Sudan University of Science and Technology

College of Graduate Studies

Association and Variation of Hematological Parameters among Sudanese Patients with Rheumatoid Arthritis in Khartoum State 2018

العلاقة والتغييرات لتعداد الدم الكامل لدى السودانيين المصابين بإلتهاب المفاصل الرثياني في ولاية الخرطوم 2018م

A dissertation submitted in partial fulfillment for the requirements for the award a degree of M.Sc in Medical Laboratory Sciences (Hematology and Immunohematology).

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الاية

بسم الله الرحمن الرحيم

(وَمَنْ أَعْرَضَ عَن ذِكْرِي فَإِنَّ لَهُ مَعِيشَةً ضَنكًا وَنَحْشُرُهُ يَوْمَ الْقِيَامَةِ أَعْمَىٰ)

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Dedication

We dedicate this work to

My parents

My lovely wife

My daughters

My friends

Abstract

This was an analytic case control study conducted in Khartoum state during period from May to July 2018 to estimate hematological parameters among Sudanese patients with rheumatoid arthritis, The study population was selected as 50 patients and 50 normal individual as control group, Also the study group classified according to their treatment types into 3 different groups whom take steroids treatment (52%), non steroids treatment (8%) and combined drugs (40%). The study was reflect significant increase in mean of TWBCs (mean of case 6.7±2.1 and control mean 5.1± 1.2) with *P* .000, significant Decrease Hb level (mean of case 11.4 ±1.3 and control mean 13.3 ± 1.8) with *P* .000, significant Decrease PCV (mean of case 36.3 ±5.0 and control mean 40.9 ± 4.5) with *P* .000, significant Decrease MCV (mean of case 81.9 ± 8.1 and control mean 92.7 ± 7.3) with *P* .000, significant Decrease MCH mean of case 25.7 ± 2.6 and control mean 29.7 ± 2.7) with *P* .000, and significant increase platelets count (mean of case 275 ±75 and control mean 243 ± 58) with *P* .020, in compare between case and control and no significance on mean of TRBCs (mean of case 4.42 ± 0.47 and control mean 4.45 ± 0.49) with *P* .707, MCHC (mean of case 31.6 ±3.1 and control mean 31.7 ± 2.3) *P* .755, also Rticulocyte count mean of case 0.58 ± 0.77 and control mean 0.40 ± 0.38) with *P* .150. the comparison between types of treatment and hematological parameter show there was significant increase on TWBCs in patient who take steroid (6.4± 1.9) an d combined drugs (7.5± 2.5) (*P* .048), significant decrease on PCV in patient who take steroid drugs (38.3± 5.2) (*P* .012), significant decrease on MCV in patient who take combined drugs (78.3± 8.0) (*P* .032), significant decrease on MCHC in patient who take steroid drugs (35.5 ± 2.9) (*P* .024), there was no significance difference on TRBCs (*P* .286), Hb level (P.v .269), MCH (*P*

.777), platelets count (*P* .382) and Rticulocyte count (*P* .291) in comparison with treatment types. Also study reflex no significance difference between CBC figures (TWBCs *P* .368, Hb *P* .898, TRBCs *P* .787, MCV *P* .998, MCH *P* .787, MCHC *P* .783, PCV *P* .768, platelets count *P* .750 and Reticulocyte count *P* .332).

مستخلص الدراسة

اجريت هذه الدراسه التحليلية الحاله والحاله الضابطة في ولاية الخرطوم في الفترة من مايو الى يوليو 2018م بقياس تعداد مؤشرات الدم الكامل لدى المرضى السودانيون المصابين بالتهاب المفاصل الرثياني.اختير 50 حاله مصابه بالتهاب المفاصل الرثياني و50 اصحاء كحاله ضابطة وايضا قسمت الحالات المرضيه على حسب نوع العلاج المستخدم الى مسخدمي الاستروديدات (52%) , غير الاستروديدات (8%) , ومستخدمي نوعي العلاج (40%).

اظهرت نتائج هذة الدراسة فروق ذات دلالة احصائية بالنسبة للمتغيرات التاليه نقصان في مستوى كريات الدم البيضاء بين الحاله (6.7±2.1) وحالة الضبط (5.1± 1.2) ايضا نقصان في خضاب الدم للحالات (11.4 ±1.3) والحاله الضابطة (13.3 ± 1.8) ،وكذلك نقصان لمعدل تكدس الخلايا للحالات (36.3 ±5.0) والحاله الضابطة (40.9 ± 4.5)، هيموغلوبين الكرية الوسطي للحالات 25.7 ± 2.6) والحاله الضابطة (29.7 ± 2.7) والحجم الكريوي الوسطي للحالات (± 81.9 8.1) والحاله الضابطة (92.7 ± 7.3), وكانت القيمه الاحتماليه لكل المقارنات اعلاه 000. وزيادة في الصفائح الدموية للحالات (275 ±75) والحاله الضابطة (243 ± 58) بقيمة احتمالية 020. . كما اظهرت الدراسة فروقات ليس لها دلاله احصائية لدى عدد خلايا الدم الحمراء للحالات (4.42 ± 0.47) والحاله الضابطة (4.45 ± 0.49) ، التركيز الوسطي لهموغلوبين الكرية للحالات (31.6 ±3.1) والحاله الضابطة (31.7 ± 2.3) وعد الكريات الشبكية للحالات (0.58 ± 0.77) والحاله الضابطة (0.40 ± 0.38) بقيمه احتمالية 510.

ايضا عند مقارنة تعداد الدم العام مع الحالات المزمنة للمرض على حسب العلاجات المستخدمة اوجدت الدراسة فروقات ذات دلالة احصائية بزيادة في كرويات الدم البيضاء لدى مستخدمي الاستروديدات(1.9 ±6.4) ونوعي العلاج (2.5 ±7.5) والقيمة الاحتمالية 048. ايضا نقصان في تكدس الدم مع مستخدمي الاسترودید (5.2 ±38.3) بقيمه 012.، ونقصان في الحجم الكريوي الوسطي لدى مستخدمي كل انواع العلاج(2.9 ± 35.5) بقيمة احتماليه 032. . وعدم وجود تغير ذو دلالات احصائية للهموغلوبين ، عدد كريات الدم الحمراء ، الصفائح الدموية ، هموغلوبين الكرية الوسطى وعدد الكريات الشبكية.

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List of abbreviations:

BFU-E: Burst Forming Unit – Erythroid.

BFU-Meg: Burst Forming Unit –Megakaryocyte

CFU-E: Colony Forming Unit Erythroid.

CFU-GEMM: Colony- Forming unit Granulocyte, Erythroid, Megakaryocyte, Macrophage

CFU-Meg: Colony Forming Unit – Megakaryocyte

CRP: C-reactive protein

Epo: Erythropoietin

EpoR: Erythropoietin Receptor

ESR erythrocyte sedimentation rate

Hb: Hemoglobin

HCT: Hematocrit

IL-11: Interleukin 11

IL-3: Interleukin 3

IL-6: Interleukin 6

PCV: Packed cell volume

RA: Rheumatoid Arthritis

RNA: Ribosomal Ribonucleic Acid

Tpo: Thrompopietin

Introduction and Literature Review

Chapter One

Chapter one

Introduction and literature review

1.1 Introduction

Rheumatoid Arthritis (RA) is the most common chronic inflammatory disorder, associated with progressive destruction of synovial joints and physical disability (Alamanos *et al*., 2006).

RA characterized by deregulation of the irnmune system, resulting in chronic activation of T–cell responses and over production of proinflammatory cytokines including tumour necrosis factor and interleukin 1. It is classified as autoimmune disease characterized by deregulation of the immune system, Resulting in Chronic activation of T-cell responses and over production of proinflammatory cytokines including Tumour necrosis factor and interleukin 1.The resultant effect of the above response is joint destruction (Okoroiwu *et al*., 2016)

RA resulting in warm, swollen, and painful joints which are typically involved symmetrically, the majority of studies estimate a prevalence of 0.5-1%, and the prevalence of RA is higher in females than males (Alamanos *et al*., 2005, Kvien *et al*., 2006).

The incidence is reported to be higher 4-5 times below the age of 50, but above 60-70 years the female/male ratio is only about 2(Kvien *et al*.,2006). Similar to many rheumatic diseases, RA has a highly variable course with activation and remission periods over time (Jayakumar *et al*., 2012).

An effective management in RA treatment depends on a closer monitoring of the disease activity. To determine the disease activity, clinicians use some clinical signs and symptoms (rheumatoid nodules, morning stiffness), radiological findings (bone erosion) and blood-derived parameters (RF

values and some acute phase response markers). C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) are the most common measuring assays to detect the acute phase response due to their reliability and cost effectiveness (Colglazier *et al*., 2005, Wollheim *et al*., 2000).

1.2 Literature review

1.2.1 Blood

Blood is only fluid connective tissue in the body, it is sticky, opaque fluid, the color of blood varies from scarlet(oxygen rich) to dark red(oxygen poor) it has slightly alkaline pH 7.35_7.45 (Marieb and Hoehn, 2013).

The average person has approximately 70 ml of blood per kilogram body weight, approximately 50_60% of the blood volume is liquid (plasma) the remainder is cells (erythrocytes, leukocytes and platelets). nearly 90% of plasma is water the remainder (10%)is include ions, glucose, amino acid and other metabolites, hormones and various protein (William, 2002).blood perform a number of functions all concerned in one way or another with distributing substance, regulating blood levels of particular substances or protecting the body (Marieb and Hoehn, 2013).

1.2.2 Site of hematopoiesis:

in fetus less than 2 months the Hematopoiesis occur in yolk sac, from 2-7 month occur in liver with minimal hematopoiesis in spleen, then after 3 months hematpoiesis start in bone marrow (BM) and in full term infant and after birth occur mainly in BM, with minimal hematopoiesis in spleen, lymph node and other lymphoid tissues) (Sood, 2009, Longo, 201., and Kawthalkar, 2013). In adult occur only in long bone (Hoffbrand *et al*., 2001).

1.2.2.1 Stem cell:

All of the cell types in the peripheral blood and some cells in every tissue of the body are derived from stem cell. Stem cells have two essential features are there capacity to differentiate into a variety of mature cell types and the capacity for self-renewal (Longo, 2010).

1.2.2.2 Erythropoiesis:

It's a process of proliferation and differentiation of erythrocyte from stem cell of bone marrow (BM) and release mature cells in to the blood (Hoffbrand *et al*., 2001).

The maturation of it begin from nucleated to a nucleated cells this development takes place in BM (Ciesla, 2007).

Erythropoiesis has two main compartment, the first one contains the erythroid progenitor cells which take erythropoiesis from stem cell to the last stage of differentiation before hemoglobin (Hb) synthesis begin, the second compartment include steps of maturation and differentiation in which Hb synthesis is major feature(Hoffbrand *et al*., 2001).

The progenitor of erythroid cell is colony- forming unit –granulocyte, erythroid, megakaryocyte, macrophage (CFU-GEMM), burst forming unit – erythroid (BFU-E) and colony forming unit erythroid (CFU-E) (Hoffbrand *et al*., 2001).

The series of erythropoiesis include pro erythroblast (pronormoblast), basophilic erythroblast (early erythroblast), polychromatic erythroblast (intermediate erythroblast), orthochromatic erythroblast (late erythroblast), reticulocyte and mature erythrocyte (Turgeon, 2012).

The red blood cell (RBC) is 8 µm in diameter, a nucleated, flexible and biconcave disc. Life span is 120 days, floating in the plasma with other cells to form blood, this cells constitute about 45% of blood volume. The main

function of red cells is to carry oxygen to the tissues and return carbon dioxide from tissues to the lungs (Osman, 2013).

The major system that control erythropoiesis is erythropoietin (Epo) hormone which produced in kidney (90%) and liver. The erythropoietin receptor (EpoR) present on BFU-E and CFU-E which in demand produce, proliferate and differentiate erythrocyte from BM (Hoffbrand *et al*., 2001).

Androgen hormone enhances Epo production and has direct stimulatory effect on the proliferation of erythroid precursors (Saxe*na et al*, 2013).

1.2.3 Reticulocytes:

Reticulocytes are juvenile red cells; they contain remnants of the ribosomal ribonucleic acid (RNA) that was present in larger amounts in the cytoplasm of the nucleated precursors from which they were derived. Ribosomes have the property of reacting with certain basic dyes such as azure B, brilliant cresyl blue or New methylene blue to form a blue or purple precipitate of granules or filaments (Dacie and Lewis, 2006).

The range of reticulocyte counts in adults and children is 50–100 _ 109/l (0.5–2.5%). At birth or in cord blood, it is 120–400 _ 109/l (2–5%) (Dacie and Lewis, 2006).

1.2.4 Leucopoiesis:

1.2.4.1 Granulopoiesis:

Is the process of formation of the granulocytes in the bone marrow which are the predominant white blood cells in the circulation. The classification of granulocyte in the neutrophil, eosinophil and basophil based on the color of these cells after staining by Romanovsky stains (Saxe*na et al*., 2013).

1.2.4.2 Lymphopoeisis:

Lymphocytes are the immunologically component cells that assist the phagocytes in defense of the body against infection and other foreign

invasion. In postnatal life the BM and thymus are the primary lymphoid organ in which lymphocytes are develop. The secondary lymphoid organs in which immune responses are generated are lymph nodes, spleen and lymphoid tissue of the alimentary and respiratory tracts (Hoffbrand *et al*., 2006).

1.2.5 Thrompopoiesis:

It's a process by which platelets (PLTs) are formed. The precursor cell of thrompopoiesis is burst forming unit –megakaryocyte (BFU-Meg) which develop into colony forming unit – megakaryocyte (CFU-Meg) (Hoffbrand *et al.,* 2001).

The series of thrompopoiesis begin with megakaryoblast, promegakaryocyte and megakaryocyte which by cytoplasmic fragmentation gives the plts (Loffler *et al*., 2004), each megakaryocyte give about 3000 plt (Hoffbrand *et al.,* 2001).

Megakaryocytes can be classified into three stages of maturation, first stage have strong basophilic cytoplasm and high N/C ratio, second stage less basophilic cytoplasm with some azurophilic granules and a lower N/C ratio, the third stage have weak basophilic cytoplasm with abundant azurophilic granules this is a mature megakaryocyte (Wahed and Dasgupta, 2015).

Thrompopoiesis regulated by thrompopietin (Tpo) and cytokines (IL-3, IL-6 and IL-11). (Hoffbrand *et al.,* 2001).

The plts are small, discoid, 2-4μm in size, the lifespan of plt 7-10 days, normally up to one third of marrow output sequestrated into spleen and have essential role in hemostasis (Hoffbrand *et al*., 2006).

1.2.6 Hemoglobin synthesis:

Hemoglobin is red dye consist of heme and polypeptide globin chains.

Embryonic Hb predominant up to third month of gestation after which fetal Hb (Hb -F) become the major form, Hb-F consist of two alpha and two gamma globin chains. Adult Hb (Hb-A) consist of two alpha and two beta globin chains (Saxena *et al*., 2013). The amount of Hb-A about 25% of the total Hb at birth, after one year Hb-A comprise about 97% and Hb-F less than 1% and the remaining amount is Hb-A_2 which consist of two alpha and two delta globin chains (Saxe*na et al*., 2013).

Heme synthesis occurs largely in the mitochondria by a series of biochemical reactions commencing with the condensation of glycine and succinyl co enzyme A under the action of the key rate limiting enzymes delta- amino laevulinic acid (ALA) synthase, vitamin B_6 is co enzyme for this reaction. Ultimately protoporphyrin combines with iron in the ferrous state to form heme (Hoffbrand, 2006).

Each molecule of which combines with globin chain made on the ribosome.A tetramer of four globin chains each with its on heme group in a packet is formed to make Hb molecule (Hoffbrand, 2006).

1.2.7 Hematological parameters:

1.2.7.1 Hemoglobin (Hb) estimation:

It is used to indirectly evaluate the oxygen-carrying capacity of the blood which makes it an important aid in detecting, evaluating blood loss, diagnosing and treating the anemias. Hb reference value in adult males 13-17 g/dL, and in adult female 12-16 g/ld. (Estridge and Reynolds, 2008)

1.2.7.2 Packed cell volume (PCV) or Hematocrit (HCT):

PCV can be used as simple screening test for anemia, as guide to the accuracy of Hb measurement also can be used in the calculation of red cell

indices. PCV can be measured by manual method or automatic method. Normal value of PCV in male (± 45%) and in female (±40%) (Dacie and Lewis, 2006).

1.2.7.3 Red blood cells (RBCS) count:

Its approximates the number of circulating RBCs which give indirect estimate of the blood's oxygen-carrying capacity also helpful in the diagnosis and treatment of many diseases mainly anemia, the normal value of adult male is 4.5-6.0 $x10^9$ cell/L and in female is 4.0-6.3 $x10^9$ cell/L (Estridge and Reynolds, 2008).

1.2.7.4 Red cell indices:

Red cell indices most frequently used in investigation and classification of anemias, they are mean cell volume (MCV), mean cell hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC) (Estridge and Reynolds, 2008).

MCV is the volume of an average red blood cell in blood sample. The MCV is calculated by using HCT and RBCs count and reported in fimtoliters (fL), the normal range is 86-98 fl (Estridge and Reynolds, 2008).

MCH estimate the average weight of Hb in RBC, the MCH is calculated using Hb value and RBCs count and expressed in pictogram (Pg), the normal range 27-32Pg.

MCHC expresses the concentration of Hb in the RBCs in relation to their size and volume, calculated by using Hb and HCT, expressed in percentage(%) and the normal range 32-37% (Estridge and Reynolds, 2008).

1.2.7.5 Red cell distribution width (RDW):

Automated instrument produce volume distribution histograms that allow the presence of more than one population of cells to be identified. Most instrument produce a quantitative measurement of the variation in cell

volume, an equivalent of the microscopic assessment of the degree of anisocytosis (Bain *et al*., 2011).

1.2.7.6 White blood cells (WBCs) count:

Is used to investigate infections and unexplained fever, monitor treatment that may cause leucopenia, normal range for adult 4-10 $x10^9$ cell/L (Cheesbrough, 2000).

1.2.7.7.1 Platelet parameters:

1.2.7.7.2 Platelets count:

PLTs count may request to investigate bleeding disorders, when patient treated with cytotoxic drugs or other drugs that may cause thrombocytopenia and the normal range is 150 - 450 x 10^9 cell/L (Cheesbrough, 2000).

1.2.7.7.3 Mean platelet volume (MPV):

Measuring of PLT size in blood sample by using impedance technology, the calculated MPV is very dependent on the technique of measurement, length and conditions of storage prior to testing the blood (Dacie and lewis, 2006).

1.2.7.7.4 Platelet distribution width (PDW):

Is a measure of PLTs anisocytosis and plateletcrit which is a product of the MPV in PLTs count by analogy with HCT by automation. PDW has been found to be of some use in distinguishing essential thrombocythaemia from reactive thrombocytosis (Dacie and lewis, 2006).

1.2.8 Rheumatoid arthritis:

Rheumatoid arthritis (RA) is a chronic systemic inflammatory disease of unknown cause, which primarily affects the peripheral joints in a symmetric pattern. Patients with RA may present constitutional symptoms such as fatigue, malaise, and morning stiffness Extra-articular involvements of the skin, heart, lungs, and eyes can be significant. RA can result in joint

destruction and thus often leads to considerable morbidity and mortality (Muddathir and Haj, 2013)

1.2.8.1 Epidemiology

Prevalence 0.5 - 1.0%, incidence (m 0.2-0.4/1000/year,k 0.1-0.2/1000/year), peak incidence 50-60, affected 2-3 times as manybwomen as man (Stengaard-Pedersen , 2002).

1.2.8.2 Etiology

Rheumatoid arthritis (RA) is one of the complex immune-mediated diseases for which an understanding of the etiology is dependent on the definition of environmental triggers that, in a restricted genetic context, may initiate immune reactions having the potential to contribute to disease development. To date, no consistent hypothesis containing all these elements has been formulated for RA or for most other complex immune diseases. However, compelling data have recently been accumulated that provide us with the possibility of formulating such a hypothesis, at least for some cases of RA (Klareskog *et al*., 2006)

1.2.8.3 Symptoms of RA

Joint swelling and tenderness, Stiffness and pain lasting more than 30 minutes in the morning, Symmetrical pattern of affected joints, most often in The wrist and finger joints, Joint malalignment and loss of motion, Fatigue and a general sense of feeling unwell (Turner *et al*., 2010)

1.2.8.4 Rheumatoid factor

Rheumatoid factors (RFs), a class of immunoglobulins (Igs) that have different isotypes and affinities, were first detected more than 70 years ago, but there is still much to discover about the mechanisms underlying their production, physiological role, and pathological effects(Ingegnoli,2013)

1.2.8.5 Diagnostic Test

Approximately 50 to 80 percent of persons with RA have rheumatoid factor, anti-citrullinated protein antibody, or both.10 Patients with RA may have a positive antinuclear antibody test result but RF is not specific for RA and may be present in patients with other diseases, CRP levels and ESR are often increased with active RA, CBC with differential and assessment of renal and hepatic function are helpful because the results may influence treatment options, Radiography of hands and feet should be performed to evaluate for characteristic periarticular erosive changes (Wasserman, 2011)

1.2.8.6 Treatment

Treatment of rheumatoid arthritis aims for tight control to achieve disease remission, as assessed by a disease activity index. Treatment with disease modifying antirheumatic drugs should be started early, because delayed initiation of therapy results in worsened outcomes. Weekly low-dose methotrexate (≤25 mg/wk) should be initiated in most patients at the time of diagnosis. If the response is inadequate after 3 to 6 months, hydroxychloroquine, sulfasalazine, or a biological agent should be substituted or added. Low-dose corticosteroids (≤5 mg/d of prednisone) may also be appropriate, although long-term side effects make their use at higher doses undesirable. Nonsteroidal anti-inflammatory drugs are used as needed to control pain. Regular exercise is safe in most patients with rheumatoid arthritis, and may improve disease and overall health status. (Turner *et al*.,2010)

1.2.9 Previous Studies:

The most important aspect of results obtained from this study was the variations in haematologic variables determined. In RA Sudanese patients, majority of haematologic parameter were estimated, had significant statistical difference from the control values ($P<0.05$). These findings were correlated with the studies by Furst DE *et al* and Wilson A *et al*.,This study concludes that, there is correlation between anemia and rheumatoid arthritis. The Hb, RBCs, PCV, MCH, were low in rheumatoid arthritis patients (Mursal *et al*., 2016).

Nkeiru *et al*. (2004) in Nigeria was equally discovered that 80% of the subjects had haemoglobin below 11.Ig/dl. 60% had white cell count of below 4.0-10.0xl09/L and 10% had platelet below 150xlO9/L. The values obtained deviated from documented normal values for elderly by and those of normal Nigerian values. Similar unusual alterations in haematologic variables were documented by Bowman (2002), who worked on haematologic manifestations in RA. Of the 214 patients, 183 (85.5%) were female and 31 (14.5%) were male. The mean age was 43.9 years (± 11.83). The mean age at disease onset was 37.11 years (± 11.61) and the mean duration of disease was 6.93 years (± 5.60). Two hundred and three patients were on DMARDs with 79.9% using one and 15% using more than one DMARD. Patients (5.1%) were not on any DMARD. NSAID were being used by 74.3% and low-dose prednisolone was being used by 26.6% of patients. Anemia was present in 151 out of 214 patients (70.6%). Agrawal in 2006Anemia found slightly more prevalent in female as compared to male patients (71% vs. 67.7%). The mean hemoglobin concentration in patients with anemia was 9.48 g/dl.

It is unlikely that the anaemia of rheumatoid arthritis was directly associated with the reticulocytosis. In two patients the reticulocyte count returned to normal before any change was observed in the haematocrit. Demann *et al*., in 1965 found no correlation was observed between the haematocrit and the reticulocyte count in the group of forty patients with active rheumatoid arthritis.

1.2.10 Objective

1.2.10.1 General Objective

-To estimate hematological parameters among Sudanese patients with rheumatoid arthritis.

1.2.10.2 Specific Objective:

-To estimate hematological parameters (TWBCs, Hb, TRBCs, MCV, MCH, MCHC, PCV, platelets and Reticulocyte count) in rheumatoid arthritis patients.

-To compare hematological parameters result between patients and normal individuals.

-To compare hematological parameters between steroids treated patients, non-steroid treated patients and who take both types of treatment.

-To compare hematological parameters between old and new cases of patients.

1.2.11 Rationale:

Rheumatoid arthritis is chronic inflammatory disease. Classified as autoimmune disease, characterized by deregulation of immune system. Can affect many hematological parameters such as RBCs, WBCS and Plts (Okoroiwu, 2016). The Platelet count was positively correlated with reactive thrompocytosis as inflammatory markers in RA patients.Therefore, it depends on laboratory analysis to diagnose cases. Rheumatoid arthritis has spread largely and very wide, with difficulty of its symptoms and complications, which can be reduced through treatment and some supplements. All of this prompted us to participate even with a little effort to describe if there is an effect RA of treatment on blood cells, and impact the supplement address this effect, and evaluate these changes in Sudanese patients with rheumatoid arthritis disease (Okoroiwn *et al*., 2016).

Materials and Methods

Chapter Two

Chapter two

Material and Method

2 .1 Study design

This was an analytic case control study conducted in Omdurman Military Hospital and Al-Amal National Hospital during period from May to July 2018.

2.2 Sample size

Fifty Sudanese rheumatoid arthritis patients and fifty healthy Sudanese individuals as control group.

2.3 Inclusion criteria

Males and females with no other medical conditions rather than rheumatoid arthritis. Healthy individuals as control group for comparison.

2.4 Exclusion criteria

Rheumatoid arthritis with any other medical conditions that may affect the results were excluded from this study such as blood or platelet transfusion, pregnancy, liver diseases, mixed infection etc.

2.5 Data collection

Data was collected using a design questioner by interviewed and some data was collected from record file to rheumatoid arthritis to obtain information that helped in study.

2.6 Sample collection

Blood is withdrawn from an antecubital vein or other visible veins in the forearm by means of 2.5ml of clean dry sterile syringe after check patients identity, Skin was been cleaned by 70% alcohol and allowed to dry before punctured. After blood is withdrawn, the blood was placed in sterial EDITA anticoagulant containers and mix gentley.

2.7 General equipment and reagents:

- Automatic hematology analyzer

- Reagents : cellpack (is a diluents used to aspirate analysis sample in order to measure an RBC count, WBC count, Hb concentration and Plts count), stromatolyser (is a reagent that lyses RBC for accurate WBC count determination, WBC tri modal size distribution analysis and Hb level measurement), cellclean (is a strong alkaline detergent used to remove lyse reagents, cellular residuals and blood proteins remaining in the hydraulics of the instrument) and eightcheck-3wp (is control blood for testing the precision and accuracy of hematology analyzers).

-EDTA container

-syringes

-tourniquet

-cotton and gauze

-disinfectant (70% alcohol).

-slides and New Methalen Blue

-microscope (OLYMPUS - CH20 JAPAN)

-waterbath

-micropippette

2.8 Preparation of Rticulocyte Stain

Dissolve 1.0 g of brilliant cresyl blue (CI 51010) or 1.0 g of New methylene blue (CI 52030) or azure B (CI52010) in 100 ml of 3% trisodium citrate-saline solution (30 g sodium citrate in 1 l saline). Filter once the dye has been dissolved. (Dacie and lewis, 2006).

2.9 Method

Withdraw 2 or 3 drops of the dye solution into a 75 _ 10 mm plastic tube by means of a plastic Pasteur pipette. Add 2–4 volumes of the patient's EDTA-

anticoagulated blood to the dye solution and mix. Keep the mixture at 37_C for 15–20 min. Resuspend the red cells by gentle mixing and make films on glass slides in the usual way. When dry, examine the films without fixing or counterstaining. (Dacie and lewis, 2006).

2.10 Complete blood count

2.10.1 Principle of Sysmex XP-300 (DC detection method):

Blood sample is aspirated, measured to a predetermined volume, diluted at the specified ratio, then fed into each transducer.

The TD chamber has a minute hole called the aperture. On both sides of aperture, there are the electrodes between which flows direct current. Blood cells suspended in the diluted sample pass current through the aperture, causing direct current resistance to change between the electrodes. As direct current resistance changes, the blood cell size is detected as electric pulses.

Blood cell count is calculated by counting the pulses, and a histogram of blood cell sizes is plotted by determining the pulse size. Also, analyzing a histogram makes it possible to obtain various analysis data (Sysmsx corporation, 2014).

2.10.2 Analysis modes:

Whole blood (WB) mode is used to analyze collected samples in the whole blood status. The tube cap is opened and the sample is aspirated through the sample probe one after another (Sysmex corporation, 2014).

2.10.3 Performance of the instrument:

Whole blood is aspirated, diluted and then divided into two samples. One sample is used to analyze the red blood cells and platelets while the second sample is used to analyze white blood cells and hemoglobin.

Electrical impedance is used to count the WBCs, RBCs, and PLts as they pass through an aperture .as each cell is drawn through the aperture, a change in electrical resistance occurs generating a voltage pulse

, the number of pulse during a cycle corresponds to the number of cells counted and the amplitude of each pulse is directly proportional to the cell volume (Sysmex corporation, 2014).

2.11 Counting Reticulocytes

The counting procedure should be appropriate to the number of reticulocytes present. Very large numbers of cells have to be surveyed if a reasonably precise count is to be obtained when only small numbers of reticulocytes were present. When the count is <10%, a convenient method is to survey successive fields until at least 100 reticulocytes have been counted and to count the total red cells in at least 10 fields to determine the average number of red cells per field. . (Dacie and lewis, 2006).

Reticulocyte count = % reticulocytes in red blood cell (RBC) population

(Normal values of all of above 0.5–1.5%)

2.11.1 Calculation

Number of reticulocytes in n fields ¼ x

Average number of red cells per field ¼ y

Total number of red cells in n fields ¼ n _ y

Reticulocyte percentage ¼ [x _ (n _ y)] _ 100% (Dacie and lewis, 2006).

2.12 Ethical approval:

Ethical approval for conducting the research was obtained from the college of Medical Laboratory Sciences-SUST also permission was obtained from the administration of Omdurman Military Hospital and Al-Amal National Hospit for the same purpose. A verbal consent was obtained from all the

participants after they had been informed about the aim of the study, expected outcome, confidentiality of the results and the procedure of blood collection.

2.13 Data analysis

Data analyzed by Statistical Package for Social Sciences (SPSS) version 16 mainly independent T test and one way ANOVA.

Results

Chapter Three

Chapter Three

Results

3 Results

This was an analytic case control study conducted in Al-Amal National Hospital and Omdurman Military Hospital during period from May to July 2018 to estimate hematological parameters (TWBCs, Hb, TRBCs, MCV, MCH, MCHC, PCV, platelets and Rticulocyte count) among Sudanese patients with rheumatoid arthritis.

The study population was selected as 50 patients and 50 normal healthy individual as control group with matched age and sex, the patient were classified into old case that has disease for more than 5 years (62%) and new case that has disease for 5 and less than 5 years (38%).

Also in our study group classified according to their treatment types into 3 different types whom take steroids treatment (52%), non steroids treatment (8%) and combined drugs (40%) and the result as flow.

Table (3.1) Frequency of Sex and Study Population

Subject	Sex		Study Population	
	Female	Male	Case	Control
Frequency	92	8	50	50%
Percent	92%	8%	50	50%
Total	100	100%	100	100%

Figure (3.1) Frequency of Study Population

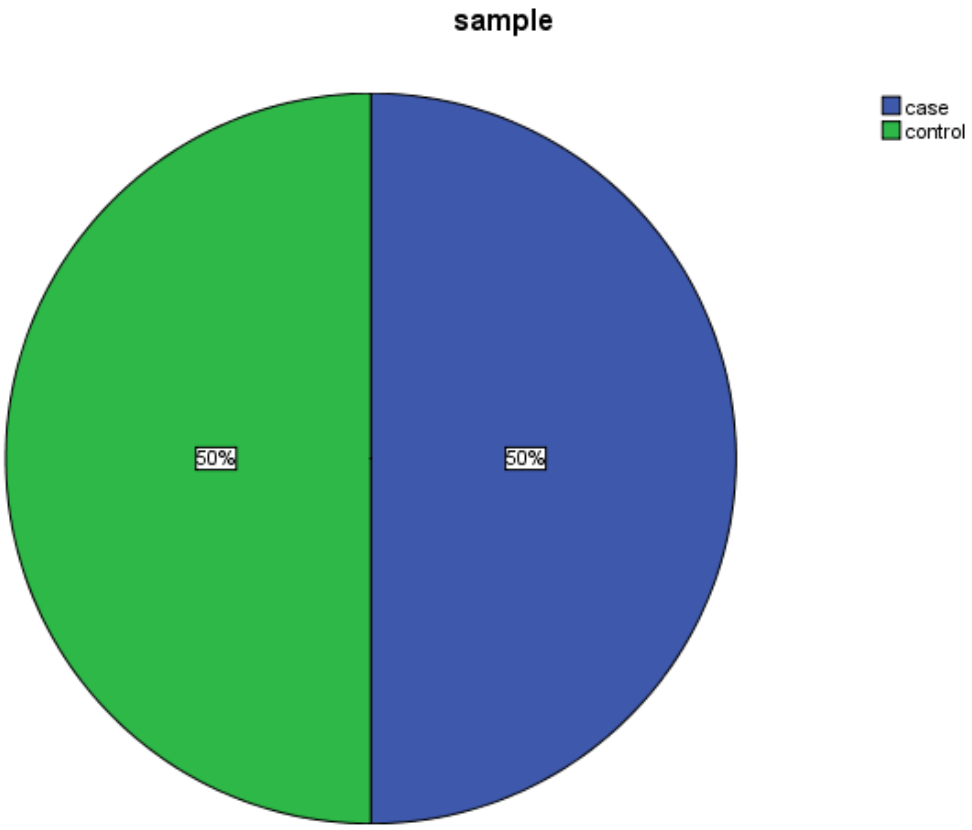

Table (3.2) Frequency of Treatments Types

Treatment	Frequency	Percent
Steroid	26	52%
Non Steroid	4	8%
Combination	20	40%
Total	50	100%

Table (3.3) Comparison of Hematological Parameter between Case and Control

	Sample	Mean	Std. Deviation	Sig
TWBCs	Case	6.7	2.2	.000
	control	5.1	1.2	
TRBCs	Case	4.42	0.47	.707
	control	4.45	0.49	
Hb	Case	11.4	1.3	.000
	control	13.3	1.8	
PCV	Case	36.3	5.0	.000
	control	40.9	4.5	
MCV	Case	81.9	8.1	.000
	control	92.7	7.3	
MCH	Case	25.7	2.6	.000
	control	29.7	2.8	
MCHC	Case	31.6	3.1	.755
	control	31.7	2.3	
Platelets	Case	275	75	.020
	control	243	58	
Reticulocyte count	Case	0.58	0.77	.150

Table (3.4) Comparison of Hematological Parameter between Cases and Types of Treatments

	Treatment	Mean	Std. Deviation	Sig
TWBCs	Steroid	6.4	1.9	0.048
	Nonsteroid	4.8	1.3	
	Combination	7.5	2.5	
TRBCs	Steroid	4.49	0.41	0.286
	Nonsteroid	4.11	0.23	
	Combination	4.38	0.56	
Hb	Steroid	11.7	1.6	0.269
	Nonsteroid	10.9	0.7	
	Combination	11.1	0.5	
PCV	Steroid	38.3	5.2	0.012
	Nonsteroid	34.6	1.6	
	Combination	34.1	4.2	
MCV	Steroid	84.3	7.4	0.032
	Nonsteroid	84.7	7.5	
	Combination	78.3	8.0	
MCH	Steroid	25.6	2.9	0.777
	Nonsteroid	26.6	1.1	
	Combination	25.6	2.6	
MCHC	Steroid	30.5	2.6	0.024
	Nonsteroid	31.5	3.3	
	Combination	33.0	3.1	
Platelets	Steroid	281	74.6	0.382
	Nonsteroid	224	62.5	
	Combination	277	79.3	
Reticulocyte count	Steroid	0.73	0.99	0.291
	Nonsteroid	0.22	0.05	
	Combination	0.44	0.43	

Discussion, Conclusion and Recommendation

Chapter Four

Chapter Four

Discussion, Conclusion and Recommendation

4.1 Discussion:

This was analytic case control study conducted in Al-Amal National Hospital and Omdurman Military Hospital during period from May to July 2018 to estimate hematological parameters (TWBCs, Hb, TRBCs, MCV, and MCH, MCHC, PCV, platelets and Reticulocyte count) among Sudanese patients with rheumatoid arthritis.

The study population was selected as 50 patients and 50 normal healthy individual as control group with matched age and sex, the patients was classified into old case that has disease for more than 5 years (62%) and new case that has disease for 5 and less than 5 years (38%).

In this study group classified according to their treatment types into 3 different types whom take steroids treatment (52%), non steroids treatment (8%) and combined drugs (40%).

In comparison of CBC parameter between case and control the study was reflect significant increase in mean of TWBCs (mean of case 6.7±2.1 and control mean 5.1± 1.2) with *P* .000 that is matched with (Syed and pinats, 1996), significant Decrease Hb level (mean of case 11.4 ±1.3 and control mean 13.3 ± 1.8) with *P* .000, significant Decrease PCV (mean of case 36.3 ±5.0 and control mean 40.9 ± 4.5) with *P* .000, significant Decrease MCV (mean of case 81.9 ± 8.1 and control mean 92.7 ± 7.3) with *P* .000, significant Decrease MCH (mean of case 25.7 ± 2.6 and control mean 29.7 ± 2.7) with *P* .000, and significant increase platelets count (mean of case 275 ±75 and control mean 243 ± 58) with *P* .020 those results were agreed with (Mursal *et al*., 2016).

On the other hand of comparison our study was given off no significance on mean of TRBCs (mean of case 4.42 ± 0.47 and control mean 4.45 ± 0.49) with *P* .707, MCHC (mean of case 31.6 ±3.1 and control mean 31.7 ± 2.3) with *P* .755 that is agreed with (Mursal *et al*., 2016) and rticulocyte count (mean of case 0.58 ± 0.77 and control mean 0.40 ± 0.38) with *P* .150 all that was agreed with (Denman *et al*., 1965).

Treatment of rheumatoid arthritis consider as difficult part of disease curable planning so different types of drugs used to get desert good prognostic outcome for that we compare between types of treatment and hematological parameter and show there was significant increase on TWBCs in patient who take steroid (6.4± 1.9) and combined drugs (7.5± 2.5) (*P* .048), significant decrease on PCV in patient who take steroid drugs (38.3± 5.2) (*P* .012), significant increase on MCV in patient who take combined drugs (78.3± 8.0) (P .032), significant decrease on MCHC in patient who take steroid drugs (35.5 ± 2.9) (P .024), these result was matched with Turner *et al*., in school of medicine in London at 2018.

Beside that there were no significance difference on TRBCs (*P* .286), Hb level (*P* .269), MCH (*P* .777), platelets count (*P* .382) (Turner *et al*., 2018) and Rticulocyte count (*P* .291) in comparison with treatment types (Denman *et al*., 1965).

4.2 Conclusion

- There was significant effect of rheumatoid arthritis in TWBC , Hb, PCV , MCH and platelets , with no effect in TRBCs , MCHC and rticulocyte count.
- Steroid treatment increased TWBC level in arthritis patient also both steroid and non steroid treatment had decrease in those patient.
- All treatment types had no effect on Hb , TRBCs , Platelets and Reticulocyt count.
- The study reflects there was no effect for duration o disease on CBC figures.

4.3 Recommendation

- More study population should be increase to get mo realistic results.
- Correlation with sub-divided treatment has to be establishing to ensure the effect of treatment the rheumatoid arthritis patient.
- Correlation between duration of disease and regulation of taken treatment and their effect on CBC parameter must be checked.
- More laboratory investigation must be done to know the type of anemia such as iron study, erythropoietin level etc…

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Chapter Five

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Appendicies

Appendices

Appendix 1: Questionnaire

Sudan University of Science and Technology

College of Graduate Studies

Department of Hematology and Immnuohematology

Questionnaire

A: General information:-

- ID: ……..…….……..
- Name: ……..……..
- Gender:

Male Female

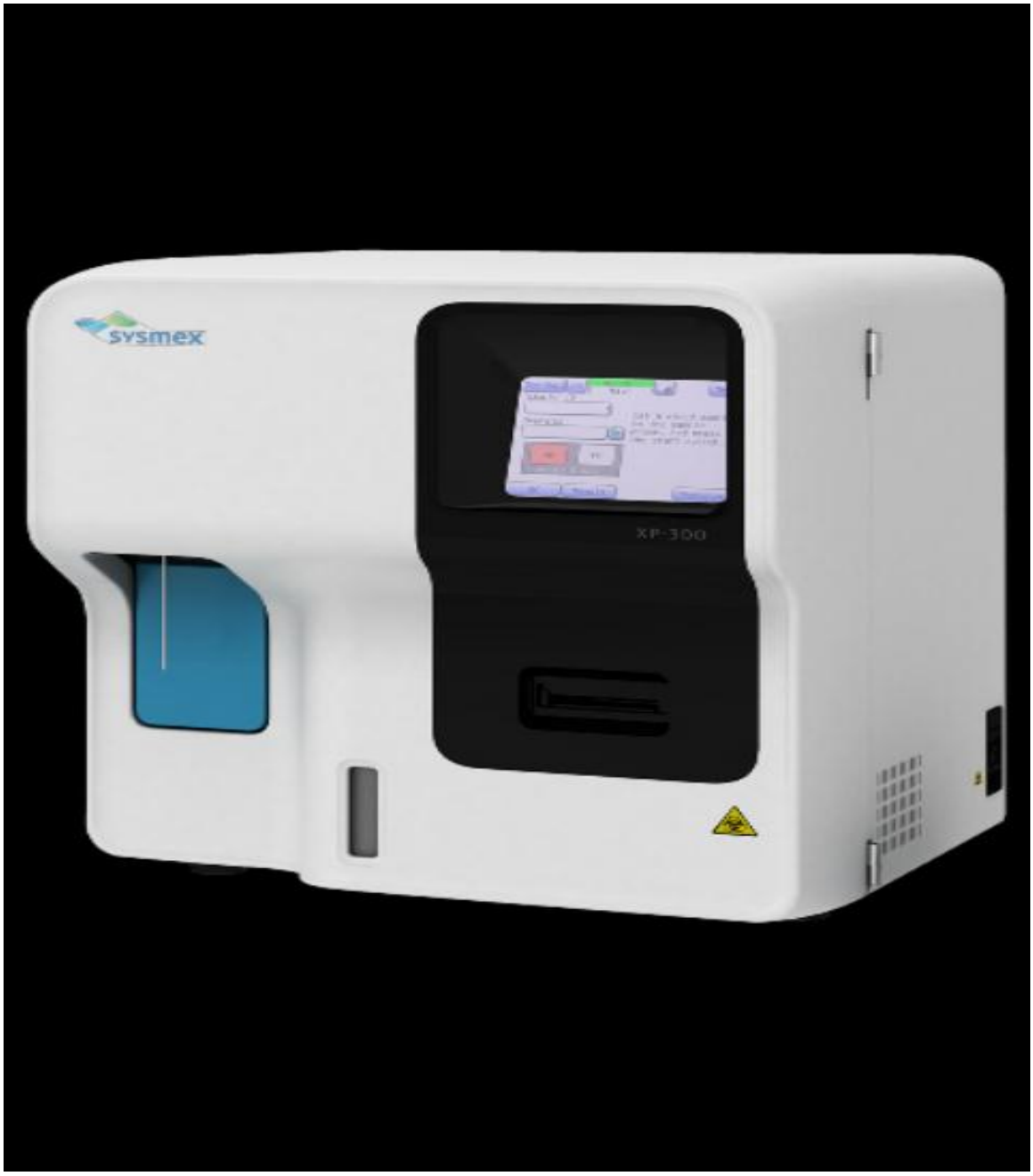
- Age: …….…….……
- Address: ……..……..
- Telephone number: ……...

B: Clinical information:-

- Onset of disease: …….
- Treatment: Yes ( ) No ( )
- Treatment course: ……
- Type of treatment:…… ……
- Underlying autoimmune disease: ……..
- Remarks: …….
- Laboratory findings: ……..

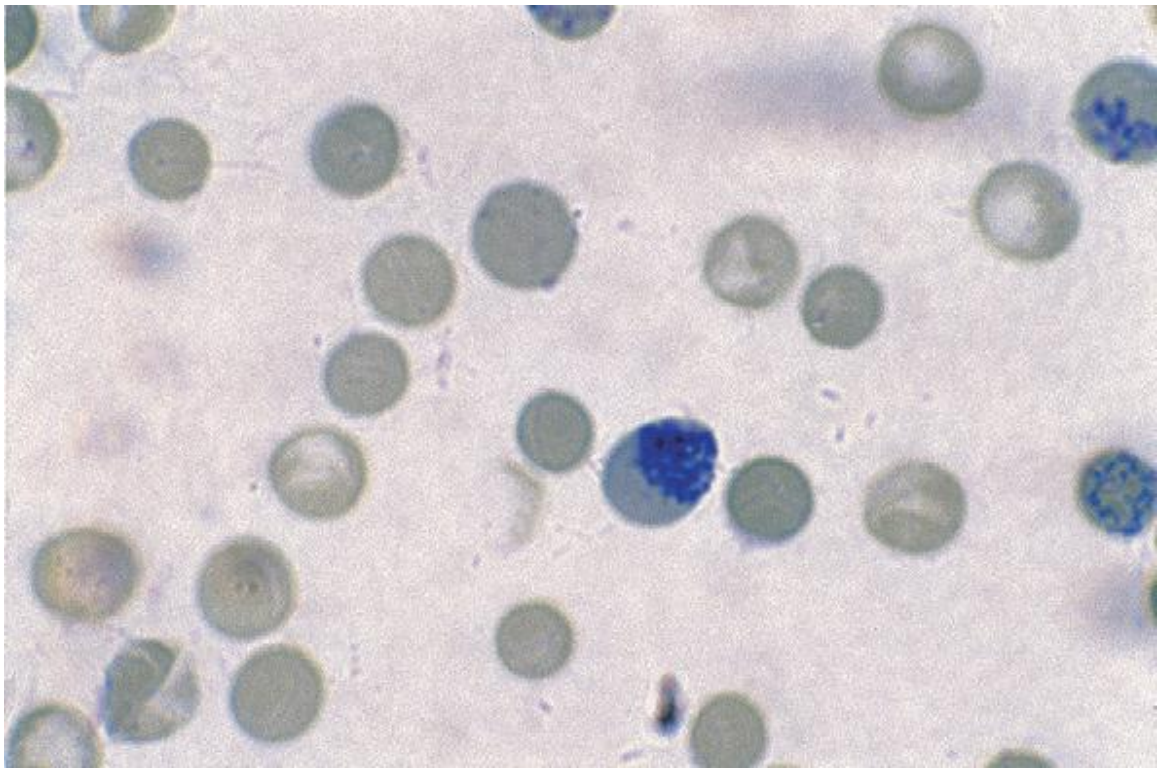
Appendix 2

. Appendix 3



Sysmex XP-300 (sysmex. com)

Appendix 4



Photomicrographs of reticulocytes showing stained supravitally by New methylene blue (Dacie and Lewis, 2006).

Appendix 5

براءة اخلاقية

اقر انا.....

موافقتي على المشاركه في هذا البحث بعد اطلاعي على اهدافه وفوائده وان المعلومات المجموعة فقط بغرض البحث ولا تستخدم لاي اهداف اخرى.

الاسم:.....

التوقيع:.....