Introduction and Literature Review

1-1Introduction:

1-1-1Complete blood count (CBC) :

The complete blood count is a series of blood tests that provides information about the components of blood including red blood cells, white blood cells, and platelets. Automated machines rapidly count the cell types. The CBC test results can help diagnose diseases and determine their severity (Braunwald *et al*.,2001).

1-1-2Rheumatoid arthritis (RA):

 is an autoimmune disorder, which causes chronic inflammation of the joints. Besides joint pain, anemia is one of the most common Rheumatoid arthritis (RA) is an autoimmune disease that causes chronic inflammation of the joints. Autoimmune diseases are illnesses that occur when the body's tissues are mistakenly attacked by their own immune system. The immune system contains a complex organization of cells and antibodies designed normally to "seek and destroy" invaders of the body, particularly infections. Patients with autoimmune diseases have antibodies and immune cells in their blood that target their own body tissues, where they can be associated with inflammation. While inflammation of the tissue around the joints and inflammatory arthritis are characteristic features of rheumatoid arthritis, the disease can also causesymptoms of RA. In fact, anemia occurs in up to 60 percent The cause of rheumatoid arthritis is unknown. Even though infectious agents such as viruses, bacteria, and fungi have long been suspected, none has been proven as the cause. The cause of rheumatoid arthritis is a very active area of worldwide research. It is believed that the tendency to develop

rheumatoid arthritis may be genetically inherited (hereditary). Certain genes have been identified that increase the risk for rheumatoid arthritis. It is also suspected that certain infections or factors in the environment might trigger the activation of the immune system in susceptible individuals. This misdirected immune system then attacks the body's own tissues. This leads to inflammation in the joints and sometimes in various organs of the body.(Majithia and Geraci,2007)

1-2 Literature Review

1-2-1 Blood:

Blood is a body fluid in animals that delivers necessary substances such as nutrients and oxygen to the cells and transports metabolic waste products away from those same cells,blood contains proteins, glucose, mineral ions, hormones, carbon dioxide (plasma being the main medium for excretory product transportation), and blood cells themselves, Albumin is the main protein in plasma, and it functions to regulate the colloidal osmotic pressure of blood, The blood cells are mainly red blood cells (also called RBCs or erythrocytes) and white blood cells, including leukocytes and platelets, The most abundant cells in vertebrate blood are red blood cells, These contain hemoglobin, an iron-containing protein, which facilitates transportation of oxygen by reversibly binding to this respiratory gas and greatly increasing its solubility in blood. In contrast, carbon dioxide is almost entirely transported extracellular dissolved in plasma as bicarbonate ion, Vertebrate blood is bright red when its hemoglobin is oxygenated, vertebrates have an adaptive immune system, based largely on white blood cells. White blood cells help to resist infections and parasites. Platelets are important in the clotting of blood. Arthropods, using hemolymph, have hemocytes as part of their immune system, Blood is circulated around the body through blood vessels by the pumping action of the heart. In animals with lungs, arterial blood carries oxygen from

inhaled air to the tissues of the body, and venous blood carries carbon dioxide, a waste product of metabolism produced by cells, from the tissues to the lungs to be exhaled. Blood accounts for 7% of the human body weight(Elert and Glenn,2012).

1-2-2:haemopoiesis:

1-2-2-erythropoietin:

also known as EPO, is a glycoprotein hormone that controls erythropoiesis, or red blood cell production. It is a cytokine (protein signaling molecule) for erythrocyte (red blood cell) precursors in the bone marrow. Human EPO has a molecular weight of 34 kDa,Also called hematopoietin or hemopoietin, it is produced by interstitial fibroblasts in the kidney in close association with peritubular capillary and tubular epithelial tubule. It is also produced in perisinusoidal cells in the liver. While liver production predominates in the fetal and perinatal period, renal production is predominant during adulthood. In addition to erythropoiesis, erythropoietin also has other known biological functions. For example, it plays an important role in the brain's response to neuronal injury.(siren *etal*.,2001),EPO is also involved in the wound healing process.(Haroon *etal*.,2003).

1-2-2-2Hemoglobin:

The mammalian hemoglobin molecule can bind (carry) up to four oxygen molecules.(Costanzo and Linda ,2007).

Hemoglobin is involved in the transport of other gases: It carries some of the body's respiratory carbon dioxide (about 10% of the total) as carbaminohemoglobin, in which CO_2 is bound to the globin protein. The molecule also carries the important regulatory molecule nitric oxide bound to a globin protein group, releasing it at the same time as oxygen.(Epstein*etal.*,1998).

Hemoglobin is also found outside red blood cells and their progenitor lines. Other cells that contain hemoglobin include the A9 dopaminergic neurons in the substantia nigra, macrophages, alveolar cells, and meningeal cells in the kidney. In these tissues, hemoglobin has a non-oxygen-carrying function as an antioxidant and a regulator of iron metabolism.(Biagioli*et al.*, 2009).

Hemoglobin is synthesized in acomplex series of steps in the mitochondria and the cytosol of immature red blood cells ,while the globin protein parts are synthesized by ribosomes in cytosol.(Retrived,2007).

1-2-2-3Blood component:

Plasma is the liquid component of blood, in which the red blood cells, white blood cells, and platelets are suspended. It constitutes more than half of the blood's volume and consists mostly of water that contains dissolved salts (electrolytes) and proteins. The major protein in plasma is albumin. Albumin helps keep fluid from leaking out of blood vessels and into tissues, and albumin binds to and carries substances such as hormones and certain drugs. Other proteins in plasma include antibodies (immunoglobulins), which actively defend the body against viruses, bacteria, fungi, and cancer cells, and clotting factors, which control bleeding ,Plasma has other functions. It acts as a reservoir that can either replenish insufficient water or absorb excess water from tissues. When body tissues need additional liquid, water from plasma is the first resource to meet that need. Plasma also prevents blood vessels from collapsing and clogging and helps maintain blood pressure and circulation throughout the body simply by filling blood vessels and flowing through them continuously. Plasma circulation also plays a role in regulating body temperature by carrying heat generated in core body tissues through areas that lose heat more readily, such as the arms, legs, and head(Alan and Lichtin,2014).

Red blood cells (also called erythrocytes) make up about 40% of the blood's volume. Red blood cells contain hemoglobin, a protein that gives blood its red color and enables it to carry oxygen from the lungs and deliver it to all body tissues. Oxygen is used by cells to produce energy that the body needs, leaving carbon dioxide as a waste product. Red blood cells carry carbon dioxide away from the tissues and back to the lungs. When the number of red blood cells is too low (anemia), blood carries less oxygen, and fatigue and weakness develop. When the number of red blood cells is too high (polycythemia), blood can become too thick, which may cause the blood to clot more easily and increase the risk of heart attacks and strokes(Alan , Lichtin,2014).

4.7 to 6.1 million (male), 4.2 to 5.4 million (female) erythrocytes(Medline and Retrieved ,2007).

Red blood cells contain the blood's hemoglobin and distribute oxygen. Mature red blood cells lack a nucleus and organelles in mammals. The red blood cells (together with endothelial vessel cells and other cells) are also marked by glycoproteins that define the different blood types. The proportion of blood occupied by red blood cells is referred to as the hematocrit, and is normally about 45%. The combined surface area of all red blood cells of the human body would be roughly 2,000 times as great as the body's exterior surface,(Robert *et al.*,2006)

White blood cells (also called leukocytes) are fewer in number than red blood cells, with a ratio of about 1 white blood cell to every 600 to 700 red blood cells. White blood cells are responsible primarily for defending the body against infection.(Alan , Lichtin,2014).

White blood cells are part of the body's immune system; they destroy and remove old or aberrant cells and cellular debris, as well as attack infectious agents (pathogens) and foreign substances. The cancer of leukocytes is called leukemia, There are five main types of white blood cells: Neutrophils, the most numerous

type, help protect the body against infections by killing and ingesting bacteria and fungi and by ingesting foreign debris, Lymphocytes consist of three main types: T cells (T lymphocytes) and natural killer cells, which both help protect against viral infections and can detect and destroy some cancer cells, and B cells (B lymphocytes), which develop into cells that produce antibodies, Monocytes ingest dead or damaged cells and help defend against many infectious organisms, Eosinophils kill parasites, destroy cancer cells, and are involved in allergic responses, Basophils also participate in allergic responses,Some white blood cells flow smoothly through the bloodstream, but many adhere to blood vessel walls or even penetrate the vessel walls to enter other tissues. When white blood cells reach the site of an infection or other problem, they release substances that attract more white blood cells. The white blood cells function like an army, dispersed throughout the body but ready at a moment's notice to gather and fight off an invading organism. White blood cells accomplish this by engulfing and digesting organisms and by producing antibodies that attach to organisms so that they can be more easily destroyed ,When the number of white blood cells is too low (leukopenia), infections are more likely to occur. A higher than normal number of white blood cells (leukocytosis) may not directly cause symptoms, but the high number of cells can be an indication of a disease such as an infection or leukemia.(Alan ,2014).

also called thrombocytes are cell-like particles that are smaller than red or white blood cells. Platelets are fewer in number than red blood cells, with a ratio of about 1 platelet to every 20 red blood cells. Platelets help in the clotting process by gathering at a bleeding site and clumping together to form a plug that helps seal the blood vessel. At the same time, they release substances that help promote further clotting. When the number of platelets is too low (thrombocytopenia), bruising and abnormal bleeding become more likely. When the number of platelets is too high

(thrombocythemia), blood may clot excessively, causing a stroke or heart attack.(Alan ,2014).

1-2-3 complete blood count (CBC):

also known as a complete blood cell count, full blood count (FBC), or full blood exam (FBE), is a blood panel requested by a doctor or other medical professional that gives information about the cells in a patient's blood. A scientist or lab technician performs the requested testing and provides the requesting medical professional with the results of the CBC,Blood counts of various types have been used for clinical purposes since the 19th century. Automated equipment to carry out complete blood counts was developed in the 1950s and 1960s,(Verso and May 1962)

Total white blood cells , All the white cell types are given as a percentage and as an absolute number per liter ,the total red blood cells , The number of red cells is given as an absolute number per litre., Iron deficiency Anemia shows up as a Low RBC count, Hemoglobin The amount of hemoglobin in the blood, expressed in grams per deciliter. A low level of Hemoglobin is a sign of anemia ,(Dugdale , 2012).

 Hematocrit: the proportion of blood volume occupied by red blood cells, is typically about three times the hemoglobin concentration measured in g/dL. For example, if the hemoglobin is measured at 17 g/dL, that compares with a hematocrit of 51%.[70]

Hematocrit or packed cell volume (PCV) – This is the fraction of whole blood volume that consists of red blood cells,(David Cand Dugdale , 2012),Red blood cell indicesinclude: Mean corpuscular volume (MCV) the average volume of the red cells, measured in femtolitres,,Mean corpuscular hemoglobin (MCH) – the average amount of hemoglobin per red blood cell, in pictograms, Mean corpuscular hemoglobin concentration (MCHC) the average concentration of hemoglobin in

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the cells, Red blood cell distribution width (RDW) the variation in cellular volume of the RBC population.(Dugdale , 2012).

A complete blood count with differential/platelet will also include:

Neutrophil granulocytes :May indicate bacterial infection. May also be raised in acute viral infections. Because of the segmented appearance of the nucleus, neutrophils are sometimes referred to as "segs." The nucleus of less mature neutrophils is not segmented, but has a band or rod-like shape, Less mature neutrophils those that have recently been released from the bone marrow into the bloodstream are known as "bands" or "stabs", Stab is a German term for rod.

Lymphocytes : Higher with some viral infections such as glandular fever and. Also raised in chronic lymphocytic leukemia (CLL). Can be decreased by HIV infection. In adults, lymphocytes are the second most common WBC type after neutrophils. In young children under age 8, lymphocytes are more common than neutrophils, Monocytes : May be raised in bacterial infection, tuberculosis, malaria, Rocky Mountain spotted fever, monocytic leukemia, chronic ulcerative colitis and regional enteritis ,Eosinophil granulocytes :Increased in parasitic infections, asthma, or allergic reaction ,Basophil granulocytes May be increased in bone marrow related conditions such as leukemia or lymphoma, A manual count will also give information about other cells that are not normally present in peripheral blood, but may be released in certain disease processes,Platelet numbers are given, as well as information about their size and the range of sizes in the blood.(Dugdale , 2012).

1-2-4Rheumatoid arthritis (RA):

is an autoimmune disease that results in a chronic, systemic inflammatory disorder that may affect many tissues and organs, but principally attacks flexible (synovial) joints. It can be a disabling and painful condition, which can lead to substantial loss of functioning and mobility if not adequately treated.

The process involves an inflammatory response of the capsule around the joints (synovium) secondary to swelling (turgescence) of synovial cells, excess synovial fluid, and the development of fibrous tissue (pannus) in the synovium. The pathology of the disease process often leads to the destruction of articular cartilage and ankylosis (fusion) of the joints. RA can also produce diffuse inflammation in the lungs, the membrane around the heart (pericardium), the membranes of the lung (pleura), and white of the eye (sclera), and also nodular lesions, most common in subcutaneous tissue. Although the cause of RA is unknown, autoimmunity plays a big part, and RA is a systemic autoimmune disease. It is a clinical diagnosis made on the basis of symptoms, physical exam, radiographs and labs.(Majithia and Geraci ,2007).

About 0.6% of the United States adult population has RA, women two to three times as often as men.(Helmick*etal.*,2008).

The name is based on the term "rheumatic fever", an illness which includes joint pain and is derived from the Greek word -rheuma (nom.), rheumatos (gen.) ("flow, current"),The first recognized description of RA was made in 1800 by Dr. Augustin Jacob Landré-Beauvais (1772–1840) of Paris.(Beauvais ,2001).

1-2-4-1Signs and symptoms:

RA primarily affects joints, however it also affects other organs in 15–25% of individuals

Arthritis of joints involves inflammation of the synovial membrane. Joints become swollen, tender and warm, and stiffness limits their movement. With time, multiple

joints are affected (it is a polyarthritis). Most commonly involved are the small joints of the hands, feet and cervical spine, but larger joints like the shoulder and knee can also be involved.[9]:1089 Synovitis can lead to tethering of tissue with loss of movement and erosion of the joint surface causing deformity and loss of function.(Majithia, Geraci ,2007).

RA typically manifests with signs of inflammation, with the affected joints being swollen, warm, painful and stiff, particularly early in the morning on waking 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 one hour, and movements induce pain caused by mechanical arthritis. The pain associated with RA is induced at the site of inflammation and classified as nociceptive as opposed to neuropathic.(Gaffo*etal.*,2006).

The joints are often affected in a fairly symmetrical fashion, although this is not specific, and the initial presentation may be asymmetrical, the pathology progresses the inflammatory activity leads to tendon tethering and erosion and destruction of the joint surface, which impairs range of movement and leads to deformity. The fingers may suffer from almost any deformity depending on which joints are most involved. Specific deformities, which also occur in osteoarthritis, include ulnar deviation(Livingston and Elsevier, 2010).

Fibrosis of the lungs is a recognized response to rheumatoid disease. It is also a rare but well recognized consequence of therapy (for example with methotrexate and leflunomide). Caplan's syndrome describes lung nodules in individuals with

RA and additional exposure to coal dust. Pleural effusions are also associated with RA. Another complication of RA is Rheumatoid Lung Disease. It is estimated that about one quarter of Americans with RA develop Rheumatoid Lung

Renal amyloidosis can occur as a consequence of chronic inflammation.(Livingstone and Elsevier, 2010).

RA may affect the kidney glomerulus directly through a vasculopathy or a mesangial infiltrate but this is less well documented (though this is not surprising, considering immune complex-mediated hypersensitivities are known for pathogenic deposition of immune complexes in organs where blood is filtered at high pressure to form other fluids, such as urine and synovial fluid.(Robbins *etal.*,2010).

People with RA are more prone to atherosclerosis, and risk of myocardial infarction (heart attack) and stroke is markedly increased.(Wolfe , Mitchell , 2006). Other possible complications that may arise include: pericarditis, endocarditis, left ventricular failure, valvulitis and fibrosis.(Gupta and Fomberstein ,2009).

1-2-4-2Causes:

RA is a form of autoimmunity, the causes of which are still not completely known. It is a systemic (whole body) disorder principally affecting synovial tissues. There is no evidence that physical and emotional effects or stress could be a trigger for the disease. The many negative findings suggest that either the trigger varies, or that it might in fact be a chance event inherent with the immuneresponse, Half of the risk for RA is believed to be genetic, It is strongly associated with the inherited tissue type major histocompatibility complex (MHC) antigen HLA-DR4 (Goeldner*etal.*,2010).

Smoking is the most significant non-genetic risk(Scott *etal.*,2010).

the RA being up to three times more common in smokers than non-smokers, particularly in men, heavy smokers, and those who are rheumatoid factor positive,(Sugiyama *etal.*,2010).

Epidemiological studies have confirmed a potential association between RA and two herpesvirusinfections, Epstein-Barr virus and Human Herpes Virus 6 .(Alvarez *etal.*,2005).

Vitamin D deficiency is common in those with RA and may be causally associated, Some trials have found a decreased risk for RA with vitamin D supplementation while others have not.(Wen and 2011).

1-2-4-3pathogensis:

Once the abnormal immune response has become established (which may take several years before any symptoms occur), plasma cells derived from B lymphocytes produce rheumatoid factors and ACPA of the IgG and IgM classes in large quantities. These are not deposited in the way that they are in systemic lupus. Rather, they activate macrophages through Fc receptor and complement binding, which seems to play an important role in the intense inflammatory response present in RA,(Boldt*etal.*,2012).

1-2-4-4diagnosis:

Blood tests

When RA is clinically suspected, immunological studies are required, such as testing for the presence of rheumatoid factor non-specific antibody, A negative RF does not rule out RA; rather, the arthritis is called seronegative. This is the case in about 15% of patients.

Because of this low specificity, new serological tests have been developed, which test for the presence of the anti-citrullinated protein antibodies (ACPAs) or anti-CCP. Like RF, these tests are positive in only a proportion (67%) of all RA cases,

but are rarely positive if RA is not present, giving it a specificity of around 95%.(Nishimura *etal.*,2012).

1-2-4-6Treatment:

Disease-modifying ant rheumatic drugs are the primary treatment for RA. They are a diverse collection of drugs, grouped by use and convention. They have been found to improve symptoms, decrease joint damage, and improve overall functional abilities(ScottDL*et al.*,2010).

The most commonly used agent is methotrexate with other frequently used agents including sulfasalazine and leflunomide. Sodium aurothiomalate and cyclosporine are less commonly used due to more common adverse effects,(Scott *et al.*,2010)

1-2-6Mechnism of anemia in RA:

Most chronic inflammatory rheumatic diseases are complicated by hematologic abnormalities, including anemia; disorders of leukocytes, platelets, and the coagulation system; and hematologic malignancies,(Hamilton ,1983).

The underlying causes of ACD are not well understood, but the inflammation that occurs throughout the body in RA may contribute to anemia. Inflamed tissues in the joints release proteins that impact the body's ability to use iron and produce red blood cells, leading to a low red blood count,Overall, 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 .(Vreugdenhil*etal.*,1990) , as confirmed by the data from Wolfe (Wolfe and Michaud ,2006) .

Evidence suggests that increased production of inflammatory cytokines such as tumor necrosis factor 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 ,Most of all, by treating RA patients with recombinant human erythropoietin (EPO) the suppression of erythropoiesis could be overcome . 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 , Investigations related to the effect of anemia on quality of life also demonstrated significant improvements during treatment with erythropoietin ,The pathogenesis of the anemia of chronic disease is incompletely understood. Two major factors appear to be important: trapping of iron in macrophages, making it relatively unavailable for new hemoglobin synthesis; and inability of the morphologically normal marrow to increase erythropoiesis in response to the anemia [6,7]. Inflammatory mediators, particularly tumor necrosis factor (TNF)-alpha, interleukin (IL)-1, IL-6, IL-10, and interferon gamma, contribute to these changes . Hepcidin, an acute phase reactant produced by the liver, may play a key role in cytokine-mediated anemia, as this protein decreases intestinal iron absorption and iron release from macrophages,TNF-alpha and polymorphisms in TNF receptor genes may be particularly important in RA .(Peeters*etal.*,1996).

TNF-inhibition with infliximab, a chimeric mouse human monoclonal antibody in RA patients with anemia of chronic disease, leads to a dose-dependent increase in

hemoglobin levels compared with placebo. These changes were also accompanied by a reduction in serum levels of both erythropoietin and IL-6 ,Evidence suggests that increased production of inflammatory cytokines (tumor necrosis factor) is linked to a decrease erythropoietin response in the bone marrow, thereby leading to inadequate erythropoiesis , As well, this inhibition could be partly reversed by increasing the concentration of erythropoietin .(Jongen*et al*.,1994) .

Most of all, by treating RA patients with recombinant human erythropoietin (EPO) the suppression of erythropoiesis could be overcome(Peeters*et al.*,1996)

 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 .(Kaltwasser*etal*.,2001).

 Investigations related to the effect of anemia on quality of life also demonstrated significant improvements during treatment with erythropoietin (Peeters*etal.*,1999).

1-2-6 Previous Studies:

1-Visser 2008 studies in rhrumatoid arthritis the objective of this studyTo develop evidence-based recommendations for the use of methotrexate in daily clinical practice in rheumatic disorders,the methodology used it is 751 rheumatologists from 17 countries participated in the 3E (Evidence, Expertise, Exchange) Initiative of 2007–8 consisting of three separate rounds of discussions and Delphi votes. Ten clinical questions concerning the use of methotrexate in rheumatic disorders were formulated. A systematic literature search in Medline, Embase, Cochrane Library and 2005–7 American College of Rheumatology/European League Against Rheumatism meeting abstracts was conducted. Selected articles were

systematically reviewed and the evidence was appraised according to the Oxford levels of evidence. Each country elaborated a set of national recommendations. Finally, multinational recommendations were formulated and agreement among the participants and the potential impact on their clinical practice was assessed,the result isA total of 16 979 references was identified, of which 304 articles were included in the systematic reviews. Ten multinational key recommendations on the use of methotrexate were formulated. Nine recommendations were specific for rheumatoid arthritis (RA), including the work-up before initiating methotrexate, optimal dosage and route, use of folic acid, monitoring, management of hepatotoxicity, long-term safety, mono versus combination therapy and management in the perioperative period and before/during pregnancy. One recommendation concerned methotrexate as a steroid-sparing agent in other rheumatic diseases.(Visser,2008).

2-Wolfe.,2006*et al* report results on the prevalence of anemia in a large cohort of 2120 consecutive patients with RA 1 . All patients with RA seen for clinical care at the Wichita Arthritis Center were investigated, with attention to the role that sex, age, and renal function play on the development of anemia. The estimated lifetime prevalence of anemia (hemoglobin, Hb, < 12 g/dl) was 51%, 34% in men and 58% in women. At lower cutpoints (Hb< 11 g/dl) the prevalence is 20% in men and 33% in women. As well, age had an effect on occurrence of anemia. The prevalence was more frequent in younger and older women, with the highest hemoglobin levels in patients about 58 years of age. The estimated annual incidence of anemia for both sexes was 7.9%. Also, a relationship between renal function and anemia could be established. A drawback of the study is that no data are available related to the cause of the anemia. And no data could be found nor an explanation for the lowered creatinine clearance of about 10 ml/min in the patients

with RA. But this study clearly illustrates that the majority of patients with RA will develop anemia during their disease, thus supporting the statement that more attention should be paid to the occurrence of anemia in our patients with RA. Moreover, the annual incidence of anemia is nearly 8%, and the lifetime prevalence of severe anemia (Hb< 10 mg/dl) is 13.7%.

1-3Rationale:

Rheumatoid arthritis of is chronic inflamtorydiseases that need to laboratory testing to identify the disease,Anemia is not considered a major problem in rheumatoid arthritis by majority of physicians ,This statement is based on the fact that studies on anemia in RA are sparse ,with few systemic reviews, and no extensive literature on its prevelance and effect on various clinical and functional outcomes, including morbility,mortality,and quality of life ,and the prevelance of anemia in large cohort of 2120 consecutive patient with RA. All patient with RA seen development of anemia.The estimated lifetime prevelance of anemia (Wolfe *et al.,*2006*),*Most choronic inflammatory rheumatic disease are complicated by heamatologic abnormalities, including anemia ,disorders of leukocytes, platelets, and the coagulation system and heamatologic malignancies,(Hmilton ,1983).For this issue conduct this study to see the hematological abnormality in Sudan.

1-4Objectives:-

1-4-1 General objective:-

To Measure complete blood count in patients with Rheumatoid arthritis.

1-4-2 Specific objectives:-

1-To Measure of (Hb, WBCS,RBCS,PCV, MCV, MCH, MCHC and platelets).in both study group.

2- To compare the result of complete blood count in rheumatoid arthritis study group with control one.

3- To correlate the result of complete blood count and risk factor gender severity anaemia.

Material & Method

2-1 Study design:

This was an analytic prospective case control study conducted in Zain Center during period from February to May 2014

2-2 Sample size:

seventy samples from rheumatoid arthritis patient and thirty healthy male and females as control group.

2-3 Inclusion criteria

Male and female with no other medical condition than rheumatoid arthritis were included in this study.

2-4 Exclusion criteria

rheumatoid arthritis with any other medical condition that effect on (Hb- PCV- TWBCs- RBCs- platelet) were excluded from this study. (e.g. SLE and pregnant women)

2-5 Plan of data collection:

Data was collected using a design questioner and som data was collected from record file to rheumatoid arthritis to obtain the following data (name, age, sex, duration of disease)

2-6Sample collection:

Five ml of venous blood was collected by using sterile 5 ml EDTA vacuntainer collection tube.

The sample collected from antecubital vein after cleaning the side by 70% alcohol.

After drying the short rubber end of needle inserted into the vacuntainer needle holder at screw side, then the other longed side of the needle inserted into the vein and the vacuntainer pushed in the holder against the rubber end of needle, the blood automatically draw in tube until 5 ml of blood and collected, then the needle removed from the vein hemostats screwed and adhesive plaster pan applied to side,

the blood immediately mixed with EDTA anticoagulant by the gentle rotation, not shaking and then labeled the container with patient name, date, number (Daci and Lewis, 2006).

2-7 Material and Method:

Automatic hematological analyzer Sysmix KX2IN for determination of complete blood count (as seen in appendix 2)

70% alcohol (Ethanol) and cotton, vacuntainer, EDTA anticoagulant container, needle.

2-8Complete blood count:

CBC was done using Sysmex KX2IN.

2-8-1 Principles of instrument (Sysmex)

Measurement of blood cells (RBCs, WBCs, and platelet) and Hb concentration obtained by aspiration of small volume of well mixed K2 EDTA blood by sample probe and mixed with isotonic diluents in nebulizer. Diluted mixture aspiration delivered to RBC aperture bath for providing information about RBC and platelet based on cell size, particles of 2 to 20 fl counted platelets, above 36 fl counted as red cells. Some portion of aspirated mixture induced into WBC bath in which hemolytic reagent (Stromatolyzer) was added to automatically to measure Hb concentration in a build calorimeter, based on cyanomethemoglobin (HiCN). Blood cell counted and size information generated in tripilicate pulses according to electronic conductivity, and translated into digital number using in build calculator programmed and designed for that RBC, WBC count hence three values were directly measured (RBC, WBC, Hb), and displayed on (LCD). Other values of red cell indices, platelet count, leukocyte differential and absolute count calculated from given information and automated constructed histograms, the result printed out according to the setting mode.

2-8-2 Method :

The reagent needed was checked for expiry date before use.

The power switch was turned and background check will be automatically performed and the vend (vend for analysis) will appear. Sample number inputted by pressing simple number, then number of sample was entered. The entered was pressed. Sample was mixed sufficiently. The tube was sited to the sample probe, and in that condition the start switch was pressed. When the LCD screen display analyzing the tube was removed. After that the unit executes automatic analysis and the result was pint out.

2-9 Ethical considerations:

Ethical clearance obtained in this study, a verbal was taken from all participated after explaining the aim of the study.

2-10 Data analysis:

Data analyzed by Statistical Package for Social Sciences (SPSS) 15

Result

seventy samples from patients with rheumatoid arthritis and thirty healthy person serve as control groups in the study to measure (Hb,WBcs,Rbcs,PCV,MCV,MCH,MCHC and platelets count).

The result showed that the mean of (Hb, WBcs ,Rbcs ,PCV,MCV,MCH,MCHC and platelets count) parameter case group were (11.6 $\pm$ 1.6mg/dl, 6.9$\pm$ 2.4/cumm , 4.4$\pm$0.5/cumm , 36.7$\pm$4.5%,83.0$\pm$ 6.9/fl, 26.0$\pm$2.8/pg , 31.0$\pm$1.6mg/dl , 325.2$\pm$106.8) respectively While the control group (13.2$\pm$ 1.1mg/dl, 6.2$\pm$1.7/cumm, 5.0$\pm$0.3/cumm, 41.0$\pm$3.5%,82.4$\pm$3.0/fl, 26.0$\pm$0.9/pg, 31.6$\pm$2.2 mg/dl, 263.2$\pm$54.6).respectively.

p.value are($P<o.ooo$, significant ,$P<0.165$ insignificant ,P.value <0.000 significant ,P.value<0.000 significant ,P.value 0.573 insignificant ,p.value<0.910 insignificant , P.value$<o$

of Chronic Rheumatoid arthritis(p.value $<o.ooo$) ,Anemia is seen even in RA due to two major factors trapping of iron in macrophages ,make it relatively not available for new Hb and inability of marrow erythropoiesis in response to inflam.236 which insignificant , P.value 0.003 significant)respectively.

Hemoglobin(Hb),Hematocrit and Red blood cell are decreased significantly in patients compared to control, this was expected since anemia is a well-recognized complication matory mediator TNF,IL-1 ,IL-6,IL-10,INFgamma .Evidence suggest that increase erythropoietin response in marrow ,production of TNF is linked to decrease it, where Hb ,<12 mg/dl and HCT<37% ,significantly

Are no decreased in MCV ,MCH,MCHC compared to control ,WBC insignificantly slightly increase as showed in the result ,platelet of patients slightly increase compared to control group.

Table (3-1)comparison of mean of Hb,Hct,MCV,MCH,MCHC,Rbcs,Twbcs,plates among study and voluntires

Test	sample	N	Mean±STD	*P value*

Hb	case	70	11.579±1.62	0.000
	control	30	13.170±1.10	
WBCs	case	70	6.914±2.46	0.165
	control	30	6.220±1.71	
RBCs	case	70	4.406±0.51	0.000
	control	30	5.013±0.31	
PCV	case	70	36.726±4.46	0.000
	control	30	41.027±3.55	
MCV	case	70	83.036±6.91	0.573
	control	30	82.473±3.01	
MCH	case	70	26.073±2.89	0.910
	control	30	26.023±1.45	
MCHC	case	70	31.091±1.65	0.236
	control	30	31.630±2.19	
Plats	case	70	325.24±106.83	0.003
	control	30	263.27±54.68	

Table(3.2):

correlation between Mean of{Hb,Hct,MCV,MCH,MCHC,Rbcs,Twbcs,plates} according topatients gender .

Test	Sex	N	Mean±STD	*P value*

Hb	Female	58	11.376±1.54	0.021
	Male	12	12.558±1.70	
WBCs	Female	58	6.962±2.51	0.725
	Male	12	6.683±2.29	
RBCs	Female	58	4.371±.50	0.214
	Male	12	4.575±.53	
PCV	Female	58	36.252±4.32	0.050
	Male	12	39.017±4.57	
MCV	Female	58	82.684±7.11	0.354
	Male	12	84.733±5.86	
MCH	Female	58	25.838±2.95	0.136
	Male	12	27.208±2.33	
MCHC	Female	58	30.900±1.67	0.033
	Male	12	32.017±1.29	
Plts	Female	58	333.34±110.92	0.165
	Male	12	286.08±76.24	

Chapter Four

Discussion

4.1 discussion:

This study showed that the mean of hemoglobin level the study group was significally decreased($p\ value<0.000$)   in all parent patient of the study group compared to to control group this Agreed with(Smith and Knight , 1992),who stated that there was decreased in hemoglobin level .patient of rheumatoid arthritis this was expected since anemia is a well-recognized complication of Chronic RA ,Anemia is seen even in RA due to two major factor trapping of iron in macrophages ,make it relatively in available for new Hb and inability of marrow erythropoiesis in response to inflammatory mediator TNF,IL-1 ,IL-6,IL-10,INFgamma .Evidence suggest that increase erythropoietin response in marrow ,production of TNF is linked to decrease hemoglobin level.

White blood cell showed that the mean of the study group was in significally increased in all($P\ value<0.165$)parent patient of the study group compared to to control group this disagreed with(jose ,Cesar*e tal* .,2005),who stated that pancytopenia.

Red blood cell showed that the mean of the study group was statically significally increased in all($P\ value<0.000$)parent patient of the study group compared to control group.

PCV also decreed in study group compared to control group with statically significant($P\ value<0.000$).

Men cell hemoglobin also no decreased in study compared to control group with statistically in significant(.$P.\ value$ 0.573).

Men cell hemoglobin concentration also no decrease in study compared to control group with statistically in significant($P.\ value$ 0.236).

Platlate also decreased in study group compared to control group with statically significant($P\ value<0.003$).

4.2Conclusions:

This study concluded the following ,hemoglobin ,red blood cell, packet cell volume of RA patients   significantly decresed ,no significant variation was

observed in MCV ,MCH,MCHC ,insignificantly increased in TWBC insignificantly increased indicating inflammation,platelate increased significantly.

4.3Recommendation

1.Recomended for further work up to involve large number of patient of rheumatoid arthritis in different state of Sudan

2.Report all cases in different hospital and clinic to conduct study in prevalence of RA and other auto immune diseases in Sudan

3.Regular checkup is very important to detect hematological changes doe to pathogenesis of disease and treatment to avoid side effect and anemia

Chapter Five

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