of inflammation (Flick *et al*.,2007).

The finding of a significant association between plasma levels of FIB and RA disease activity may be a consequence of increased synovialis and articular cartilage injury accompanying with increased FIB concentration in RA patients (Rooney *et al*.,2011).

The positive correlation between RF and CBC emerging evidence indicates that hematological parameters, in addition to white blood cells (WBCs), including hemoglobin (Hb) and platelets, are associated with chronic inflammation (Ganzet al.,2019). Some authors have found lower Hb levels and mean platelet volumes (MPVs) but higher neutrophil-to-lymphocyte ratios (NLRs) and platelet counts (Işık *et al*.,2014).

In RA patients with high disease activity than in those with disease remission. One prior study also reported high NLR as a predictor of RA relapse however, such a study was limited by its small sample size and showed only a modest predictive performance (Chandrashekara *et al.*,2015).

Considering that the complete blood count (CBC) is a simple and inexpensive laboratory test, using parameters of CBC might be a useful adjunctive tool in assessing RA disease activity. To date, no studies have investigated whether multiple hematological parameters could be incorporated into a prediction model for identifying patients with RA remission. The objective of this study was to evaluate the levels of hematological parameters, including Hb, red blood cell (RBC), WBC, and platelet components, among RA patients with different disease activity levels. The performance of combined hematological parameters in predicting the remission phase of RA was also determined.

Conclusions

Form the present study, there are many facts appear clearly as follow- :

1. Increase incidence of RA proportionate with ages and high BMI.

2. Women appear more susceptible to affected with RA than of men and this is because of sexual hormones differences.

3. Rheumatoid arthritis is accompanied with anemia of patients with RA.

4. Increase of WBCs and other inflammatory component in RA is prominent evidence for systemic inflammation

Recommendation

1. Studding the effects of hormone replacement therapy on incidence and progressive of RA

2. Relationship between inflammatory Markers and oxidative stress in RA patients

3. Molecular study on Rf encoding genes and other cytokine such as IL1, MCF and growth factor to explain their role in incidence of RA

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الخلاصة

ان مرض التهاب المفاصل الرثوي يعد احد اصناف الامراض المناعية والذي يتميز بالتهاب معظم مفاصل العظام وبصورة متوالية . صممت الدراسة الحالية لتقييم بعض المعايير الدموية وبعض المؤشرات الحياتية في مرضى التهاب العامل الرثوي من الرجال و النساء.

كان الافراد الذين شاركوا في الدراسة الحالية 80 شخصاً من المرضى والاصحاء وقد تم تصنيفهم وفقا" للجنس الى فئتين فرعيتين , الاولى تضمنت 40 ذكرا" تم تقسيمهم الى 20منهم مرضى و 20 اصحاء كمجموعة سيطرة اما عند النساء فكانت 40 منهن 20 مصابات بألتهاب المفاصل الروماتودي و 20 أصحاء كمجموعة سيطرة تراوحت جميع اعمار اشخاص الدراسة بين 40 الى 79 سنة وقد قسموا الى اربعة مجامع طبقا الى اعمارهم المجموعة الاول (40 – 49) المجموعة الثانية (50 - 59) سنة والمجموعة الثالثة (60- 69) المجموعة الرابعة (70-79) سنة .

اشارت البيانات الديموغرافية التي تم الحصول عليها من هذه الدراسة بان النساء اكثر عرضة للمرض من الرجال حيث كانت نسبة النساء المصابات 60% اعلى من الرجال المصابين حيث شكلوا 40% من مجموع المرضى الكلي.

بالاضافة الى ذلك وفان توزيع المرض طبقاً إلى الفئات العمرية فقد شكلت النسب التالية المجموعة الاولى 30%, المجموعة الثانية 30% المجموعة الثالثة 25% ، والمجموعة الرابعة 15% على التوالي .

بالاضافة الى ذلك فان تأثيرمؤشر كتلة الجسم BMI أعلى عند نسبة المصابين فقط لوحظ بان المرضى ذات BMI 18 كغم/م ^{2} 20% أقل من المرضى ذات BMI 25- 29.9 كغم/م 2 30% أقل من المرضى الذين لديهم BMI يترواح بين 39.9 30-كغم/م. 45% .

اكد التغيرات الحاصلة في المعايير الدموية ارتفاعا معنويا P<0.01 في مستوى العدد الكلي لكريات الدم البيضاء WBCs ومعدل ترسيب كريات الدم الحمر ESR لدى

أ

المرضى المصابين بالروماتيزم من الرجال والنساء عند مقارنتهم مع الاشخاص الاصحاء في حين لوحظ انخفاضا معنويا P<0.05 في عدد كريات الدم الحمر RBCs وتركيز الهيموغلوبين والنسبة المئوية للخلايا اللمفاوية لدى مرضى الروماتيزم بالمقارنة مع الاشخاص الاصحاء.

حققت معاملات الارتباط Correlation coefficients بين المعايير الدموية المدروسة ما يلي حيث لوحظ وجود ارتباط معنوي ايجابي (r=0.52,sig=0.001) بين مستوى ESR والصفيحات في مرضى الروماتيزم ,كذلك لوحظ ارتباط معنوي ايجابي (r=0.42,sig 0,005) بين ESR والنسبة المئوية للخلايا الحبيبية Granulocyte وبنفس الوقت حققت قيم ESR ارتباطا معنويا ايجابيا (r=0.42,sig=0.007) مع قيم WBCs لكن لوجظ حصول ارتباط معنوي سلبي (r=-0.43,sig=0.006) بين قيم ESR والنسبة المئوية Lymphocyte عند الاشخاص المصابين بالتهاب المفاصل الرثوي.

وبخصوص المعايير الحيوية Biomarkers والتي شملت عامل الروماتيزم RF وبروتين الطور الحاد C-RP و الفايبرينوجين وعامل التنخر الورمي TNFα والعامل المحفز لمستعمرات الخلايا الحبيبية والاحادية GM-CSF فقد حققت ارتفاعا معنويا P<0,05 في جميع الفئات المدروسة من مرضى المصابين بالتهاب المفاصل الرثوي عند المقارنة بالاشخاص الاصحاء.

بينت معاملات الارتباط للمعايير الحيوية المدروسة ما يلي اذ وجدت حصول ارتباط معنوي ايجابي بين TNF-α و ESR , ESR و GM-CSF , ESR و Fibrinogen , ESR و RF , ESR و CRP و ESR , GM-CSF و Platelets , GM-CSF و WBCs , GM-CSF و Fibrinogen , GM-CSF و RF , GM-CSF , GM-CSF و CRP , GM-CSF و TNF-α , RF و TNF-α , CRP و TNF-α, Fibrinogen و RBCs , WBCs و CRP , Fibrinogen و Platelets , RF و Platelets, CRP و Platelets , RF و WBCs, CRP و RF لدى جميع المرضى المصابين بالتهاب المفاصل الرثوي.

من الدراسة الحالية يمكن استنتاج ان التهاب المفاصل الروماتويدي مرتبط بتطور فقر الدم anemia, وذلك بسبب الالتهاب الجهازي و الاجهاد التأكسدي , بالاضافة الى زيادة المؤشرات الحيوية .

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