Assessment of the Serological Markers and Hematological Parameters among Sudanese Patients with Rheumatoid Arthritis

A thesis submitted in fulfillment for the requirement of the M.Sc. degree in Medical laboratory sciences (medical Hematology)

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Dedication

To the greatest lights in life:

My Family (source of tender love)

My sweetest son (Maaz) and grandchildren (Mazin, Hala and to my lovely twins Mohamed / Ahmed)

My friends (source of interest in life)

To all patients suffering from Rheumatoid Arthritis (source of hope to solve problems)
Acknowledgements

I would like to express my greatest gratitude to Dr. Hiba BadrEldin Khalil Ahmed, my supervisor, (Alneelain University, Faculty of Medical Laboratory Sciences) for supervising this work to layout.

I would like to express my deep thanks to Rheumatologist Dr. Hamza Khidir Maki for his providing patients and clinical diagnosis in this work.

Great thanks to Dr. Salih AbdElgadir Elmahdi (Ribat University, Faculty of Medical Laboratory sciences), for his supporting my ideas as a first reference in Sudan studied in RA patients.
My thanks are extended to my lovely son Maaz Ahmed Hashim for helping me in Computer work.

Last but not least, I will never forget the unlimited assistance, support and care I got from all my family, and so my deepest thanks go to them.

List of contents

Dedication................................................................................................................I
Acknowledgment....................................................................................................II
List of content........................................................................................................III
List of Figure...........................................................................................................V1
List of Tables.........................................................................................................V111
Abstract..................................................................................................................1X
ملخص الأطروحة....................................................................................................X1
Chapter one.............................................................................................................1
1. Introduction........................................................................................................1
1.1 Rheumatoid Arthritis .....................................................................................1
1.1.1 Incidence
1.1.2 Etiology and pathophysiology
1.1.3 Signs and symptoms
1.1.4 Complication of RA
1.1.5 The diagnosis of RA
1.1.5.1 Rheumatoid Factor (RF)
1.1.5.2 Anti-cyclic Citrullinated Peptide (ACCP)
1.1.5.3 C-Reactive Protein (CRP)
1.1.5.4 Complete blood count
1.1.5.5 Erythrocyte Sedimentation Rate (ESR)
1.1.5.6 Hemoglobin (Hgb)
1.1.5.7 Hematocrit (Hct)
1.1.5.8 Anemia and rheumatoid arthritis
1.1.5.9 Platelets (thrombocytes)
1.2 Previous studies of serological markers and haematological parameters in RA
1.3 Rationale and objective
1.3.1 Rationale
1.3.2 Objective
1.3.2.1 Main objective
1.3.2.2 Specific objective
Chapter two
2. Materials and methods
2.1 Materials
2.2 Methods
2.2.1 Complete Blood Count ............................................................................43
2.2.2 Erythrocyte Sedimentation Rate by fast detector protocol (ESR) ..................46
2.2.3 IgM Rheumatoid Factor protocol (IgM-RF) ................................................47
2.2.4 Quantitative measurement of C-Reactive Protein (CRP) ...........................47
2.2.5 Anti-CCP ELISA (IgG) ...........................................................................48
2.2.6 Statistical analysis .....................................................................................49

3. Chapter three ..................................................................................................50
3. Results .............................................................................................................50

3.1 Rheumatoid Factor .......................................................................................50
3.2 C-Reactive Protein .......................................................................................51
3.3 Anti-CCP .......................................................................................................52
3.4 ESR ................................................................................................................53
3.5 Haemoglobin (Hb) ........................................................................................53
3.6 Red Blood Cell count (RBCs) .......................................................................54
3.7 White Blood Cells count (WBCs) ..................................................................55
3.7.1 WBCs differential count .............................................................................56
3.7.2 Neutrophil percentage ..............................................................................56
3.7.3 Lymphocyte percentage ..........................................................................57
3.7.4 Monocyte percentage ..............................................................................58
3.7.5 Eosinophil percentage ..............................................................................59
3.7.6 Basophil percentage ..................................................................................60
3.9 Platelet ...........................................................................................................60
3.9 Clinical features ............................................................................................61
3.9.1 Knee joint pain .........................................................................................61
3.9.2 Warm full swelling ...................................................................................62
3.9.3 Wrist pain ..................................................................................................62
3.9.4 Moring stiffness .......................................................................................62
3.9.5 Deformability ............................................................................................62
3.9.6 Impaired movement ..................................................................................64
3.9.4 Symmetric arthritis ...................................................................................66
3.9.5 Ankle –Albow pain ..................................................................................69
3.10 Disease duration per months .....................................................................69
List of Figures

Figure 1.1 Pathogenesis of RA.................................................................2
Figure 1.2 Clinical features .................................................................4
Figure 3.1 The mean of RF in patient and control according to sex...........50
Figure 3.2 The mean of CRP in patient and control according to sex ........................................51
Figure 3.3 The mean of ACCP in patient and control according to sex ....................................52
Figure 3.4 The mean of Hb in patient and control according to sex .......................................54
Figure 3.5 The mean of RBCs in patient and control according to sex .....................................55
Figure 3.6 The mean of WBCs in patient and control according to sex ....................................56
Figure 3.7 The mean of Neutrophil percentage in patient and control .................................57
Figure 3.8 The mean of Lymphocyte percentage in patient and control ..............................58
Figure 3.9 The mean of Monocyte percentage in patient and control .................................59
Figure 3.10 The mean of Eosinophil percentage in patient and control ..............................60
Figure 3.11 The mean of platelet count in patient and control according to sex ..................61
Figure 3.12 The correlation between Joint deformability and RF .........................................63
Figure 3.13 The correlation between Joint deformability and ACCP .................................64
Figure 3.14 The correlation between Impaired movement and RF ......................................65
Figure 3.15 The correlation between Impaired movement and ACCP ...............................66
Figure 3.16 The correlation between Symptoms in symmetrical way and RF ......................67
Figure 3.17 The correlation between Symptoms in symmetrical way and ACCP ............68
List of Tables

Table 1.1 Revised American Association Criteria for classification of RA .................7

Table 1.2 Laboratory test Association findings .......................................................8
Abstract

Rheumatoid arthritis (RA) is a common chronic inflammatory autoimmune disease of unknown origin. It affects 1% of the population and causes the irreversible functional and anatomical joint damage. Early diagnosis with the help of the new diagnostic tools is the main standpoint of modern rheumatology as it is absolutely necessary for early treatment with Disease-Modifying Anti Rheumatic Drugs (DMARDs). (1) The etiology of rheumatoid arthritis is not fully understood. Evidence points to a complex interplay between environmental and genetic factors. Although laboratory testing and imaging
studies can help confirm the diagnosis and track disease progress, rheumatoid arthritis primarily is a clinical diagnosis and no single laboratory test is diagnostic. This study aimed to assess and correlate the hematological parameters and serological markers with the clinical features in RA. A total of 60 RA Sudanese patients: 23 males and 37 females age ranging between 30 and 60 years. Also a 60 healthy controls were involved in this study; ages ranging between 30 to 60 years. Five ml of venous blood were collected from each patient and healthy control. A 2.5 ml were added in EDTA containers for complete blood count (CBC), erythrocyte sedimentation rate (ESR), rheumatoid factor (RF) and C-reactive protein (CRP) performance ,while the other 2.5 ml were kept in plain container for the ACCP performance. Fourty six percent only (46%) of patients were really diagnosed by Rheumatoid Arthritis. This study concluded that; No single Laboratory test will confirm the diagnosis of RA. ACCP, CRP, ESR, RF and Hematological parameters should be performed as panel for diagnosis, detect disease activity, prognosis and treatment management among RA patients. Females were affected more than males (3:1).The range of age in patients 44-49 years. ACCP and RF were higher in females rather than males. CRP was higher in males rather than females. Directional significant correlation between ACCP, CRP, ESR and RF. Directional significant correlation between ACCP, CRP, RF and increased in lymphocytes and neutrophils .Absent of either normocytic normochromic or microcytic hypochromic anemia among RA patients. Mild leukocytosis in patients compared with healthy control. Knee joint, wrist pain and stiffness pain was common sign in all patients either ACCP positive or negative. Deformability, impaired movement and
symmetrical arthritis were associated and with positive ACCP and RF. Females were showed complications in 35 months without treatment (good prognosis). Males were showed complications in 21 months (not poor prognosis). The immunofluorescent protocol of RF and CRP by i-Chroma was revealed reliable results, but high in cost compared with ELISA. To our knowledge, this was the first study in Sudan aimed to compare between sex and baseline Rheumatoid Arthritis status outcome like hematological /immunological parameters and clinical features.

ملخص الأطروحه

التهاب المفاصل الروماتويدي مرض مزمن ذاتي المناعة يصيب المفاصل ويؤثر على الغشاء المبطن للمفاصل (الغشاء الزليمي) مسببًا التهابًا به مما يؤدي إلى تأكل الغضاريف والعظام محدثًا تشويها دائماً وأحياناً تدميراً مما يسبب إعاقة الحركة.

هذا المرض يصيب 1% من عامة سكان العالم من عمر 30 إلى 50 عاماً مع زيادة نسبة حدوثه عند النساء 3:1 وأسباب حدوث هذا المرض غير واضحة لكن من المرجح أن يكون السبب نتيجة مزيج من عدة عوامل وراثية وعوامل تتعلق بنمط الحياة وعوامل بيئية.

يعتمد تشخيص المرض على الفحص السريري بواسطة أخصائي الروماتيزم ومن ثم الأشعة والفحوصات المخبرية التي تشمل فحص الموسميات المصلية التي تفحص وجود أضداد جسم مضاد مسببة للروماتويد وهي الروماتويد فاكر RF وأضداد مضادة لبيبتيدات السترويلين الدوروية ACCP وفحص
Complete Haemogram (CBC)بالإضافة لفحص خلايا الدم والهيموقلوبين (CRP) وفحص ترسب كريات الدم الحمراء (ESR).

منهجية البحث:

هذه الدراسة المستعرضة الوصفية شملت الحالات المرضية (Patients) والحالات الصحية الضابطة (Control) والتي أجريت في مركز النقيب الطبي مدينة نيالا ولاية جنوب دارفور. لتحديد القيم القياسية (RF, ACCP, CRP) للموسمات المرضية (ESR, CBC) ومقارنة هذه القيم مع الأعراض السريرية للمريض وجنس المريض (Sex). شملت الدراسة 60 فردًا من المرضى منهم 37 إناث و23 ذكر بالإضافة إلى 60 ممثلو الحالات الصحية الضابطة (Control). تم قياس الأجسام المضادة الذاتية (ACCP) فحص CRP, RF, CBC بينما تم قياس كل من ESR, BC3000 وفقًا بالنسبة لELIZA بجهاز ACCP بينما تم قياس كل من ESR, BC3000 وفقًا بالنسبة لELIZA بجهاز ACCP بينما تم قياس كل من ESR, BC3000 وفقًا بالنسبة لELIZA بجهاز ACCP بينما تم قياس كل من ESR, BC3000 وفقًا بالنسبة لELIZA بجهاز ACCP بينما تم قياس كل من ESR, BC3000 وفقًا بالنسبة لELIZA بجهاز ACCP بينما تم قياس كل من ESR, BC3000 وفقًا بالنسبة لELIZA بجهاز ACCP بينما تم قياس كل من ESR, BC3000 وفقًا بالنسبة لELIZA بجهاز ACCP بينما تم قياس كل من ESR, BC3000 وفقًا بالنسبة لELIZA بجهاز ACCP بينما تم قياس كل من ESR, BC3000 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قياس كل من ESR, BC3000 وفقًا بالنسبة لELIZA بجهاز ACCP بينما تم قياس كل من ESR, BC3000 وفقًا بالنسبة لELIZA بجهاز ACCP بينما تم قياس كل من ESR, BC3000 وفقًا بالنسبة لELIZA بجهاز ACCP بينما تم قياس كل من ESR, BC3000 وفقًا بالنسبة لELIZA بجهاز ACCP بينما تم قياس كل من ESR, BC3000 وفقًا بالنسبة لELIZA بجهاز ACCP بينما تم قياس كل من ESR, BC3000 وفقًا بالنسبة LIZA بجهاز ACCP بينما تم قياس كل من ESR, BC3000 وفقًا بالنسبة LIZA بجهاز ACCP بينما تم قياس كل من ESR, BC3000 وفقًا بالنسبة LIZA بجهاز ACCP بينما تم قياس كل من ESR, BC3000 وفقًا بالنسبة LIZA بجهاز ACCP بينما تم قياس كل من ESR, BC3000 وفقًا بالنسبة LIZA بجهاز ACCP بينما تم قياس كل من ESR, BC3000 وفقًا بالنسبة LIZA بجهاز ACCP بينما تم قياس كل من ESR, BC3000 وفقًا بالنسبة LIZA بجهاز ACCP بينما تم قياس كل من ESR, BC3000 وفقًا بالنسبة LIZA بجهاز ACCP بينما تم قياس كل من ESR, BC3000 وفقًا بالنسبة LIZA بجهاز ACCP بينما تم قياس كل من ESR, BC3000 وفقًا بالنسبة LIZA بجهاز ACCP بينما تم قياس كل من ESR, BC3000 وفقًا بالنسبة LIZA بجهاز ACCP بينما 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خلاصة البحث:

لعلنا أن هذه أول دراسة في السودان تدرس العلاقة بين قيم الموسمات المصلية وقيم مكونات CBC مع الأعراض السريرية للمرض وجنسي المرضى (Sex).

تخلص هذه الدراسة إلى أن هناك فقط 46% من المرضى تم تشخيصهم كمرضى الالتهاب المفاصل الروماتويدي وأن نسبة إصابة الإناث أعلى من الذكور 3:1 كما تخلص الدراسة أنه لا يوجد فحص واحد لتشخيص المرض بل يجب أن يشخص المرض بكل هذه الفحوصات ACCP,RF,CRP,ESR,CBC. كما تخلص الدراسة إلى أن ACCP ذو خصوصية عالية في تشخيص المرض وذو تأثير فعال خاصة إذا أضيف مع RF.

كما تخلص الدراسة أن هناك زيادة بسيطة في عدد كريات الدم البيضاء في المرضى كما أنه لا توجد أنيميا وسط المرضى رغم أن نسبة الهوموقلوبين في المرضى أقل منها في الكنترول. وأيضاً تخلص الدراسة إلى أن هناك ارتباط إيجابي بين الإعاقة في الحركة Impaired والتشوهات المفصلية Joint deformability وظهور الأعراض المتزامنة بين المفصل شمال واليمن Symptoms in symmetrical way) كما أنه ليس هناك علاقة ACCP و RF وبين كل من الـ RF,ACCP وبين كل من الـ RF,ACCP في المفاصل في الصباح الباكر.

Chapter one

1. Introduction

1.1. Rheumatoid Arthritis (RA)
Rheumatoid arthritis (RA) is a common chronic inflammatory autoimmune disease of unknown origin. It affects 1% of the population and causes the irreversible functional and anatomical joint damage. Early diagnosis with the help of the new diagnostic tools is the main standpoint of modern rheumatology as it is absolutely necessary for early treatment with Disease-Modifying Anti Rheumatic Drugs (DMARDs).

1.1.1 Incidence

About 1% of the world’s population is affected by RA women three times more often than men (3:1 ratio). Onset is most frequent between the ages of 30 and 50, but people of many ages can be affected.

1.1.2 Etiology and pathophysiology

The etiology of rheumatoid arthritis is not fully understood. Evidence points to a complex interplay between environmental and genetic factors. In monozygotic twins, there is a more than 30 percent concordance rate for rheumatoid arthritis development, and 80 percent of whites with rheumatoid arthritis express the HLA-DR1 or -DR4 subtypes. These and other regions of the Major Histocompatibility Complex may confer susceptibility to more severe disease by causing a specific arthrogenic peptide to be presented to CD4+ T cells. Joint damage in RA begins with the proliferation of synovial macrophages and fibroblasts after a triggering incident, possibly autoimmune or infectious. Lymphocytes infiltrate perivascular regions, and endothelial cells
proliferate. Neovascularization then occurs. Blood vessels in the affected joint become occluded with small clots or inflammatory cells. Over time, inflamed synovial tissue begins to grow irregular. Forming invasive pannus invades and destroys cartilage and bone. Multiple cytokines, interleukins, proteinase, and growth factors are release, causing further joint destruction and development of systemic complications (3). (Fig 1.1)
1.1.3 Signs and symptoms

Rheumatoid arthritis typically manifests with signs of inflammation, with the affected joints being swollen, warm, painful and stiff, particularly early in the morning on walking or following prolonged inactivity. Increased stiffness early in the morning is often a prominent feature of the disease and typically lasts for more than an hour. Gentle movements may relieve symptoms in early stages of the disease. These signs help distinguish rheumatoid from non-inflammatory problems of the joints, often referred to as osteoarthritis or "wear-and-tear" arthritis. In arthritis of non-inflammatory
causes, signs of inflammation and early morning stiffness are less prominent with stiffness typically less than 1 hour, and movements induce pain caused by mechanical arthritis (4). In RA, the arthritis of the joints known as synovitis, the joints are often affected in a fairly symmetrical fashion, although this is not specific, and the initial presentation may be asymmetrical. As the pathology progresses the inflammatory activity leads to tendon tethering and erosion and destruction of the joint surface. Most patient, symptoms emerge over weeks to months, starting with one joint and often accompanied by prodromal symptoms of anorexia, weakness, or fatigue. In approximately 15 percent of Joints most commonly affected are those with the highest of synovia to articular cartilage. The wrists are nearly always involved, as are the proximal ratio interphalangeal and metacarpophalangeal joints. The distal interphalangeal joints and sacroiliac joints tend not to be affected (5). Rheumatoid joints typically are boggy, tender to the touch, and warm, but they usually are not erythematous. Some patients complain of "puffy" hands secondary to increased blood flow to inflamed areas. Prominent epitrochlear, axillary, and cervical lymph nodes may be noted. Muscles near inflamed joints often atrophy. Weakness is commonly out of proportion to pain on examination. Low-grade fever, fatigue, malaise, and other systemic complaints may arise, especially in an acute presentation. The rheumatoid nodule, which is often subcutaneous, is cutaneous feature most characteristic of RA (5, 6). (Figure1.2)
1. Deformability

2. Swelling

3. Nodules

Figure 1.2: Clinical features

1.1.4. Complication of RA

Anemia: Correlates with erythrocyte sedimentation rate and disease activity; three fourths of patients have anemia of chronic disease; one fourth of patients respond to iron therapy. Cancer: May be secondary to treatments; lymphomas and leukemia two to three times more common in patients with rheumatoid arthritis; increased risk for various solid tumors; genitourinary cancer risk is reduced in rheumatoid arthritis,
perhaps because of nonsteroidal. *Cardiac complication:* Pericarditis-one third of patients may have asymptomatic pericardial effusion at diagnosis; atrioventricular block-rare; myocarditis-diffuse inflammation can occur, may or may not be symptomatic.

*Cervical spine disease:* Tenosynovitis of transverse ligament can lead to instability of atlas on axis. Caution must be used during endotracheal intubation; may see loss of lordosis of the neck and decreased range of motion; C4-C5 and C5-C6 subluxations are possible; may see joint space narrowing on lateral cervical spine films; avoid flexion films until odontoid fracture ruled out if injury is suspected; myelopathy can occur, with gradual onset of upper extremity weakness.

*Hand joint deformities:* Ulnar deviation at metacarpophalangeal joints; boutonniere deformity-flexed PIP (Proximal Inter Phalangeal) and hyperextended DIP (distal interphalangeal); swan neck deformity- the reverse of boutonniere, with flexed DIP and hyperextended PIP; thumb hyperextension; increased risk of tendon rupture.

*Other joint deformities:* Frozen shoulder may develop; popliteal cysts can arise; carpal and tarsal tunnel syndromes common.

*Respiratory complications:* Lung nodules can coexist with cancers and form cavitory lesions; cricoarytenoid joint inflammation can arise, with hoarseness and laryngeal pain; pleuritis-present in 20 percent at onset of disease; not usually associated with pleuritic pain; interstitial fibrosis-rales may be noted on lung examination. *Rheumatoid nodules:* Often have necrotic tissue in their centers; found in 20 to 35 percent
of patients with rheumatoid arthritis; usually found on extensor surfaces of the limbs or other pressure points; may form nearly anywhere, including on the sclera, vocal cords, sacrum, or vertebral bodies.

*Vasculitis*: Forms include distal arteritis, pericarditis, peripheral neuropathy, cutaneous lesions, arteritis of viscera, and coronary arteritis; increased risk of developing if male sex, high rheumatoid factor titers, treatment with steroids, number of disease-modifying ant rheumatic drugs prescribed; associated with increased risk of myocardial infarction.

*Other complication include*, eye problems in form of episcleritis, and fistula formation which is a cutaneous sinuses form near affected joint, connecting bursa the skin\(^5\).

### 1.1.5. The diagnosis of RA

Although laboratory testing and imaging studies can help confirm the diagnosis and track disease progress, rheumatoid arthritis primarily is a clinical diagnosis and no single laboratory test is diagnostic. Complication of RA may begin to develop within months of presentation; therefore, early referral to or consultation with a rheumatologist for initiation of treatment disease-modifying anti rheumatic drugs is recommended. Diagnosis of RA is difficult most of the time, as revised American College of Rheumatology (ACR) criteria (1987) For diagnosis of RA are based mostly on clinical disease manifestation\(^6\). *(Table 1.1)*
Other criteria included are the presence of rheumatoid factor (RF) and radiological finding. Radiological findings characteristic of RA appear late in the course of the disease. Also, RF may not be detected in many cases (particularly in early disease). Many workers have agreed that only 52–80% of early RA patient’s fulfill ACR criteria for diagnosis of RA \(^7,^8\). On the other hand, early intervention is crucial for preventing irreversible to Progression joint damage \(^9,^10\). No single diagnostic test definitively confirms the diagnosis of rheumatoid arthritis. However, several tests can provide objective data that increase diagnostic certainty and allow disease progression to be followed. The American College of Rheumatology Subcommittee on Rheumatoid Arthritis (ACRSRA) recommends that baseline laboratory evaluations include a
complete blood cell count with differential, rheumatoid factor, and erythrocyte sedimentation rate (ESR) or C-reactive protein (CRP). Baseline evaluation of renal and hepatic function also is recommended because these findings will guide medication choices. (Table 1.2) \(^{(5,11)}\).

Table 1.2: Laboratory and Imaging Findings

<table>
<thead>
<tr>
<th>Laboratory test</th>
<th>Associated findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-reactive protein*</td>
<td>Typically increased to &gt;0.7 picograms per mL; may be used to monitor disease course.</td>
</tr>
<tr>
<td>Erythrocyte sedimentation rate*</td>
<td>Often increased to &gt;30 mm per hour; may be used to monitor disease course.</td>
</tr>
<tr>
<td>Hemoglobin/hematocrit*</td>
<td>Slightly decreased; hemoglobin averages around 10 g per dL (100 g per L); normochromic anemia, also may be normocytic or microcytic.</td>
</tr>
<tr>
<td>Liver function*</td>
<td>Normal or slightly elevated alkaline phosphatase</td>
</tr>
<tr>
<td>Platelets*</td>
<td>Usually increased</td>
</tr>
<tr>
<td>Radiographic findings of involved joints*</td>
<td>May be normal or show osteopenia or erosions near joint spaces in early disease; wrist and ankle films are useful as baselines for comparison with future studies.</td>
</tr>
<tr>
<td>Rheumatoid factor*</td>
<td>Negative in 30 percent of patients early in illness; if initially negative, can repeat six to 12 months after disease onset; can be positive in numerous other processes (e.g., lupus; scleroderma; Sjögren’s syndrome; neoplastic disease; sarcoidosis; various viral, parasitic, or bacterial infections); not an accurate measure of disease progression.</td>
</tr>
<tr>
<td>White blood count*</td>
<td>May be increased</td>
</tr>
<tr>
<td>Anticyclic citrullinated peptide antibody</td>
<td>Tends to correlate well with disease progression; increases sensitivity when used in combination with rheumatoid factor; more specific than rheumatoid factor (90 versus 80 percent); not readily available in many laboratories.</td>
</tr>
<tr>
<td>Antinuclear antibody</td>
<td>Limited value as a screening study for rheumatoid arthritis</td>
</tr>
<tr>
<td>Complement levels</td>
<td>Normal or elevated</td>
</tr>
<tr>
<td>Immunoglobulins</td>
<td>Elevated alpha-1 and alpha-2 globulins possible.</td>
</tr>
<tr>
<td>Joint fluid evaluation</td>
<td>Consider if an affected joint can be tapped and diagnosis is uncertain; straw-colored fluid with fibrin flecks often seen; fluid may clot at room temperature; 5,000 to 25,000 white blood cells per mm³ (5 to 25 × 10⁶ per L) with 85 percent polymorphonuclear leukocytes a common finding; in rheumatoid arthritis, cultures are negative, there are no crystals, and fluid glucose level typically is low.</td>
</tr>
<tr>
<td>Urinalysis</td>
<td>Microscopic hematuria or proteinuria may be present in many connective tissue diseases.</td>
</tr>
</tbody>
</table>

*—Recommended for initial evaluation for rheumatoid arthritis.

Note: Renal function, although not as likely to change as a direct effect of disease, should be followed to assess renal effects of drug therapy.

Rheumatoid arthritis must be differentiated from a number of other disorders. Infection-related reactive arthropathies, seronegative spondyloarthropathies, and other connective tissue diseases such as systemic lupus erythematosus may have symptoms in common with rheumatoid arthritis, as may an array of endocrine and other disorders.
Gout rarely coexists with rheumatoid arthritis, and a joint aspiration should be considered if gout is suspected.

Table 1.3: Differential Diagnosis of RA

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Connective tissue diseases</td>
<td>Such as scleroderma and lupus</td>
</tr>
<tr>
<td>Fibromyalgia</td>
<td>Evaluate for trigger points.</td>
</tr>
<tr>
<td>Hemochromatosis</td>
<td>Iron studies and skin coloration changes may guide diagnosis.</td>
</tr>
<tr>
<td>Infectious endocarditis</td>
<td>Rule out murmurs, high fever, and history of intravenous drug use.</td>
</tr>
<tr>
<td>Polyarticular gout</td>
<td>Joints often erythematous; podagra commonly found; gout and rheumatoid arthritis rarely coexist; but calcium pyrophosphate deposition disease can accompany rheumatoid arthritis.</td>
</tr>
<tr>
<td>Polymyalgia rheumatica</td>
<td>Rheumatoid arthritis, unlike polymyalgia rheumatica, rarely presents with pain in the proximal joints of the extremities only.</td>
</tr>
<tr>
<td>Sarcoidosis</td>
<td>Granulomas likely, as are hypercalcemia and chest film findings.</td>
</tr>
<tr>
<td>Seronegative spondyloarthropathies, reactive arthritis</td>
<td>Tend to be more asymmetric than rheumatoid arthritis. More commonly involve the joints of the spine. Evaluate for history of psoriasis, Reiter's comorbidities, inflammatory bowel disease. Reactive arthritis can be postinfective, sexually acquired, or related to gastrointestinal disorders.</td>
</tr>
<tr>
<td>Still's disease</td>
<td>Tends to present with fever, leukocytosis with left shift, sore throat, splenomegaly, liver dysfunction, and/or rash.</td>
</tr>
<tr>
<td>Thyroid disease</td>
<td>Consider thyroid-stimulating hormone level depending on symptoms.</td>
</tr>
<tr>
<td>Viral arthritis</td>
<td>Consider parvovirus, hepatitis B.</td>
</tr>
</tbody>
</table>

1.1.5.1. Rheumatoid factor (RF)

Rheumatoid factors (RF) are autoantibodies directed against the Fc portion of IgG. The rheumatoid factor (RF) as initially described by Waaler and Rose\(^{(13)}\) in 1940 and as currently measured in clinical practice is an IgM RF, although other immunoglobulin types, including IgG and IgA, have been described. The presence of RF can be detected by a variety of techniques such as agglutination of IgG-sensitized sheep red cells or bentonite or latex particles coated with human IgG, radioimmunoassay, enzyme linked immunosorbent assay (ELISA) or nephelometry \(^{(13,14)}\). RF is taken as a non-specific marker of RA because it is also seen in other collagen vascular diseases like SLE and Sjogren’s syndrome as well as in normal healthy individuals. Measurement of these
non-IgM RFs is not widely available. However, they may be of prognostic value, since there is evidence suggesting that IgG, IgA, and 7S IgM RFs are associated with more severe disease\(^{(15)}\). The reported sensitivity of the RF test in RA has been as high as 90 percent. However, population-based studies, which include patients with mild disease, have found much lower rates of RF-positive RA (26 to 60 percent)\(^{(16)}\). A positive RF test can be found in rheumatic disorders, non-rheumatic disorders, and healthy subject\(^{(17)}\).

Rheumatic disorders Patients may have detectable serum RF in a variety of rheumatic disorders, many of which share similar features, such as symmetric polyarthritis and constitutional symptoms. These include; Rheumatoid arthritis (26 to 90 percent) Sjögren's syndrome (75 to 95 percent), Mixed connective tissue disease (50 to 60 percent), Mixed cryoglobulinemia (types II and III) (40 to 100 percent), and lupus erythematosus (15 to 35 percent).

1.1.5.1.1 Pathophysiology of RF

The origin of RF is incompletely understood.\(^{(18,19)}\) An abnormal immune response appears to select, via antigenic stimulation, high-affinity RF from the host's natural antibody repertoire\(^{(20)}\). This may occur in rheumatic diseases, such as rheumatoid arthritis (RA), and in a number of inflammatory diseases characterized by chronic antigen exposure, such as sub-acute bacterial endocarditis (SBE). The development of RF after such infections has suggested that they represent an antibody response to antibodies that have reacted with microbes. This possibility is supported by experimental evidence showing that mice immunized with IgM coated VSV (vesicular
stomatitis virus) develop rheumatoid factors (21). Normal human lymphoid tissue commonly possesses B lymphocytes with RF expression on the cell surface. However, RF is not routinely detectable in the circulation in the absence of an antigenic stimulus. Modified IgG could be a stimulus to RF production and could be an important component of RA pathogenesis; this concept is supported by studies that observed an association of RF and more severe RA with autoantibodies to advanced glycated end product-damaged IgG or agalactosyl IgG (22,23). Costimulation of B cells, perhaps mediated by toll-like receptors (TLRs), may allow B cells with low affinity receptors for IgG to become activated. TLRs are components of the innate immune system, and they provide signals after engaging various bacterial and viral products (24,25). Studies in patients with RA have enhanced our understanding of the origin of RFs: CD14-positive cells (monocytes) from the bone marrow stimulate RF-producing B cells (26). Furthermore, RF found in the peripheral blood probably originates within the bone marrow (27). Synovial fluid RF may be produced by synovium-derived CD20-negative, CD38-positive plasma cells (28). Circulating B cells require interleukin-10 (IL-10) for RF production (29). In RF-negative patients with RA, B cells capable of RF production are fewer in number and less responsive to T cell help than in RF-positive patients with RA. In one study, for example, the frequency of RF+ : IgM+ B cells was increased more than 50-fold in seropositive patients (7 to 20 percent of IgM+ B cells versus well under 1 percent in normal; patients with seronegative RA had intermediate values (1.5 to 6 percent of IgM+ B cells) (30). Production of RF is also associated with the shared epitope of HLA DRB1*0401 (31). Cigarette smoking, a risk factor for more severe RA, is associated
with an increased prevalence of RF \(^{(32)}\). RFs possess significant heterogeneity related to mutations within heavy and light chain genes \(^{(33)}\). Thus, IgM RFs from patients with RA react with a variety of antigenic sites on autologous IgG \(^{(34)}\). They also react against immunoglobulins found in many cellular and tissue antigens, but may have different biologic activity in different hosts and anatomic locations. As an example, one report of RF derived from synovial tissue lymphocytes in a patient with RA found specificity for gastric gland nuclei and smooth muscle; in contrast, RF derived from a control patient's peripheral blood did not show this pattern of reactivity \(^{(35)}\).

1.1.5.1.2. Possible function of RF

Possible functions include

1- Binding and processing of antigens embedded immune complexes.

2- Presentation of antigens to T lymphocytes in the presence of HLA molecules.

3- Immune tolerance.

4- Amplification of the humoral response to bacterial or parasitic infection.

5- Immune-complex-clearance.

The role of RFs in the pathogenesis and perpetuation of RA or other rheumatic diseases is unknown. A high correlation for RF has been noted among identical twins with RA, suggesting that genetic factors influence both RF function and disease development \(^{(36)}\). However, some studies have shown that patients with RF-negative RA have HLA
susceptibility alleles similar to those in RF-positive patients \(^{(37,38)}\). There may therefore be a similar immunogenetic predisposition to RA in these patients that is independent of RF. IgG and IgA RFs are occasionally present in patients with RA in the absence of IgM RF \(^{(39,40)}\). Measurement of these non-IgM RFs is not widely available. However, they may be of prognostic value, since there is evidence suggesting that IgG, IgA, and 7S IgM RFs are associated with more severe disease \(^{(41,42)}\). This risk appears to be independent of HLA alleles associated with severe disease \(^{(43)}\).

1.1.5.1.3. The prevalence of Rheumatoid Factor

The prevalence of positive RF has been reported to be high in Pima Indians \(^{(44)}\), related to the high incidence of RA among the Pima \(^{(45)}\) and declining in line with the temporal trends in RA \(^{(46)}\). The prevalence of RA is 0.5–1% among adults in Europe, but it seems to be much lower in some Asian and African populations \(^{(47)}\).

1.1.5.2. Anti-cyclic-citrullinated peptide (ACCP)

An enzyme linked immunosorbent assays (ELISA) was developed to detect antibodies directed against filaggrin derived from human skin and has high specificity and sensitivity for the diagnosis of RA \(^{(48)}\). The target amino acid in filaggrin is citrulline, a post-translationally modified arginine residue \(^{(49)}\). Subsequently, an ELISA assay for the detection of antibodies to a cyclic peptide containing citrulline was made commercially available, which was easier to standardize, and also had high sensitivity and specificity for the diagnosis of RA. This became the assay for the detection of anti-cyclic
citrullinated peptide (anti-CCP) antibodies. Citrullinated proteins and peptides Anti-citrullinated protein antibodies are highly specific for RA\(^{50}\). The citrullination is catalyzed by peptidyl arginine deiminase; arginine residues on fibrin and fibrinogen may be favored sites for deimination within rheumatoid joints \(^{51,52}\). Intracellular citrullinated proteins colocalized with the deimidase in 59 percent of RA synovial samples versus 17 percent of control samples \(^{53}\). However, citrullinated proteins may also be found in the synovium of other forms of arthritis, in non synovial tissue from patients with RA (e.g. pulmonary rheumatoid nodules), in the lungs of patients with interstitial pneumonitis, in brain from patients with multiple sclerosis, and in normal brain\(^{54,55}\). The RA-associated HLA-DRB1*0404 allele is also associated with production of antibodies to citrullinated fibrinogen, and T cell proliferation in response to fibrinogen peptides is frequent in RA patients but rare in controls \(^{56}\). In contrast, in another study the shared epitope was associated with antibodies to a citrullinated peptide derived from vimentin but not from fibrinogen-derived citrullinated peptide \(^{57}\). A strong association between cigarette smoking, a known risk factor for RA, and the presence HLA-DBR1*0404 or other HLA alleles comprising the shared epitope has been noted for RA patients who have anti-citrulline antibodies \(^{58,59}\). Measurement of anti-CCP antibodies also may be useful in the differential diagnosis of early polyarthritis \(^{60}\). Although anti-CCP antibody testing is more specific than RF for RA \(^{61}\), positive result can occur in other diseases, including tuberculosis and several autoimmune rheumatic diseases \(^{62,63,64}\). Anti-CCP antibody-positive patients with early RA are at increased risk of progressive joint damage \(^{65,66,61,67,68}\). ELISA assays that detect anti-CCP antibodies
have a sensitivity and specificity of 47 to 76 and 90 to 96 percent for RA, respectively (49,62). The best data come from a meta-analysis of 87 studies in which the pooled sensitivity and specificity for the diagnosis of RA were 67 percent (95% CI 62-72 percent) and 95 percent (95% CI, 94-97 percent) for anti-CCP antibodies, compared to 69 percent (95% CI 65-73 percent) and 85 percent (95% CI, 82-88 percent) for IgM RF (61). Test performance is dependent upon the characteristics of the assay kit. Higher values of sensitivity and specificity have been reported with alternate generation assay compared to the original assay (60,69,70).

1.1.5.2.2. Combination of Rheumatoid Factor and Anti-CCP Antibodies

Testing for the combination of anti-CCP antibodies and IgM RF may be better for excluding the diagnosis of RA than is achievable by testing for either antibody alone (71). Those with early arthritis who are RF or anti-CCP antibody positive are at an increased risk of developing RA and erosive joint disease, while those with neither of these markers are less likely to develop joint damage (67). Thus, earlier intervention with disease modifying anti-rheumatic drug (DMARD) therapy may be warranted in those with positive markers, while symptomatic treatment (e.g. with nonsteroidal anti-inflammatory drugs) may be appropriate for those lacking both RF and anti-CCP antibodies when first seen.

1.1.5.3.1. C - Reactive protein
C-Reactive protein (CRP) was first described in 1930 at the Rockefeller Institute by Tillet and Francis (72). These investigators observed that the serum of patients diagnosed with pneumonia precipitated when brought into contact with a soluble extract (the C-polysaccharide) of Streptococcus pneumonia. Upon this observation, this substance was called “fraction-C” a name that was later changed into CRP. Interestingly, the precipitation reaction disappeared when the pneumonia resolved but remained positive in patients with a fatal outcome. Later, it became clear that serum precipitation not only occurred with extracts from S. pneumonia but also with other bacteria and fungi. No precipitation was however seen with viruses.

1.1.5.3.1.2. CRP as an acute-phase protein

CRP is one of the most important acute-phase proteins. Stimuli that induce an acute-phase reaction can be of various origins: infectious (bacterial, fungal, mycobacterial, or severe viral), inflammatory, stress, tissue necrosis, trauma, childbirth, and neoplasia. CRP is produced almost exclusively by hepatocytes. The main stimulus for production is IL-6. This response is enhanced in combination with IL1 and TNF. CRP has a half-life of 19 hours that is independent of any physiological or pathophysiological circumstances or of the concentration of CRP in the serum. Therefore, the synthesis rate of CRP by the liver is the only factor determining the plasma CRP concentration. Consequently, only liver failure or therapies affecting the acute phase stimulus may decrease CRP. Under normal conditions, the baseline concentration of CRP in the plasma is around 0.8 mg/L (73). In the presence of an acute-phase stimulus, CRP
production is rapidly (within hours) up-regulated and may reach concentrations that are 500- to 1,000-fold higher than under basal circumstances. The short half-life of CRP also ensures that the concentrations quickly decrease once the acute-phase stimulus disappears, making CRP a very valuable marker to detect and follow-up inflammation, and this in contrast to other acute phase proteins as for instance fibrinogen\(^\text{(74)}\). The CRP concentration is thus a very useful nonspecific biochemical marker of inflammation, measurement of which contributes importantly to (a) screening for organic disease, (b) monitoring of the response to treatment of inflammation and infection, and (c) detection of infection in immunocompromised individuals, and in the few specific diseases characterized by modest or absent acute-phase responses\(^\text{(77)}\). (\textbf{Table 1.4})

\begin{table}[ht]
\centering
\caption{Majer CRP acute phase response}
\end{table}
The CRP Entrez gene cytogenetic band located on the first chromosome: 1q21-q23. CRP is a 224-residue protein with a monomer molar mass of 25106 Da. The protein is an annular pentameric disc in shape and a member of pentraxin family\(^{(75)}\).

Function of CRP: Displays several functions associated with host defense it promotes agglutination, bacterial capsular swelling, phagocytosis (CRP initiates the activation of the complement cascade and binds Fc gamma RI (CD64) and Fc gamma RIIA (CD32a).
on phagocytes to activate phagocytic responses) and complement fixation through its calcium-dependent binding to phosphoryl choline. It can interact with DNA and histones and may scavenge nuclear material released from damaged circulating cells (76).

CRP is used mainly as a marker of inflammation measuring and charting CRP values can prove useful in determining disease progress or the effectiveness of treatment. Blood, usually collected in a serum-separating tube, is analyzed in a medical laboratory or at the point of care. Various analytical methods are available for CRP determination, such as ELISA (Enzyme-linked immune sorbent assay ELISA can perform other forms of ligand binding assays instead of strictly "immune" assays, though the name carried the original immune" because of the common use and history of development of this method. The technique essentially requires any ligating reagent that can be immobilized on the solid phase along with a detection reagent that will bind specifically and use an enzyme to generate a signal that can be properly quantified. In between the washes only the ligand and its specific binding counterparts remain specifically bound or "immune sorbed" by antigen antibody interactions to the solid phase, while the nonspecific or unbound components are washed away. Unlike other spectrophotometric wet lab assay formats where the same reaction well (e.g. a cuvette) can be reused after washing, the ELISA plates have the reaction products immune sorbed on the solid phase which is part of the plate and thus are not easily reusable immune turbidimetry (Method This reagent is intended for the in vitro quantitative determination of CRP concentration in serum or plasma on automated clinical chemistry analyzers) rapid
immune diffusion (is a diagnostic involves diffusion through a substance such as agar. Two commonly known forms double immune diffusion and radial immune diffusion) and visual agglutination quantitative slide method and semi quantitative diluted method)\(^{(77)}\). There are two different tests for CRP. The standard test measures a much wider range of CRP levels but is less sensitive in the lower ranges. The high-sensitivity CRP (hs-CRP) test can more accurately detect lower concentrations of the protein (it is more sensitive), which makes it more useful than the CRP test in predicting a healthy person's risk for cardiovascular disease\(^{(75)}\).

1.1.5.4. Complete blood count

The component of complete blood count (CBC) include a hemogram and differential white blood cell (WBC) count. The hemogram includes the enumeration of WBCs, red blood cells (RBCs), and platelets; it also provides determinations of hemoglobin, hematocrit, and RBC indices. The WBC count with differential enumerates the different WBC types. Together, the components of the CBC evaluate primary diseases of the blood and bone marrow, which include disorders such as anemia, leukemia, polycythemia, thrombocytosis, and thrombocytopenia. The CBC also evaluates medical conditions that secondarily affect the blood and bone marrow resulting in hematologic manifestation such as infection, inflammation, coagulopathies, neoplasms, and toxic substance exposure. In many instances, specific symptomatology of a medical condition may not be present and hematologic changes on the CBC may be the only finding present. These changes prompt investigation to then identify the medical condition\(^{(78)}\).
1.1.5.4.1 General indications for CBC test

General indication for a CBC that are considered medically reasonable and are accepted by med care as follow:

The hemogram should be evaluated for any patient with signs, symptoms, or conditions associated with anemia or polycythemia. The platelet count should be evaluated for patients with findings or conditions associated with increased or decreased platelet production, destruction, or disfunction. The WBC differential should be evaluated for any patient with signs, symptoms, or conditions associated with infections, inflammatory processes, bone marrow alterations, and immune disorders. The WBC count has also been recently identified as a possible risk stratification tool for mortality in acute coronary syndromes (79).

1.1.5.4.2 WBC in Inflammation and Infection

The inflammatory process is triggered by cell injury, which can be caused by a variety of conditions such as trauma, burns, ischemia, surgery, snakebite, caustic chemicals, and extremes in heat and cold, as well as infectious microorganisms. It is important to remember that although all infections are accompanied by inflammation, not all inflammation is accompanied by infection. Any damage to the vascular endothelium or the mast cell will trigger an inflammatory response, which is orchestrated by inflammatory cytokines. Cytokines are hormone like protein mediators responsible for the cell-to-cell communication that regulates local and systemic physiologic and
pathologic interactions. The cells of the vascular endothelium have been recently identified as a major player in the inflammatory process. The mast cell (cellular bag of granules) is another important activator of the inflammatory response. Mast cells are found in connective tissues intimately surrounding blood vessels and in mucosal surfaces. Once endothelial or mast cells are injured or damaged, they release inflammatory cytokines, which orchestrate the manifestations of inflammation. Manifestations of inflammation include a short period of vasoconstriction to limit bleeding followed by vasodilation. Vasodilation increases blood flow to the area, bringing nutrients and large amounts of WBCs. Vasodilation also results in hyperemia (redness and warmth). Another manifestation is increased capillary permeability, which allows for the immigration of WBCs from the blood vessel to the interstitial spaces where they can phagocytize unwanted organisms and debris. The WBCs also release cytokines to call more WBCs to the area and to perpetuate the inflammatory response. Increased capillary permeability also allows for the exudation of plasma and plasma proteins resulting in edema. The edema may cause pressure on the nearby nerves resulting in pain.

1.1.5.4.3. The WBCs Count with Differential

The WBC count with differential determines the total number of WBCs (also called leukocytes) with a percentage of each type. The major function of the WBC is to defend the body against organisms and injury. WBCs are the main players in infectious/inflammatory and immune responses. WBCs can be divided into 2 main
groups: phagocytes and immunocytes. Although Phagocytes are WBCs that have the capability to attach to, engulf, and release enzymes to kill and degrade unwanted microorganisms and debris. The WBCs that are phagocytic include neutrophils, eosinophils, basophils, and monocytes. Immunocytes include the lymphocytes, WBCs that drive the immune response. A more common manner in which WBCs are divided is by the presence of granules in the cytoplasm. Those WBCs that contain granules in their cytoplasm are neutrophils, eosinophils, and basophils. WBCs that do not contain granules in their cytoplasm include monocytes and lymphocytes For the purpose of this discussion, WBCs will be divided into granulocyte and a granulocytes. Granulocytes get their name from the granules present in their cytoplasm. These granules contain biochemical mediators that serve inflammatory and immune functions. Granulocytes also contain enzymes in their cytoplasm capable of destroying microorganisms and catabolizing debris ingested during phagocytosis. They take about one week to develop in the bone marrow. They circulate for only about 6 to 12 hours in the blood stream and 2 to 3 days after entering the tissue.

**Neutrophils**

Neutrophils are a type of granulocyte and are mature cells that account for more than half of all the WBC sub types in circulation. They are also called segmented neutrophils (segs) or polymorphonuclear neutrophils (PMNs) or polys because the nucleus of these cells consists of 3 to 5 lobes connected by thin strands. Highly motile, these cells are the first to arrive (usually within 90 minutes) in response to acute inflammation or
infection; they migrate out of the capillaries and into the inflated tissue site in a process called diapedesis or emigration. The neutrophils ingest microorganisms and debris and then die, forming purulent exudate, which is removed by the lymphatics or through the epithelium. When there is an increased demand for neutrophils, as in response to acute infection, immature neutrophils may be released from the bone marrow. These immature nuclei that resemble bands or rods. Thus, immature neutrophils are called bands or stabs. They are normally found only in very low percentages in circulating blood.

**Eosinophils**

Eosinophils function principally to ingest and kill multicellular parasites. They are also effective in detoxifying antigen-antibody complexes that form during allergic reactions. People with chronic allergic conditions such as atopic rhinitis and extrinsic asthma typically have elevated circulating eosinophil counts. Eosinophils are believed to play a role in down regulating hyper-sensitivity responses by neutralizing histamine, inhibiting mast cell degranulation, and inactivating slow-reacting substances (SRS) of anaphylaxis.

**Basophils**

Basophils are associated with systemic allergic reactions. Similar to mast cells, basophils have granules that contain pro inflammatory chemicals such as histamine, serotonin, bradykinin, and heparin. They release their granules in response to stimulation by
immune cells. Basophils circulate in the blood stream. Whereas mast cells are found in connective tissue. The average basophil has a life span of days, but the mast cell can live weeks to moths. Non granulocytes, as mentioned earlier, are WBCs that do not have granules in their cytoplasm. Inclusive in this group are monocytes and lymphocyte.

**Monocyte**

Macrophage monocytes are the largest of the WBCs and are young cells found freely circulating in blood or en route to a tissue location. Once the young monocyte leaves the blood stream and enters tissue, it transforms into a mature macrophage. Macrophages live within tissue spaces in widespread locations. These cells have different names related to the particular tissue in which they are found, ie, the Kupffer cells are macrophages that live in the liver. Because of the complex connection of these cells to the bloodstream and the tissue, monocytes and macrophages are described as one system, called the mononuclear phagocyte.

Macrophages arrive on the scene in about 5 hours after injury and become the predominant leukocyte within 48 hours. Because macrophages lie within the tissue spaces, they are usually the first cell to engulf and process the antigen and present it to the immune cells (lymphocytes) in a manner that will stimulate a specific immune response to that particular antigen. In other words, the macrophage, in a special process, can destroy the organism while keeping its cell surface markers to give to the lymphocytes so that they can always identify that particular organism and mount a specific defense against it.
**Lymphocytes**

Lymphocytes are also non granulocytes and are responsible for immune responses to specific organisms. They are the most numerous circulating WBC after neutrophils. There are 2 major classes of lymphocyte: the T lymphocyte (T cell) and the B lymphocyte (B cell). Both T and B cells can be sorted into subtypes based on characteristic surface molecules on them called cluster of differentiation (CD). Cluster of differentiation surface molecules assist in defining the function of the different lymphocyte subtypes.

**T-cell**

The T-cell matures in the thymus and is responsible for cell-mediated immunity. The T cell has several subtypes that can be divided into regulator or effector cells. Regulator T cells are so called because of their regulatory functions of turning on or off the immune response. There are 2 types of regulator T cells: the helper T cell and the suppressor T cell. The helper T cell is considered the master switch of the immune system. These cells are surveyors, and when a specific antigen is presented to them, they release mediators that influence and stimulate the production of other immune cells including B cells. Helper T cells have CD4 surface molecules on them. Suppressor T cells suppress the immune response once the infection is controlled. Effector cells are T cells that have a direct action. The 2 types of effector cells are the cytotoxic T cell and the memory T cell. The cytotoxic T cell carries the CD8 molecule on its surface. It attaches to identified infected cells and cancer cells and releases enzymes to destroy these cells. Cytotoxic T cells are particularly effective at destroying virally infected cells, foreign cells, and
mutant cells\(^{(80)}\). Memory T cells are produced after invasion by a specific organism. They provide long lasting immunity against that particular organism and then wait to rapidly respond to a second attack by the same organism. Their average survival rate is about 5 years.

**B cells**

The B cell matures in the bone marrow and is responsible for humoral, also known as antibody-mediated, immunity. When an antigen (foreign body) is presented to the B cell, either by a macrophage or helper T cell, the B cell becomes activated to produce plasma cells. The plasma cell then releases antibodies specific for that specific antigen.

**Natural killer cells**

There is a third class of lymphocyte that does not have T- or B-cell markers called natural killer (NK) cells. NK cells are nonspecific and can therefore respond to a variety of antigens. They are very effective against tumor cells and virally infected host cells.

1.1.5.4.4. Evaluating the WBC Count with Differential

The white count differential is expressed in cubic millimeters and in percentages. An elevation in the total WBC count (WBC 11,000/ L) is called leukocytosis. Leukocytosis most commonly identifies infection, tissue inflammation, or tissue necrosis associated with disorders such as acute myocardial infarction, burns, gangrene, leukemia,
radiation exposure, extremes in heat or cold, or lymphoma. AWBC count of greater than 10,000 has been associated with increased mortality rates in patients with acute coronary syndromes and is now being used by some as a predictor of adverse outcomes in these patients. The role of inflammation in the pathogenesis of ischemic stroke is also currently being studied. Patients with elevated WBC counts during the stroke event have been found to have a greater relative risk of subsequent ischemic stroke than did those with lower WBC counts. Thus, an elevated WBC count is being looked at as a predictor of ischemic stroke. Severely elevated total WBC counts (100,000), as seen in leukemia, promotes circulatory sludging and increased blood viscosity. Venous thromboembolism (VTE) prophylaxis is required in these situations. Leukocytosis may also occur in response to physical and emotional stressors such as over-exertion, seizures, anxiety, anesthesia, and epinephrine administration. With stress leukocytosis, however, the WBC will return to normal within an hour. Certain medications such as corticosteroids, lithium and Beta-agonists may also cause leukocytosis.

**Neutrophilia**

Neutrophilia is an increase in the total neutrophil count more than 70%. Neutrophilia is most commonly caused by an acute bacterial infection. Neutrophil counts will rise 4 to 6 hours after an invasion by microorganisms. If findings do not suggest infection, a myeloproliferative disorder may be the cause. Myeloproliferative disorders include polycythemia vera and chronic myelocytic leukemia, which increases stem cell proliferation in the bone marrow. Elevations in neutrophil counts are also associated
with obesity and cigarette smoking. Additionally, neutrophil counts can increase after the stress of surgery, but in this case, counts will quickly return to normal if no infection is present. An elevation in segmented neutrophils is considered a “shift to the right.” During tissue breakdown from injuries such as burns, arthritis, myocardial infarction, hemorrhage, or electric shock, neutrophils are called in to clean up the damage or the dead cell. An elevation in bands is referred to as a “shift to the left,” which means that there is an increased number of immature neutrophils released from the bone marrow and circulating in the blood. This occurs in response to overwhelming infection when the numbers of mature neutrophil reserves have been depleted. Clinically, the term shift to the left specifies an acute bacterial infection has depleted the normal reserves of mature neutrophils, and the bone marrow has had to resort to releasing immature ones.

**Eosinophilia**

Eosinophilia describes an absolute eosinophil count greater than 0.5\( \times 10^9 \)/L. This count has been found to increase with parasitic infections such as toxoplasmosis and with infections by gastrointestinal parasites. Elevations have also been noted with bronchoallergic reactions such as asthma, allergic rhinitis, and hay fever. Eosinophilia has also been noted with skin rashes.

**Basophilia**

An absolute basophil count greater than 0.2 \( \times 10^9 \)/L maybe observed with acute hypersensitivity reactions, chronic infections, and inflammatory disorders (tuberculosis,
rheumatoid arthritis, ulcerative colitis) or with viral infections (influenza, varicella). Many patients with myeloproliferative disorders (chronic, polycythaemia vera) present with basophilia which may increase in severity as the disorder progresses. Mast-cell leukaemia is a rare syndrome characterised by the presence in the peripheral blood of large numbers of mast cells of atypical appearance, associated with leukocytosis and Lymphocytosis.

Lymphocytosis

Lymphocytosis occurs in acute viral infections such as mononucleosis, cytomegalovirus, measles, mumps, and rubella. Elevated lymphocyte counts will also be noted in patients during chronic infections and early in human immunodeficiency virus (HIV) disease. Severely elevated levels would be seen with chronic lymphocytic leukemia (CLL).

Monocytosis

Increased monocyte counts, occur late during the acute phase of the infection and with chronic infections such as tuberculosis and sub-acute bacterial endocarditis (SBE). Monocytosis also occurs with Hodgkin’s disease, multiple myeloma, some leukemias, and systemic lupus erythematosus.

Decreased Counts/Levels

A decrease in the total WBC count (4,500/L) is called leukopenia. Leukopenia results from decreased production of total WBCs in the bone marrow or increased destruction...
of WBCs. Total counts will usually fall with radiation therapy and chemotherapy. Neutropenia is clinically defined as a neutrophil count of less than 2,000/L. Aetiology of neutropenia occasional bacterial infection such as typhoid, and overwhelming sepsis HIV infection, Aplastic anaemia, marrow infiltration, megaloblastic anaemia, hypersplenism, immune destruction and drugs.

**Other Reductions**

Reductions in eosinophil (eosinopenia) and basophil (basopenia) counts are uncommon because so few of these cells normally circulate in the blood. Monocytopenia is a rare occurrence but has been seen with glucocorticoid therapy, hairy-cell leukemia, and aplastic anemia. Lymphopenia, a decreased lymphocyte count, occurs normally as a person ages. Lymphopenia is most significant with HIV and acquired immunodeficiency syndrome (AIDS). A CD4 count (remember the helper T lymphocyte has the CD4 marker on its surface) of less than 200 is one indicator of conversion from HIV to AIDS.

**1.1.5.4.5. Erythrocyte (RBC)**

The main function of the RBC is to carry oxygen (O₂), which it picks up in the lungs, to the cells of the body, and to transport carbon dioxide from the cell to the lungs for excretion. Essentially, RBCs are containers for hemoglobin (Hgb). Hgb is the oxygen-carrying protein of the RBC. The average life span is approximately 120 days. The mature RBC is a biconcave disk. This unique shape allows for a greater surface area for
oxygen to combine with Hgb. RBCs have no nucleus, and therefore cannot divide. Like
the WBCs the RBC is derived from the PSC in the bone marrow. The production of
RBCs by the bone marrow is stimulated by low oxygen levels in peritubular cells of the
kidney in a process called erythropoiesis. During erythropoiesis, renal erythropoietic
factor (an enzyme) is secreted in response to peritubular cell hypoxia. This factor
interacts with a plasma protein to form erythropoietin, a hormone that circulates to the
bone marrow to stimulate stem cells to produce more RBCs. RBCs are released from the
bone marrow as reticulocytes and then become mature RBCs in one day. There is a
small percentage of reticulocytes released into the bloodstream that accounts for
approximately 0.5% to 1.5% of the total RBC count. An increased count indicates the
bone marrow is attempting to replace sudden RBC loss from hemorrhage or
destruction. A decreased count would indicate bone marrow hypo function. This count
is normally increased in pregnancy. Vitamin B12, folic acid, and iron are also needed
for RBC metabolism. Vitamin B12 and folic acid are needed for cell growth, DNA
synthesis, and for reproduction. Iron is needed for Hgb synthesis. RBCs count is the part
of the CBC that determines the number of RBCs found in a cubic centimeter of blood. It
is also expressed in International Units, which is the number of RBCs per liter of blood.
RBC indices are calculated mean values that are used to define the size, weight, and
Hgb content of the RBC. They are mainly used to classify anemias. RBC indices consist
of mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean
corpuscular hemoglobin concentration (MCHC).

1.1.5.4.6. Hemoglobin (Hgb)
Hgb’s primary function is to carry oxygen to the cells and remove carbon dioxide from the cells. Hgb is a complex protein made up of heme and globin. It is produced in the immature RBC. Synthesis stops once the cell matures in circulation. Hgb is measured in grams per deciliter.

1.1.5.4.7. Hematocrit (Hct)

Hct represents the percentage of the total volume of RBCs relative to the total volume of whole blood in a sample. “Hematocrit” means “to separate blood with today’s method of automated cell counting, Hct is calculated rather than centrifuged. The RBC count, Hct, and Hgb are closely related. Alterations in one are usually associated with alterations in the others.

*Increased Levels*

An increase in the number of RBCs can be described as erythrocytosis. Erythrocytosis seen in polycythemia either primary polycythemia or secondary polycythemia. Dehydration also causes a relative increase in RBC, Hgb, and Hct because of a decrease in plasma volume. This is clinically referred to as hemoconcentration.

*Decreased Levels*

Decreased levels of RBCs, Hgb, and Hct are associated with hemodilution and anemia.

1.1.5.4.8. Anemia and Rheumatoid Arthritis
Anemia in RA is classified as an anemia of chronic disease (ACD). ACD is considered the most frequent cause of anemia in RA; however, iron deficiency due to gastrointestinal blood loss or a combination of both should be considered in patients with RA developing anemia. In various cross-sectional studies, ACD has been reported to be present in 30% to 70% of patients with RA\(^{88,89}\) as confirmed by the data from Wolfe\(^1\). The anemia develops slowly during the first month of illness\(^89\) and has been found to be associated with a higher degree of disease activity. ACD is usually mild and non-progressive, characterized by decreased plasma iron, decreased total iron-binding capacity, decreased iron saturation of transferrin, decreased bone marrow sideroblast, and normal or increased reticuloendothelial iron\(^90\). The diagnosis of ACD is made by exclusion. The main problem in differential diagnosis of ACD in RA is the presence of concomitant iron deficiency. The most reliable characteristic for the detection of iron deficiency is stainable iron content in bone marrow aspirate \(^{90,91}\). Extensive studies have demonstrated that for daily clinical practice a combination of serological characteristics was able to make differentiation between an ACD and iron deficiency. The presence of low serum ferritin (< 50 g/l) in combination with high transferrin levels (50 g/l) and decreased mean corpuscular volume (MCV) of erythrocytes (80 fl) results in 100% sensitivity and specificity for the detection of iron deficiency \(^93\). Recently, serum transferrin receptor levels were proposed as a sensitive characteristic for detection of iron deficiency\(^{93,94}\). In other words, patients with RA with anemia and with elevated serum ferritin level (> 50 g/l), excluding iron deficiency will have an ACD. By using these characteristics, it is possible to avoid many invasive investigations
(colonoscopy, gastroscopy, bone marrow). To date, however, these findings have not been applied. Evidence suggests that increased production of inflammatory cytokines (tumor necrosis factor-α) is linked to a decrease erythropoietin response in the bone marrow, thereby leading to inadequate erythropoiesis (ex vivo experiments). As well, this inhibition could be partly reversed by increasing the concentration of erythropoietin \(^{95}\). Most of all, by treating RA patients with recombinant human erythropoietin (EPO) the suppression of erythropoiesis could be overcome\(^{96}\). Within 6 weeks a significant increase in hemoglobin levels was obtained in the RA patients treated with EPO. It was very surprising that sustained benefit was also apparent for RA disease activity. Of patients in the EPO group, 32% showed a Paulus 20% response, compared to 8% of the placebo treated patients. A beneficial effect was also observed on secondary disease activity characteristics for the number of swollen joints, pain score, and patient’s global assessment of disease activity. These findings were confirmed by other studies\(^{97}\). Investigations related to the effect of anemia on quality of life also demonstrated significant improvements during treatment with erythropoietin \(^{97,98}\). These results suggest that anemia is associated with a negative impact on both RA symptoms and quality of life. Thus the question needs to be raised of why so little research on anemia-related outcomes has been conducted. This gap in the literature is strange because anemia is a common comorbidity in patients with RA. Additional large-scale studies on prevalence and anemia-related outcomes are needed to support the importance of anemia screening and treatment in RA.
1.1.5.4.9. Platelets (Thrombocytes)

Platelets are the smallest of the cells found in blood. They are un nucleated, flattened disk shaped structures that can be round or oval. They have a lifespan of 9 to 12 days. Platelets play a vital role in hemostasis; they, along with the coagulation factors, are responsible for hemostasis in small and medium-size arteries and veins. Platelets aggregate or stick together to form the initial plug where there is damaged endothelium. Clotting factors are then triggered to form fibrin strands throughout the plug to firmly hold the plug together. For the capillaries, platelets plug and stop bleeding by themselves, thereby sealing the multitude of minute ruptures that occur on a daily basis. A platelet plug forms within 3 to 5 minutes. The platelet count only provides the number of circulating plates; it does not describe how adequately they function. The most indicative test of platelet function is the “bleeding time.”

Increases in the platelet count or thrombocytosis are usually asymptomatic until counts reach greater than 1,000,000 /L, where increased viscosity and inappropriate clotting may occur. A transient thrombocytosis with platelet counts of 450,000 to 600,000 /L can be seen as a physiologic response to physical stress, exercise, trauma, infection, and ovulation. Counts greater than 600,000 /L may be associated with myeloproliferative disorders of the stem cells in the bone marrow. Thrombocytopenia or decreased platelet counts defined as a count of less than 150,000 /L. Causes include depressed production by the bone marrow or increased consumption or destruction as seen with idiopathic thrombocytopenia. Bleeding usually does not occur until counts fall below 50,000 /L if
platelets are functioning normally. Small hemorrhagic areas under the skin called purpura may occur at this level (78).

1.1.5.5. Erythrocyte Sedimentation Rate (ESR)

This test was invented in 1897 by the Polish doctor Edmund Biernacki. In some parts of the world the test continues to be referred to as Biernacki's Reaction. The erythrocyte sedimentation rate (ESR) is the rate of sedimentation of RBCs and is used often as a non-specific measure in monitoring disease activity and assisting in the diagnosis of many inflammatory disorders. The ESR is not a well-understood phenomenon and has been described as occurring in 3 phases: RBC aggregation, precipitation, and packing. RBC aggregation is a critical factor for the sedimentation and is facilitated by the presence of certain plasma proteins called agglomerins, which include fibrinogen, IgM, and α2-macroglobulin. Any factors affecting these 3 phases, including those in the number and shape of RBCs and plasma viscosity, can affect the sedimentation rate. The ESR is expressed as millimeters per hour and varies between age and sex. (99)

Committee for Standardization in Hematology (ICSH) recommends the use of the Westergren method (100). While the role of acute phase reactants and cytokines in inflammatory responses is well-established (101). ESR measurement remains the method of choice in evaluating different clinical conditions (102). The ESR has also been found to be of clinical significance in the follow-up and prognosis of non-inflammatory conditions such as prostate cancer, (103) coronary artery disease (104) and
Therefore, the ESR is important in the diagnosis of inflammatory conditions and in the prognosis of non-inflammatory conditions.

1.1.5.5.1. Methods and factors affecting the test

The International Committee on Standardization in Hematology Reference Procedure accepts the Westergren method (100). Ethylene-diamin-etetra-acetic acid (EDTA) anticoagulated blood sample is preferably diluted in a large bore tube before using the Westergren tube (100). With this modified Westergren’s method (100,107), there is an excellent correlation with the ICSH reference. Several modifications to the existing method, including automated instruments, have been introduced during recent years to minimize human contact with blood products and to improve turnaround time (99). Blood samples can be stored for up to 24 hours at 4°C, but not at room temperature, without affecting the Westergren level (109). Erythrocyte aggregation is affected by two major factors: red cell surface charges and frictional forces around the red cell. The erythrocytes normally have net negative charges and, therefore, repel each other. High molecular weight proteins, especially when positively charged, increase viscosity and favor rouleaux formation and thus would raise the ESR (109,110). Fibrinogen, the most abundant acute phase reactant, has the greatest effect on the elevation of ESR when compared with other acute phase proteins (101,102,110). Paraproteins are positively charged molecules and when abundantly present as in multiple myeloma or Waldenstromis macroglobulinemia will increase the ESR levels by enhancing rouleaux formation and elevating plasma viscosity (101,102,110). For this reason, plasma viscosity measurement
correlates with the ESR, but it is not as reliable as that of ESR since it is marginally affected by short-term changes in acute phase responses. On the other hand, a change in the frictional forces around the red blood cell can affect the ESR. A drop in the red cell number, as in anemia, slightly elevates the ESR since this also physically interferes with rouleaux formation. Macrocytosis with a small surface-to-volume ratio have charge relative to their mass and thus sediment more rapidly. In general, normal values are 15 mm/hr or less for men and 20 mm/hr or less for women. Normal ESR values increase with age.

1.1.5.2. The ESR in clinical practice

ESR can aid in the diagnosis of RA, but it cannot be used solely for diagnosing RA. It is very useful when used with other parameters as outlined in the American College of Rheumatology Guidelines in the diagnosis and follow-up of RA patients. Wolfe and Michaud showed that the ESR can be elevated when RA is quiescent clinically and vice versa. The authors concluded that the ESR role in the diagnosis and follow-up of RA patients may not be accurate. An editorial comment on the study emphasized that the ESR should not be used as an isolated test, but as part of a group of clinical criteria to diagnose and follow patients with RA and inflammatory arthritis. The ESR is also helpful in the follow-up of systemic lupus erythematosus, but of questionable value, if any, inflammatory myopathy or spondyloarthropathy. The ESR is almost always elevated in both temporal arteritis and polymyalgia rheumatica. In temporal arteritis it may exceed 100 mm/hr. Multiple Myeloma and Other
Paraproteins. The importance of ESR parallels that of plasma viscosity in these conditions \(^{(107)}\). While an increased ESR is helpful in suspecting these conditions, the diagnosis depends on criteria such as monoclonal spike or serum electrophoresis, marrow plasmacytosis, and lytic bone lesions \(^{(112)}\). The ESR may have an additional role in follow-up of patients with otitis media, osteomyelitis, sickle cell disease, HIV, pelvic inflammatory disease, intravenous drug users, prostate cancer, coronary artery disease, and stroke. The ESR can be helpful in patients with symptoms. The ESR, however, should only be used as a guide. The clinician, when ordering an ESR, should realize that this test is only one parameter that could be helpful in the diagnosis and follow-up of certain inflammatory conditions. The ESR can also have an important prognostic role in non-inflammatory conditions such as prostate cancer, stroke, and coronary artery disease \(^{(113)}\).

1.2 A Previous studies of serological markers and haematological parameters in RA

In 2014, Binesh F. Salehabadi et al showed that anti-CCP antibodies indeed were good serological markers for RA. Anti-CCP antibodies were well suited as a front line diagnostic test for RA and especially early RA. In RF seronegative patients, anti-CCP positive can be helpful in confirming the diagnosis of RA. This does not mean that anti-CCP can replace RF in diagnostic and prognostic testing for RA. The study was offer a combination of anti-CCP and RF tests rather than anti-CCP or RF to get the best results in RA diagnosis \(^{(114)}\).
In 2009, Usha Singh et al, concluded that RF was still gold standard test for diagnosis of RA. Anti-CCP2 Ab was neither sensitive marker nor a marker for early diagnosis of RA but anti-CCP Ab has additional advantage of increasing the sensitivity when it is added in panel of investigations for RA along with RF\(^{(115)}\).

In 2005 Sigita Stropuvienė, et al, concluded that ACCP was a new and valuable serological marker for RA diagnosis, and the sensitivity and specificity of the method were high enough to suggest it for routine clinical practice. Performed together with RF, (ACCP (97%),Aka (92%), RF-IgM (80%) and RF-IgA (87%) it supports the early diagnosis at early stages and gives implications for early treatment. When tests for ACCP and RF were used in combination, they demonstrated the highest specificity for RA\(^{(1)}\).

In (2004), Nielen et al. Showed the appearance of anti-CCP antibody in the circulation may occur several years before RA onset and anti-CCP antibody represents a marker of future RA\(^{(116)}\).

In 2004, Kadir Yildirim, et al found serum CRP level was the best biochemical indicator of disease activity in RA patients\(^{(117)}\).

In 1996 Syed. Khalid.M.J and Pinal S.Roberts, observed that the count of WBCs was greater than 10,000mm\(^3\) which found in 40% from 98 patients with RA .The WBCs elevation was primary caused by an increase in neutrophil .Patients with leukocytosis tend to have more active arthritis\(^{(118)}\).
1.3 Rationale and Objectives

1.3.1 Rationale

Rheumatoid arthritis (RA) is a common chronic inflammatory autoimmune disease of unknown origin. Although laboratory testing and imaging studies can help confirm the diagnosis and track disease progress, rheumatoid arthritis primarily is a clinical diagnosis and no single laboratory test is diagnostic. Complications of RA may begin to develop within months of presentation; therefore, early referral or consultation with a rheumatologist for initiation of treatment disease-modifying anti rheumatic drugs is recommended. This study was aimed to assess and correlate the hematological parameters and serological markers with the clinical features in RA. Patients will able to make beneficial effect in diagnosis, follow up, supportive treatment and progress the quality of life.

1.3.2 Objectives

1.3.2.1 Main Objective

Assessment of the serological markers and hematological parameters among Sudanese patients with rheumatoid Arthritis
1.3.2.2 Specific Objectives

1. Estimation of complete blood count and ESR among Sudanese patients with Rheumatoid Arthritis.

2. Assessment of serological markers (RF, ACCP and CRP) among Sudanese patients with Rheumatoid Arthritis.

3. Correlation and exploration the association between the hematological parameters and serological markers among Sudanese patients with Rheumatoid Arthritis.

4. Correlation and assessment the hematological parameters and serological markers in Sudanese patients with Rheumatoid Arthritis according to sex, age and clinical presentation.

5. Correlation and comparison the hematological parameters and serological markers between patients and controls.
Chapter Two

2. Materials and Methods

2.1 Materials

A cross-sectional-prospective study was performed at National Ribat University, Khartoum, Sudan during 2013 to 2015. The clinical diagnosis, examination and diagnostic work-up have been conducted at the Alngaib Clinic referred by Clinical Rheumatologist (Dr. Hamza Khidir) in Nyala, Darfour state, Sudan. The study was included all patients who had been diagnosed as clinically Rheumatoid Arthritis with different clinical features. The practical work performed in Alnagib Medical Laboratory, Nyala, Sudan. Clinical data for each patient had been recorded in a pre-designed questionnaire (Appendix 1). An informed consent was obtained from each patient. A total of 60 RA Sudanese patients: 23 males and 37 females age ranging between 30 and 60 years. Also a 60 healthy controls were involved in this study; ages ranging between 30 to 60 years. Five ml of venous blood were collected from each patient and healthy control. A 2.5 ml were added in EDTA containers for complete blood count (CBC), erythrocyte sedimentation rate (ESR), rheumatoid factor (RF) and C-reactive protein (CRP) performance, while the other 2.5 ml were kept in plain container for the ACCP performance.
2.2 Methods

2.2.1 Complete Blood Count

The counting of cellular elements in blood sample was done by full automated hematology analyzer (Mindray- BC 3000 plus) based on impedance cemetery technique. This technique was based on the modification of the impedance of a calibrated aperture soaking in an electrolyte and going through a constant course delivered by two electrodes located on both sides of the aperture. A vacuum was applied on side of the aperture allows the cells passage. They opposed their physical volume to the course passage. A voltage impulse was registered at the electrodes terminal. The height of this impulse was proportional to the cell volume. The innovative optical detection system was covered by two patents pending. This technology called: OCHF (for optical cytometer hydro focus free) was based on a unique and innovative concept of an active sample flow and passive sample flow was introduced in the flow cell under pressure and the sheath was only dedicated to maintain it. This principle was unable to introduced a large quantity of sample and to use a great dilution rate (which allows to do haemoglobin measurement with the same dilution).For each cell throwing the optical detection area, to pulses were generated , when for the axis loss light (ALL) measurement and for the forward side scatter (FSC) measurement. The result of those two axis of measurement was high dilution matrix that unable to identify the WBC population, the five part different was obtained by the optical matrix analysis after
action of the lytic reagent (banding pattern). The reagent destroys the RBCs and their stromas, composes the oxyhaemoglobin chromogen and product the white blood cell membrane to keep it in closed native state. The haemoglobin measurement was directly done in the WBCs chamber by spectrophotometer at 555 nm. Haemoglobin is detected by formation a chromogenoxy haemoglobin type (cyanide free technique). A measurement of blank haemoglobin was done for each analytic cycle and during the startup raising step. Leucocytes analysis is done by impedancemetry in the WBCs counting chamber, the other ten parameters were obtain by flowcytometry measurement. The erythrocyte analysis was done by impedancemetry in RBCs counting chamber and by analysis of the haemoglobin inside the WBCs chamber as previously described. Seven parameters are obtained, RBCs, HGB, HCT, MCV, MCH, MHC, RDW, the red cell indices are calculated. Platelet analysis was made by impedancemetry in the RBC counting chamber at same time with red blood cell, four parameters are obtained, platelet, MPV, PDW, P-LCR. The protocol was reported by Mindray company, China.

**Normal range**:

<table>
<thead>
<tr>
<th>Red blood cell count</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td>5.0 ± 0.5 × 10^{12}/l</td>
</tr>
<tr>
<td>Women</td>
<td>4.3 ± 0.5 × 10^{12}/l</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>--------------------------</td>
<td>--------------------</td>
</tr>
<tr>
<td><strong>Haemoglobin</strong></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>150 ± 20 g/l</td>
</tr>
<tr>
<td>Women</td>
<td>135 ± 15 g/l</td>
</tr>
<tr>
<td><strong>Packed cell volume (PCV) or Haematocrit (Hct)</strong></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>0.45 ± 0.05 (l/l)</td>
</tr>
<tr>
<td>Women</td>
<td>0.41 ± 0.05 (l/l)</td>
</tr>
<tr>
<td><strong>Mean cell volume (MCV)</strong></td>
<td></td>
</tr>
<tr>
<td>Men and women</td>
<td>92 ± 9 fl</td>
</tr>
<tr>
<td><strong>Mean cell haemoglobin (MCH)</strong></td>
<td></td>
</tr>
<tr>
<td>Men and women</td>
<td>29.5 ± 2.5 pg</td>
</tr>
<tr>
<td><strong>Mean cell haemoglobin concentration (MCHC)</strong></td>
<td></td>
</tr>
<tr>
<td>Men and women</td>
<td>330 ± 15 g/l</td>
</tr>
<tr>
<td><strong>Red cell distribution width (RDW)</strong></td>
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</tr>
<tr>
<td>As coefficient of variation (CV)</td>
<td>12.8 ±1.2%</td>
</tr>
<tr>
<td>As standard deviation (SD)</td>
<td>42.5 ± 3.5 fl</td>
</tr>
<tr>
<td>---------------------------</td>
<td>----------------</td>
</tr>
<tr>
<td>White blood cell count</td>
<td>4.0–10.0 × 10⁹/l</td>
</tr>
</tbody>
</table>

**Differential white cell count**

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Count</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutrophils</td>
<td>2.0–7.0 × 10⁹/l</td>
<td>40–80%</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>1.0–3.0 × 10⁹/l</td>
<td>20–40%</td>
</tr>
<tr>
<td>Monocytes</td>
<td>0.2–1.0 × 10⁹/l</td>
<td>2–10%</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>0.02–0.5 × 10⁹/l</td>
<td>1–6%</td>
</tr>
<tr>
<td>Basophils</td>
<td>0.02–0.1 × 10⁹/l</td>
<td>&lt;1–2%</td>
</tr>
<tr>
<td>Platelet count</td>
<td>280 ± 130 × 10⁹/l</td>
<td></td>
</tr>
</tbody>
</table>

**2.2.2 Erythrocyte sedimentation rate by fast detector protocol (ESR)**

A citrated venous blood sample was added to ESR vacuum tube and gently mixed. Then, it was incubated vertically onto the fast detector rack room for 30 minutes and then the result was read aligning the upper surface of erythrocyte to the scale on the detector. The protocol was reported by IMPROVE MEDICAL Technology Co.Ltd, ESR Fast Detector.
**ESR Normal range**

Male: up to 15mm.

Female: up to 20mm.

2.2.3 IgM Rheumatoid Factor protocol (IgM –RF)

The Rheumatoid Factor level was measured by quantitative immunoassay using IgM RF i-CHROMATM kit and reader. A sandwich immune detection method, such that the detection antibody in buffer *(A detection buffer containing fluorescence labeled anti-human IgM antibody, labeled with biotin fluorescence BSA stabilizer and sodium azide as a preservative in PBS.)* was bound to human RF-IgM in the plasma sample and antigen-antibody complexes were captured by antibodies that have been immobilized on the test strip as sample mixture migrates through nitrocellulose matrix.

*Reference value < 20 IU/mL.*

2.2.4 Quantitative Measurement of C-reactive protein (CRP)

The i-CHROMATM CRP Test is based on fluorescence immunoassay technology. The i-CHROMATM CRP Test uses a sandwich immune detection method, such that by mixing detector buffer with blood specimen in test vial, the fluorescence-labeled detector anti-CRP antibody in buffer binds to CRP antigen in blood specimen. As the sample mixture is loaded onto the sample well of the test device and migrates the nitrocellulose matrix of test strip by capillary action, the complexes of detector antibody and CRP are captured to anti-CRP sandwich pair antibody that has been immobilized on test strip. Thus the more CRP antigen is in blood specimen, the more complexes are
accumulated on test strip. Signal intensity of fluorescence of detector antibody reflects amount of CRP captured and is microprocessed from i-CHROMATM Reader to show CRP concentration in blood specimen. The default result unit of i-CHROMATM CRP Test is displayed as an mg/L from i-CHROMATM Reader.

Reference Range: > 5 mg/L may reflect an acute-phase response to infectious diseases or disorders characterized by acute inflammation. (Refer to i-CHROMA-TM Reader Operation Manual for the complete instructions on use of the Test. REF Catalog No. FR-203).

2.2.5 Anti-CCP ELISA (IgG)

The Anti-CCP ELISA (IgG) test kit (EUROIMMUN Medizinische Labordiagnostika A G, USA) was intended for the qualitative or semi-quantitative determination of IgG class antibodies against cyclic citrullinated peptides (CCP) in human serum and plasma. It was used as an aid in the diagnosis of rheumatoid arthritis, in conjunction with other laboratory and clinical findings. The test kit contained 12 microtiter strips each with 8 break-off reagent wells coated with synthetic cyclic citrullinated peptides. In the first reaction step, diluted patient samples, calibrators and controls were incubated in the wells. Anti-CCP antibodies were bind to the antigens coated in the microtiter wells. The wells were washed to remove any unbound proteins and non-specific antibodies. In a second reaction step, rabbit anti-human IgG HRP enzyme conjugate was added to each well. The enzyme conjugate was bind to any wells that have human IgG binding to the CCP antigen. The wells were washed to remove any unbound HRP enzyme conjugate. Then 3,3,5,5 tetramethylbenzidine (TMB) enzyme substrate was added. If the HRP
enzyme is present in the well (positive reaction), the HRP enzyme was react with the TMB substrate and produced a blue color. After an additional incubation time to allow the color development, a stop solution was added which turns the blue color yellow and inhibits further color development to allow for a stable spectrophotometric reading. The test strips were placed in a microplate reader and the optical density of the color was measured. The amount of antigen specific bound antibody was proportional to the color intensity.

**Reference Range : < 1 RU/ml**

**2.2.6 Statistical analysis**

The analysis of the data was based on 120 cases (patients and controls), analyzed as non-experimental exploratory method using SPSS package. Cross tabulation, Chi-square tests, Test and ANOVA for RA patients and controls *sex, RA patients and controls *age, RA patients and controls *Serological markers, RA patients and controls *Haematological parameters , Clinical symptoms * hematological/immunological markers were used.
Chapter Three

3. Results

3.1 Rheumatoid Factor

Of the 60 patients with clinically Rheumatoid Arthritis, there were 27 (45%) patients had RF positive (>20 IU/ml). The means of Rheumatoid Factor results in females and males were 62.7 IU/mL and 35.3 IU/mL respectively, while in the 60 controls the mean for males was 10.2 IU/mL and 10 IU/mL for females. A significant statistical difference was found between the means of RF in patients and control. (P.value=0.0001). Moreover, a statistical significant correlations were found between RF and disease duration; ACCP; CRP; ESR, Neutrophil count and Lymphocyte count (P.value<0.05) (Figure 3.1).
Figure 3.1 Shows the mean of RF in patients and control according to sex

3.2. C-Reactive Protein (CRP)

Of the 60 patients with clinically Rheumatoid Arthritis, there were 56 patients (93.3%) had CRP positive (> 5 mg/L). The means of CRP results in females and males were 20.8 mg/L and 45.7 mg/L respectively, while in the 60 controls the mean for males was 3.6 mg/L and 4.4 mg/L for females. A significant statistical difference was found between the males and females in CRP result (P. value=0.02). Also a significant statistical difference was found between the means of CRP in patients and control (P. value=0.000). Moreover there were significant statistical correlations between CRP and ACCP; RF; Platelets; neutrophils and lymphocytes (P≤ 0.05). (Figure 3.2)
Figure 3.2 Shows the mean of CRP in patients and control according to sex

3.3 Anti-CCP

Of the 60 patients with clinically Rheumatoid Arthritis, there were 28 patients (46.7%) had ACCP positive (>1 RU/ml). The means of ACCP results in females and males were 4.2 RU/ml and 1.7 RU/ml respectively; while in the 60 controls the mean for males was 0.4 RU/ml and 0.5 RU/ml for females. A significant statistical difference was found between the males and females in ACCP result (P. value=0.005). Also a significant statistical difference was found between the means of ACCP in patients and control (P.
value=0.000). Moreover there were significant statistical correlations between ACCP and RF; CRP; Hb; RBC and disease duration (P.value ≤ 0.05). (Figure 3.3)

Figure 3.3 Shows the mean of ACCP in patients and control according to sex

3.4 ESR

Of the 60 patients with Rheumatoid Arthritis, the means of ESR results in both females and males were 72 mm \ hour, while in the 60 controls the mean for males was 14 mm\h and 18 mm\h for females. A significant statistical difference was found between the means of ESR in patients and controls (P. value=0.000). Moreover there were significant statistical correlations between ESR and RF; Hb (P ≤ 0.05).
3.5 Haemoglobin (Hb)

Of the 60 patients with Rheumatoid Arthritis, the means of Hb results in females and males were 12.2 g/dl and 13.3 g/dl respectively; while in the 60 controls the mean for males was 14.8 g/dl and 13.5 g/dl for females. A significant statistical difference was found between the males and females in Hb result (P. value=0.00). Also a significant statistical difference was found between the means of Hb in patients and control (P. value=0.00). Moreover there were significant statistical correlations between Hb and RBCs, ESR, ACCP and platelets (P.value ≤ 0.05). (Figure 3.4)

Figure 3.4 Shows the mean of Hb in patient and control according to sex
3.6 Red Blood Cells Count (RBCs)

Of the 60 patients with Rheumatoid Arthritis, the means of RBCs results in females and males were 4.8 $\times 10^{12}$ /l and 5 $\times 10^{12}$ /l respectively; while in the 60 controls the mean for males was 5.3 $\times 10^{12}$ /l and 4.7 $\times 10^{12}$ /l for females. No significant statistical difference was found between the males and females in RBCs result ($P$-value=0.1). Also, no significant statistical difference was found between the means of RBCs in patients and control ($P$-value=0.4). Moreover there were significant statistical correlations between RBCs count and Hb; platelets count ($P$-value ≤ 0.05). (Figure 3.5)

Figure 3.5 Shows the mean of RBCs in patients and control according to sex
3.7 White Blood Cells Count (WBCs)

Of the 60 patients with Rheumatoid Arthritis, the means of WBCs results in females and males were $8.6 \times 10^9 /l$ and $8.1 \times 10^9 /l$ respectively; while in the 60 controls the mean for males was $5.3 \times 10^9 /l$ and $5.7 \times 10^9 /l$ for females. No significant statistical difference was found between the males and females in WBCs result (P. value=0.7). While a significant statistical difference was found between the means of WBCs in patients and control (P. value=0.002). Moreover there were significant statistical correlations between WBCs and Monocytes count; Lymphocytes count and eosinophil count (P ≤ 0.05). (Figure 3.6)

Figure 3.6 Shows the mean of the WBCs count in patient and control according to sex.
3.7.1 WBCs differential count

3.7.2 Neutrophil Percentage

Of the 60 patients with Rheumatoid Arthritis, the means of Neutrophil percentage in females and males were 54 and 56 respectively; while in the 60 controls the mean for males was 50 and 52 for females. No significant statistical difference was found between the males and females in Neutrophil percentage (P. value=0.5). While a significant statistical difference was found between the means of Neutrophil percentage in patients and control (P. value=0.02). Moreover there were significant statistical correlations between Neutrophils and RF, CRP, lymphocytes and eosinophils (P≤ 0.05). (Figure 3.7)

Figure 3.7 Shows The mean of neutrophil percentage in patient and control
3.7.3 Lymphocyte Percentage

Of the 60 patients with Rheumatoid Arthritis, the means of Lymphocyte percentage in females and males were 37 and 35 respectively; while in the 60 controls the mean for males was 41 and 39 for females. No significant statistical difference was found between the males and females in Lymphocyte percentage (P. value=0.8). While a significant statistical difference was found between the means of Lymphocyte percentage in patients and control (P. value=0.03). Moreover there were significant statistical correlations between Lymphocytes and RF, CRP, WBCs and neutrophils (P≤ 0.05).

(Figure 3.8)
Figure 3.8 shows the mean of lymphocyte percentage in patient and control according to sex.

3.7.4 Monocyte percentage

Of the 60 patients with Rheumatoid Arthritis, the means of Monocyte percentage in females and males were 6 and 8 respectively; while in the 60 controls the mean for males was 8 and 7 for females. A significant statistical difference was found between the males and females in Monocyte percentage (P. value=0.006). While, no significant statistical difference was found between the means of Monocyte percentage in patients and control (P. value=0.1). Moreover there were significant statistical correlations between monocytes eosinophils count WBCs count (P ≤ 0.05). (Figure 3.9)
3.7.5 Eosinophil Percentage

In the 60 patients with Rheumatoid Arthritis, the range of Eosinophil percentage in females and males were (0-2 %) and (0-10 %) respectively; while in the 60 controls the range for both males and females were (0-2%). No significant statistical difference was found between the males and females in Eosinophil percentage (P. value=0.1). Moreover no significant statistical difference was found between the means of Eosinophil percentage in patients and control (P. value=0.3). Moreover there were significant
statistical correlations between eosinophil and WBCs, monocytes and neutrophils (\(P \leq 0.05\))(Figure 3.10).

3.7.6 Basophil Percentage

Of the 60 patients with Rheumatoid Arthritis and 60 healthy control, the range of Basophil percentage was (0-2). No significant statistical difference was found between the basophil percentage and the hematological/immunological parameters (P. value ≥ 0.05).

![Bar Chart](image)

Figure 3.10 Shows the mean of Eosinophil percentage in patient and control

3.8. Platelets count
Of the 60 patients with Rheumatoid Arthritis, the means of Platelets count in females and males were $342 \times 10^9 /l$ and $324 \times 10^9 /l$ respectively; while in the 60 controls the mean for males was $269 \times 10^9 /l$ and $283 \times 10^9 /l$ for females. No significant statistical difference was found between the males and females in Platelets count ($P$-value=0.3). While, a significant statistical difference was found between the means of Platelets count in patients and control ($P$-value=0.001). Moreover, a statistical correlations were found between platelets count and CRP, Hb and RBCs ($P$-value <0.05). (Figure 3.11)

![Figure 3.11 Shows the mean of platelets count in patient and control](Image)

3.9 Clinical features

3.9.1 Knee joint pain
Of the 60 patients with Rheumatoid Arthritis, 59 of them had knee joint pain. The frequency in females and males were 37 and 23 respectively. No significant statistical correlation was found between the knee joint pain and sex; hematological/immunological markers (P. value=0.3).

### 3.9.2 Warm full swelling

Of the 60 patients with Rheumatoid Arthritis, 46 of them had warm full swelling. The frequency in females and males were 29 and 17 respectively. No significant statistical correlation was found between the sex and warm full swelling and hematological/immunological markers (P. value=0.46).

### 3.9.3 Wrist pain

Of the 60 patients with Rheumatoid Arthritis, 57 of them had wrist pain. The frequency in females and males were 35 and 22 respectively. No significant statistical correlation was found between the sex and wrist pain; hematological and immunological markers (P. value=0.6).

### 3.9.4 Morning stiffness

Of the 60 patients with Rheumatoid Arthritis, 53 of them had morning stiffness. The frequency in females and males were 32 and 21 respectively. No significant statistical correlation was found between the sex and morning stiffness (P. value=0.4). While a significant correlation between lymphocytosis and morning stiffness (P.value = 0.03).
3.9.5 Joint deformability

Of the 60 patients with Rheumatoid Arthritis, 25 of them had joint deformability. The frequency in females and males were 14 and 11 respectively. No significant statistical correlation was found between the sex and joint deformability (P. value=0.3). On the other hand a significant correlations were found between RF, ACCP and platelets count and joint deformability (P.value ≤ 0.05).(Figure 3.12 and 3.13)
Figure 3.12 shows the correlation between Joint deformability and RF
3.9.6 Impaired movement

Of the 60 patients with Rheumatoid Arthritis, 34 of them had impaired movement. The frequency in females and males were 22 and 12 respectively. No significant statistical correlation was found between the sex and impaired movement (P. value=0.38). On the other hand, a significant correlations were found between RF, ACCP and impaired movement (P.value = 0.004). (Figure 3.14 and 3.15).
Figure 3.14 shows the correlation between the impaired movement and RF
3.9.7 Symptoms in symmetric way

Of the 60 patients with clinical Rheumatoid Arthritis, 42 of them had symptoms in symmetric way. The frequency in females and males were 28 and 14 respectively. No significant statistical correlation was found between the sex and Symmetric arthritis (P.value=0.17). On the other hand a significant correlation between symptoms in symmetric way and RF ; ACCP (P.value = 0.000). (Figure 3.16 and 3.17)
Figure 3.16 Shows the correlation between Symptoms in symmetrical way and RF
Figure 3.17 Shows the correlation between Symptoms in symmetrical way and ACCP n
3.9.8 Ankle –Elbow pain

Of the 60 patients with Rheumatoid Arthritis, 57 of them ankle-elbow pain. The frequency in females and males were 35 patients and 22 patients respectively. No significant statistical correlation was found between sex and ankle –elbow pain (P. value=0.67).

3.10 Disease duration per months

The disease duration in females and males was 35 months and 21 months respectively. A significant statistical difference was found between the sex and disease duration (P. value=0.007). Moreover, a statistical significant correlations were found between the duration and RF and ACCP (P<0.05).
Chapter Four

4. Discussion

To our knowledge, this was the first study in Sudan aimed to compare between sex and baseline Rheumatoid Arthritis status outcome like hematological / immunological parameters and Clinical features. We focused on the difference between the laboratory outcomes according to the sex and that to investigate whether this would provide additional diagnostic and prognostic informations in Rheumatoid Arthritis patients. According to sex we observed that, females were affected more than males. The frequency of positive RA females (22 patients) to positive RA males (6 patients) was approximately 3:1. These findings were closely matched with Firestein GS, 2005, who observed that, the women were affected more often than men (3:1 ratio) and the most frequent age between 30 years and 50 years (2).

On the other hand, the females showed a longer disease duration (35 months) rather than males (21 months) with significant difference (P.value = 0.007). When we estimated the IgM - RF quantitative level, we found also, the females revealed the higher level (62.7 U/ml) rather than males (35.3 U/ml), with significant difference between sex. Moreover, for baseline ACCP levels, we found that females showed the higher frequency (4.2 RU/ml) rather than males (1.7 RU/ml). A significant correlation and
matching in positivity between RF (positive in 27 patients) and ACCP (positive in 28 patients). This finding supported and agreed with Binesh F. et al, those who recommended that, a combination of anti-CCP and RF tests rather than anti-CCP only or RF single test to get the best results in RA diagnosis. Moreover, the study confirmed the conclusion of Usha S, in 2009, who concluded that ACCP Ab has additional advantage of increasing the sensitivity when it is added in panel of investigations for RA along with RF. In addition, IgM-RF was significant correlated with ESR; ACCP; CRP; Neutrophil count and Lymphocyte count. These positive correlations were agreed with the recommendation of the American College of Rheumatology Subcommittee on Rheumatoid Arthritis (ACRSRA), who recommended that baseline laboratory evaluations for RA must include a complete blood cell count with differential, rheumatoid factor, and erythrocyte sedimentation rate (ESR) or C-reactive protein (CRP). On the other hand, the CRP results were showed the higher level in males (45.3 mg/l) rather than females (20.8 mg/l).

According to the clinical features in this study, a significant correlation were found between appearance of deformability (in 25 patients) and impaired movement (in 34 patients) with RF, ACCP and platelets count. This findings were agreed with Ronnelid, J in 2005 who concluded that Anti-CCP antibody-positive patients with early RA are at increased risk of progressive joint damage.

The hematological parameters in our study showed that ESR result was matched between males and females (72 mm/h in both). Moreover, in ESR result, there was
significant directional correlation was found between ESR and RF, while a reversible correlation with Hb. Although the haemoglobin level within normal in females patients (12.2 g/dl) and slightly decreased in males patients (13.3 g/dl), but we found a significant difference between them, and moreover a significant difference between females control (13.5 g/dl) and males control (14.8 g/dl). The exclusion of anemia of chronic disease might be due to the healthy lifestyle of the western population in Sudan. Also the red blood cells count revealed the same results mentioned above concerned the Hb beside more significant correlation with platelets instead of ACCP. All these findings might disagreed with Harris ED in (2005) who mentioned that most of RA patients had normocytic normochromic anemia.

Another finding was the white blood cells count, all patients showed mean count 8,000 cu/mm and the control showed 5,000 cu/mm, with a significant difference (P.value=0.002). That mean a mild leukocytosis within the 21 months to 35 months of disease duration without treatment and these finding was agreed with Scottish in 2000, who summarized that WBCs count may be increase in RA patients. Moreover we found a significant correlation with the positivity of ACCP, CRP and RF with the decreased in lymphocytes count. About the platelets count in our study, there was a mild increase in patients (335,000 cu/mm) comparing with the control (275,000 cu/mm).

On the other hand, we observed that all the clinical features including knee joint pain, warm full swelling, wrist pain, morning stiffness, deformability, impaired movement, symmetric arthritis and ankle–elbow pain were increased in females rather than males.
Our study also agreed with Hariss ED, in 2005 in disease duration time, we found the mean of disease duration time was above 20 months. The females were diagnosed after longer duration (35 months) without treatment, while the males were diagnosed within 21 months. No single diagnostic test definitively confirms the diagnosis of rheumatoid arthritis. The present study concluded that ACCP, RF, ESR, CRP and Hematological parameters definitely should be performed as panel for confirming the diagnosis, prognosis, activity and treatment management of RA from other inflammatory diseases. Our study agreed with Binesh F.S et al in 2014, who showed that anti-CCP antibodies indeed were good serological markers for RA, and in RF seronegative patients, anti-CCP can be helpful in confirming the diagnosis of RA. And this does not mean that anti CCP can replace RF in diagnostic and prognostic testing for RA. Binesh also offered a combination of anti-CCP and RF tests rather than anti-CCP or RF to get the best results in RA diagnosis (114).
4.1 Conclusions and Recommendations

4.1.1 Conclusions

1. Forty six percent only (46%) of patients were really diagnosed as Rheumatoid Arthritis.

2. No single Laboratory test will confirm the diagnosis of RA. ACCP, CRP, ESR, RF and Hematological parameters should be performed as panel for diagnosis, detect disease activity, prognosis and treatment management among RA patients.

3. Females were affected more than males (3:1).

4. ACCP and RF were higher in females rather than males.

5. CRP was higher in males rather than females.

6. Directional significant correlation between ACCP, CRP, ESR and RF.

7. Directional significant correlation between ACCP, CRP, RF and decrease in lymphocytes.
8. Absent of either normocytic normochromic or microcytic hypochromic anemia among RA patients.

9. Mild leukocytosis in patients compared with healthy control.

10. Knee joint, wrist pain and stiffness pain was common sign in all patients either ACCP positive or negative.

11. Deformability, impaired movement and symmetrical arthritis were associated with positive ACCP and RF.

12. Females were showed complications in 35 months without treatment.

13. Males were showed complications in 21 months (not poor prognosis).

14. The immunoflurescent protocol of RF and CRP by i-Chroma was revealed reliable results, but high in cost compared with ELISA.

4.1.2 Recommendations

1. Detection of HLA-DR 1&4 genes and CD4 in RA patients for disease management.

2. X-ray, CT-scan and MRI in primary diagnosis to exclude the OA patients.

3. Perform LFT &RFT (Liver &Renal function tests) to correlate the disease activity, prognosis and treatment management with the immunological and hematological parameters.

4. Determine the time of morning stiffness (more than hour or less).
5. Increase sample size and search in other different tribes and populations in Sudan with different life style.

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