EFFECTS OF HIGH FIBER MULTIGRAIN SUPPLEMENTATION ON CLINICAL DISEASE MEASURES, INFLAMMATORY BIOMARKERS, NUTRITIONAL STATUS AND QUALITY OF LIFE AMONG MODERATE TO SEVERE RHEUMATOID ARTHRITIS PATIENTS

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by

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LIST OF SYMBOLS

° C	Celsius
%	Percentage
µ	Micro
µl	Micro litre
g	Gram
g/dL	Gram per decilitre
g/L	Gram per litre
kg	Kilogram
M	Molar
mcg	Microgram
mg	Milligram
min	Minute
ml	Mililitre
mm/h	Milimeter per hour
mm/rev	Milimeter per revolution
mmol/L	Milimole per liter
ng/ml	Nanogram per mililiter
nm	Nanometer
nmol/L	Nanomole per litre
pg/ml	Picogram per millilitre
U/L	Unit per liter
U/ml	Unit per mililitre

LIST OF ABBREVIATIONS

AC	Ambrotose complex
ACPA	Anti-citrulinated protein/peptide antibody
AIMS	Arthritis impact measurement scales
ALK	Alkaline phosphatase
ALP	Alkaline phosphatase
AMDI	Advanced medical and dental institute
ARC	Animal research complex
AST	Aspartate aminotransferase
ATP	Adenosine triphosphate
bDMARDs	Biological disease-modifying antirheumatic drug
BIA	Biometric impedance analysis
BMI	Body mass index
BMR	Basal metabolism
BPM	Beats per minute
cDMARDs	Conventional disease-modifying antirheumatic drug
CC	Calf circumference
COX	Cyclooxygenase
CRP	C-reactive protein
Cu	Copper
DAS-28	Disease activity score
DHODH	Dihydroorotate dehydrogenase
DHQ	Dietary history questionnaire
DMARDS	Disease modifying anti-rheumatic drugs
EBNA-1	Epstein-Barr Nuclear Antigen 1
EDTA	Ethylenediaminetetraacetic acid
eGFR	Estimated glomerular filtration rate
EI	Energy intake
ELISA	Enzyme-linked immunosorbent assay
ESR	Erythrocyte sedimentation rate
EULAR	European league against rheumatism
FeSOD	Iron Superoxide dismutase

FFQ	Food frequency questionnaire
GA	Arabic gum
GCs	Glucocorticoids
GSH	Gluthathione peroxidase activity
H_20_2	Hydrogen peroxide
HO_2	Perhydroxyl radical
HAQ	Health assessment questionnaire
HAQ-DI	Health assessment questionnaire disability index
Hb	Haemoglobin
HbA1c	Glycated haemoglobin
HC	Hip circumference
HDL	High density lipoprotein
HLA-DRB1	Human leukocyte antigen
HRP	Avidin-horseradish peroxidase
hs-CRP	Histidine c-reactive protein
HUSM	Hospital Universiti Sains Malaysia
IFCC	International Federation of Clinical Chemistry's
IO_2	Singlet oxygen
IL-1	Interleukin-1
IL-6	Interleukin-6
Kcal	Kilocalories
LD	Lactate dehydrogenase
LDL	Low density lipoprotein
LOO	Lipoperoxide
Malay-HAQ	Malay version of the health assessment questionnaire
MCP	Metacarpophangeal joints
MD	Mediterranean diet
MDA	Malondialdehyde concentration
MDG	Malaysian dietary guideline
MHC	Histocompatibility complex
MMP-3	Matrix metalloproteinase-3
Mn	Manganese
MUAC	Mid-upper arm circumference

NAD	Nicotinamide adenine dinucleotide
NADH	Nicotinamide adenine dinucleotide + hydrogen
NO	Nitric oxide
NOS	Nitric oxide synthases
NSP	Non-starch polysaccharides
NSAID	Non-steroidal anti-inflammatory drug
O_2^-	Superoxide anion radical
OD	Optical density
OH	Hydroxyl
OH^-	Hydroxyl radical
$ONOO^-$	Peroxynitrite anion
PC	Protein carbonyls
PIP	Proximal interphangeal joints
p-NPP	*p*-nitrophenyl phosphate
PTPN22	Protein tyrosine phosphatase non receptor type 22
PUFA	Polyunsaturated fatty acid
RA	Rheumatoid arthritis
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
ROO^-	Peroxyl radical
RF	Rheumatoid factor
SLE	Systemic lupus erythematosus
SOD	Superoxide dismutase activity
SST	Serum separating tubes
TAC	Total antioxidant capacity
TC	Total cholesterol
TCA	Trichloroacetic acid
TG	Triglyceride
TNF- α	Tumor necrosis factor- α
TNF- α -R2	TNF- receptor 2 signal
tsDMARDs	Targeted synthetic disease-modifying antirheumatic drug
USM	Universiti Sains Malaysia
VAS	Visual analogue scale
WBC	White blood cell

WC	Waist circumference
WHO	World health organization
WHR	Waist hip ratio
XO	Xanthine oxidase
Zn	Zinc

LIST OF APPENDICES

KESAN SUPLIMENTASI BIJIRAN PELBAGAI TINGGI SERAT TERHADAP UKURAN PENYAKIT KLINIKAL, BIOMARKER INFLAMASI, STATUS PEMAKANAN DAN KUALITI HIDUP DALAM KALANGAN PESAKIT ARTRITIS REUMATOID SEDERHANA HINGGA TERUK

ABSTRAK

Artritis Reumatoid (RA) adalah penyakit kronik reumatik autoimun radang kronik yang mengakibatkan keradangan dan kemusnahan sendi secara progresif. RA dianggap sebagai penyakit kompleks, mengalami perubahan klinikal yang dikaitkan dengan gabungan faktor genetik dan persekitaran. Kini, tiada kaedah penyembuhan yang diketahui untuk penyakit ini, namun rawatan ubat semasa untuk RA boleh membantu memperlahankan perkembangan penyakit. Disebabkan oleh kesan sampingan ubat, ramai pesakit RA beralih kepada rawatan alternatif lain. Dengan perkara yang dinyatakan di atas, kajian ini bertujuan untuk menilai keberkesanan suplementasi bijirin serat tinggi keatas (1) gejala penyakit secara klinikal, (2) status keradangan, (3) tahap tekanan antioksidan dan oksidatif (4) status pemakanan, kualiti hidup dan fungsi dalam pesakit RA. Lima puluh satu pesakit RA sederhana hingga teruk telah diambil secara rawak diberikan sama ada suplementasi bijirin serat tinggi (n=25; ubat reumatik piawai + 80g/hari suplementasi) atau kumpulan kawalan (n=26; ubat reumatik piawai) selama 12 minggu. Pemeriksaan secara rawak terhadap penilaian klinikal terbukti berkurangan dengan ketara dalam kumpulan suplementasi bijirin serat tinggi dalam skor DAS 28 ($p<0.05$), skala pagi ($p<0.01$), skala sendi ($p<0.05$) dan skala kesakitan ($p<0.01$). Di samping itu, kumpulan suplementasi menunjukkan penurunan ketara IL-1β ($p<0.01$), TNF-α ($p<0.01$),

MMP-3 ($p<0.01$), IL-6 ($p<0.0001$), dan hs-crp ($p<0.0001$) selama 12 minggu pengambilan suplementasi bijirin serat tinggi. Peningkatan ketara dalam kepekatan TAC ($p<0.0001$) dan SOD ($p<0.0001$) telah ditunjukkan, manakala tahap MDA ($p<0.0001$) dan Protein Carbonyl ($p<0.0001$) menurun dalam kumpulan suplementasi. Kumpulan suplementasi bijirin serat tinggi menunjukkan kualiti hidup yang lebih baik dari sudut fizikal ($p<0.05$) dan sosial ($p<0.05$), mengurangkan ukur lilit pinggang ($p<0.05$), ukur lilit pinggul ($p<0.05$), dan komposisi lemak visceral ($p<0.05$). Pematuhan terhadap suplementasi adalah memuaskan mencapai (80%) dengan aduan gastrousus yang minimum. Secara kesimpulannya, suplementasi bijirin serat tinggi boleh bertindak sebagai agen pemakanan aktif untuk mengawal RA sederhana hingga teruk.

EFFECT OF HIGH FIBER MULTIGRAIN SUPPLEMENTATION ON CLINICAL DISEASE MEASURES, INFLAMMATORY BIOMARKERS, NUTRITIONAL STATUS AND QUALITY OF LIFE AMONG MODERATE TO SEVERE RHEUMATOID ARTHRITIS PATIENTS

ABSTRACT

Rheumatoid Arthritis (RA) is a chronic inflammatory, autoimmune rheumatic disease, resulting in progressive joint inflammation and destruction attributed by a combination of genetic and environmental factor. The current RA drugs may help slow the disease's progression, but they may cause side effects. Due to the potential side effects, many RA patients swifted to other alternative remedies. Dietary nutritional components have been demonstrated to influence inflammation, oxidative stress, and disease progression. With the aforementioned, this study is aimed to evaluate the effect of high fiber multigrain supplementation on (1) clinical disease symptoms, (2) inflammation status, (3) antioxidant and oxidative stress level (4) nutritional status, quality of life and functionality in RA patients. Fifty-one RA patients were recruited from Rheumatology Unit, Hospital Universiti Sains Malaysia, and randomly assigned into either high fiber multigrain supplement (n=25; standard rheumatic medication + 80g/d multigrain) or control (n=26; standard rheumatic medication) groups for 12 weeks. Clinical assessments were improved significantly in the supplement group; as evident by reductions in disease activity score (DAS 28) ($p<0.05$), morning stiffness rating scale ($p<0.01$), joint scale ($p<0.05$) and pain scale ($p<0.01$). In addition, supplement group showed significant lowered IL-1β ($p<0.01$), TNF-α ($p<0.01$), IL-6 ($p<0.0001$), MMP-3 ($p<0.01$) and hs-crp ($p<0.0001$) over 12 weeks high fiber multigrain

supplementation. Significant improvements in TAC ($p<0.0001$) and SOD ($p<0.0001$) concentration were demonstrated, while the level of MDA ($p<0.0001$) and Protein Carbonyl ($p<0.0001$) were reduced in supplementation group. High fiber multigrain supplementation group showed better QoL physical ($p<0.05$), QoL social ($p<0.05$), reduce indices of WC ($p<0.005$), HC ($p<0.05$), and visceral fat composition ($p<0.05$). The compliance towards the supplement was satisfactory (80%) with minimal gastrointestinal complaints. Conclusively, high fiber multigrain supplementation could act as an active precision nutrition agent to combat moderate to severe RA.

CHAPTER 1

INTRODUCTION

1.1 Research background

Rheumatoid Arthritis (RA) is a common chronic autoimmune disease. It weakens the immune system and causes chronic pain with a high morbidity and mortality rate (Ben-Hadj-Mohamed et al., 2017). The aetiology is still unclear (Kourilovitch et al., 2014), and demonstrated with the unusual symptoms and prolonged duration of morning stiffness, swelling, tenderness, and destruction of polyarthritis (Heidari, 2011). However, some studies included genetic factors and environmental factors as the causes of RA (Abqariyah, 2012; Kourilovitch et al., 2014; Kurkó et al., 2013). The global prevalence of RA was reported to be increase between 1980 to 2019, up to 460 per 100,000 individuals (Almutairi, et al., 2021)and 26% of the RA cases were accompanied with a greater functional disability compared to the healthy population (Myasoedova et al., 2019).

Adults in the US reported the prevalence of RA ranged from 0.41% to 0.52% from 2004 to 2014 (Hunter et al., 2017). In 2010, Australia had the highest prevalence (0.46%), followed by Western Europe (0.44%) and North America (0.45%). East Asia (0.16%), Southeast Asia (0.16%), and the Middle East (0.16%) have the lowest RA prevalence (Cross et al., 2014). From 1990 to 2010, the region's RA prevalence was 0.63 % (Neovius et al., 2011). In 2008, Sweden had 0.77 % RA prevalence, with female dominated patients of all ages (Neovius et al., 2011). Polish adult RA prevalence was 0.9 %, with more females (1.06 %), and 56 % of them were diagnosed in the last 5 years (Batko et al., 2019). From 2004 to 2014, the prevalence of RA increased from 0.53 % to 0.55 % in the United States (Hunter et al., 2017). Typically, female RA patients increased from 0.56 % in 2004 to 0.71 % in 2014, whereas the

male RA patients remained consistent from 0.23 % in 2004 to 0.26 % in 2014 (Neovius et al., 2011). In Malaysia, study conducted in Sarawak General Hospital reported 84 new RA patients, where 66 of them were female (78.6 %) (Wan et al., 2020). Another study conducted in the Hospital Raja Permaisuri Bainun (HRBP) found that females (85.2%) dominated males (33.6%) over 129 RA patients (Sulaiman et al., 2009). The most susceptible group was female (88.6%), which dominated by Malays (31.4%), Chinese (11.6%), indigenous (1.2%), and others (1.3%) (Shahrir et al., 2008).

The RA mortality rate was twice as high as than the general healthy population, with life expectancy reduced by up to 15 years (Jeffery, 2014). RA is gradually associated with systemic complications, social instability, economic burden and psychological disorders (Dougados et al., 2014; Kourilovitch et al., 2014). Current treatment for RA involves the prescription of disease modifying anti-rheumatic drugs (DMARDs) therapy as part of the immediate treatment. Monotherapy, combination of DMARDs therapy, or biological therapy, including the steroid use, were also adopted as part of the therapy regimen (Heidari, 2011). Apart from the use of DMARDs and non-steroidal anti-inflammatory drugs (NSAIDs), complementary therapies, such as dietary modifications and herbal treatment are highly recommended (Gioia et al., 2020). These alternative regimens are frequently been used, as complications of comorbid conditions related with drug treatment, such as osteoporosis and cataracts (steroid use), gastrointestinal ulcers (NSAIDs use), infections and melanoma (NSAID use) (Nyhäll-Wåhlin et al., 2009). Meanwhile, prolonged use of drug medications also cause vitamin and mineral deficiencies among the RA patients. Such deficiency is attributed to the effects of DMARDs and NSAIDs intake, systemic inflammation, and an increase in the requirement in certain nutrients (Silva et al., 2016).

The most often used dietary patterns or diets to treat RA are as follows: Mediterranean-style and vegetarian diets, ‘elemental’ eating plans and ‘elimination’ diets combined with periodical fasting. Mediterranean-style diet, which is rich in fruit, vegetables, unrefined cereals, and legumes, with a moderate quantity of red meat and a lot of fish and olive oil consumption, improved several dimensions of the SF-36 score, including physical function, body pain, global score, physical and mental components among the RA patients (García-Morales et al., 2020). Whole grains, including wheat, corn, brown rice, millet rice, oats and sorghum, contain natural antioxidants that provide beneficial effects (Tian et al., 2019). The Mediterranean diet (MD), which is high in the recommended consumption of whole grains, legumes, fruits and vegetables, is shown to be helpful in improving inflammatory markers, lipid profile and blood pressure (Esposito et al., 2004). Particularly, increasing dietary fiber intake is associated with a lower levels of inflammatory biomarkers in postmenopausal women (Ma et al., 2008). High fiber supplementation containing ground flaxseed, oat flakes, psyllium husk, inulin, arrowroot flour, guar gum, coconut and hemp flour in RA patients at 15g/d for 14 days, followed by 30g/d for another 14 days observed an increase in the circulating regulatory T cell number, favourable Th1/Th17 ratios, and lower bone erosion, as well as improved RA clinical outcomes values (Häger et al., 2019).

1.2 Problem statement and study justification

RA is an autoimmune, chronic inflammatory rheumatic disease that causes immunopathological alterations in RA. It is interrelated and driven by a complex network of cellular and biochemical events that are influenced by multi-interdependent systems, including both endogenous and exogenous variables, in addition to being under a high level of genetic control (Kobayashi et al., 2008). Exogenous influences have a major impact, accounting for 40-50 percent of the risk. Infectious agents, smoking, sex hormones, and food are all predisposing environmental variables (Abqariah, 2012; Chang et al., 2014; Kurkó et al., 2013; Liao et al., 2009; Marchand et al., 2021; Ye et al., 2021). The latter is interesting, given the current research into the link between biological activities and potential beneficial effects of dietary supplements, such as dietary grains.

There is no recognized cure for this condition at the moment. Current RA medications (e.g. methotrexate) may help delay the progression of the disease, but they may have potential adverse effects such as folate malabsorption (Endresen & Husby, 2001; Whittle & Hughes, 2004). As a result of these potential side effects, many RA sufferers seek relief through alternative therapies such as specialize diets and/or dietary supplements. While the processes behind the pathogenesis of joint illnesses are mostly unclear, a number of dietary components, both food and non-nutrient, have been found to influence the inflammatory process and, more specifically, clinical disease development.

Additionally, patients with RA may be nutritionally deficient due to the difficulty associated with meal preparation. Due to the disease's impact on nutritional status and capacity to perform activities of daily life, RA may have a significant impact on the patient's ability to recover from the underlying condition. RA patients

may lack the motivation to purchase, prepare, or consume food due to a lack of appetite, tiredness, and impaired mobility (Kershner & Lasswell, 1992). Nutritional deficit is common in RA patients, particularly the elderly, and was significantly demonstrated by rheumatologic manifestations such as muscle wasting (15.5%), spooning of nails (9.2%), night blindness (13.4%), glossitis (16.9%), tetany (11.3%) and loss of appetite (18%) (Elshebini et al., 2021). However, the nutritional status of RA patients has been poorly defined, and only a few robust dietary intervention studies using nutrients known to have anti-inflammatory properties (e.g. n-3 polyunsaturated fatty acids) (Rajaei et al., 2015; Remans et al., 2004) have been conducted to determine their effect on symptom relief.

The previous studies on the benefits of dietary intake were mainly focused on the type of diet and the intake of vitamins, herbs, fatty acids, and prebiotics (Bitler et al., 2007; Ghavipour et al., 2017; Javadi et al., 2017; Kamal et al., 2018; Park et al., 2013). Currently, the benefits of dietary manipulation, such as vegetarian, Mediterranean, elemental, and elimination diets, on RA symptoms (e.g. pain, stiffness, and mobility) are unknown due to the limitations in the design of previous experiments (Philippou et al., 2021; Rojahn, 2011). These were deemed to be small, single-study designs with a low to moderate risk of bias. For example, two types of diets – fasting followed by a vegetarian eating plan – considerably reduced pain but had no effect on functional status or joint stiffness. When dietary interventions were compared to a control diet (i.e., subjects were requested to continue eating normally), a greater dropout rate was seen in the diet intervention groups, implying that these diets may be difficult for RA patients to adhere to. The majority of dietary interventions share the following characteristics: an increase in fruit and vegetables and fiber; a decrease in saturated fatty acids; and an increase in dietary antioxidants.

Such traits are indicative of a balanced, nutritious diet. Thus, shifting from a 'less healthy' (i.e. insufficient fiber consumption) to a 'healthier' diet may account for part of the reported improvements in RA symptoms with varied dietary patterns.

This is the first trial in the Asia-Pacific region to use high-fiber multigrain supplementation as part of the habitual dietary regimen among RA patients. The current study is undertaken to examine the effects of high fiber multigrain supplements on clinical disease severity measures, blood inflammatory markers, oxidative stress, nutritional status, physical functionality, and quality of life changes in RA patients to that of conventional pharmacological therapies.

1.3 Objectives

1.3.1 General objective

To determine the effect of high fiber multigrain supplementation on clinical disease measures, inflammatory biomarkers, peripheral antioxidant status, oxidative stress level, nutritional status, quality of life and physical functionality in moderate to severe RA patients in a randomized, open-labelled clinical trial for 12 weeks.

1.3.1 Specific objectives

1.3.1(a) Specific objective 1

To evaluate the effect of high fiber multigrain supplementation on the level of clinical disease severity among RA patients.

1.3.1(b) Specific objective 2

To determine the effect of high fiber multigrain supplementation on circulating inflammatory biomarkers.

1.3.1(c) Specific objective 3

To assess the effect of high fiber multigrain supplementation on peripheral antioxidant status and oxidative stress level.

1.3.1(d) Specific objective 4

To determine the effect of high fiber multigrain supplementation on nutritional status, quality of life and physical functionality.

1.3.1(e) Specific objective 5

To determine the safety, compliancy and tolerability towards the high fiber multigrain program.

1.4 Research hypotheses

i. High fiber multigrain supplementation will improve the clinical disease severity measures.
ii. High fiber multigrain supplements will improve anti-inflammatory status.
iii. High fiber multigrain supplementation will enhances on peripheral antioxidant status.
iv. High fiber multigrain supplementation will improve overall nutritional status, quality of life, and physical functionality.
v. High fiber multigrain supplementation are safe and well tolerated.

1.5 Null hypotheses

i. High fiber multigrain supplementation may not improve the clinical disease severity measures.

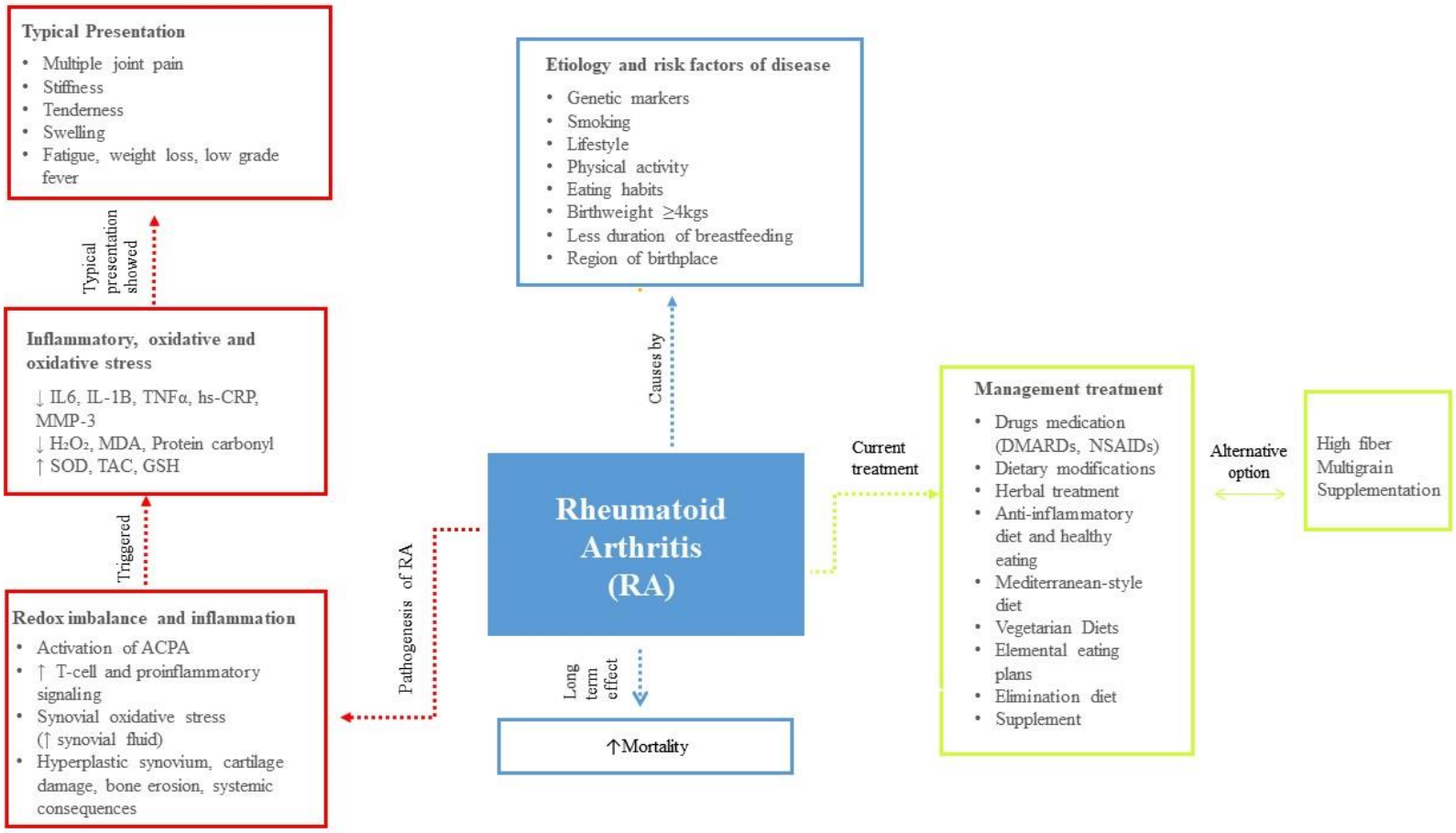
ii. Anti-inflammatory effects of high-fiber multigrain supplements may be ineffective.

iii. High fiber multigrain supplementation does not enhance peripheral antioxidant status.

iv. High fiber multigrain supplementation does not improve overall nutritional status, quality of life, and physical functionality.

v. High fiber multigrain supplementation is unsafe and cannot be tolerated.

1.6 Conceptual framework



CHAPTER 2
LITERATURE REVIEW

2.1 Rheumatoid Arthritis

Rheumatoid Arthritis (RA) is a chronic autoimmune disease that results in progressive joint damage and systemic complications. It affects both sides of the body by affecting smaller joints first and then progressing to larger joints later on. Frequently, the ligaments and cartilage of the joints weaken, causing problems with the skin, eyes, heart, kidneys, and lungs (Bullock et al., 2019). RA is more prevalent among the females compared to male counterpart. The onset and development of RA are a multistage process, which involve an immunological and glycosylation alterations (Alavi & Axford, 2008; Albrecht et al., 2014; Reiding et al., 2017; Scherer et al., 2010). The alterations in total immunoglobulin G (IgG) and anti-citrullinated protein antibody (ACPA) galactosylation (Van de Geijn et al., 2009) might cause inflammation and subsequent recruitment and targeting of immunomodulating cells inside the affected joints (Alavi et al., 2011). A complex network of cellular and metabolic processes, driven by both endogenous and external stimuli, interconnects these immunopathological alterations in RA (Kobayashi et al., 2008). Innate immunity and autoimmunity are altered owing to gut dysbiosis caused by poor nutrition, smoking, and stress (Masuko, 2018). RA is managed by the introduction of highly effective medications, such as methotrexate, leflunomide, and biological agents in long-term prognosis targeting that treatment may help to lower disease activity or remission (Bullock et al., 2019).

2.1.1 RA sub types

There are two main types of RA. Seropositive RA and seronegative RA are defined according to the blood basis result of the presence or absence of autoantibodies specific for autoantigens modified by ACPA and autoantibodies specific for self IgG-Fc that are recognized as rheumatoid factor (RF). Another type of RA is juvenile RA that only affects children.

2.1.1(a) Seropositive RA

The presence of these antibodies defines the seropositive form of RA, that s accounts for almost two-thirds of all RA cases and associated with accelerated joint destruction. It signals that one's body is responding to normal tissues with an immunological response (Malmström et al., 2017).

2.1.1(b) Seronegative RA

Seronegative RA defined when there are no RF or ACPA antibodies but the patient is diagnosed with RA based on other tests such as clinical symptoms, x-rays, or blood routine tests. People with seronegative RA had milder effect than the seropositive RA (Pratt & Isaacs, 2014; Reed et al., 2020).

2.1.1(c) Juvenile RA

Juvenile RA is a term used to describe arthritis that begins before the age of 16 and lasts longer than 6 weeks. Juvenile RA has higher disability rate and the pathogenesis is unknown but mostly linked to the genetic predominant and environmental factors (Hahn & Kim, 2010).

2.2 Prevalence

The global prevalence of RA was 460 per 100,000 people between 1980 and 2019 (Liang et al., 2021). Global prevalence of RA since 1990 to 2010 was reported 0.24% and two times higher in females (0.35%) compared to men (0.13%) (Cross et al., 2014). In 2010, was highest prevalence was recorded in the Australian region (0.46%), followed by Western Europe (0.44%) and North America (0.44%). Asia has the lowest RA prevalence, with East Asia (0.16%), Southeast Asia (0.16%) and Middle East (0.16%) (Cross et al., 2014). Crude prevalence of RA in Africa region was 0.63% in 1990 to 2010. Sweden recorded 0.77% RA prevalence in 2008, which was dominated by female patients in all age groups (Neovius et al., 2011). Specifically, in Poland diagnosed with new European League Against Rheumatism (EULAR) classification criteria stated that RA prevalence was 0.9% estimates for Europe adult Polish population with higher number of females RA patients (1.06%), and 56% of them were diagnosed within the last 5 years (Batko et al., 2019). An observational retrospective, cross-sectional study in the United States reported that the prevalence of RA has increased from 2004 to 2014, from (0.53% to 0.55%) (Hunter et al., 2017). The prevalence of female RA patients were seen to increase from 0.56% in 2004 to 0.71% in 2014, while men RA patients remained stable over the year from 0.23% in 2004 to 0.26% in 2014 (Neovius et al., 2011).

In Malaysia, study from the Hospital Raja Permaisuri Bainun (HRBP), Ipoh has reported that 129 RA patients were dominated by females (85.2%) compared to males, and highest among the Chinese ethnicity (33.6%), followed by Indian (32.8%), Malay (27.3%) and other ethnicities (6.3%) (Sulaiman et al., 2009). Another multicenter study from Selayang, Putrajaya, Taiping, and Seremban hospitals showed similar data, with female (88.6%) as the most susceptible group, and Indian (54.5%)

ethnicity reported the highest number of RA patients followed by Malays (31.4%), Chinese (11.6%), indigenous (1.2%) and others (1.3%) (Shahrir et al., 2008).

2.3 Pathogenesis

RA is an association between human leukocyte antigen (HLA)-DRB1 in the presence of rheumatoid factor (RF) and ACPA antibodies (Guo et al., 2018) that causes microbial protein, increase T-cell and pro inflammatory signalling (Iain & Georg, 2011). It is related with phenotype gene-environment interaction such as exposure to smoking and bronchial stress due to exposure to silica (Symmons et al., 1997).

Pathogenesis of RA involves four stages: 1) triggering stage; 2) maturation stage; 3) targeting stage; and 4) fulminant stage (Guo et al., 2018).

2.3.1 Triggering stage

The triggering step began with the presence of ACPA, which resulted in abnormal antibody responses to a range of citrullinated proteins throughout the body, including fibrin, vimentin, fibronection, Epstein-Barr Nuclear Antigen 1 (EBNA-1), type II collagen, and histones. ACPA is associated with the gene-environment risk factor, and the strongest risk factors are known as shared epitopes. Epigenetic regulation combined with environmental factors, such as lung exposure to noxious agents including smoke, silica dust, nano-sized silica, or carbon derived nanomaterials and smoking triggered RA immune reaction. The protein tyrosine phosphatase non receptor type 22 (PTPN22) acts as potent inhibitor for T cell activation and turn effect in the ACPA production. Another associated factors triggered ACPA producing is because of dysbiosis. Meanwhile, gut microbiota may contributes to the pathogenesis

of RA via multiple molecular mechanism as a result from the abundance of certain rare bacterial lineages (Guo et al., 2018).

2.3.2 Maturation stage

Maturation stages start at the site of secondary lymphoid tissues of bone marrow. The development of the immune response to endogenous epitopes result to the release of antigens. The epitope spreads and the ACPA concentration gradually increases which lasts for years before joint symptoms appear (Van Der Woude et al., 2010) that press pain , bone loss and inflammation in RA (Krishnamurthy et al., 2016; Wigerblad et al., 2016). Other possible factors on the activation of ACPA induce and gradual development of targeted joints encompass of the biological characteristics of the target autoantigen, local microvascular, neurological, and biomechanical factors, and mechanisms related to micro trauma may further play a role (Iain & Georg, 2011).

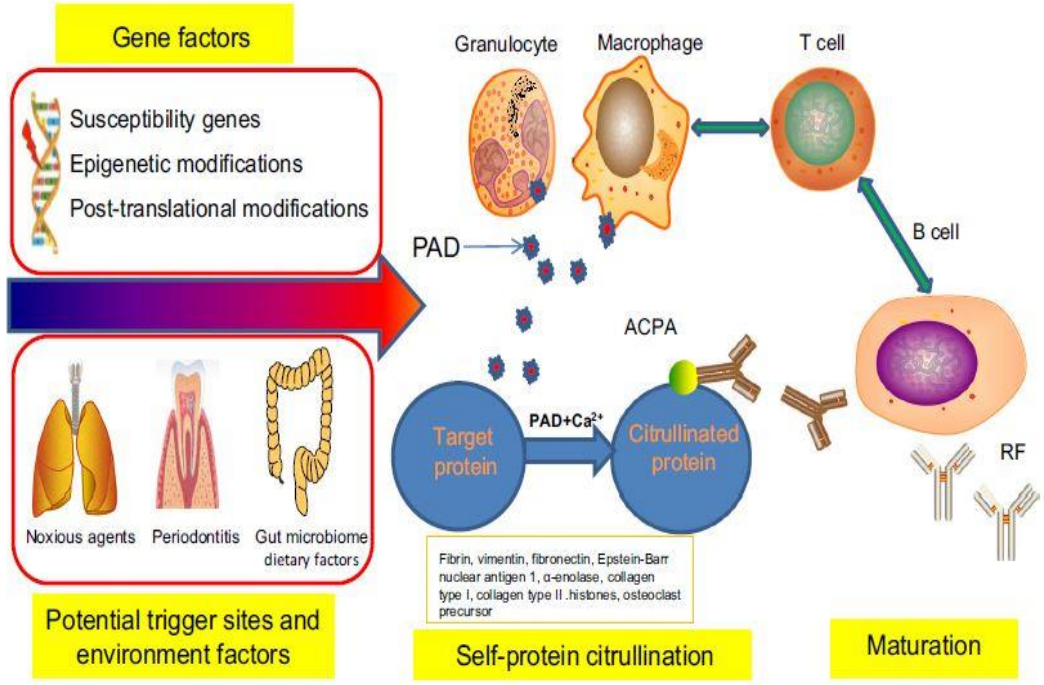


Figure 2.1 Trigger and maturation phases in RA
(Source: Guo et al., 2018)

2.3.3 Targeting stage

The nitial phase of the targeting stage involves the activation of T lymphocytes and B cells together with the cytokines as pro inflammatory cytokines such as TNF-α, IL-1 and IL-17 that simulate inflammation and may degrade bone and cartilage (Mateen et al., 2016). Pro-inflammatory mediators interact to produce inflammation through the interaction of fibroblast-like synoviocytes with the cells of the innate immune system including macrophages, mast cells, and adaptive immune system T lymphocytes and humoral immunity B cells (Choy, 2012)

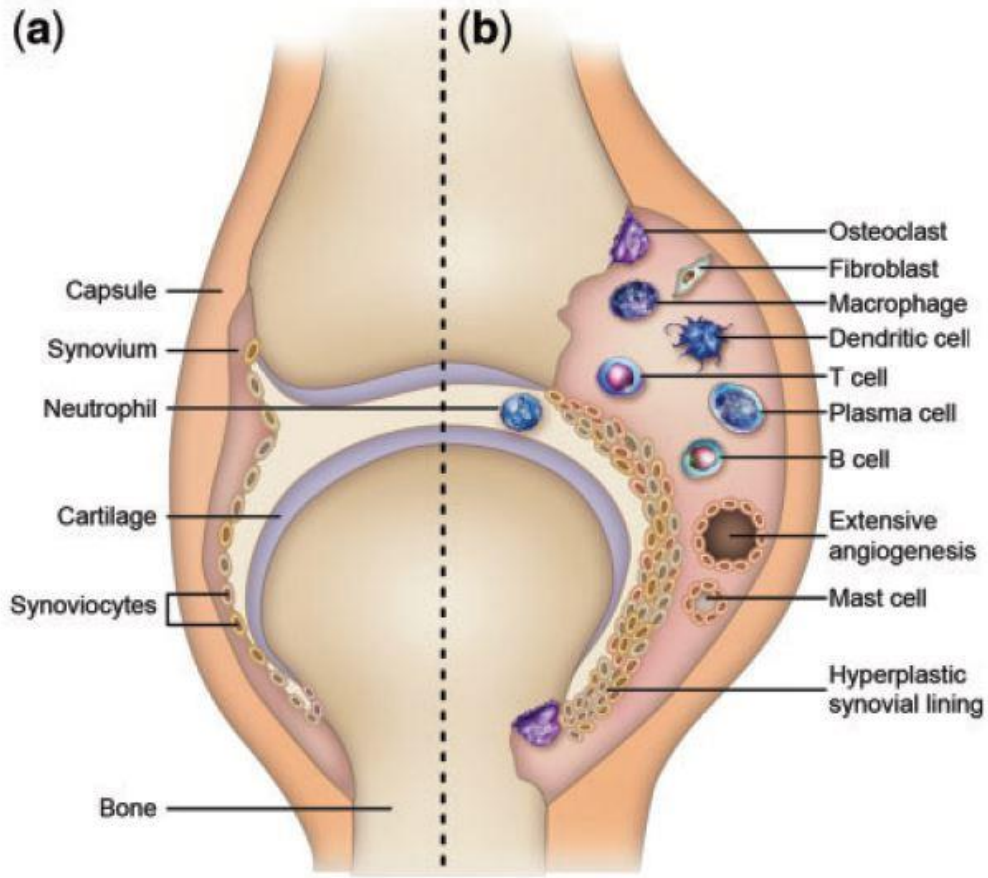


Figure 2.2 Schematic view of (a) normal joint and (b) RA affected joint (Source: Choy, 2012; Smolen & Steiner, 2003)

2.3.4 Fulminant stage

Cytokines play a role in RA joint effects. It causes hyperplastic synovium, cartilage damage, bone erosion, and systemic consequences such as cardiovascular events, fibrotic disease, secondary Sjogren's syndrome, sarcopenia, and osteoporosis in RA patients (Guo et al., 2018)

2.3.4(a) Hyperplastic synovium

Hyperplastic synovium refers to the increase of cellularity of the synovial membrane and caused thickening of synovial fluid. It occurs as after effect of fibroblast-like synoviocyte (FLS) dysfunction. The abnormal growth of FLS induces inflammation by inflammatory cytokines and proteinases, and allows T cell and B cell accumulations that prolong joint destruction (Filer et al., 2006).

2.3.4(b) Cartilage damage

Hyperplastic synovium indirectly causes major damage to the cartilage by direct adhesion, invasion and inflammatory signal (Guo et al., 2018). Under the influence of cytokines, cartilage damage occurs when TNF-α, IL-1, Il-6, IL-17A and reactive nitrogen activates synoviocytes resulting in secretion of matrix metalloproteinases (MMPs) into the synovial fluid and activate chondrocytes that secrete extra MMPs into the cartilage (Smolen & Steiner, 2003).

2.3.4(c) Bone erosion

The main cause of bone erosion is the development of additional growth in the joints of RA patients called pannus. It causes pain, swelling, and damage to bones, cartilage, and other tissues (Choy, 2012). There are two possible mechanisms for bone loss, which involved the formation of immune complex and Fc-receptor-mediated osteoclast differentiation and development of anti-citrullinated vimentin antibodies.

The combination of ACPA and human osteoclast antecedent induces osteoclastogenesis, bone resorption and bone loss. The effect is brought by the release of TNF-α from osteoclast cell numbers with enhanced expression of activation and growth factor receptors (Harre et al., 2012).

2.3.4(d) Systemic consequences

Cytokines activation may increase the endothelial activation and possibility for atheromatous plaques (Guo et al., 2018). The changes in the concentration of certain plasma protein during acute-phase response alter protein synthesis within heptocytoces.

Alteration of the cytokine levels such as IL-1, IL-6, c-reactive protein (CRP), and so on may worsen tissue damaged conditions and impact on further complications such as cardiovascular disease, anemia, osteoporosis, fatigue, and depression (Choy, 2012).

2.4 Etiology and risk factors

The etiology of the disease is still under investigation. However, epidemiological studies have shown that genetic markers and environmental factors such as smoking, lifestyle, physical activity, and eating habits are closely related to RA (Abqariah, 2012; Chang et al., 2014; Kurkó et al., 2013; Liao et al., 2009; Marchand et al., 2021; Ye et al., 2021). In Malaysia, smoking, occupational silica exposure work related to stone dust, rock drilling or stone crush are associated to increased risk of developing ACPA positive RA (Abqariah, 2012; Liao et al., 2009). Other factors such as increase dosage of smoking, birth weight ≥ 4kg, less duration of breastfeeding and region of birth has found were also contributed to risk factors of RA (Liao et al., 2009). Genetic susceptibility may also associated with the risk of RA

(Deane et al., 2017; van der Helm-van Mil & Huizinga, 2008; Yarwood et al., 2015). The influence of lower intake of omega-3 fatty acids and excessive intake of pro-inflammatory food choices such as red meat, salt, and excessive caloric intake were also recognized as risk factors for RA pathogenesis (Deane et al., 2017; Manzel et al., 2014; Oliviero et al., 2015). An observational study by Dai and Zhang (2018) has related the effect of fiber intake on the gut microbiome and the lowering risk and/or delaying RA disease progression as another option for RA treatment.

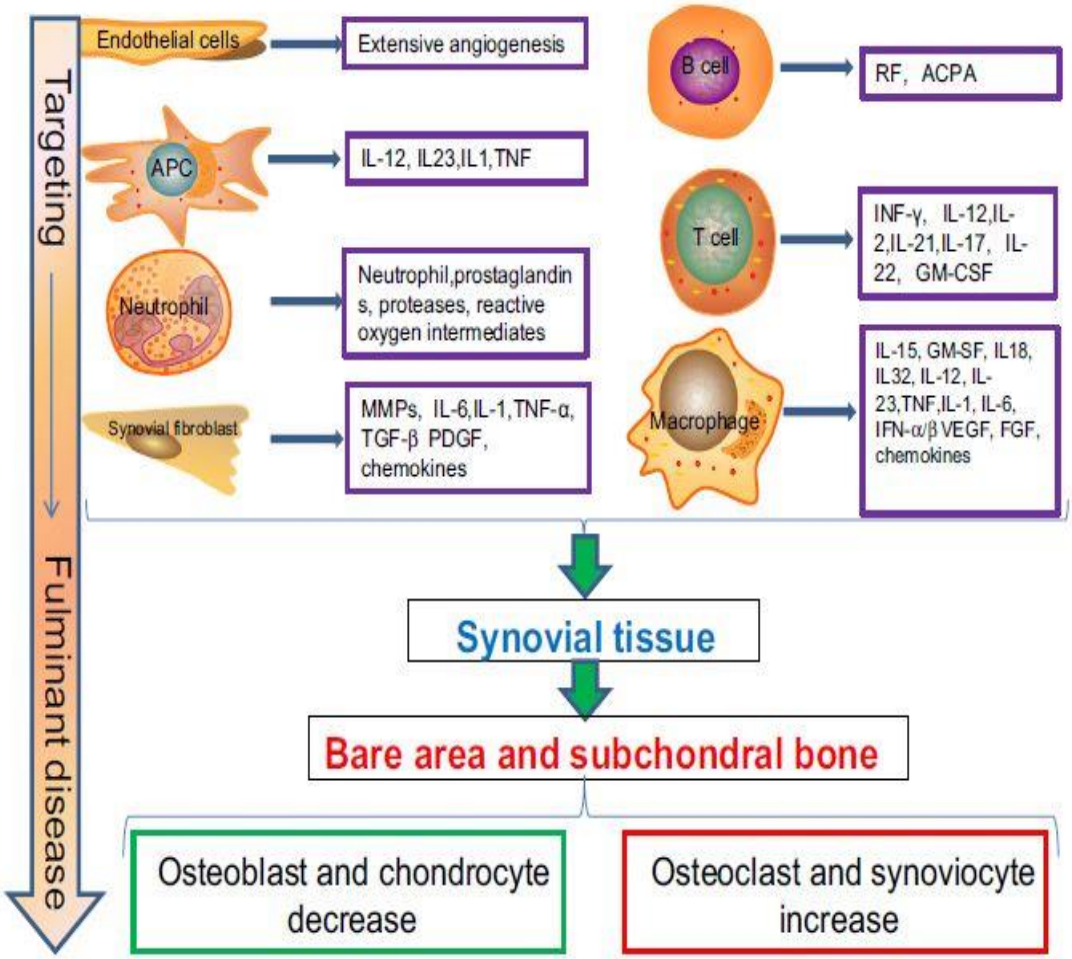


Figure 2.3 Targeting and fulminant phases in RA
(Source: Guo et al., 2018)

2.5 Criteria to diagnose RA

RA diagnosis at early stages are important to prevent the development of further joint erosion or stop progression of erosive disease. Method of diagnosis of RA is not simple and direct. RA sign and symptoms mimic many other disease such as gout or systemic lupus erythematosus (SLE), fibromyalgia or osteoarthritis, especially in the early stages.

2.5.1 Typical presentation

Patients with RA have multiple joint pain and stiffness, especially on the wrist, proximal interphalangeal joints and metacarpophalangeal joints. Besides, RA patients mostly presented with morning stiffness that lasting more than an hour. Prolonged period of symptom duration, symmetric arthritis, hand arthritis, larger number of swollen joint and painful joint are monitored. Most of the RA patients may have joint swelling due to synovitis or subtle synovial thickening detected during the joint examination. Other symptoms seen on during the disease activity becoming active such as fatigue, weight loss, and low grade fever (Wasserman, 2018).

2.5.2 Diagnostic criteria

The most commonly used method according to the American College of Rheumatology and European League Against Rheumatism to classify RA criteria (Wasserman, 2018) (Table 2.1).

Table 2.1 The 2010 American College of Rheumatology/ European League against Rheumatism Classification Criteria for RA

Domains	Description	Score
Joint involvement	One large joint	0
	Two to ten large joints	1
	One to three small joints	2
	(with or without involvement of large joints)	
	Four to ten small joints	3
	(with or without involvement of large joints)	
	> Ten joints (at least one small joint)	5
Serology	Negative RF and negative ACPA	0
	Low positive RF or low positive ACPA	2
	High positive RF or high positive ACPA	3
Acute phase reactants	Normal CRP and normal ESR	0
	Abnormal CRP or normal ESR	1
Duration of symptoms	< six weeks	0
	≧six weeks	1

ACPA= anti-citrulinated protein; CRP= C-reactive protein; ESR= Erythrocyte sedimentation rate; RF= Rheumatoid factor.

Persistent RA predictors are monitored and diagnosed with the duration of morning stiffness (in minutes), and Health Assessment Questionnaire (HAQ) percentage of the change after 3 months disease duration (de Rooy et al., 2011; El Miedany et al., 2008) (Figure 2.4).

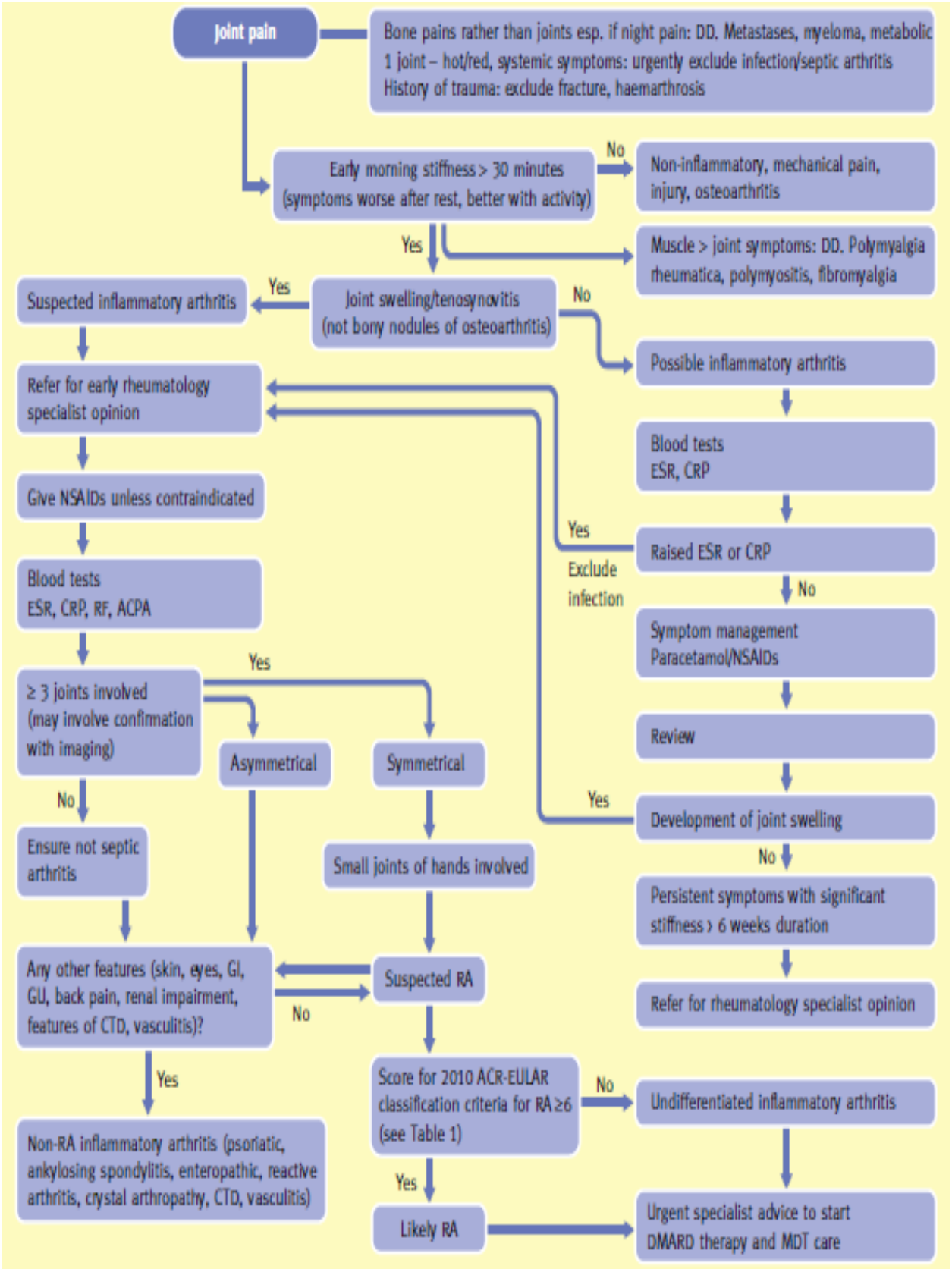


Figure 2.4 Algorithm of diagnosis for early RA
(Source: Jeffery, 2014)

2.5.3 Diagnostic test

As RA is an autoimmune disease that relates to autoantibodies, RF, ACPA, CRP, and ESR are measured (Wasserman, 2018). ACPA is more specific for RA compared to RF that is not specific only for RA, but also for other disease such as hepatitis C (Balsa et al., 2010). RF and ACPA showed high diagnostic specificity and produce accurate prediction in undifferentiated RA patients (Heidari, 2011; Van Der Helm-Van Mil et al., 2008; Van Gaalen et al., 2004; Van Venrooij et al., 2008). Abnormal values of ESR and CRP indicated acute phase response (Heidari, 2011). Higher level of CRP values together with radiographic changes are significantly linked to severity of disease (Gulati et al., 2018).

Other laboratory variables suggested to be tested for RA diagnosis include imaging tests of radiography and magnetic resonance imaging (MRI). Plain radiographic is the standard method in diagnosis anatomic changes in RA. Soft tissue inflammation and mild adjacent osteoporosis may be the initial features of early joint imaging of the hand with RA (Heidari, 2011; Jeffery, 2014). In comparison, sonography and MRI are better diagnostic methods to be sensitive and promising than radiography. Sonography is more reliable to measure bone erosion in early RA (Heidari, 2011), as it can measure more number of erosions and larger scale of patients than radiography (Heidari, 2011). Figure 2.5 shows the bone erosion detection using the sonography technique. Whereas, MRI imaging detects synovitis of hand and wrist in RA and detect patients with true RA compared to ACR diagnostic (Heidari, 2011). It helps in differentiate between RA and non-RA disease. Current trend use MRI in the detection of early sign of arthritis as the sensitivity is higher compared to the conventional radiography and ultrasound (Rahmani et al., 2010).

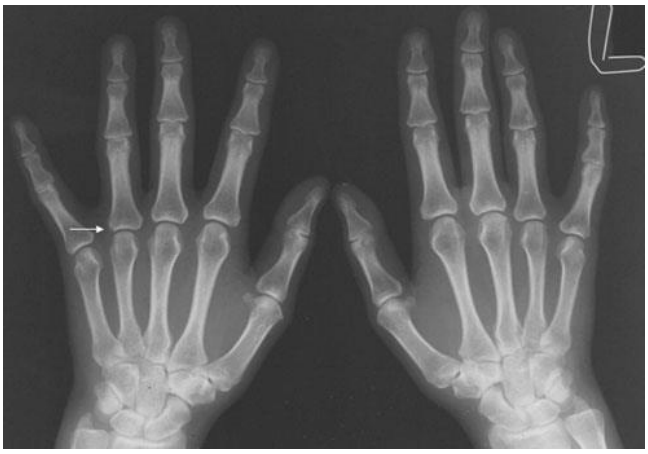
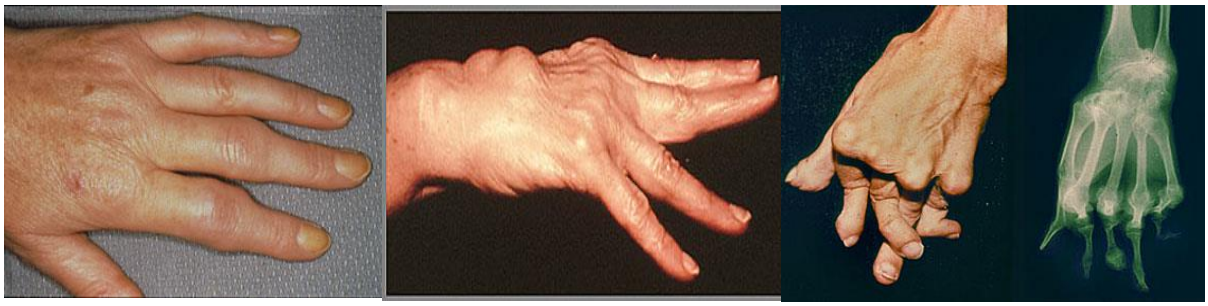


Figure 2.5 Signs of small bone erosion in right 4th MCP in posterior–anterior CR view of a patient with early RA
(Source: Rahmani et al., 2010)

2.6 Clinical Manifestations

RA typically presented with polyarticular joint pain, stiffness, tenderness and swelling of joint, usually in symmetrical pattern. It usually presented on small joints of hand and feet at the metacarpophalangeal (MCP), proximal interphalangral (PIP) and metatarsophalangeal joints, followed by wrist and ankle, elbow, shoulders and knees with most of joint get affected (Figure 2.6). The distal interphalangeal (DIP) joint are generally spared (Wasserman, 2018). Moreover, RA may also affect temporomandibular and crico-arytenoid joints that control mouth opening, chewing, speech and breathing (Jeffery, 2014).

Other nonspecific systemic symptoms can be determined, and these include the occurrence of fatigue, loss of appetite, weight loss and low grade fever (37-38 °C) (Jeffery, 2014). Intrigo and team reported that the clinical manifestations among the RA patients were morning stiffness (76%), fatigue (60%), loss appetite (54%), weight loss (44%), xerophthalmia (34%), xerostomia (32%), myalgias (32%), fever (30%) and raynaud (2%), which most commonly typical among women (Intriago et al., 2019).



(a) Early (b) Later (c) Later with advance

Figure 2.6 Clinical manifestation of RA with swelling of the PIP and MCP joint of the hand, (a) early stages, (b) later stages, (c) later with advance stages. (Source: Rahmani et al., 2010)

2.7 Extra-articular manifestations

Extra-articular manifestations of RA often occur among the seropositive severe RA patients. Even though RA is mostly seen in the joints, other organ systems may also be involved and show symptoms as the disease gets worse. Extra-articular RA features are as listed in Table 2.2 (Jeffery, 2014).

2.8 RA and inflammation

RA has three stages: an asymptomatic period of hereditary risk, a preclinical phase in which RA-related autoantibodies can be found, and a clinical phase with signs and symptoms of acute inflammatory arthritis, like pain and swelling in the joints (Deane et al., 2010). One of the signs of RA is synovitis that lasts for a long time (Heidari, 2011). This is caused by an ongoing flow of immune cells into the joints. Effector T cells, B cells, and other innate effector cells work together in this environment to make pro-inflammatory cytokines, which in turn activate the resident fibroblast-like synoviocytes and lead to cartilage and bone destruction (McInnes & Schett, 2007; Schett et al,. 2013). Neutrophils, mast cells, and macrophages (Haringman et al., 2005) are playing roles in the development of synovitis by