Sudan University of Science and Technology
College of Graduate Studies

Effect of Ramadan Fasting on Interleukin-6 and Some Hematological Parameter in Rheumatoid Arthritis Patients

تأثير صيام رمضان في الانترلوكين - 6 وبعض المقاييس الدموية لدي مرضى التهاب المفاصل الروماتويدي

A thesis submitted in Partial Fulfillment for the Requirement of M.Sc. degree in Medical Laboratory Sciences

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الايـــــة

بسم الله الرحمن الرحيم

قال الله تعالى:

﴿قَالُواْ سُبْحَانَكَ لاَ عِلْمَ لَنَا إِلاَّ مَا عَلَّمْتَنَا إِنَّكَ أَنتَ الْعَلِيمُ الْحَكِيمُ﴾

سورة البقرة – الآية (32).

Dedication

To

Who give love, kindness. and power supply

Our parents

To

Those who put sun in my mind

Our teachers

To

Our sisters and brothers

To

Our friends

Acknowledgement

Praise to Allah who give my health and power to finish this work.

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Abstract

This was analytical cross section study conducted out in Omdurman military hospital and Alrian laboratory in Khartoum state. During the period from May 2019 to December 2019, the study aimed to investigate the effect of intermittent fasting Ramadan (14-16) hours on IL-6 and some hematological parameter in rheumatoid arthritis patients, 60 rheumatoid arthritis patients were selected randomly, and divided into fasting rheumatoid arthritis patient (n=30) and non- fasting rheumatoid arthritis patient (n=30) as control. Three ml of venous blood samples were collected from each subject in EDTA container. Complete Blood Count (CBC) was performed for All blood samples using sysmex s310, the plasma separate by using centrifugations. IL-6 level was measured in plasma samples by using ELISA kit (human IL-6, biolegand's ELISA MAX$^{TM)}$ (Western Blotting technique). The data were analyzed using Statistical Package for Social Science (version18) using independent T-test. IL-6 level was significantly decrease in fasting rheumatoid arthritis (143.29±123.75) compared with non-fasting rheumatoid arthritis (256.27 ± 253.52), (P.value = 0. 0 23). Hematological parameters (WBC, RBC ,Hb and HCT) were not affected significantly by fasting (P.value 0.400, 0.917, 0.140 and 0.203) respectively.

This is study concluded that: intermittent fasting is favorable behavior for rheumatoid arthritis patients indicated by reduction of inflammatory marker IL6.

المستخلص

أجريت هذه الدراسة المقطعية التحليلية في مستشفى أم درمان العسكري ومختبر الريان بولاية الخرطوم. هدفت الدراسة ، خلال الفترة من مايو 2019 إلى ديسمبر 2019 ، إلى تقصي أثر صيام رمضان المتقطع (14-16) ساعة على الإنترلوكين 6 وبعض المقاييس الدموية في مرضى إلتهاب المفاصل الروماتويدي ، وتم إختيار60 مريضاً بإلتهاب المفاصل الروماتويدي عشوائياً وتقسيمهم. مرضى إلتهاب المفاصل الروماتويدي الصائمين (ن=30) ومرضى إلتهاب المفاصل الروماتويدي غير الصائمين (ن=30). تم جمع ثلاثة مل من عينات الدم الوريدي من كل شخص في حاوية EDTA .تم إجراء تعداد الدم الكامل (CBC) لجميع عينات الدم بإستخدام sysmex s310 ، فصل البلازما بإستخدام الطرد المركزي. تم قياس مستوى IL-6 في عينات البلازما بإستخدام مجموعة ELISA(IL-6 البشري ، ELISA MAXTM). تم تحليل البيانات بإستخدام الحزمة الإحصائية للعلوم الإجتماعية (الإصدار 18) بإستخدام إختبار T المستقل. إنخفض مستوى IL-6 إنخفاضاً ملحوظاً في مرضى إلتهاب المفاصل الصائمبن (143.29 ± 123.75) مقارنة مع مرضى إلتهاب المفاصل الروماتويدي غير الصائمين (256.27 ± 253.52) ، (القيمة = 230.0). لم تتأثر المقاييس الدموية (WBCو RBCو Hb وHCT) تأثراً ملحوظاً بالصيام (القيمة =0.400و0.917 و0.140 و 0.203) على التوالي .خلصت هذه الدراسة إلى أن: الصيام المتقطع هو السلوك المفضل لمرضى إلتهاب المفاصل الروماتويدي ، وذلك بتقليل مؤشر الإلتهاب IL-6

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Abbreviation

AA	Alpecia areata
ACR/ EUIAR	American collage of rheumatology/European league Aganist Rheumatism
AKT	All kinds things
AOI	Anemia of inflammation
CD4T	Cluster of differentiation
CD8	Cluster of differentiation
CLC	Cardiotrophin-like cytokine
CNTF	Ciliaryneutrophic factor
CRP	C-reactive protein
CTLs	Cytotoxic T lymphocyte
DAMPS	Damage related molecular patterns
ESR	Erythrocyte sedimentation rate
Fe^{++}	Ferrous
Fl	femto liter
Gp	Glycoprotien
GP130	Glycoprotien130
Hb	Hemoglobin
HLA DR	Human leukocyte antigen locus
HRP	Horse radish peroxidase
IGG FC	Immunoglobulin G fragment ;crystallizable region
IGM	Immunoglobulin M

IL-15	Interleukin-15
IL-17	Interleukin-17
IL-1B	Interleukin-1 beta
IL-23	Interleukin-23
IL-27	Interleukin-27
IL-6	Interleukin-6
IL-6R	Interleukin-6 receptor
JAK	Janus kinase
LIF	Leukemia inhibitory factor
MCH	Mean cell hemoglobin
MCHC	Mean cell hemoglobin concentration
MCV	Mean cell volume
MPS	Mucopolysaccharides
MQ	Macrophage
MRI	Magnetic resonance imaging
OSM	Oncostatin M
p.v	p.value
P3K	Phosphatidylinositol 3 kinase
PAMPS	Pathogen molecular patterns
PCV	Package cell volume
Pg	pico gram
RA	Rheumatoid arthritis
Raf	Rapidly Accelerated fibro sarcoma

RANK	Receptor activation of nuclear facter kappa
RANKL	Receptor activation of nuclear factor kappa B-ligend
Ras	Rat sarcoma
SAA	Serum Amyloid A
sIL-6R	Serum interleukin-6 receptor
SPSS	Statistical package for social science
STAT	Signaltransducer and Activator transcription
TGF-B	Transforming growth factor beta
TH-17	T-helper 17
TLR3	Toll-like receptor3
TNF-a	Tumer necrosis alpha
VEGF	Vascular endothelial growth factor
VEGF	Vasculer endothelial growth factor beta
WBC	White blood cell count
WHO	World Health organization

Chapter I

Introduction

1. Introductions

1.1.General introduction

Ramadan is the ninth month of the Islamic lunar calendar and is strictly observed by millions of Muslims worldwide. Fasting is about 12-19 hours a day, depending on the season which Ramadan falls in and the geographical position of the country (Trepanowski and Bloomer,2010).

Rheumatoid arthritis is an autoimmune disorder affect the total blood count. In RA person's immune system errors, the body's healthful tissue for overseas invaders and the immune machine response inflammation happen in target tissue or organ. (Crostein,2007).

Rheumatoid arthritis (RA) generally affect the palms and feet first, but it can happen in any joint .it typically includes the equal joints on both facets of the body. Common symptom includes stiff joints, especially upon getting up in the morning or after sitting down for a while. Some people often ride fatigue and common feeling of being unwell. (Niams,2007). Rheumatoid arthritis is associated with a high risk of morbidity and premature death secondary to the earlier development of cardiovascular, lung disease and malignant (Crostein,2007).

Interlienkin-6, is pro-inflammatory cytokine, promptly and transiently produced in response to contamination and tissue, injuries,contributes to host defense through the stimulation of acute section response hematopoiesis and immune reaction.

Dysregulated chronic synthesis of IL6 play pathological impact on inflammation of autoimmunity (Scott,1981) .

The people death by Rheumatoid arthritis in 2015 are 30,000 (Wang et al.,2015).

1.2.Rationale

Rheumatoid arthritis is an autoimmune disease, involving inflammation and progressive destruction joints of affect hands, feet, cervical spine cause loss function, characteristic, movement and lead to deformity. Finally, cause premature death. The measuraty of patients with RA have one or more comorbid condition, the most common being cardiovascular disease , osteoporosis and depression, the presence of which are associated with poorer clinical outcome.

Interleukin-6 is pro-inflammatory cytokine, performs multiple and essential functions in immune regulation, inflammation . IL-6 act locally promote joints inflammation and destruction and in the circulation to mediate extra-articular manifestations of RA including pain, fatigue, morning stiffness, anemia and weightloss. Intermittent fasting Ramadan reductions IL-6 and therefore decrease severity of RA disease.

In Sudan there is no published data about impact of intermittent fasting Ramadan on IL-6 and hematological parameter in Rheumatoid arthritis patients.

1.3. Objectives

1.3.1. General objective

To determination the effect of intermittent fasting (Ramadan) in interleukin-6 and some hematological parameter in RA patients.

1.3.2.Specific objective

To compare level of interleukin-6 among fasting and non -fasting RA patients.

To compare hematological parameter among fasting and non -fasting RA patients.

Chapter II

Literature Review

2. Literature review

Inflammation is the body's immune system's response to an harmful stimuli such as pathogen, irritant and foreign objective such as a splinter in your finger. (Kaspers et al.,2015)

2.1. Rheumatoid arthritis

Rheumatoid arthritis (RA) is a multisystem continual inflammatory sickness, principally affecting peripheral joints in a symmetric trend and many times leading to cartilage destruction, bone erosions and joint deformities. Its motive is variable; extra-articular manifestations, such as vasculitis and subcutaneous nodules, are current in 10-25% of the patients.

Women are affected more than guys, with a woman: male ratio of 3:1; the sickness onset reaches its apex between 35-50 years. RA has a worldwide distribution, with an incidence of about 1%. It typically ends result in warm, swollen, and painful joint(Niams,2007). RA is characteristically associated with the HLA DR4 and DR1, alleles. relaxation hand is involving, with equal joint generally involve in both facet of the body-pentameric IMG rheumatoid component is the serological hallmark of two RA. Pain and stiffness frequently irritate flowing rest (Niams,2007).

2.2. Pathophysiology of RA

The initial website of disease in the synovial membrane, the place swelling and congestion lead to infiltration by way of immune system. There phase of development of RA are :-

Initiation segment (due to non-specific inflammation), an amplification phase (due to T-cell activation) and Chronic irritation segment, with tissue damage resulting from cytokines, IL-1, TNF-alpha and IL-6 (Shah and Ankur,2012).

2.2.1. Initiation phase

No precise irritation element permitting immune device response. Once initiated, become permanent and chronic these thing is genetic disorder which alternate regulation of adaptive immune response (Smolen et al.,2016). Genetic element interacts with surroundings threat elements for RA, with cigarette smoking as the most sincerely defined risk issue (Sugiyama et al.,2010). and hormonal component may provide an explanation for high risks for women. B-cell produce immune complicated and T-cell

products in infection in RA has persevered for 30years, but neither telephone is fundamental at web site of inflammation, only autoantibody to IgG FC, known as rheumatoid thing and ACPA, having an 80% specificity for analysis RA (Hua et al.,2017)

2.2.2. Amplification in the synovium

Once the generalized bizarre immune response has emerge as established. which may additionally take countless years before any symptom occur-plasma telephone derived from B-cell produce rheumatoid factor and ACPA of the IgG and IgM lessons in massive portions. These active macrophages via FC receptor and complement building, which is part of the intense infection in RA (Boldt et al.,2012).

2.2.3. Chronic inflammation phase

The ailment progresses with the aid of forming granulation tissue at the edge of synovial lining, pannus with huge angiogenesis and enzyme inflicting tissue damage (Elshabrawy et al.,2015). The synovium thickens, cartilage and underlying bone crumble and the joint deteriorates and raised calprotectin stage serving as a biomarker of these events (Abidrup et al.,2015). Cytokine and chemokine appeal to and accumulate immune cell, i.e activated T-cell B-cell, monocyte and macrophage from activated fibroblasts, in the joint space .by signaling thru RANKL and RANK the ultimately trigger osteoclast production, which degrades bone tissue (Chiu and Ritchlin, 2017). (TNF. α) Tumor necrosis issue alpha: play important function and countless theories exist on how TNF launch take place in RA.

TNF. α two is a pro-inflammatory cytokine that play: a pivotal position in regulation the inflammatory response in (RA) rheumatoid arthritis if TNF launch encouraged via B-cell merchandise in shape of RA or ACPAcontaining immune complex's, through activation of immune globulin FC receptors, then RA can been considered as a form of type ш hypersensitivity (Chaffar and Abdul,2016). if TNF release is motivated by means of T-cell products such as IL-17 it might be nearer to kind IV hypersensitivity(Holmes,1999). Although TNF appears to be the dominant chemical mediator other cytokine are involved in inflammation in all tissue, particularly lung disease and nodules may get worse.

Blocking IL-1, IL-15 and IL-6 have beneficial effect and IL-17 may be important (Gaffen,2009).

2.3. Sign and symptoms of RA

RA primarily affect joints. But it additionally affects other organs in extra than 15-25% of cause (Turesson et al.,3003). Most normally concerned are the small joints of the hand, feet, and cervical spine, but larger joints like the shoulder and knee can additionally be involved (Walker et al.,2014). Synovitis can lead to tethering of tissue with loss of movement and erosion of the joint surface inflicting deformity and loss of characteristic (Majithia and Geraci,2007). Associated problems include cardio vascular disease, osteoporosis, interstitial lung disease, infection, cancer, felling worn-out, depression, intellectual difficulties, and bother working (Cytolo et al.,2014). Warm, swollen, painful joints, low purple blood telephone count, irritation around the lungs, and irritation round the heart, fever and low power may additionally be existing (Niams,2007).

Often, symptoms come on gradually over week to month (Majithia and Geraci,2007). As the pathology progresses the infection endeavor lead to tendon tethering and erosion and destruction of the joints surface, which impairs stages of motion and lead to deformity. Specific deformities, which additionally show up in osteoarthritis, include ulnar deviation, boutonniere deformity, swan neck deformity. (Walker et al.,2014). The hammer toe deformity perhaps seen. in the worst case, joints are recognized as arthritis multilane due to the mutilating nature of the deformities (Shah and Ankur,2012).

2.4. Complication of RA

Rheumatoid nodule, which is sometime in the skin, is the most frequent non-joint function and appear in 30% of humans who have RA(Turesson,2013). A number of varieties of vasculitis appear in RA, however are basically considered with long. Standing and untreated ailment, rheumatoid vasculitis can for that reason Commonly existing with skin ulceration and vasculitis nerve infarction acknowledged as mononeuritis multiplex (Genta et al.,2006). Diffuse alopecia aerate (diffuse AA) take place more frequently in people with rheumatoid arthritis (Khan-Mohammed
,2018). RA is additionally considered extra regularly in these with relative who have AA(Khan-Mohammed,2018).

Lung fibrosis is cognized complication of rheumatoid arthritis. It is also a rare however well-recognized consequence of therapy (for instance with methotrexate and leflunomide). Caplan’s syndrome describes lung nodules in man or woman with RA and

extra exposure to coal dust. Exudative pleural effusion are also companion with RA (Kim et al.,2009).

People with RA are more susceptible to atherosclerosis, and danger of myocardial contamination (heart attack) and stroke is markly make bigger (Avina-Zubieta et al.,2008). Other viable problems that can also occur encompass: pericarditis, endocarditis, left ventricular failure, valvulitis and fibrosis (Gupta and Fomberstein,2009).

Renal amyloidosis can occur as a consequence of untreated chronic inflammation (Degroot,2007).

Liver problem in people with rheumatoid arthritis maybe due to underlying disease process or as a result of the medication use to treat the disease (Selimi et al.,2011). Liver disease such as primary biliary cirrhosis or autoimmune hepatitis (Selimi et al.,2011).

Rheumatoid disease of the spin can lead to myelopathy. the most common problem is carpal tunnel syndrome caused by compression of the median nerve by swelling around the wrist (Wasserman et al.,2011).

Periodontitis and tooth loss are common in people with rheumatoid arthritis (Paola,2009).

The threat of non-melanoma skin cancer is enlarge in humans with RA in contrast to the ordinary population and affiliation feasible due to the use of immune suppression dealers for treating RA. The incidence of lymphoma is make bigger, an even though it is amazing and associated with the persistent inflammation, now not the cure of RA (Paola,2009).

2.5. Classification criteria of RA

The classification criteria of RA proposed by way of the American collage of rheumatology in 1987 differentiated established RA from other rheumatic disease. The new criteria proposed by using the ACR/EU LAR in 2010 permits to classify RA on previously stages. That permit to prevent bone destruction and radiological development thanks to the use of diseasemodifying capsules (Vanderkooij et al.,2009).

ACR/EULAR 2010 standards does not decide any other strategies for diagnostic of synovitis without of clinical examination, however insist on a presence of at least one articulation with definitive synovitis (Colebatch et al.,2013). EULAR recommendation

for the use of imaging in rheumatoid arthritis states that when there is diagnostic doubt, conventional radiography, ultrasound or MRI can be used to improve the sure bet of a analysis of two RA above medical criteria alone, MRI and ultrasound can be used to product the development from undifferentiated inflammatory to scientific RA, and due to the fact of ultrasound and MRI is most beneficial to scientific examination in the detection of joint inflammation, these strategies ought Tobe considered for extra correct evaluation of inflammation (Colebatch et al.,2013).

2.6. Cytokines

Proteins that are produced and secreted with the aid of many exclusive mobile kind and mediate infection and immune reactions, cytokine mediators of communication between cell of immune system (Abbas et al.,2015). Cytokine have many exceptional names rather than cytokine encompass lymphokine, monokine, chemokine and interleukin. (cytokine made by using one leukocyte and performing on other leukocyte).

2.7. Interlenkin-6

Interlenkin-6 is a monokine with a quantity of organic activities, which are intimately related to inflammatory response (Swaak et al.,1998). IL-6 is a cytokine with a massive range of organic activities. It is mediator for immunoglobulin category substituting and to alter the acute phase response. It is additionally irritation indicator inside body. For the occurrence of bacteremia IL-6 can use as investigative marker. (Swaak et al.,1998). IL-6 is an endogenous biochemical which is energetic for the duration of B-cell maturation and method of infection. It can act as pyrogen and can motive fever all through infection, non-infection and autoimmune sickness. Either infection chronic or acute it is produced and situation is cancers, trauma, burns and infection.IL-6 is additionally assumed to cause increased susceptibility to systemic from of juvenile rheumatoid arthritis and diabetes mellitus (Dalrymple et al.,1996).

IL-6 is produce by using macrophage and monocyte in reaction to different infection cytokines which incorporate tumor necrosis factor(TNF)beta and interleukin -11. In resting segment the receptor of IL-6 is present on everyday activated B-cell-cell in hepatic and Myeloid telephone lines and regular T-lymphocytes (Kubistova et al.,2012). Inflammatory response is produced by IL-6 by using initiating the transcription factors that are present on multiple inflammation path ways. It beginning happen with protein

kinase C, CAMP / protein kinase A and launch of calcium take place (Maeda et al.,2010). IL-6 has a number of characteristic and shape on the foundation of its manufacturing and additionally has pleiotropic activity. When non-infection inflammation takes place such as nerve-racking injury or burn then damage related molecular patterns (DAMPS) from the injury web site prompts the TLRs to produce the IL-6 (Swaak et al., 1998). In the host defense the acute IL-6 expression plays a predominant function through activates the different cellphone population IL-6 initiates the extensive range of acute. Phase proteins such as serum Amyloid A(SAA), fibrinogen, hatoglobin, c-reactive protein, such as serum Amyloid A(SAA), fibrinogen, hatoglobin ,c-reactive protein ,hebeidin and ant chymotrypsin when appearing on hepatocyte and lessen the cytochrome p450, transferrin ,fibronectin and albumin (Eto et al.,2011).

For clinical laboratory test CRP (c-reactive protein) is an accurate biomarker of infection and expression is associated to interleukin-6 (Eto et al.,2011). If the level of hepcidin that is activated the IL-6 can iron transporter ferroportin in intestine epithelial hepatocytes and macrophage it can lead to anemia of a continual inflammation and hypoferremia. TGF-B with the IL-6 beautify the differentiation of IL-17 manufacturing T-helper cells that have important role initiating autoimmune tissue injury (Eto et al.,2011).

Induction of CD8+T-cell via the IL-6 is useful to produce the T-cell. Activation of hematopoietic stem phone and maturation of megakaryocytes into platelets is inducing by the IL-6 in hematopoiesis. Receptor activation of NF-kappa B ligand two is activated by means of the IL-6 manufacturing in bone marrow stromal cellphone that is essential for the activation and differentiation of bone resorption and osteoporosis (Grossman et al.,1989). Additionally IL-6 is infected lesion such as synovium tissue of the rheumatoid arthritis is due to immoderate vascular endothelial growth issue (VEGF) that make bigger the angiogenesis. Autoimmune pores and skin sickness occur two due to the collagen manufacture in dermal fibroblasts and also enhance increase of mesangial mobile and plasmacytoma occurs (Turesson,2013).

2.7.1. Structure of interleukin-6 (IL-6)

Signaling of IL-6 is started by relation of IL-6 and IL-6R (IL-6RA,

CD126) with gp130 dimerization induction and gp130 protein (IL6RB,CD130) that result in a complex of hexameric structure that is capable of signaling .In the body fluids IL-6R is present in soluble form and it has the ability to bind with IL-6 that leads to trans signaling process of IL-6 so that expression of gp130 occurs (Gruys *et al.*,2005).

Signaling of IL-6 activated the Ras-Raf, P3k/AKT and JAK/STAT pathways by activation of different pathways regulation of pro-tumorigenic and antiapoptotic activities occur.it is most ubiquitously derestricted cytokine in cancer so it is involve in differentiation and growth of various malignant tumor cell. Tumorigenesis involve in different tumor models such as lunge, ovarian, colon and breast cancer that is due to signaling of IL-6-JAK-STAT pathways (Escobar *et al.*,2003).

At the binding line of IL-6-IL-6R-GP130 hexameric complex there are fourteen oncogenic mutations are present (Guven *et al.*,2004).

2.7.2. Interleukin-6 family cytokines

The IL-6 family cytokines is a group of cytokines consisting of IL-6, IL-11, ciliary neutrophic factor (CNTF), leukemia inhibitory factor (LIF), oncostatin M (OSM),cardiotrophin- like cytokine (CLC),and IL-27 (John,2018). There a groped into one family because the receptor complex of each cytokine contain tow (IL-6 and IL-11) or one molecular of the signaling receptor sub unite gp130 (John,2018). IL-6 family cytokines have overlapping but also distinct biologic activities and are involved in regulation of hepatic acute phase reactions in B-cell stimulation, in regulation of balance between regulatory and effect or T-cell, in metabolic regulation, and in many neural function. Blocked of IL-6 family cytokine has been shown to be beneficial in autoimmune disease, and help to minimize side effects during therapeutic blockade (John,2018).

2.8. Role of IL-6 in the pathophysiology of RA

2.8.1. Adaptive immune response

IGM and IGG rheumatoid factor along with antibodies to citrullinated peptides are characteristically increase in RA demonstrates that impact of Bcell activity on synovial inflammation and joint damage. IL-6 stimulates Bcell to differentiate into plasma cell to produce immunoglobulins (Muraguchi et al.,1988).IL-6 induces B-cell differentiation (Jogo and Bataille,2001). B-cell antibody production (Dienz et al.,2009).and influences T-cell development by stimulating the proliferation and differentiation of Tlymphocyte into

TH-17 cell which produce IL17 (Chizzolinic et al.,2008). They reported that in murine models of autoimmune disease in the presence of IL-6 and transforming growth factor beta (TGF-b), naive T-cell develop into this cell. In human this pathway is driven by IL-6 in combination with IL-1b and IL-23 rather than TGF-beta (Chizzolinic et al.,2008).
all of this would suggest that IL-6 has an important role in the development of the adaptive immune response and maybe involved in pathogenesis of RA.

Neutrophil migration from blood to tissue is a characteristic feature of inflammation. Upon entry ,activated neutrophils release protolytic enzyme and reactive oxygen intermediates resulting in tissue destruction and joint damage in RA. Neutrophils express membrane-bound IL-6R and are activated by IL-6 (Lindemann et al.,2004).When endothelial cell were cocultured with fibroblasts isolated from the synovium of RA patients,IL-6 level increase and neutrophils adhered to the endothelium neutrophil release SIL-6R as they reach the size of inflammation resulting in local recruitment of leukocytes through activation of adjacent endothelium cell and subsequent chemokine release (Lindemann et al.,2004).

2.9. IL-6 in development of systemic symptom of RA

2.9.1. Acute phase response

During an acute phase there is release of pro-inflammatory cytokine and alteration in level of acute phase proteins in the plasma (Castell *et al.*,1989). IL-6 is principle stimulator of acute phase proteins synthesis through hepatocyte stimulation .in patients with RA, serum IL-6 level correlate with CRP level (Madhok et al.,1993).

2.9.2. Anemia of chronic inflammation

Anemia of chronic in inflammation is present in more than a third of RA patients and in a quarter of patients within the first year of disease (Nikolaisen et al.,2008). Anemia is an independent factor contributing to physical disability in patients with RA (Han et al.,2007). Transport and the release of iron from macrophages (Genz,2003). IL-6 stimulate induced hepcidin expression has been noted in vitro studies of human hepatoma cell, this along with the observation that a rapid increase in hepcidin secretion occur following IL-6 infusion in healthy volunteers suggest an important role of IL-6 in the anemia of inflammation observed in many patients with RA (Nemeth et al.,2004).

2.10. Autoimmunity

The possibility that an individual's immune system may react against autologous antigens and cause tissue injury. Because inflammation is a prominent component of these disorder, they are sometime grouped under immune -mediated inflammatory disease (Abbas et al., ,2015).

2.11. Hematological manifestations

Patients with RA can also current with hematological abnormalities both at time of analysis ,or at some point of the course of their sickness . Hematological manifestation in RA can be greatly classified into areas of anemia, neutropenia, thrombocytopenia,thrombocytosis, eosinophilia, and hematological malignancies (Walker et al.,1990).

Anemia is through far, one of the most common extra-articular symptom of RA. the cause of the anemia in RA is multifactorial-disease exercise, druginduced, nutritional, gastrointestinal bleeding, bone marrow suppression and ineffective erythropoiesis (Agrawal et al.,2006).

Anemia of continual disorder is determined in RA, where it generally correlates with the disorder exercise, particular the degree of articular inflammation. It is normochromic normocytic. Eosinophilia in RA displays energetic disorder or hypersensitivity to capsules (Wilson et al.,2004). thrombocytosis is a familiar finding in lively infected joints (Ustun et al.,2002).

lymphadenopathy is the now and again found in RA, usually presenting on biopsy as benign follicular hyperplasia (Agarwal et al.,2004).

Chapter III

Material Methods

3. Material and Method

3.1. Study design

Analytical cross section study was designed to determine impact of fasting Ramadan on IL-6 and some hematology parameter in RA patients.

3.2. Study area

Study was conducted in Omdurman military hospital and Alrian laboratory.

3.3. Study Duration

During the period from May to December, 2019.

60 RA patient, 30 fasting Ramadan and 30 non - fasting were include in the study.

3.4. Inclusion criteria

Rheumatoid arthritis patients with rheumatoid factor positive who are fasting Ramadan, control group non-fasting rheumatoid arthritis included.

3.5. Exclusion criteria

The study excluded any other autoimmune disease that can effect on cytokines level physiological factors such as smoking and alcoholism. Infectious disease as well as allergy, hypersensitivity.

3.6. Ethical consideration

Permission to carry out the study was take from Sudan University Collage ofMedical Laboratory science, Omdurman military hospital and Alrian lab. The patient or copatient were informed about the purpose of the study before collection of specimen and verbal consent was taken.

3.7. Sampling

Non probability convenience sampling method was used.

3.7.1 Sample size

60 RA patient, 30 fasting Ramadan and 30 non fasting according to formula.

3.8. Data collection

Personal and clinical data were obtained by direct interviewing questionnaire from patient.

3.9. Sample processing

A total of 60 blood specimen (n:30) from fasting and (n:30) for nonfasting RA patient were collected in sterile EDTA container. the plasma sample were preserved at-20

ċ until processing. All the blood sample collected were tested for CBC using Sysmex S310 &serum IL-6 level by using ELISA kit (human IL-6 , bio legend's ELISA MAX™).

3.9.1 Hematological Technique

3.9.1.1. Sysmex automatedPrinciple

The cells sxuspended in an electrically diluents increase the resistance between two electrodes when passing through a sensing aperture. The impedance of the direct current (DC) creates measurable voltage. The size of pulse generated by the cell proportion to it is volume .The cell cunt is determined by number of pulse generated (Anne et al.,1998).

3.9.1.2 Procedure of complete blood count

3.9.1.3 Sysmex Reagent

The reagents that was used by analyesd cetrimide, 10% formaldehyde, glacial aceticacid, NaCl and water (Bain *et al.*,2017).

3.9.1.4 Quality control of sysmex

Quality control is intended to ensure that measurement are sufficiently precise day by day or batch by batch within establish limit .Result on patients sample should not be issued until it is clear from the control data that there has been not significant in the analytical proceder. (Bain *et al.,* 2017).

The best known method is to test a control sample at intervals a long side the routine specimens and to plot the results on a leavy-jenning control chart. This linear graph showing the mean and limits of standard deviation (SD) at 1 SD and 2SD. The result sequential (daily) measurements are plotted on the graph, when system is in good control, not more than 1 in 20 measurement should fall outside 2SD. When two or more consecutive measurement are outside this limits there is likely to have been a random error, whereas several consecutive values within 2 SD, but all on one side of the mean, indicate a consistent bias. A wildly deviant result outsides 3SD may occur as a result of a gross error ("blunder") (Bain *et al.*, 2017).

3.9.2 Eliza

3.9.2.1 Principle

Biolegend's ELISA MAX^{TM} Deluxe Set is a sandwich ELISA Linked Immunosorbent Assay (ELISA). A human IL-6 specific monoclonal antibody is first coated on 96-well plat. Standers and samples are added to the wells ,and IL-6bind to the immobilized capture antibody Next ,a biotinylated anti-human IL-6 detection antibody is added ,producing an antibody-antigen-antibody "sandwich"Avidin-hoursereadish peroxidase is subsequently added ,followed by TMB Substrata Solution ,producing a blue color in proportion to the concentration of IL-6 present in the sample .Finally ,the stop Solution changes the reaction color from blue to yellow, and the micro well absorbance is read at 450nm with a microplate reader.

3.9.2.2 Procedure

Add 100 µL capture antibody solution to each well, seal the plan and incubate overnight between 2ċ and 8ċ. Wash plate 4times*, block the by adding 200µL 1*Assay Diluent A to each well, seal plate and incubate at room temperature for 1 hour with shaking on a plate shaker (e.g . 500 rpm with a 0.3cm circular orbit). All subsequent incubation with shaking should be performed similarly. Wash plane 4times*, add 100µL diluted standards and samples to the appropriate wells. seal the plate and incubate at room temperature for 2 hours with shaking. Wash plate 4times*, add 100 µL diluted detection antibody solution to each well , seal the plate and incubate at room temperature for 1 hour with shaking. Wash plate 4times*, add 100µL diluted Avidin-HRP solution to each well, seal the plate and incubate at room temperature for 30minutes with shaking. Wash plate 5times*, soaking for 30 seconds to 1 minute per wash. add 100µL of freshly mixed TMB substrate solution to each well and incubate in the dark for 15 minutes. Finally Add 100µL stop solution to each well. Read absorbance at 450nm and 570nm within 15 minutes. the absorbance at 570nm can be subtracted for the absorbance at 450nm.

3.9.3 Data Analysis

Data were analyzed by statistical package for social science (SPSSs), (version 18.0) using independent T-test for test difference significance.

Chapter IV

Result

4. Results

60 subject of age (11-73) years old were enrolled in this study,30 subject were fasting rheumatoid arthritis (cases) and 30 subject were non fasting rheumatoid arthritis (control). The mean of age was 48.53 and 51.2 years old in case and control respectively. The gender contribution was 16.7% for male,83.3% for female, while similar in gender contribution for non- fasting rheumatoid arthritis controls.

The data obtained in table (4.1) showed that the mean of IL-6(pg/ml) were 143.29±123.75 in fasting rheumatoid arthritis compared 256.27+253.52 in non- fasting rheumatoid arthritis ,with statisticaly significant difference (p.value: 0.023).

Table (4-1)Compare IL-6 between fasting and non-fasting rheumatoid arthritis:-

Test	Fasting RA	Non-fasting RA	P. Value
IL-6	143.2946739	256.2702333	0.023

Significant p. v ≤ 0.05

The data obtained in table (4.2) showed that the mean level of Hb (g/dl) was 11.97±1.59 in case when compared with 12.40±1.1 control, with no significant difference (p.value:0.140).Mean count of WBCs($\times l0^3/\mu L$) was 7.40±2.38 in case when compared to 7.91±3.39 in control, with No significant difference (p.value:0.400).Mean level of HCT(٪) was 35.3±4.2 in case compared to 36.4±3.1 in control, with no significant difference (p.value: 0.203).Mean count of RBCs was 4.42±0.59 in case compared to 4.41±0.61 in control ,with no significant difference (p.value :0.917).

Table (4-2) compare of hematological parameter among study subject

Variables	Fasting (mean ± SD)	Non-fasting (mean ± SD)	P. Value
WBC ($\times 10^9$ / L)	7.40±2.38	7.91±3.39	0.400
RBC ($\times 10^{12}$ / L)	4.42±0.59	4.41±0.61	0.917
Hb (g/dL)	11.97±1.59	12.40±1.1	0.140
HCT %	35.3±4.2	36.4±3.1	0.203

Significant p. v $\leq$ 0.05

Chapter V

Discussion, Conclusion, Recommendation and

5. Discussion, Conclusion and Recommendations

5.1. Discussion

Intermittent fasting Ramadan is more safe in RA patiants was evaluated by blood analysis of cytokines .the result showed that it is safe. In this study plasma level of IL6(pg/ml) is significantly decrease in fasting RA compared to non-fasting (p.value 0.023). Therefor fasting causes favorable immune modulatory effect by decrease interleukin-6.This finding agree with previous similar studies including Fraser et al.,(2000). Who found significantly reduction of cytokines in fasting rheumatoid arthritis compared to non fasting (p.value 0.003),our finding is also strongly supported by Fehime et al., (2007) who demonstrate reduction in level of IL-6 concentration in fasting with (p.value< o.oo1) .Also , Khalil et al., (2010) in Diyala university in Iraqi reported significant differences between fasting and non -fasting RA patients (p.value< 0.05).Similar studies have been reported by Moez et al., (2012) and Safia et al., (2012).

In this Study ,red blood cell count ,hemoglobin concentration, total leukocyte count ,hematocrit remained unchanged (p.value 0.917, 0.140, 0.400,0.203) respectively , this finding is consistent with Kamal and Waleed,(2003). Who found unchanged in hematological parameters (p, value 0.60, 0.1, 0.5, 0.08) respectively. Which consistent with (Azizi and Rasouli ,1986; Azizi, 2002). The finding also agree with Sarraf et al., (2000).In contrast ,other studies showed a slight reduction of hem concentration and red blood count in fasting compared to non- fasting patients ,but other hematological parameter showed no significant change (El-Hazmi et al.,1987) ,Dewanti et al., (2006)showed a significant reduction in hemoglobin and hematocrite.

This finding was consistent with Husain (1987). Red blood cell (p, value <0.01), hemoglobin (p. value <0.05), hematocrite (p. value 0.001), total white blood cell (p. value <0.05).These controversial result may be due to geographical ,climatic differences nutritional variations ,economical factors.

5.2. Conclusion

Intermittent fasting during Ramadan is the favorable behavior for RA petients indicated by reduction of inflammatory marker IL-6.There was no significant different in hematological parameter(WBC, RBC ,Hb, HCT) between fasting and non-fasting rheumatoid arthritis.

5.3. Recommendation

Intermittent fasting in Ramadan is recommended for RA patients as it's associated with IL-6 levels reduction.

Regular measurement of serum IL-6 level and complete blood count to access rheumatoid arthritis patients.

Large sample size could be used in future studies.

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Appendixes

Appendix(1):ELISA reader

Appendix (2):ELISA washer

Appendix(3):ELISA plate

Appendix(4):ELISA Kits

Appendix(5):sysmex XP300

Appendix (6): Questionnaire

Sudan University of Science and Technology

Faculty of Medical laboratory Science

Department of hematology

Questionnaire form

Effect of fasting Ramadan in IL-6 and some hematological parameter in RA patients

-Name:.....Patient No.....

- Age.....

-Gender:

Male.....Female.....

-Marital status.....

-Occupation:.....

-Do you fasting Ramadan?

Yes.....No.....

-Do you have anemia?

Yes.....No.....

-Do you have any complication of other disease?

Yes.....No.....

-Lab data:

RF.....Hb.....

RBC.....HCT.....

WBC.....