Chapter One

Introduction and Literature Review

1.1. Introduction

Rheumatoid Arthritis (RA) is a chronic, progressive, systemic, autoimmune disease that affects 0.3-1% of the world population (WHO, 2019). It affects the joints, connective tissues, muscle, tendons, and fibrous tissue, characterized by persistent synovitis, systemic inflammation, and auto-antibodies (Scott *et al.*, 2010). RA tends to develop during adulthood between the ages of 20 to 40 years (WHO, 2019). In Sudan, the mean age of the onset of the disease is 43 years, with a predilection of females (Elshafie, 2016). Uncontrolled active rheumatoid arthritis causes disability and decreases the quality of life.

Fasting is the willing partial or total abstinence from eating and drinking, for a period of time, it is performed mainly for religious reasons. Besides, it has been found that deprivation of food for a period of time has a numerous health benefits ranging from improved digestion, sleep and weight loss to change in the immunological and metabolical responses of the body (El-banna and El-Garawany, 2016). Fasting has also has been relied upon as an alternative strategy in improving health and increasing longevity in certain diseases without the need for pharmacological interventions. The three most commonly studied fasting strategies are: Caloric Restriction (CR), Dietary Restriction (DR) and Intermittent Fasting (IF) (Ahmed *et al.*, 2019).

Intermittent fasting is an eating pattern that involves fasting for varying periods of time, typically for 12 hours or longer (Anton *et al.*, 2018). In IF individuals usually fast for 14-16 hours a day, restricting their "eating window" (Leonard, 2018), which has shown a great benefit in rheumatoid arthritis patients, it has been found to reduce the inflammation (Dalton, 2018).

Hence, Ramadan fasting, which is classified as a type of Intermittent fasting, comprises fasting for 14-18 hours a day. It has been found to be useful in reducing inflammation and disease severity in rheumatoid arthritis patients in a number of growing literature (Darlington and Ramcy, 1993). This study is designed to scientifically investigate the impact of fasting on the disease process in rheumatoid arthritis patients.

1.2. Literature Review

1.2.1. Blood

Blood is a fluid connective tissue that flows through our veins, arteries and capillaries to transport oxygen, nutrients, and waste products to and from tissues, it also carries cells that have specialized functions and antibodies that fight infections, and it has other homeostatic functions. It forms around 7 to 8 % of our body weight. It is comprised of a matrix termed plasma (55%) and formed constituents (45%). Plasma is composed of 92% water, 7% plasma proteins, 1% of other solutes. It's main function is to carry nutrients, waste products, antibodies, chemical messengers, such as hormones and proteins (Jackson and Ahmed, 2007).

The formed constituents part is composed of cells, and is mainly comprised of Red Blood Cells, which contains hemoglobin, that carries and delivers Oxygen from the lungs, and Carbon di oxide to the lungs to be exhaled (American Society of hematology, 2019). White Blood Cells (leukocytes), which are divided into granulocytes, which function in fighting infections and allergy, and, agraulocytes, which also functions in fighting infections, and has immunological functions, like producing antibodies, cytokines and take part in chronic inflammation. And platelets which also comprises a small part of

the cellular segment, they are small cellular fragments that functions in the blood clotting process and preventing leakage of blood (American Society of hematology, 2019).

1.2.2. Immunity

Immunity is the processes that takes place to defend the body against foreign organisms or molecules. It is divided into: Innate_Immunity, which is the natural immunity, doesn't need prior exposure. It has several mechanisms: First line of defense. Such as, Physical Barriers (Skin, Mucus), Chemical Barriers (Lysozymes, low PH). Second line of defense. Such as: Phagocytosis, which is ingesting the microorganism and digesting it by phagocytic cells. Inflammation, which is a non-specific response by the body to trap and eliminate the agent causing tissue injury. Complement activation, in which certain proteins cascade are activated leading to a product formation, which stimulates inflammatory response, enhances phagocytosis and lyses foreign cells (Cuilla and Lehman, 2010). Adaptive Immunity, which is the acquired immunity, which occurs, after exposure or encountering a certain agent, and responds specifically to this agent. It has two types: Humoral mediated immunity, through producing antibodies. Antibodies which are specific host self-proteins produced in response to a specific foreign non-self-protein or intolerated self-proteins. And, Cellular mediated, through producing a number of cells, such as, Natural Killer cells, T-helper cells, Cytotoxic T-lymphocytes (Chaplin, 2010, Cuilla and Lehman, 2010).

1.2.3. Inflammation

Inflammation is a local response to cellular injury that is marked by capillary dilatation, leukocytic infiltration, redness, heat, pain, swelling, and often loss

of function, it serves as a mechanism initiating the elimination of noxious agents and of damaged tissue (Kumar, *et.al.* 1997).

1.2.3.1. Types of inflammation:

Kumar *et. al* (1997) explained and classified the types of inflammation into: Acute Inflammation, which is of a short duration, lasting for few minutes or up to days, and it is characterized by fluid and plasma protein exudation, and mostly the accumulation of Neutrophils. And to Chronic Inflammation, which is of a longer duration, and is manifested histologically by an influx of lymphocytes and macrophages and by tissue destruction and repair.

In both the acute and chronic inflammation, cytokines are developed from a variety of cell types (mainly Lymphocytes and Monocytes) and are involved in the regulation of the magnitude and duration of the inflammatory response; they are also known as inflammatory mediators, such as Interleukins 1, 6 and Tumor Necrotic Factor-Alpha and Beta, Interferon-Gamma, and each has its own role in stimulating inflammation process (Zhang and An, 2009, Kumar, *et al.* 1997).

The Author also added that, during inflammation certain plasma proteins are also elevated in the body, such as, the acute-phase reactants, which are the C-reactive proteins, complement proteins, fibrinogen, haptoglobins...Etc. (Kumar, *et al.* 1997).

1.2.4. Autoimmune disease

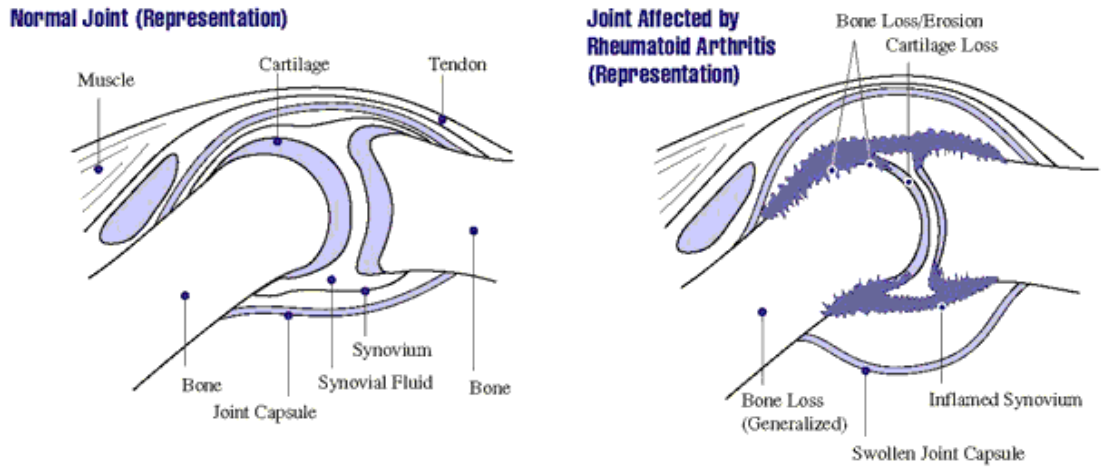
An autoimmune disease occurs when an individual produces antibodies against his/her own antigens. It is due to loss of self-tolerance, in which the body attacks its own cells (Actor, 2014).

1.2.5. Rheumatoid Arthritis

Arthritis is a common worldwide health problem, it affects more than 350 million people. It is a leading cause of disability, leading to premature mortality. Arthritis is a term that includes different types of joint diseases that affect people of all ages and ethnicities, but, rheumatoid arthritis is the most common form of arthritis here in Sudan (Kvien, 2004, Elshafie *et al.*, 2016). Rheumatoid arthritis (RA) is an autoimmune inflammatory arthritis, affecting approximately 1% of the population. Its etiology is unknown but it is presumed to be an immunologic disease with contributing genetics, such as "HLA-DRB1" which was identified as a gene locus that is associated with RA, and environmental factors, such as cigarette smoking, and reproductive factors in women (hormonal factors), bacterial overgrowth in the digestive tract, immune system hyper-activity. Rheumatoid Arthritis is a systemic disease that can affect several other systems in the body, such as lungs, heart, blood vessels, eyes and skin (Fuhrman, 2016).

In RA, the autoimmune process causes the synovium in certain joints to become inflamed. The tissue swells and becomes painful with every movement of the affected joints according to Ezerioha (2018). He also added that the uncontrollable joint inflammation leads eventually to joint erosion, loss of motion, and joint damage, leading to swelling, pain and stiffness.

Representation of joints affected by rheumatoid arthritis:



This figure was acquired from Rheumatoid Arthritis Organization webpage (Freeman, 2018)

1.2.5.1. Pathogenesis:

The pathogenesis of RA has not been fully clarified, it is however thought to be due to an environmental trigger stimulating an immune response in a genetically susceptible host, this will then result in an inflammatory cascade in synovial tissue, eventually, leading to joint destruction. Disease expression is not limited to the joints, it can extend to other organs. Extra-articular manifestations occur in the lungs, eyes, skin, and the cardiovascular system ending in morbidity and premature mortality (Detrick, *et al.*, 2006, Duckworth, 2018). Moreover, numerous studies show substantial irreversible joint damage that occurs within the first years of disease onset. Risk factors that are associated with RA such as, female sex, Caucasian race, tobacco use, industrial pollutants, bacterial and viral infections were also proposed. Familial studies have also identified specific human leukocyte antigen (HLA) genotypes that are associated with the disease development (Detrick, *et al.*, 2006, Anic and Mayer, 2014, Duckworth, 2018). HLA-DR4 has been

recognized for more than 20 years as a risk factor. More recent data suggests a stronger association with particular DR4 alleles known as the "shared epitope," which confers the risk for more aggressive disease (Detrick, *et al.*, 2006).

1.2.5.2. Types of rheumatoid arthritis:

Rheumatoid Arthritis is mainly categorized by researchers, to types and sub-types depending on the onset, the symptoms, the patients experience, the laboratory data, in addition to other factors. The commonly used classification is:

1.2.5.2. 1 . Seropositive:

Rheumatoid arthritis patients who are classified as seropositive have the presence of anti-cyclic citrullinated peptides (anti-CCPs) in their blood test results. These are specific antibodies that attack the body and produce the symptoms of rheumatoid arthritis.

1.2.5.2. 2 . Seronegative:

In this type patients develop rheumatoid arthritis without the presence of antibodies in their blood. This is referred to as seronegative type of rheumatoid arthritis. These are the patients who test negative for the anti-CCP or another antibody called rheumatoid factor (Duckworth, 2018).

Seronegative patients who lack the antibodies can still be diagnosed with rheumatoid arthritis in a number of ways. These include the demonstration of clinical rheumatoid arthritis symptoms, or through X-ray results that indicates patterns of cartilage and bone deterioration (Duckworth, 2018).

– Juvenile Rheumatoid Arthritis: Juvenile rheumatoid arthritis is another type that affects patients under the age of 17 years old. It is also known as juvenile idiopathic arthritis. Some patients may experience rheumatoid arthritis symptoms for the rest of their lives (Duckworth, 2018, Nordberg, *et al.*, 2018).

1.2.5.3. Stages of rheumatoid arthritis:

According to the Rheumatoid Arthritis organization, there are four distinct stages of RA progression:

Stage One, is the early stage of RA that involves the initial inflammation in the joint capsule and swelling of synovial tissue. The swelling causes the symptoms of joint pain, swelling, and stiffness. Stage Two, is the moderate stage of RA, in which the inflammation of the synovial tissue becomes severe enough that it creates cartilage damage, and symptoms of loss of mobility and decreased motion of the joint. Stage Three, is considered severe RA inflammation in the synovium that begins to destroy not only the cartilage but the bone as well, with increased pain and swelling and a further decrease in mobility and muscle strength. Physical deformities of the joint may start to develop as well. Stage Four, is the end stage of RA, the inflammatory process ceases and joints stop functioning altogether. Pain, swelling, stiffness and loss of mobility are still the primary symptoms in this stage (Freeman, 2018).

1.2.5.4. Treatment of rheumatoid arthritis:

The treatment of rheumatoid arthritis is carried out mainly by assessing the disease activity and subjecting the patient to NsAIDs and glucocorticoids which are used to manage and control pain and inflammation. DMARDs, are

also used as first line of defense to manage and treat in early RA, such as: methotrexate, Infliximab, rituximab, which are known as Disease-Modifying Anti-Rheumatic Drugs, given according to their Disease Activity Score (DAS) (Arthritis foundation, 2019).

New alternative remedies had been suggested in treating RA, beside the NsAIDs for chronic RA patients, which have side effects, ranging from headaches and stomach pain to serious side effects such as stomach ulcers, bleeding, allergic reactions, high blood pressure, cardiovascular disorders, liver and kidney damage (Arthritis foundation, 2019), these alternative remedies include fasting, which is a natural way that has been proven to be effective in a number of literature.

1.2.6. Fasting:

Fasting is defined as the partial or total abstinence from food and drinks, for a certain period of time, in which the calories are restricted, and the food and fluid intake is discontinued, followed by the eating window, in which fast is broken and food or drinks are consumed (Youngson, 2005).

1.2.6.1. Types of fasting:

Intermittent Fasting (IF): Is the fast that involves cycling between eating and fasting periods, ranging from hours to days. It has four types: Alternate day fasting, which involves fasting every other day. In which the calories are limited on the fasting days, and on the non-fasting days, the regular diet is resumed. The twice-a-week method, which is the 5:2 method, where you fast for 2 non-consecutive days, or restrict your calorie intake and in the next 5 days, you resume a healthy diet. The time-restricted eating: The 16/8 or 14/10 method. Where you fast for 16 hours and are allowed to eat for only 8 hours,

or, you fast for 14 hours and are allowed to eat for only 10 hours. And this is the most convenient method of IF and is recommended by the nutritionists. And the other type is the: Extended fasting: which involves fasting for a full 24 hours once or twice a week (Fung and Moore, 2016).

1.2.7. Ramadan fasting:

Ramadan is the ninth month of the Muslim calendar, it is a holy month in which Muslims fast for an entire month, from dawn until sunset. In Sudan people usually fast for 12-14 hours. Which is considered as one of the Intermittent fasting techniques (i.e: 14/10 time-restricted fast). Besides its spiritual and religious benefits, Ramadan has been mentioned in a number of literature to have health benefits to patients suffering from autoimmune diseases (Sharma, *et al.*, 2011, Dalton, 2018)

1.2.8. Effects of fasting in rheumatoid arthritis pathology

Fasting in RA patients has been studied to have beneficial effects mainly in reducing inflammation and altering the gut microbiota following long-term fast. As to, significantly reducing the DAS score (Kjeldesen-Kragh, 1991, Abendroth, *et al.*, 2010).

1.2.9. Diagnosis of rheumatoid arthritis

1.2.9.1. Rheumatoid Factor

Rheumatoid Factor is a type of antibody found in most of rheumatoid arthritis patients, which is directed against the fc portion of immunoglobulin G. It’s an antibody that attacks healthy tissue leading to joint inflammation potentially resulting in the development of rheumatoid arthritis symptoms.

This is in addition to testing positive for anti-CCPs (Ingegnoli, *et al.*, 2013, Nazario, 2018).

1.2.9.2. Anti-Cyclic Citrullinated Peptides

Anti-cyclic citrullinated peptide (anti-CCP) is a specific antibody present in rheumatoid arthritis patients. It is going to be used in this study to select the sero-positive rheumatoid arthritis patients. Between 60 and 80 % of rheumatoid arthritis patients test positive for the presence of anti-CCPs, meaning it is a reliable indicator for diagnosis as well as prognosis and evaluation of treatment outcome (Chou, *et al.*, 2007). The presence of these antibodies can even be detected as early as 5 to 10 years before clinical rheumatoid arthritis symptoms appear (Nazario, 2018), making it a consistent parameter.

1.2.9.3. Complete Blood Count

The involvement of hematological parameters, namely, the White Blood Cells, which are main key players in the inflammation process (Chen, *et al.*, 2018), and the alteration of their count during the fasting state, has been suggested in previous literature.

1.2.9.4. Total Leukocytes Count (TLC)

White blood cells are produced from pluripotent stem cells that are located within the bone marrow. Their growth is stimulated by many growth factors including interleukin-1 (IL-1), IL-3, IL-5 (for Eosinophils), IL-6, IL-ll, granulocyte macrophage colony-stimulating factor (GM-CSF), granulocyte CSF (G-CSF) and monocyte CSF (MCSF), these factors are involved in this maturation and differentiation process of the pluripotent stem cell into different lineages of cells, each one exerting different characteristics and

functions. Their development is also governed by other external stimuli including cytokines, matrix proteins, and other cellular products within the marrow environment (Hoffbrand *et al.*, 2016, Scheiermann, *et al.*, 2015).

Leukocytosis is defined as the increase of Total White Blood Count, above 10x109/L (Hoffbrand *et al.*, 2016).

1.2.9.5. Lymphocytes Absolute Count

Lymphocytes are white blood cells derived from the lymphoid lineage from the common lymphoid progenitor cell (CLP) which is derived from the hemopoetic stem cell. There are three classes of lymphocytes, the B cells, T cells and NK cells. T cells are generated in the thymus and are divided into CD4+ or CD8+ T cells. While the B cells are generated from within the bone marrow. B cells develop into plasma cells which are responsible of secreting immunoglobulin (Antibodies). And, the Natural killer (NK) cells which develop from within the bone marrow (AbulAbbas, 2012).

The total Lymphocyte count is going to be studied, hence fasting has shown to cause immunosuppression in RA patients (Fraser, *et al.*, 1999).

Absolute lymphocytosis is defined as the increase of Lymphocytes count above 3.5 × 109 /L in adults (Hoffbrand *et al.*, 2016 and AbulAbbas, 2012).

1.2.9.6. Monocytes Absolute Count

Monocytes are granulocytes that spend only a short time in the marrow and circulate in the blood for only 20-40 hours, then they leave the blood to reside in the tissues where they mature and carry out their principal functions. Their extravascular lifespan after their transformation to macrophages may be

as long as several months or even years. Then they assume specific functions in different tissues (e.g. skin, gut, liver) (Hoffbrand *et al.*, 2016).

Monocytes has many functions, such as, chemotaxis, which is cells mobilization and migration to the sites of infection or inflammation, sensing their local environment for pathogens, phagocytosis, killing and digesting foreign bodies, , producing chemokines which are proinflammatory cytokines, that act on other cells, they also contribute to tissue repair. Such as neutrophils, and also their immunological role in stimulating immunological reactions by producing cytokines such as alpha TNF, Interlukin-1, Interlukin-6 (Boyette, *et al.*, 2016).

Rheumatoid arthritis is listed as one of the causes of Monocytosis. Monocytosis is characterized by a rise in blood monocyte count above 0.8 x 109/L (Hoffbrand *et al.*, 2016).

They also play a specific role in rheumatoid arthritis, by producing, IL-1, TNF-a, IL-6 and matrixmetalloprotinases (MMPs), which are immunological cytokines, leading to endothelial cell activation, acute phase reactions, and cartilage damage. Furthermore, monocytes have the ability to polarize CD4+Tcells and can differentiate into osteoclasts, which may contribute to their role in RA pathogenesis. As such, monocytes and their parallel macrophages are viewed as relevant therapeutic targets in RA (Roberts, 2015).

1.2.9.7. Neutrophils Absolute Count

Neutrophils are the most abundant of leukocytes, they are increased in infections, inflammation and a number of other conditions as well. (Hoffbrand *et al.*, 2016).

Neutrophils are the most abundant leukocytes in the inflamed joints as they play an essential role in the initiation and progression of rheumatoid arthritis disease (Rosas, 2017).

1.2.9.8. Inflammation markers

Several inflammation markers are going to be assessed in this study to study the impact of fasting on these markers, as it has been suggested in previous literature:

1.2.9.9. Erythrocyte Sedimentation Rate (ESR)

The Erythrocyte Sedimentation Rate measures the rate at which the red blood cells fall and settle at a bottom of a tube. The rate is measured in millimeters per hour. A high/fast sedimentation rate indicates there is inflammation, infection, changes in plasma proteins, particularly increases in fibrinogen, immunoglobulins, and C-reactive proteins. In rheumatoid arthritis patients, due to the accumulation of proteins and infiltration of the joints will lead to raised erythrocyte sedimentation rate. ESR is also used to evaluate disease activity and treatment efficacy (Chou *et al.*, 2007, Nazario, 2018).

Here, the ESR is going to be used to measure the level of inflammation in a rheumatoid arthritis patient by inspecting the rate of sedimentation while the patient is in a fasting state.

The normal range in men 17–50 years of age is ≤10, while men

51–60 years, the ESR is ≤12.

The normal range is women 17–50 years ≤12, while women 51–60 years the ESR is ≤19.

But in active rheumatoid arthritis disease the ESR is elevated (Nazario, 2018).

1.2.9.10. High Sensitivity- C-Reative Protien (Hs-CRP)

C-reactive protein (CRP) is a protein that is produced by the liver. It increases in the blood with inflammation and infection as well as following a heart attack, surgery, or trauma. CRP is used to evaluate disease activity and treatment efficacy in rheumatoid arthritis (Chou *et al.*, 2007, Nazario, 2018).

The hs-CRP test accurately measures low levels of CRP to identify low but persistent levels of inflammation it detects lower levels of the protein than the standard CRP test. It measures CRP in the range from 0.5 to 10 mg/L.

Normal hs- CRP is between 0-1 mg/dl (Nazario, 2018).

Reduction in levels of Anti-CCP, ESR most of the time accompanies clinical improvement in RA, suggesting the control of pathophysiologic mechanisms. Laboratory measures have contributed invaluably to understanding of pathogenesis and to development of new treatments for RA. They appear considerably of value in understanding the pathology of the disease rather than the changes in other measures of RA status, such as tender joint counts or patient questionnaire scores for pain. (Pincus, 2014).

Previous studies have been conducted that studied the effect of fasting and diet change on rheumatoid arthritis patients:

A study performed by Fraser, *et al.* (1999), investigated the effect of a 7 day fast with acute starvation of ten rheumatoid arthritis subjects, after which, immunological, hormonal, laboratory and clinical evaluations were carried out on day 0, 7, and 21, concluded that the 7 day fast significantly decreased the Erythrocyte Sedimentation Rate, C-reactive protein level, and the CD4+ and CD8+ lymphocytes. In a study performed in Norway by Jajic, *et al.*, (1998), in which 46 subjects who suffered from RA, between 3 to 20 years

were included, these patients showed an improvement in the erythrocytes sedimentation rate, c-reactive protein and white blood cell count after fasting for 7-10 days, and stated that reduction of diet has improved their quality of life and had a positive effect on their symptoms.

Kjeldsen-Kragh, *et al.* (1991) performed a controlled trial of a 7-10 days of fasting and a one year of vegetarian diet, determined that after 1 month of diet there was a significant reduction in the erythrocyte sedimentation rate (ESR) ($p < 0.002$), C-reactive protein (CRP) ($p < 0.005$), and WBC count ($p < 0.0001$) which were maintained even after 1 year of the administration of this diet. In a published report, written by Zolfaghari, *et al.* (2015), about the Islamic Fasting and presentations of rheumatoid arthritis disease, they confirmed the association of B and T lymphocytes and Monocytes with disease progression in rheumatoid arthritis, and has provided literature that fasting decreases the immune and inflammatory responses of RA. And has found that Rheumatoid Factor, leukocytes, ESR and CRP and monocytes and macrophages, decreases following the Islamic Ramadan Fasting, besides its effectiveness in diminishing stress and depression levels. They also recommended that Fasting could be used as a complementary treatment beside the conventional method, with the suitable diet, could help in reducing the anti-inflammatory drugs medications dosage and treating the symptoms and improving health of RA patients.

A clinical research, carried by Fuhrman, *et al.* (2002), in which medically supervised fasting, followed by a vegan diet, was implemented on 3 cases suffering from rheumatoid arthritis, who fasted from 1 up to 3 weeks, and were off the medications. At the end of the fast, patients were free of symptoms, and symptoms did not recur after carefully following the diet, and

the ESR was normalized. Reduction in excessive lymphocyte activity and inflammatory activity has been suggested.

1.3. Rationale:

Due to the incurable, chronic and progressive nature and the severity of the symptoms which causes the patients suffering results from the progression and the severity of the inflammation, therefore some patients try to influence their symptoms by using means of dietary modifications.

All the mechanisms of the disease and complications of its treatment can reduce patient's quality of life and cause psychiatric diseases and sleep disorders, leading to patient dissatisfaction and depression (Mindham, *et al.*, 1981). And, moreover, the economical and socio-economical burden of this disease, in developing countries such as Sudan, concerning the annual costs of anti-inflammatory drugs, and the reality of this disease which affects the working class, having deleterious effects on individual's employment status and work-productivity.

Furthermore, some evidences suggest that RA patients also have altered microbiota that is responsible for the pathogenesis and disease severity, which could be treated by introducing certain diets or by monitored fasting, which has shown long term effects. (Muller, 2001, Sundqvist, 2009)

Therefore in this study, I will test and examine certain hematological and inflammatory markers to try and understand the pathogenesis of this disease and if fasting diet can be scientifically proven to reduce inflammation, and use of anti-inflammatory drugs, besides its religious benefits, mainly in improving the quality of life for these patients.

1.4. Objectives

1.4.1. General Objective

To determine the impact of fasting in certain hematological parameters and the inflammatory response in Rheumatoid Arthritis patients.

1.4.2. Specific Objectives

- To compare changes in TLC, Abs Neutrophil, Abs Monocytes, Abs Lymphocytes count between fasting and non-fasting Rheumatoid Arthritis patients.
- To compare changes in Hs-CRP and ESR between fasting and non-fasting Rheumatoid Arthritis patients.
- To compare the hematological parameters and inflammatory markers between the males and females.
- To correlate age with the hematological parameters and inflammatory mediators.

Chapter Two

Material and Method

2. Material and Method

2.1. Study design:

This is a crossectional case-control study, designed to compare between two groups of confirmed rheumatoid arthritis patients, who are on follow-up, the fasting (case group) and the non-fasting (control group).

2.2. Study area and duration:

This study was carried out at Omdurman Military Hospital and Rayan Specialized Center in Khartoum state, through the period of May to July 2019.

2.3. Study population:

The total of the participants in this study was 62 rheumatoid arthritis patients. 30 fasting patients, designated as the case group, and 30 non-fasting patients, designated as the control group.

2.4. Inclusion criteria:

- Patients with chronic disease on follow-up
- Patients over 40 years of age
- Subjects fasting at least for 14 days of Ramadan or until the day of sampling, without missing a day.
- Patients with positive Anti-CCP and RF.

2.5. Exclusion criteria:

- Patients on any anti-inflammatory or anti-rheumatic drugs during the acquisition of samples.

- Patients suffering from other inflammatory diseases.
- Patients suffering from any kind of infection.
- Patients suffering from hyperproteinemia or any disease.

2.6. Data collection

Data was collected through the hospital from confirmed RA patients following up at the hospital, from May to July. A structured questionnaire was used to collect demographic and clinical data from the study subjects. The Fasting group (Case group) samples were collected 15 days following the beginning of Ramadan. The Non- Fasting group (Control group) samples were collected 30 days following the end of the month of Ramadan.

2.7. Methodology

2.7.1. Sample collection:

Patients with a history of RA were identified by the hospital staff and selected for blood collection. The phlebotomist placed the tourniquet on the patient's arm about 3-4 inches above proposed collection site. The tourniquet was placed for no longer than one minute. They used their index fingers to palpate and trace the path of the vein. The personnel put on a clean pair of gloves. The vein puncture site was cleansed with an alcohol prep pad. The needle was uncapped and turned so that the bevel is facing up. Then they punctured the vein with an angle of approximately 15 degrees. The plunger was pulled to fill the syringe with blood by negative pressure. The tourniquet was removed as soon as the blood appeared in the syringe. And normal blood flow was restored to the arm then a piece of cotton was placed on top of the needle as they gently removed it from the arm. Then a bandage was put over

the collection site after bleeding had stopped. They separated the needle from the syringe body and threw it in a sharp objects safety container. And emptied the blood into an EDTA container for CBC and a plain container for the other tests and an ESR container. Then they labeled the container with the appropriate patient name and lab number.

EDTA container which contains Ethylene Di amine Tetra Acetic Acid was used as an anticoagulant, as it functions in preserving the CBC blood sample by chelating calcium. Plain Container contains no additives which was used for the Anti CCP and RF tests. ESR container contains sodium citrate as an anticoagulant, was used in the Erythrocyte Sedimentation Rate (ESR) Test.

2.7.2. Automated cell-analyzer (Sysmex kx-21n):

A 3-part differential hematological analyzer is used, it provides a Complete Blood Count with 18 reportable parameters.

Blood collected in EDTA tube is mixed well, and the tube is inserted in the sucking probe of the device. A button is clicked and a suitable amount of blood is taken into the device. Automatic discriminators separate the cell populations using Hydro focusing techniques and as well as complex algorithms. The intensity of the electronic pulse from each analyzed cell is proportional to the cell volume (Sysmex, 2007). Results visible on the screen are checked and then recorded.

2.7.3. Thin blood film:

From a well-mixed EDTA anti-coagulated blood, a drop of blood was taken using a capillary tube and placed at one of the edges of a clean glass slide. Using a clean, smooth piece of cover glass for spreading the drop of blood, the blood was allowed to extend along the edge of the spreader, then

spread along the slide making a thin film with a smooth tail on the glass slide. The blood on the slide was allowed to dry before staining it. The slides were then fixed using Methanol alcohol for 1-2 minutes on a rack at Room Temperature, according to Cheesbrough (2006).

2.7.4. Staining method (Leishman stain):

- The blood film was covered with undiluted stain using a dropper.
- Add twice the volume of the stain, pH buffered water (i.e. twice the number of drops as stain). The water is well mixed with the stain by mixing the stain and water using a dropper.
- Allow to stain to sit at Room Temperature for 10 minutes.
- Wash off the stain with filtered tap water.
- Wipe the back of the slide clean and stand it in a draining rack for the smear to dry.

The blood film should appear neither too pink nor too blue (check results microscopically). And should be read by a microscope using a x100 oil immersion lens and after choosing an ideal area in the tail of the Blood film (Cheesbrough, 2006).

2.7.5. Differential White Blood Cells Count

A drop of immersion oil on the lower third of the blood film and cover with a clean cover glass. The blood film was examined microscopically using the x10 objective, and after checking the staining and distribution of cells. Afterwards, the film was moved to a part where the red cells are just beginning to overlap and shifted to x40 objective. The blood film was examined systematically and the different white cells seen in each field were counted,

preferably using an automatic differential cell counter, readings of each cell type were recorded. The absolute number of monocytes was calculated by multiplying the number of cells counted (expressed as a decimal fraction) by the total WBC count (Cheesbrough, 2006)

2.7.6. ESR

Blood was collected into the ESR container and mixed well. Then it was immediately placed in the Reader device and left to stand for 30 minutes. And then the rate of the sedimentation of the red blood cells in the plasma was read, by pulling the tube until the plasma line is adjacent to the 0mm/hr on the scale of the reader and then take the sediment RBCs reading.

2.7.7. IgM Rheumatoid Factor by ELISA (Model- Euroimmun Analyser-12P):

A fully Automated ELISA device was used, using Euroimmun IgM RF kits to test the Rheumatoid Factor.

2.7.8. Anti-Cyclic Citrullinated Peptides (IgG) by ELISA (Model-Euroimmun Analyzer-12P):

A fully Automated ELISA device was used, using Euroimmun IgG Anti-CCP kits to test the Anti-CCP.

2.7.9. High Sensitivity C-reactive Protiens:

A fully Automated ELISA device was used, using Aptec CRP kit.

Serum samples were also used in this test. A fully automated ELISA device was used for the estimation of hs-CRP.

Method: Measurement of antigen-antibody reaction by the end-point method:

- First, the samples and control were uploaded into the wells, around 16 ul from each.
- Reagent 1 was added, mixed and incubated for 2 minutes, the Absorbance was read using wavelength 340nm.
- Reagent 2 was added immediately after the first reading, incubated and the absorbance was read after 5 minutes using wavelength 340 nm.

2.8. Data analysis

Data was analyzed by using the Statistical Package of Social Sciences program (SPSS) version 20.0. The analysis was performed using the Independent T-test. The data obtained were presented as mean $\pm$ SD.

2.9. Ethical clearance

Verbal consent was acquired from patients and the permission to collect patient's data was acquired from the Medical Administrator at Omdurman Military Hospital and Rayan Medical Center.

Chapter Three

Results

3. Results

3.1. Demographic data:

This analytical case-control study aimed to investigate the effect of fasting in rheumatoid arthritis fasting patients during the third and fourth week of Ramadan, following a 15 days, 14 hours fast. Sixty-two Sudanese RA following-up patients were enrolled in the study, thirty-one were fasting for 3 weeks, and thirty-one were non-fasting as a control group.

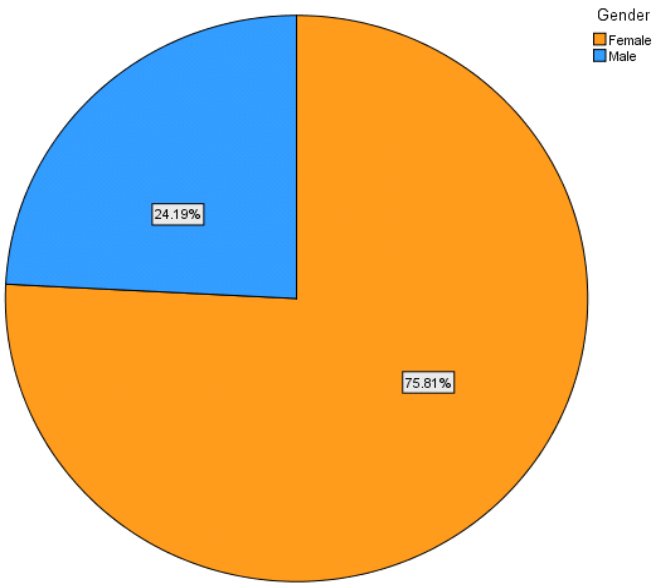
The mean age of the fasting group was 66$\pm$11 years, and the non-fasting group was 65 $\pm$10 years, as revealed in table (1).

Table (1): The mean Age between the fasting and non-fasting RA patients:

Groups	N	Minimum	Maximum	Mean	Std. Deviation
Fasting	31	45	83	65.97	11.119
Non-Fasting	31	45	88	65.19	10.824

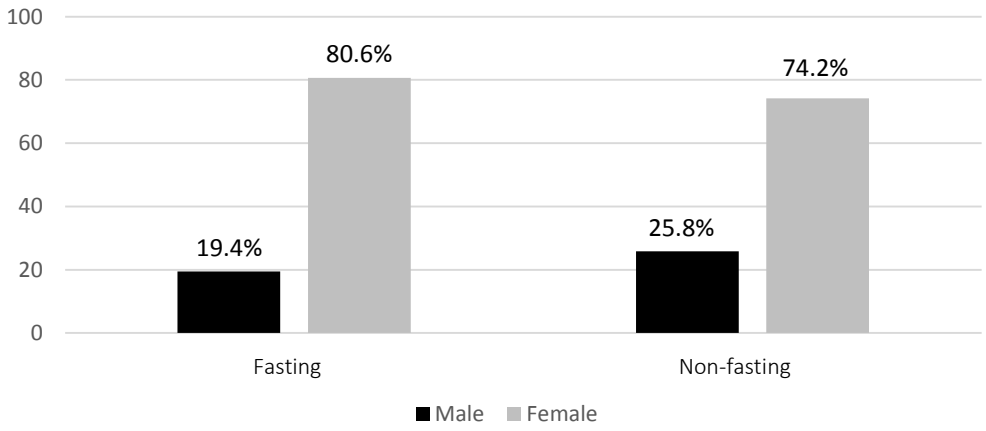
Of all Rheumatoid Arthritis patients (n=62), around 24.2% were males, and 75.8% were females, see (figure 1).

Figure (1): Gender distribution among RA patients:



In the Fasting group 19.4% were males (n=6) and 80.6% were females (n=25), while in the non-fasting group 25.8% were males (n=8) and 74.2% were females (n=23), approximately, as revealed in figure (2).

Figure (2): Shows gender distribution among the fasting and non-fasting groups:



3.2. Laboratory data:

The mean and standard deviation of the tlc, abs lymphocyte count, abs monocyte count, abs neutrophil count, hs-CRP, ESR for both the fasting and non-fasting groups were summed in table (2).

The mean value of the TLC was (7.5±2.4) and (7.5±3.4) for case and control. No significant difference in the TLC (p-value=0.942) between the fasting and non-fasting patients.

The abs lymphocyte count had a mean value of (2.4±0.5) and (2.4±0.9) for case and control. There was no significant difference between the fasting and non-fasting patients in the abs lymph count (p-value=0.877).

In the abs neutrophil count, the mean value for the case and control was (3.8±2.0) and (4.2 ±2.3) respectively. The data didn't reveal a significant difference in the abs neutrophil count (p-value=0.451) between the fasting and non-fasting groups.

Regarding the mean value of the abs monocyte count, was (0.44±0.19) and (0.89±0.93) for case and control group respectively. The abs monocyte count (p-value=0.013) was significantly lower in fasting patients.

However, the mean value of the hs- CRP between the case and control was (7.4±2.5) and (20±17). The hs-CRP values between the fasting and non-fasting groups were strikingly significant (p-value= 0.00).

The mean values of the ESR was (68±19) and (82±24) for the case and control. Fasting group had significantly (p-value= 0.014) lower ESR values.

Table (2): Comparison of the mean of TLC, Absolute lymphocyte count, Absolute monocyte count, Absolute neutrophil count, HS-CRP, ESR between the fasting and non-fasting groups, using the Independent T test:

		N	Mean	Std. Deviation	Sig.
TLC	Fasting	31	7.535	2.448	0.942
	Non-fasting	31	7.590	3.402	
Abs Lymph#	Fasting	31	2.413	0.5845	0.877
	Non-fasting	31	2.445	0.9869	
Abs Neut#	Fasting	31	3.848	2.0078	0.451
	Non-fasting	31	4.268	2.3337	
Abs Mono #	Fasting	31	0.442	0.1963	0.013
	Non-fasting	31	0.894	0.9359	
Hs-CRP	Fasting	31	7.429	2.5618	0.000
	Non-fasting	31	20.797	17.097	
ESR	Fasting	31	68.71	19.016	0.014
	Non-fasting	31	82.90	24.521	

The compared mean between the males and females, of the TLC (p-value= 0.675), abs lymphocyte count of (p-value= 0.66), abs monocyte count (p-value= 0.907), abs neutrophil count (p-value= 0.949), hs-CRP (p-value= 0.512) and ESR (p-value 0.45) was insignificant (p-value is more than 0.05). Indicating there is no significant difference between the two genders in the fasting group, as shown in table (3).

Table (3): Comparison between mean among the males and females of the fasting group:

		N	Mean	Std. Deviation	Sig.
Age	Male	6	70	10.373	0.331
	Female	25	65	11.273	
TLC	Male	6	7.15	2.341	0.675
	Female	25	7.63	2.511	
Lymph#	Male	6	2.317	0.407	0.66
	Female	25	2.436	0.6242	
Mono #	Male	6	0.433	0.2422	0.907
	Female	25	0.444	0.1895	
Neut#	Male	6	3.8	1.9677	0.949
	Female	25	3.86	2.0571	
Hs-CRP	Male	6	6.8	2.6046	0.512
	Female	25	7.58	2.5821	
ESR	Male	6	63.33	14.024	0.45
	Female	25	70	20.052	

The correlation between the Age and the TLC (p-value= 0.62), absolute lymphocyte count (p-value=0.41), absolute monocyte count (p-value= 0.322), absolute neutrophil count (p-value=0.12), ESR (p-value= 0.90), hs-CRP (p-value= 0.83) was insignificant (p-value more than 0.05). It also revealed negative correlation between the tlc, abs neut count, ESR and hs-CRP and age, indicating that these parameters decrease with aging, as revealed in table (4).

Table (4): Correlation of the age with TLC, Abs lymphocyte count, Abs monocyte count, Abs neutrophil count, ESR, Hs-CRP variable:

		TLC	Lymph#	Mono #	Neut#	ESR	Hs-CRP	Age
Age	Pearson Correlation	-0.092	0.15	0.184	-0.279	-0.023	-0.038	1
	Sig. (2-tailed)	0.624	0.419	0.322	0.129	0.902	0.838	
	N	31	31	31	31	31	31	31

Chapter Four

Discussion, Conclusion, Recommendation

Discussion

This study investigated the changes in TLC, abs lymphocyte count, abs monocyte count, abs neutrophil count, ESR and CRP in fasting and non-fasting RA patients.

In the hematological parameters, the TLC was not affected by fasting (p-value= 0.94) unlike Jajic, *et al.*, (1998) who has found an improvement in the total white blood cells count, following a 7-10 days fasting protocol, agreeing with Kjeldsen-Kragh, *et al.*, (1991), and Zolfaghari, *et al.*, (2015) who also found a reduction in the leukocytes count that was maintained for a year, following the traditional Ramadan fast. This was perhaps due to the different diets and eating habits followed in Sudan, since the nutrition consumed in the eating window can have some adverse effects, such as high-fat diets (Skoczynska, 2018), and the duration of the fast itself, here the fast proceeded for 12-14 hours, and for more than 2 weeks.

On the contrary, the Abs monocyte count was significantly affected by fasting (p-value=0.013), agreeing with the results reported by Zolfaghari, *et al.*, (2015), who has confirmed the association of monocytes with RA progression, concluded that following Ramadan fast monocytes were reduced as well, and patients have shown clinical improvement.

Absolute lymphocyte count, was not affected by fasting (p-value=0.87). Disagreeing with the studies carried by Fraser, *et al.*, (1999), who has found that fasting has a significant effect on the lymphocytes count, both the B-cells and T-cells. This variation in results could be explained by the 7-days acute starvation approach that he followed in which loss of body weight was shown to affect the CD4+ cells. While Fuhrman (2002) has found

a decrease in lymphocytes activity but, not the count. Siadat, *et al.*, (2014), which has carried out a study in the lymphocytes population during the month of Ramadan and after Ramadan, agrees with this study's result of lymphocytes being unaffected by Ramadan fasting (Intermittent fasting).

ESR was of higher significance (p-value= 0.000) in fasting patients, conforming to the results reported by Fraser, et al., (1999), Jajic, *et al.*, (1998), Kjeldsen-Kragh, *et al.*, (1991), Fuhrman, *et al.*, (2002) and Zolfaghari, *et al.*, (2015), indicating that the inflammation can be resolved, thus, explaining the clinical improvement as reported by the previous literature.

Harmoniously, the hs-CRP, which is also an inflammatory marker, showed high significance (p-value= 0.000) between the fasting and non-fasting groups, indicating a decrease in inflammation following the fasting process, thus, agreeing with the results reported by Kjeldsen-Kragh, *et al.*, (1991), Fuhrman, *et al.*, (2002), and Zolfaghari, *et al.*, (2015), Jajic, *et al.*, (1998), Fraser, *et al.*, (1999), also explaining the declining in morning stiffness and the improvement of symptoms reported by the study subjects.

There was no any significant variation in results between the males and females, in Tlc (p-value=0.675), Abs neutrophil count (p-value= 0.949), Abs lymphocyte count (p-value= 0.66), Abs monocyte count (p-value= 0.907), Hs-CRP (p-value=0.512) and ESR (p-value= 0.45), which proposes that Gender has no variation on the inflammation reduction and clinical remission process between males and females of this study group, as was proposed by Sokka, *et al.*, (2009) who has found that men had better remission scores than females.

The results also revealed that there was no correlation between the age and TLC (p-value= 0.62), Abs neutrophil count (p-value= 0.129), Abs

lymphocyte count (0.419), Abs monocyte count (p-value= 0.322), Hs-CRP (p-value= 0.838), and the ESR (0.902). Which nullifies that age has an effect on disease or inflammation attenuation in RA patients.

Conclucion:

There was a statistically significant reduction in the Absolute monocytes count, ESR and Hs-CRP in the fasting rheumatoid arthritis patients, which indicates that fasting could be effective in reducing the inflammatory response in patients suffering from incurable Chronic Rheumatoid Arthritis.

Recommendation:

- According to these results, intermittent fasting can be suggested to RA patients suffering from chronic disease are recommended to fast, but after returning to their physicians to monitor the fast.
- Monitored diets should be studied and introduced by dieticians, as innovative ways, for treatment of incurable autoimmune diseases. And studies involving the clinical effects (DAS-28) as well as the specific immunological changes (Interlukins and alpha-TNF) in RA patients.
- Further experiments should be implemented to study the specific effect of fasting and diet on each parameter in RA patients.

References

Abendroth, A., Michalsen, A., Ludetke, R., Ruffer, A., *et al.* (2010). Changes of intestinal microflora in patients with rheumatoid arthritis during fasting or a Mediterranean Diet. *Forsch Komplementmed*, 17(6):307-13. [doi: 10.1159/000322313]

Abul Abbas, K., Litchman, A. H. and Pillai S. (2012).*Cellular and molecular immunology*. (7th edition). Philadelphia, Saunders, p: 4-9.

Actor, J. (2014). *Introductory to Immunology*. Massachusetts, Academic Press, p: 104.

Ahmed, A., Saeed, F., Arshad, M.U., *et al.* (2019). Impact of Intermittent fasting on human health: an extended review of metabolic cascades. *International Journal of Food Properties* 21(1). [doi: https://doi.org/10.1080/10942912.2018.1560312]

American Society of Hematology. (2019). Blood Basics. Available: https://www.Hematology.org/Patients/Basics/

Anic B, Mayer M. (2014). Pathogenesis of rheumatoid arthritis. *Reumatizam*, 61(2):19-23.

Anton, S.D., Moehl, K., Donahoo, W.T., *et.al.* (2018). Flipping the Metabolic Switch: Understanding and applying the health benefits of fasting. *Obesity (Silver Spring)* 26(2): 254-268. [doi: 10.1002/oby.22065]

Arthritis Foundation. (2019). DMARDs: Benefits and Risks. Available from: https://www.arthritis.org/living-with-

arthritis/treatments/medication/drug-types/disease-modifying-drugs/benefits-risks-dmards.php [Accessed: September, 2019]

Boyette, L.B., Macedo, C, Hadi, K, *et al.* (2017). Phenotype, function, and differentiation potential of human monocyte subsets. *PLoS One*, 12(4): e0176460.

Chaplin, D.D. (2010). Overview of the immune response. *J Allergy Clin. Immunol.*, 125(2 Suppl 2): S3–23.

Cheesbrough, M. (2006). *District Laboratory Practice in Tropical Countries Part2*. Cambridge University Press, New York, p: 319-322.

Chen, L., Deng, H., Cui, H., *et al.* (2018). Inflammatory responses and inflammation-associated diseases in organs. *Oncotarget*, 9(6): 7204–7218.

Chou, C., Liao, H., Chen, Ch., *et al.* (2007). The Clinical application of Anti-CCP in Rheumatoid Arthritis and Other Rheumatic diseases. *Biomark Insights*, 2: 165-71.

Ciulla, A.P. and Lehman, D.C. (2010). *Success in Clinical Laboratory Sciences- Immunology and Serology*. (Fourth edition). New Jersey: Pearson Education, p: 322-323.

Dalton, T. (2018). "Intermittent Fasting Benefits for Autoimmune Disorders". Healthtide. Available from: https://healthtide.com/intermittent-fasting-benefits-for-autoimmune-disorders/ [Accessed: August, 2019]

Darlington, L.G., and Ramcy, N.W. (1993). Review of Dietary therapy for rheumatoid arthritis. *Br J Rheumatol* 1932:507-14.

Detrick, B., Hamilton, R.G. and Folds, J.D. (2006). *Manual of Molecular and Clinical Laboratory Immunology*. (7th Edition), p: 1033.

Duckworth, H. (2018). RA Types: What distinguishes types of Rheumatoid Arthritis?. 27 Oct 2018. Available from: https://www.rheumatoidarthritis.org/ra/types/ .

Elshafie, A.I., Elkhalifa, A.D., Elbagir, S., *et al.* (2016). Active Rheumatoid Arthritis in Central Africa: A Comparative Study between Sudan and Sweden. *The journal of Rheumatology*, 43 (10). 1777-1786.

El-banna, S. and El-Garawany, O. (2016). 'Fasting for religion is common, but are there health benefits'. *The Irish Times*. 16 July, 2016. Available from: https://www.irishtimes.com/life-and-style/health-family/fasting-for-religion-is-common-but-are-there-health-benefits-1.2722043. [Accessed 15/09/2019]

Ezerioha, M. (2018). Rheumatoid Arthritis. 13 Sept, 2018. Available from: https://www.rheumatoidarthritis.org/ra/ .

Fraser D., Theon J., Reseland J., *et al.* (1999). Decreased CD4+ lymphocyte activation and increased IL-4 production in peripheral blood of rheumatoid arthritis patients after acute starvation. *Clin. Rheumatol*, 18 (5), 394-401.

Freeman, J. (2018, a). RA causes: What are the top causes of Rheumatoid Arthritis. 27 Oct 2018. Available from: https://www.rheumatoidarthritis.org/ra/causes/ .

Freeman, J. (2018, b). RA progression: What are the signs of Rheumatoid Arthritis progression?. 27 Oct, 2018. Available from: https://www.rheumatoidarthritis.org/ra/symptoms/progression/.

Fuhrman, J. (2016). A progressive approach to Rheumatoid Arthritis. Available from: https://www.drfuhrman.com/get-started/eat-to-live-

blog/119/a-progressive-approach-to-rheumatoid-arthritis (Accessed Sept, 2019).

Fuhrman, J., Sarter, B., Calabro, D.J. (2002). Brief case reports of medically supervised, water-only fasting associated with remission of autoimmune disease. *AlternTherHealthMed,* 8:112, 110-111.

Fung, J. and Moore, J. (2016).*The complete guide of fasting: Heal your body through Intermittent, Alternate-Day, and Extended fasting.* Canada: Victory Belt Publishing, p: 200.

Hoffbrand, A.V., Moss, P.A.H., Pettit, J.E. (2006). *Essential Hematology*. (Fifth Edition). Oxford: Blackwell publishing, p: 94-104.

Ingegnoli, F., Castelli R and Gualtierotti R. (2013). Rheumatoid factors: clinical applications. Dis. Markers, 35(6): 727-34.

Jajic, Z., Vuksic, D., Jajic, I. (1998). Effect of nutrition on the clinical picture of RA. *Reumatizam* 46:27-30.PMC.

Jackson, M.J. and Ahmed, W. (2007). Surface Engineered Surgical Tools and Medical Devices. New york: Springer Science Business Media. P: 350.

Kjeldesen-Kragh, J., Haugen, M., Borchgrevink, C.F., *et al.* (1991). Control trial of fasting and one year vegetarian diet in rheumatoid arthritis. *Lancet* 338:899-902.

Kumar, V., Cotran, R.S., Robbins, S.L. (1997). *Basic Pathology*. (Sixth edition), Philadelphia: Saunders, p: 26-32.

Leonard, J. (2018). "Seven ways to do intermittent fasting". Medical News Today. Available from:

https://www.medicalnewstoday.com/articles/322293.php. (Accessed: September, 2019)

Mindham, R.H., Bagshaw, A., James, S.A., Swannell, A.J. (1981) Factors associated with the appearance of psychiatric symptoms in rheumatoid arthritis. J Psychosom Res 25(5):429–35.

Muller, H., de Toledo, F.W., Resch, K.L. (2001). Fasting followed by vegetarian diet in patients with Rheumatoid Arthritis: a systemic review. Scand J RHeumatol 30(1):1-10.

Nazario, B. (2018). Blood tests for RA and other Autoimmune conditions. 09 Apr, 2018. Available from: https://www.webmd.com/rheumatoid-arthritis/blood-tests.

Nordberg LB, Lillegraven S, Aga AB, Sexton J, *et al.* (2018). Comparing the disease course of patients with seronegative and seropositive rheumatoid arthritis fulfilling the 2010 ACR/EULAR classification criteria in a treat-to-target setting: 2 year data from the ARCTIC trial. RMD Open, 4(2): e000752.

Pincus, T., Gibson, K.A., Shmerling, R.H. (2014). An evidence-based approach to laboratory tests in usual care of patients with rheumatoid arthritis. Clinical and Experimental Rheumatology. *Clin. Exp. Rheumatol.,* 32(Suppl. 85):S23-S28.

Roberts, C.A., Dickinson, A.K., Taams, L.S. (2015). The Interplay between Monocytes/Macrophages and CD4+ Tcell Subsets in Rheumatoid Arthritis. *FrontImmunol.,* 6:571. [DOI: 10.3389/fimmu.2015.00571].

Rosas, E.C., Correa, L.B. and Henriques, M.G. (2017). Neutrophils in Rheumatoid Arthritis: A Target for Discovering New Therapies Based on

Natural Products. Available from: https://www.intechopen.com/books/role-of-neutrophils-in-disease-pathognesis/neutrophils-in-rheumatoid-arthritis-a-target-for-discovering-new-therapies-based-on-natural-products (Accessed: Oct 1, 2019).

Rheumatoid Arthritis Organization. (2019). Available from: https://www.rheumatoidarthritis.org.

Scott, D.L., Wolfe, F. and Huizinga, T.W. (2010).Rheumatoid Arthritis. *Lancet* 25;376(9746):1094-108.

Sharma, M., Veena, S., Gupta, M., Tailang, M. and Pathak, A.K. (2011). Quantitative change in circulating immune complexes during Ramadan in Rheumatoid Arthritis patients. *J. Pharm. Res.*, 4(5):1306.

Scheiermann, C., Frenette, P. S. and Hidalgo, A. (2015). Regulation of leucocyte homeostasis in the circulation. *Cardiovac Res.* 107(3): 340–351.

Siadat, Z., Rastin, M., Tabasi, N., Rezaee, A., *et al.* (2014). Evaluation of the effects of Ramadan Fasting on the subpopulations in a Two- year follow-up. *J. fasting Health.* 2(1): 31-36.

Sokka, T., Toloza S, Cutolo M, Kautiainen H, *et al.* (2009). Women, men, and rheumatoid arthritis: analyses of disease activity, disease characteristics, and treatments in the QUEST-RA Study. *Arthritis Res. Ther*, 11(1):R7.

Sundqvist, T., Lindstrom, F., Magnusson, K.E., Skoldstam, L., *et al.* (2009). Influence of Fasting on Intestinal Permeability and Disease Activity in Patients with Rheumatoid Arthritis. *Scandinavian Journal of Rheumatology*, 11(1): 33-38 [DOI: 10.3109/03009748209098111].

Sysmex. (2007). Sysmex KX-21N Automated Hematology Analyzer. Available from: https://www.sysmex.com/us/en/Products/Hematology/3PartDiff/Pages/KX-21-Hematology-Analyzer.aspx. (Accessed on: Sept, 2019)

Kvien, T.K. (2004). Epidemiology and burden of illness of rheumatoid arthritis. *Pharmacoeconomics*, 22 (2Suppl1):1-12.

WHO (World Health Organization). (2019). Chronic rheumatic conditions. Available from: https://www.who.int/chp/topics/rheumatic/en. (Accessed: July 2019)

Youngson, R.M. (2005). *Collins dictionary of Medicine.* (Fourth Edition). Glasgow: Harper Collins, p: 144.

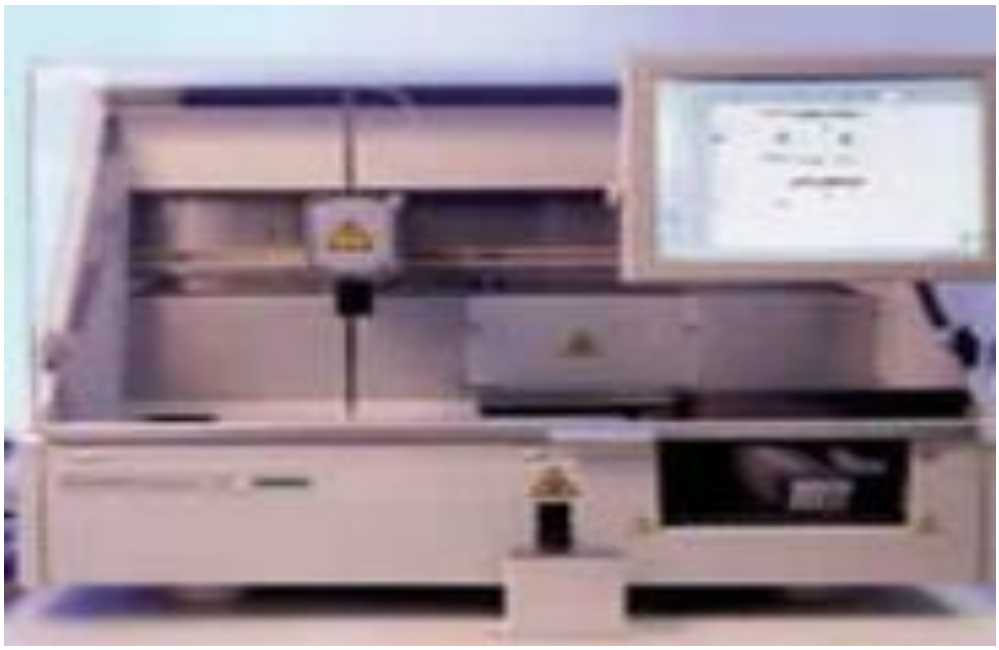
Zhang, J.M. and An, J. (2009). Cytokines, Inflammation and Pain. International Anesthesiol Clin., 45(2): 27–37. PMC.

Zolfaghari, S., Pourazizi, M. and Kokhaei, P. (2015). Islamic Fasting and Presentations of Rheumatoid Arthritis. *Middle East J. Rehabil. Health* 2(1): e24843 [DOI:10.17795/mejrah-24843].

Appendices

Appendix A:

Automated ELISA (Euroimmun analyser-12p):



Appendix B:

Staining of blood smears

Appendix C:

Sudan University of Science and Technology

College of Graduate Study

(Questionnaire for research)

Impact of Fasting on certain Hematological and Inflammatory Responses in Sudanese Rheumatoid Arthritis patients

-Date…… -Sample number……..

-Gender……. -Age…….

-History of patient:

……

-Grade of Disease:

Mild [ ]

Moderate [ ]

Severe [ ]

-Presence of other inflammatory disease:

-SLE [ ] -Other: ……..

-Presence of other conditions:

-Hypertension Yes [ ] No [ ] -Malaria Yes [ ] No [ ]

-Heart disease Yes [ ] No [ ] -Other……..

-Medications:

-NSAIDS Yes [ ] No [ ] -Steroids Yes [ ] No [ ]

-Other Medications: ……..

-Other relevant information:

……..……

-Signature……..