Effectiveness of high fiber multigrain supplementation among the rheumatoid arthritis (RA) patients: A randomized, open-label clinical trial

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1.0 STUDY JUSTIFICATION

Rheumatoid Arthritis (RA) is a chronic inflammatory, autoimmune rheumatic disease, resulting in progressive joint inflammation and destruction. Although its pathogenesis has not yet been fully elucidated, RA is considered to be a complex, clinically variable disease that is influenced by a combination of genetic and environmental factors (Oliver & Silman, 2009). The initiation and progression of RA is a multistep process involving several intermediate stages, including key immunological and glycosylation changes (Alavi & Axford, 2008; Scherer et al., 2010). The glycosylation changes centre on marked changes in peripheral and intra-articular total immunoglobulin G (IgG) and ACPA galactosylation (van der Geijn et al., 2009), which can trigger inflammation and subsequent recruitment and targeting of immunomodulating cells within affected joints (Alavi et al., 2011). These and the other immunopathological changes in RA are interconnected and governed by a complex network of cellular and biochemical events which, in addition to being under a high level of genetic control, are also influenced by multi-interdependent systems involving both endogenous and exogenous factors (Kobayashi et al., 2008). The impact of exogenous factors is significant and accounts for 40-50% of the risk. The predisposing environmental factors include infectious agents, smoking, sex hormones and diet. The latter of which is interesting, given the current research into the link between biological activities and potential beneficial effects of dietary supplements, such as dietary grains.

At present, there is no known cure for this disease. Current drug treatments for RA (e.g. methotrexate) may help slow the development of the disease; however, they may have potential side effects such as malabsorption of folate. As a consequence of these potential side effects many RA patients turn to other alternative remedies such as specialised diets and/or dietary supplements in a bid to relieve their symptoms. Although the mechanisms underlying the pathogenesis of joint diseases are for the most part unknown, a number of nutrient and non-nutrient dietary components have been shown to affect the inflammatory process and, in particular, to influence clinical disease progression. Evidence from limited observational studies suggest that the nutrient status of RA patients, in general, is poor (Woolf & Manore 2008; Rossini et al. 2010). Additionally, patients with RA may be nutritionally compromised because of the difficulty in preparing meals. However, to date, the nutritional status of RA patients has been poorly characterised and only a small number of robust dietary intervention studies with nutrients that are known to exert anti-inflammatory effects (e.g. n-3

polyunsaturated fatty acids) have been carried out to determine their effects on symptom relief (Miles & Calder 2012).

In addition, the effects of dietary manipulation, including vegetarian, Mediterranean, elemental and elimination diets, on symptoms of RA (e.g. pain, stiffness and movement) are uncertain because of limitations in the design of the trials carried out to date. These were considered to be small, single studies with a moderate to high risk of bias (Smedslund et al. 2010). For example, two types of diets – fasting followed by a vegetarian eating plan and a Cretan Mediterranean diet – significantly reduced pain, but had no effect on functional status or joint stiffness. When the dietary manipulations were compared with an ordinary diet (i.e. control diet where subjects were asked to continue their normal diet) a higher dropout rate was found in the diet intervention groups, suggesting difficulty for RA patients following these diets. They also had a higher weight loss. Weight gain can also be a side effect of certain anti-rheumatic drugs. Overweight and obesity can be harmful to joint health and may increase pain, stiffness and swelling, decrease quality of life and increase the risk of co-morbidities that include diabetes mellitus and hypertension. Thus, strategies that promote healthy weight maintenance are likely to be successful. In summary, some studies investigating the role of different dietary patterns in the treatment of RA have reported improvement in a number of symptoms, but the potential mechanisms of action are still unclear. Common characteristics for most dietary interventions are an increase in fruit and vegetables and fibre; a reduction in saturated fatty acids; and an increased dietary antioxidant intake. Such characteristics are typical of a healthy, balanced diet. Thus, changing from a diet that is 'less healthy' (e.g. high in saturated fatty acids) to a 'healthier' diet may explain some of the positive changes in RA symptoms observed with different dietary patterns (Smedslund et al. 2010).

Therefore, the current study will be conducted to examine the effectiveness of high fiber multigrain-enriched supplementation on the level of clinical disease severity measures, blood inflammatory molecules, together with the oxidative stress and nutritional status changes in RA patients, as compare with conventional drug treatments.

2.0 LITERATURE REVIEW

2.1 Pathophysiology of Rheumatoid Arthritis (RA)

2.1.1 Inflammation

One key inflammatory cascade includes overproduction and overexpression of tumor necrosis factor (TNF) (Feldman et al., 1996). This pathway drives both synovial inflammation and joint destruction. TNF overproduction has several causes, including interactions between T and B lymphocytes, synovial-like fibroblasts, and macrophages. This process leads to overproduction of many cytokines such as interleukin 6, which also drives persistent inflammation and joint destruction (Choy et al., 2002). Overproduction of other proinflammatory cytokines (eg, interleukin 1) differs from the process for IL-6 in that production is either less marked or is specific to one or more disease subsets, as best shown by the effects of IL-1 blockade in subforms of juvenile idiopathic arthritis or adult-onset Still's disease.

2.1.2 Synovial cells and cartilage cells

The dominant local cell populations in joints affected by RA are synovial and cartilage cells. Synovial cells can be divided into fibroblast-like and macrophage-like synoviocytes. Overproduction of proinflammatory cytokines is believed to be led predominantly by macrophage-like synoviocytes. Fibroblast-like synoviocytes show abnormal behaviour in RA. In experimental models, co-implantation of fibroblast-like synoviocytes with cartilage leads to fibroblasts invading cartilage (Müller-Ladner et al., 1996), behaviour that correlates with joint destruction. Considerable information has accumulated about joint destruction and the role of osteoclast activation as a key process leading to bone erosion. This association is proven because specific inhibition of osteoclast activation can reduce joint destruction yet not affect joint inflammation (Cohen et al., 2008). One argument for RA starting in the joint is the observation that fibroblast-like synoviocytes showing altered behaviour can spread between joints, suggesting how polyarthritis might develop. Regulation of immune inflammation depends on balances between the number and strength of different cell types. Control of arthritogenic immunoresponses has been studied in mice in which the specific antigen is known. Infusion of low numbers of T cells with specific characteristics ameliorates arthritis in a rodent model of the disease, showing T cells can be protective (Charbonnier et al., 2010).

2.1.3 Autoantibodies

Rheumatoid factor is the classic autoantibody in RA. IgM and IgA rheumatoid factors are key pathogenic markers directed against the Fc fragment of IgG. Additional (and increasingly important) types of antibodies are those directed against citrullinated peptides (ACPA). Although most, but not all, ACPA-positive patients are also positive for rheumatoid factor, ACPA seem more specific and sensitive for diagnosis and seem to be better predictors of poor prognostic features such as progressive joint destruction (van der Linden et al., 2009). Ongoing research aims to identify antibody specificities relevant for different patients' subsets and disease stages. 50–80% of individuals with RA have rheumatoid factor, ACPA, or both. Composition of the antibody response varies over time, with limited specificities in early RA and a mature response-in which more epitopes are recognised and more isotypes used-in late disease (Fig. 1) (Ioan-Facsinay et al., 2008). Evidence from animal models and in-vivo data suggest that ACPA are pathogenic on the basis of induction of arthritis in rodent models and because immunological responses are present in ACPA-positive patients in a citrullinespecific manner (Uysal et al., 2009; Schuerwegh et al., 2010). Findings of clinical studies show that patients with RA and both rheumatoid factor and ACPA (autoantibody-positive disease) differ from individuals with so-called autoantibody-negative disease. For example, histologically, people with ACPA-positive disease have more lymphocytes in synovial tissue, whereas those with ACPA-negative RA have more fibrosis and increased thickness of the synovial lining layer (van Oosterhout et al., 2008).

2.1.4 Genetics

50% of risk of developing RA is attributable to genetic factors (van der Woude et al., 2009). Much progress has been made in identification of genetic regions tagged by structural variation (single nucleotide polymorphisms); more than 30 genetic regions are associated with RA (Plenge 2009). At present, apart from PTPN22 and HLA genes, no major pathogenic insights have come from these genetic associations. However progress is shown by the realisation that from a putative 2 m of DNA harbouring candidate variants, these 30 regions are all contained within 2 mm of DNA. With current sequencing methodology, 2 mm of DNA allows sequencing in large cohorts. Many risk alleles discovered in recent years are fairly common in the population as a whole; individually, they have modest effects on the risk of RA. However, ongoing research suggests that several risk loci are linked to other autoimmune diseases, and some genes fall within discrete biological pathways that are driving inflammation.

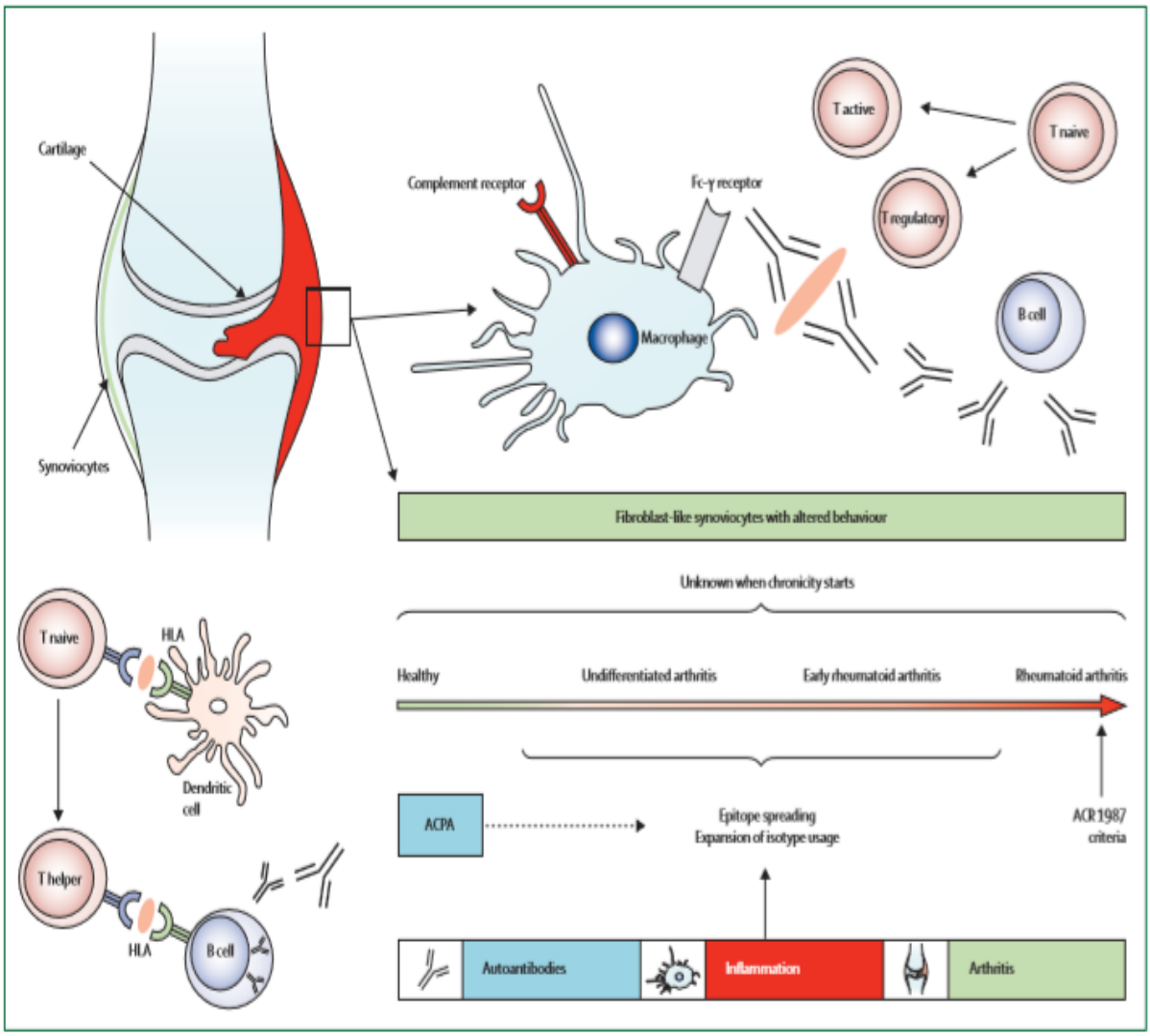

Fig.1 Key pathological changes in the synovium in RA (Ioan-Facsinay et al., 2008)

Findings of genetic studies show differences in ACPA status of patients with RA, related to the number of specific HLA-DRB1 alleles (Fig 1). These HLA alleles share a common motive, which is known as the shared epitope. Currently, antigens are believed to be modified by a process called citrullination; this step entails post-translational modification of the amino acid arginine to citrulline. This change is thought to allow antigens to fi t in the HLA alleles that harbour this shared epitope. The end result is breaking of tolerance that allows antibody formation against these antigens (Hill et al., 2003).

2.2 Classification and diagnosis

2.2.1 Early Arthritis

The American College of Rheumatology (ACR) 1987 criteria are limited by poor sensitivity and specificity for classification of patients with early inflammatory arthritis as having RA

(Banal et al., 2009). Effective treatment in early arthritis averts or delays patient fulfilling these 1987 criteria, and two criteria—erosive joint damage and extra-articular disease, are late changes prevented by modern treatment. Prediction models have been developed from prospective observational studies of treated patients with early arthritis. These models are designed to forecast outcomes in individuals with early arthritis who do not currently meet the 1987 criteria (Fig. 2) (Emery et al., 2010; Tamai et al., 2009). In the presence of inflammatory arthritis, evidence of systemic inflammation, shown by high acute-phase reactants and prolonged morning stiff ness and autoantibodies in serum, particularly ACPA and rheumatoid factor, increases the likelihood of individuals having RA.

2.2.2 Revised Diagnostic Classification

As a result of these concerns and developments, the ACR and European League Against Rheumatism (EULAR) have devised new classification criteria for early arthritis (Aletaha et al., 2010), which assess joint involvement, autoantibody status, and acute-phase response and symptom duration (Fig. 2). These criteria were developed in three phases. Phase one was a data-driven approach, based on cohorts of patients with early arthritis, to identify factors and their relative weights associated with the decision by a doctor to start methotrexate. Phase two was a consensus-driven approach refining these factors with a series of paper patients (ie, written summaries of anonymised cases that provide sufficient information to make decisions about the patient's diagnostic classification) to allow input of current clinical thinking. Phase three summarised all data to arrive at a prediction model and cut-off for the probability score. The effect on diagnosis and management of these new criteria will become clear over the next few years.

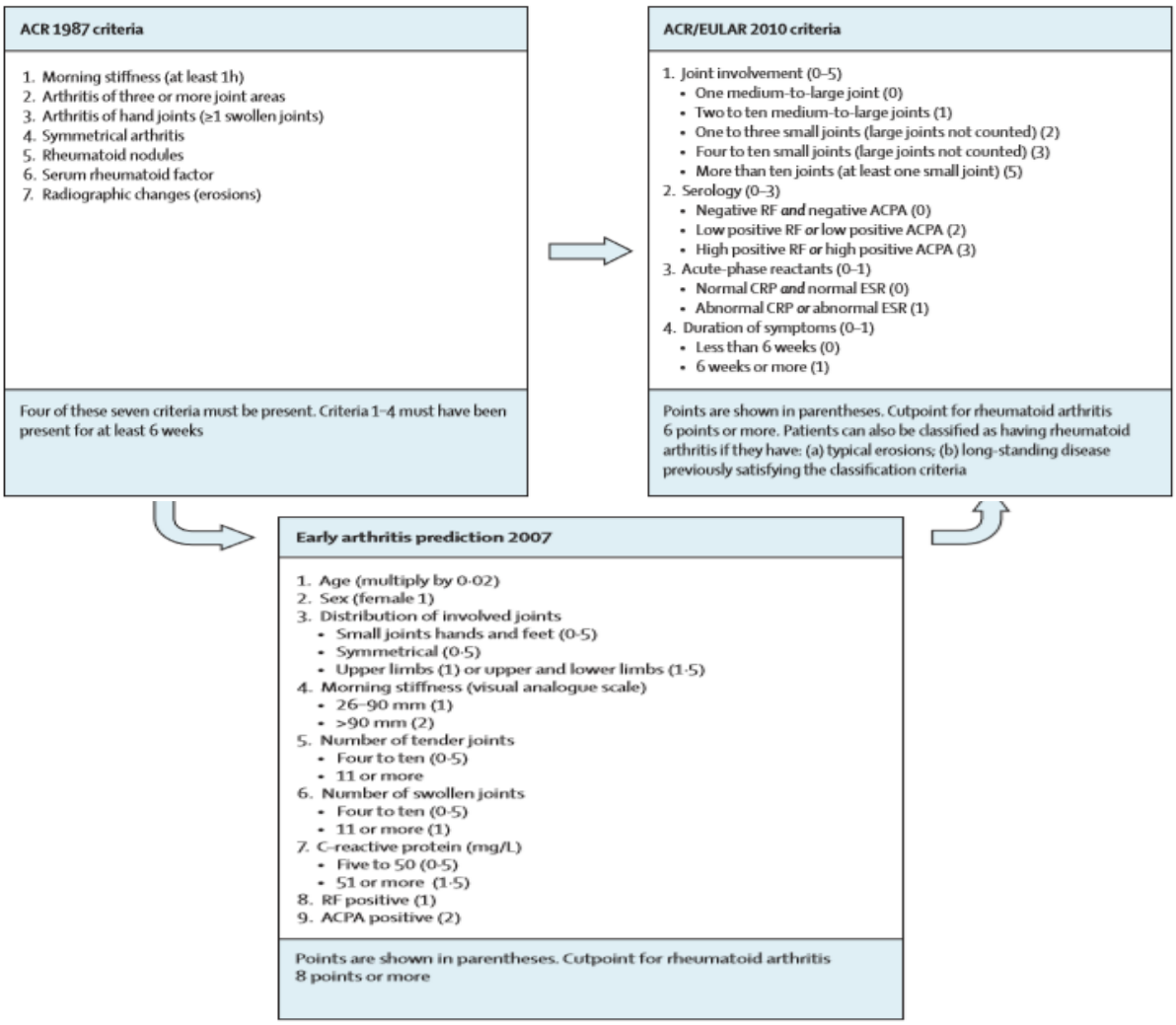

Fig. 2 Conventional and new classification criteria for RA

ACR=American College of Rheumatology. EULAR=European League Against Rheumatism. RF=rheumatoid factor. ACPA=antibodies against citrullinated antigens. CRP=C-reactive protein. ESR=erythrocyte sedimentation rate. ACR 1987 criteria 3 (left panel) were designed to classify established RA. 2010 ACR/EULAR criteria (right panel) are intended to classify both early and established disease. Prediction models such as the van der Helm model (lower panel) represent an intermediate phase; they were designed to identify patients with early undifferentiated arthritis who are most likely to subsequently meet criteria for RA; such models are somewhat more complex than the new criteria.

2.3 Epidemiology

2.3.1 Frequency

Findings of population-based studies show RA affects 0.5-1.0% of adults in developed countries. The disease is three times more frequent in women than men. Prevalence rises with age and is highest in women older than 65 years, suggesting hormonal factors could have a pathogenic role (Symmons et al., 2002). Estimates of the frequency of RA vary depending on the methods used to ascertain its presence. Incidence ranges from 5 to 50 per 100 000 adults in developed countries and increases with age (Carbonell et al., 2008; Pedersen et al., 2009).

Prevalence of RA varies geographically. The disease is common in northern Europe and North America compared with parts of the developing world, such as rural West Africa (Kalla & Tikly, 2003). These variations are indicative of different genetic risks and environmental exposures. Some evidence suggests incidence of RA might be declining, with onset happening later in life.

In Malaysia, RA affects about 5 in every 1000 people. The most common age for the disease to start is in between 30 to 50, and women are more commonly affected than men (Arthritis Foundation of Malaysia, 2017). A local cross-sectional study has demonstrated that there were no statistically significant differences in the sero-prevalence of rheumatoid factor (RF) isotypes and anti-CCP between the three major ethnic groups. In that preliminary study, anti-CCP2 was the most prevalent auto-antibody across the three ethnic groups, while IgA RF was found to be the least prevalent, being present in approximately 18–27% of the population (Gomez et al., 2011).

2.3.2 Environmental risk factors

Smoking is the dominant environmental risk factor and doubles risk of developing RA (Charlens et al., 2010). Its effect is restricted to patients with ACPA-positive disease. Although the risk factor is very important, on a population level, the risk is too low to be clinically relevant. Other potential environmental risk factors include alcohol intake, coffee intake, vitamin D status, oral contraceptive use, and low socioeconomic status, although supporting evidence for these other factors is weak (Liao et al., 2009).

2.4 Clinical Assessment

2.4.1 Core measures

Assessments in RA mainly look at joint inflammation (Dougados et al., 2007). Doctor-based reviews include swollen and tender joint counts and global assessment (ie, overall estimates of disease activity and health status). Standard joint counts focus on 28 joints in the hands, upper limbs, and knees; joints in the feet, although important, are omitted. Some experts prefer extended 66 and 68 joint counts, which include the feet. Laboratory measures encompass erythrocyte sedimentation rate, C-reactive protein, or both. Patient-based measures appraise pain, global assessment, and disability (Wells, 2009). The health assessment questionnaire (HAQ) measures disability. Patients record other relevant areas, such as fatigue and depression. Patient-based measures are especially important because they measure the individual's perspective of the burden of their RA.

2.4.2 Combined Indices

The disease activity score 28 (DAS 28) combines 28 swollen and 28 tender joints (hands, arms, and knees), patient's global assessment, and erythrocyte sedimentation rate to indicate the patient's current status. Because calculation of DAS28 entails application of a complex mathematical formula, simplified variants have been devised. The simplified disease activity index uses 28 tender and swollen joint counts, doctors' and patients' global assessments, and C-reactive protein (Aletaha & Smolen, 2009). The clinical disease activity index is similar but omits C-reactive protein. ACR improvement criteria, which gauge change in status in clinical trials, include falls in joint counts and several other measures (patient's and doctor's global assessments, erythrocyte sedimentation rate, pain, and HAQ). They record 20% (ACR20), 50% (ACR50), and 70% (ACR70) improvements in five of the seven measures. Combined indices need cautious interpretation because high scores can show active arthritis or high pain levels (Wolfe et al., 2005).

2.4.3 Imaging

Juxta-articular erosions characterise progressive established RA and are usually irreversible. They are identified readily by radiography of the hands and feet. Two typical erosions are sufficient for diagnosis (Thabet et al., 2009). Extensive damage seen on radiographs suggests RA is inadequately controlled, and rapid progression of joint damage needs intensive treatment. Scoring systems in which damage seen on radiographs is recorded are mainly used in research.

Extensive interest has arisen in new imaging modalities, particularly ultrasound and MRI, which can assess irreversible and reversible structural changes (Kubassova et al., 2009). Striking inter observer variability has somewhat restricted their value in routine practice, despite wide use in research. One exception is negative ultrasound findings; these have useful negative predictive value in patients with high pre-test probabilities of development of RA (Gaojoux-Viala et al., 2009).

2.5 Outcomes

2.5.1 Assessment

Key outcomes in RA are persistent joint inflammation, progressive joint damage, and continuing functional decline (Scott & Steer, 2007). Other important outcomes include extra-articular features (eg, vasculitis), comorbidities (eg, cardiac disease and infections), and patient-related factors (eg, fatigue) (Wolfe, 2004). The key treatment goal in RA is remission with no active joint inflammation and no erosive or functional deterioration. 10–50% of patients with early RA achieve remission. Frequency of remission depends on how remission is defined, and the intensity of treatment for RA affects development of remission (van Tuyl et al., 2010). Other important goals are reduced disease activity and pain, maintenance of function, and preservation of work and recreational activities. Generic measures, such as short form 36, capture the effect of RA on patients' overall health and quality of life (Lempp et al., 2009). Work disability (loss of employment) is an important personal and societal indicator of the burden of disease (Allaire et al., 2009). Finally, RA increases mortality, although its effect on death rates varies across patients' populations and over time (Sokka et al., 2008).

2.5.2 Improvement

The severity of RA might be lessening. Inflammatory markers such as erythrocyte sedimentation rate and extra-articular features such as vasculitis are declining. Admissions to hospital and joint replacement rates for RA are decreasing. Previously high mortality rates, particularly in severe cases of disease, may be falling. Changes in care delivery could account for some improvements; for example, identification of more ACPA-negative patients with mild RA improves average outcomes (Alcorn et al., 2009; Abelson et al., 2009; Bartels et al., 2009; Louie & Ward, 2010). However, better treatment seems the dominant factor. Since improvements preceded widespread use of biological agents, better conventional treatment seems especially important.

2.6 Management

2.6.1 Treatment of Symptoms

Analgesics reduce pain, and non-steroidal antiinflammatory drugs (NSAIDs) lessen pain and stiff ness. Both groups of drugs are used widely to control symptoms of RA. Evidence for use of analgesics is modest but uncontroversial (Wienecke & Gøtzsche, 2004); support for use of NSAIDs is considerably stronger. NSAIDs have lost their historical role as first-line treatment because of concerns about their limited effectiveness, inability to modify the long-term course of disease, and gastrointestinal and cardiac toxic effects (Scott et al., 2007). These agents should be given with proton-pump inhibitors for gastroprotection, with short-acting drugs administered for short periods to minimise risks.

2.6.2 Disease Modifying Antirheumatic Drugs (DMARs)

Disease-modifying antirheumatic drugs (DMARDs) are a heterogeneous collection of agents grouped together by use and convention. They are the mainstay of treatment for RA. Their diverse mechanisms of action are incompletely understood. They reduce joint swelling and pain, decrease acute-phase markers, limit progressive joint damage, and improve function.

Methotrexate is the dominant DMARD. Sulfasalazine and leflunomide are also widely used. Their efficacy has been established in placebo-controlled trials (Donahue et al., 2008). Hydroxychloroquine and chloroquine have DMARD-like properties. Gold (rINN sodium aurothiomalate) and ciclosporin are additional DMARDs; their use is limited by toxic effects.

DMARDs are sometimes combined, and several combinations of DMARDs have proven efficacy. An example is methotrexate, sulfasalazine, and hydroxychloroquine-termed triple therapy (Choy et al., 2005). Use of DMARD combinations varies across different countries; in some regions they are used rarely.

Adverse effects of DMARDs include those that are minor (eg, nausea) and serious (eg, hepatotoxicity, blood dyscrasias, and interstitial lung disease) (Salliot & van der Heijde, 2009). Monitoring of adverse effects requires pretreatment screening and subsequent safety recording of blood counts and liver function tests.

2.6.3 Biological Agents

TNF inhibitors were the first licensed biological agents, followed by abatacept, rituximab, and tocilizumab: they are highly effective. Caution is needed when comparing treatments because populations of patients with RA in various trials are dissimilar. Effects of biological agents can be especially striking in the subset of inadequately treated or non-responsive patients selected

for trials. Uncertainty exists about the extent to which the strongly positive trial results for use of these agents translates into routine clinical practice, when drugs can be given to people with less active disease who will have diminished responses (Sokka and Pincus, 2003).

Biological agents are combined conventionally with methotrexate. Initially, this combination was to reduce antibody formation (Svenson et al., 2007), but it potentially increases efficacy. Leflunomide can replace methotrexate (Strangfeld et al., 2009). Some biological agents are self-injected at twice weekly to monthly intervals; others are given by infusion. The long-term risks of biological agents have been studied by meta-analysis of trials and routine-practice registries. Infection is the main concern. Risk spans bacterial infections (eg, sepsis, cellulitis, and abscesses), fungal infections (eg, candidiasis), and viral infections (eg, herpes zoster). Concerns have also been raised about demyelination and cancer; lymphoma risk in particular has been investigated in detail. Risk of lymphomas is increased in severe RA, and these patients are most likely to receive biological agents. No convincing evidence supports the idea that these drugs increase risk of lymphoma above that of RA (Strengfeld et al., 2010).

2.6.4 Glucocorticoids

The striking entry of steroids into management of RA more than 60 years ago was followed by uncertainty about their value. Short-term glucocorticoids reduce synovitis. In the long term they decrease joint damage (Kirwan et al., 2007) but incur substantial adverse risks, such as infections and osteoporosis, and their overall risk/benefit ratio is deemed unfavourable. Glucocorticoids can be especially useful in two settings. First, short-term use during flare-ups in disease can lead to rapid improvement and allow other treatments, such as DMARDs, which have a slower onset of action-to be adjusted. Use of steroids in this way is low risk. Oral or intramuscular glucocorticoids are administered by many centres in this setting. Second, intra-articular glucocorticoids are a highly effective local treatment for individual active joints (Goossens et al., 2000).

2.6.5 Supportive Treatments

Effective non-drug treatments span exercise, joint protection, foot care, and psychological support (Hurkmans et al., 2009). Patients' education is also of crucial importance. All these strategies are best delivered by a multidisciplinary team of rheumatologists, nurses, therapists, and podiatrists.

Management of comorbidities is important; they reflect both the disease process and its treatment. Comorbidities include cardiac disease, bone disease, and depression. Conventional guidance recommends annual reviews to detect and treat comorbidities. Systemic complications such as Sjögren's syndrome, lung disease, and vasculitis, need specific treatments, which range from eye drops to cytotoxic drugs. Surgical treatment, particularly joint replacement surgery, is vital to maintain function when joints fail, and collaboration with orthopaedic specialists is required.

2.7 Complications

2.7.1 Death and comorbidities

Patients with RA continue to have increased risks of mortality, mostly from cardiovascular disease and infection. The major causes of mortality mirror rises in specific comorbid disorders. Risks of both myocardial infarctions and strokes are amplified in individuals with RA (Lévy et al., 2008). Although this increase could indicate inflammation-associated vascular damage, identification and treatment of cardiovascular risk factors is important; some evidence shows that methotrexate reduces cardiovascular risks in patients with RA. Comorbid disorders are associated with increased disability and frequent medical consultations (Michaud and Wolfe 2007).

A slightly elevated risk of lymphoma and lymphoproliferative malignant disease is associated with RA activity (Kaiser, 2008). Prevalence of lung cancer is also raised, potentially due to increased cigarette smoking in patients with RA (Khurana et al., 2008).

2.7.2 Treatment-associated comorbidities

Treatment-associated comorbid disorders include osteoporosis and cataract (steroids), gastrointestinal ulceration (NSAIDs), and infections and melanoma (biological agents and steroids). Many of these associations are confounded by RA activity (Nyhall-Wahlin, 2009).

2.8 Dietary Pattern and Rheumatoid Arthritis

The most common dietary patterns or diets that have been used to treat RA include: Mediterranean-style and vegetarian diets, 'elemental' eating plans and 'elimination' diets combined with a period of fasting. Studies have looked at the effects of vegetarian diets on RA such as vegan diets or lacto-ovo-vegetarian (which include dairy products and eggs) diets.

A Mediterranean-style eating pattern is typically high in fruit, vegetables, cereals (traditionally unrefined) and legumes, and contains moderate amounts of red meat and high amounts of fish and olive oil (Trichopoulou et al. 2005). Skoldstam et al. (2003) suggested that this dietary pattern may be beneficial to health because of the relatively high level of antioxidants and unsaturated fatty acids. Hagfors and colleagues (2002) investigated the efficacy of a Mediterranean diet (MD) compared to ordinary western diet for suppression of disease activity in patients with RA. 26 RA patients received MD diet throughout the three weeks of the Outpatient based rehabilitation programme (ORP). Then, for the next nine weeks all patients returned home and back with their everyday life are instructed to continue with the MD diet prepared by themselves. MD group need to follow Creatan Mediterranean diet were they are allowed to used olive oil and canola oil in food preparation, baking and in salad dressing. They were instructed to reduce the amount of yogurt, cheese, milk or choose low fat products and were encouraged to drink green or black tea. Whereas, another 25 patients were in control group needs to continue with their regular diet. Study showed decrease in DAS 28 score ($p<0.001$), decrease in HAQ ($p=0.020$) and in SF-36 Health Survey; increase in vitality ($p=0.018$) and decrease in "compared with one year earlier (p=0.016) in MD group. No significant changes seen in control group at the end of study.

The same authors investigated the effect of Mediterranean diet on the antioxidant intake, plasma antioxidant and oxidative stress status of the patients with RA (Hagfors et al., 2003). Creatan Mediterranean diet, which contain large amount of fruit, vegetables, pulses, cereal, fish, nut, high omega 3 fatty acid, high content of α-linolenic acid and moderate and regular alcohol consumption especially red wine. Significantly higher intake of vitamin C ($p=0.014$), vitamin E ($p=0.007$) and selenium ($p=0.004$) and lower intake of retinol ($p=0.049$) has been reported in the MD group, as compared to the CD group. There is no changes in urine MDA or in the plasma level of antioxidants, from baseline to the end of the study. The levels of retinol, vitamin C and uric acid were negatively correlated to disease activity variables. No correlation found between antioxidant intake and the plasma level of antioxidants in RA patients.

The efficacy of a dietary plant-derived polysaccharide supplement in patient with RA has been evaluated by Alavi et al. (2011). 33 RA patient received powder AC contained Ambrotose Complex, a dietary supplement approved as source of dietary fiber, taken 1.3g/day orally. The main active ingredient of AC contains aloe vera gel extract, arabbinogalacton, gum ghatti, gum tragacanth and glucosamine. Whereas, another 33 RA patients in a placebo group received rice flour powder with identical texture and appearance as AC supplement. All patient consumed supplementation for 8 weeks. Study findings showed there is no significant overall effect on patient outcomes in AC group. AC supplementation resulted in 12% reduction in serum-N- glycosylation (GoF) (p=0.035) whereas the placebo group did not result in any significant changes. There was a decrease in DAS score in AC group compared to placebo group (p=0.009).

RCTs or clinical controlled trials that compared different diets with an ordinary/ usual diet and assessed the impact on a range of primary outcomes, e.g. pain, functional status (i.e. the capacity to perform everyday tasks), joint stiffness and fatigue, as well as any possible adverse effects (such as the number of withdrawals because of adverse events and unwanted weight loss) were included. Overall, changing from a diet that is 'less healthy' (e.g. high in saturated fatty acids) to a 'healthier' diet may explain some of the positive changes in RA symptoms observed with different dietary patterns. In summary, studies investigating the role of different dietary patterns in the treatment of RA have reported improvement in a number of symptoms, but the potential mechanisms of action are still unclear.

There is limited number of human clinical trial involving RA patients and dietary fiber intake. Thus, this study findings will help to figure the effect of high fiber consumption among RA patient on their disease severity, blood inflammatory molecules, antioxidant level, oxidative stress and nutritional status changes.

2.8.1 Fiber intake and inflammatory process

Dietary changes have been proposed to be linked to improvements in RA through a number of mechanisms, such as a decrease in the inflammatory process associated with the condition, an increase in antioxidant levels, altered lipid profiles and/or possible changes in gut microbiota composition (Hagen et al. 2009). However, the mechanisms underlying the pathogenesis of joint diseases are for the most part unknown. It has, nevertheless, been demonstrated that diet and lifestyle can play a role in their pathogenesis as well as improve the course of RA. Rich in complex carbohydrates, fiber is able to promote saccharolitic microbiota favouring short-chain

fatty acid production. These metabolites have been shown to possess positive immune-modulating activity by modifying the cytokine production profile of TH cells, promoting intestinal epithelial barrier integrity, resolving intestinal inflammation, and regulating the acetylation of lysine residues, a covalent modification that affects proteins involved in a variety of signalling and metabolic processes (Hagen, 2009).

Inappropriate dietary intake are related to the insufficient intake of carbohydrate dietary fiber may severe RA symptoms by triggered the cytokines production. The immune cell produce large production of immune mediator such as tumor necrosis factor-alpha (TNF-α), interleukin 1 (IL-1), interleukin 6 (IL-6) and T-cells. The volume of synovial fluid increase the narrow joint space causing inflamed tendon, thinning of cartilage, swelling, bone erosion and impaired mobility. Thus, increasing dietary fiber intake from fruits, vegetables and wholegrain may help to alleviated RA symptoms by reduce inflammation.

Dietary fiber is a carbohydrate polymers which neither digested or nor absorbed in human intestine are subjected to bacterial fermentation in gastrointestinal tract which affect the gastrointestinal balance and changes. This include composition of bacterial, microbial metabolic activities that include the production of fermentative end-products. Diet rich in fiber (> 30g/day) seen to have shifts in bacterial diversity and production of microbial derived fecal fermentative as end products within 24 hours in humans study that switch meat based diet to diet rich in fiber (David et al,. 2013).

Human enzymes only capable to degrading few glycosidic linkages present in a subset of carbohydrates including starch polysaccharide via the action of pancreatic and salivary amylase, and disaccharides sucrose and lactose via the brush border disaccharides, sucrose and lactase (Cantarel et al,. 2012). However carbohydrate that escaped digestion by human enzymes are substrate for bacterial fermentation within the gastrointestinal tract. The major bacterial fermentative end product of complex carbohydrate are short chain fatty acid ($SCFA_s$) Hannah, 2017). It is notable also as acetate, propionate and butyrate (Cummings et al., 1987). $SCFA_s$ is an important indicator for bacterial fermentation in colon. $SCFA_s$ may influence gastrointestinal epithelial cell integrity, glucose homeostasis, lipid metabolism, appetite regulation and immune function (Koh et al., 2016).

Butyrate is the primary energy source for colonocytes and protect again cancer and inflammation by inhibiting histone deacetylases (HDACs) (Flint et al., 2012). HDACs inhibitor reported used for cancer therapy due to its function as anti-inflammatory or immune suppression (Johnstone, 2002). Butyrate suppresses proinflammatory effector in lamina propria macrophages (Chang et al., 2014) and differentiation of dendritic cells from bone marrow stem

cells (Singh et al., 2010) via HDAC inhibition. Thus, immune system become hyporesponsive to the beneficial of the bacterial. SCFA help to regulate cytokine expression in T cells and generate T cell regulation thorough HDCs inhibition. Effector T cells then enhanced glycolysis process and inhibition of glycolysis promotes cell generation (Shi et al., 2011). This help in induces tolerogenic and anti-inflammatory profile of T cell and reduce inflammation.

Another study showed that gum Arabic has immune modulator effect on RA. Gum Arabic fermentation by colonic bacteria increases serum butyrate concentrations. Result significantly showed significant decrease in level of serum TNF α (p value 0.05), ESR (p value 0.011) and number of swollen and tender joints in RA patients significant decrease in disease severity score DAS 28 (p value 0.001) after 12 week consumption (Ebtihal et al., 2018). Thus, the bacteria release does help reduce the inflammation that are the main trigger for symptoms of RA.

3.0 OBJECTIVES

General Objective

- To determine the effectiveness of high fiber multigrain supplementation among the rheumatoid arthritis patients

Specific objectives:

In the current study we would like to test the hypothesis that high fiber multigrain supplementation among the rheumatoid arthritis patients would:

(a) improve the level of clinical disease severity measures
(b) improve the blood inflammatory molecules and antioxidative status
(c) improve the overall nutritional status

4.0 RESEARCH DESIGN

This is a single center, with balanced randomisation [1:1], parallel group, open labelled study. The allocation ratio is 1:1.

5.0 SAMPLE SIZE ESTIMATION

The results from the previous study (Jalili et al. 2014) among RA patients will be used to determine the trial sample size. The following formula will be used to calculate the sample size for each arm:

$$n = \frac{2x\left[z_{(1-\alpha/2)} + z_{(1-\beta/2)}\right]^2}{\Delta^2}$$

Thus, for this study,

n = 21 subjects

Where n = sample size,

Z = 0.8416 (80% power; 95% confidence interval)

Z = 1.96

= mean difference/standard deviation

With consideration that the dropout rate is 20%, thus the sample size would be 21 + 4 = 25 participants for each arm.

6.0 SAMPLING METHOD

Rheumatoid Arthritis patients will be sampled from the Medical Clinic, Hospital Universiti Sains Malaysia. Convenient sampling, under the niche of non-probability sampling method, will be adopted in the study. Accessible population are registered medium to severe stage RA patients. The study will take place at the Clinical Trial Ward of HUSM in Kubang Kerian, Kelantan, Malaysia from 30th May 2019 to March 2020.

7.0 INCLUSION AND EXCLUSION CRITERIA

a. Inclusion criteria

Inclusion criteria for the clinical trial are:

- RA patients according to the American College of Rheumatology (ACR)

- Moderate (DAS 28 = 3.2 – 5.1) and severe (DAS 28 > 5.1) RA manifestation
- Chronological age: 21 years and above
- Stable RA patients who are receiving NSAIDs, glucocorticoids/DMARDs (for example leflunomide, methotrexate, sulfasalazine and hydroxychloroquine) for at least 3 months prior to entering the study
- Not taking antioxidant/antiinflammatory supplements

(Example of antioxidant: vitamin C, vitamin E, grape seed extract, garlic capsule, ginkgo biloba)
(Example of anti-inflammatory supplement: fish oil, curcumin extract, ginger extract, spirulina)

b. Exclusion criteria

Exclusion criteria for the clinical trial are:

- Having liver (chronic liver failure, cirrhosis, all types of hepatitis), kidney (chronic kidney disease, haemodialysis) or haematological (anaemia, thalassemia, haemophilia) disorders
- Active gastric/duodenal ulcer
- Psychiatric disease/mental retardation (bipolar disorder, depression, schizophrenia)
- Cancer (all types), Diabetes Mellitus (Type I and II), uncontrolled hypertension (BP at 140/90 mmHg for the past 3 months), and endocrine disorders (Cushing's disease, gigantism and hyperthyroidism).
- Alcohol and drug abuse (self-mentioned or as recorded in the medical card)
- Other autoimmune/inflammatory diseases (systemic lupus erythematosus, multiple sclerosis, gout, psoriasis, chronic inflammatory demyelinating polyneuropathy)
- Pregnancy/lactation
- Hormone replacement therapy (for at least 3 months prior to entering the study)
- Herbal remedies (any parts from the plants such as flowers, rhizome, seeds, roots, leaves, fruits, stems)
- Vegetarian patient (pure vegan)
- Gluten intolerance
- Participations from another supplementary program

8.0 VULNERABILITY OF THE SUBJECT/PARTICIPANT

The principle investigator and co-investigators will comply with the applicable regulatory requirement(s) related to the reporting of unexpected serious adverse drug reactions to the

regulatory authorities and the institutional review board. Any complaints (particularly gastrointestinal discomfort) from the subject will be recorded, and they will be arranged to receive immediate medical care at HUSM. If the complaints persist during supplementation period, the subject will be stopped by the investigator from the trial. The affected subject will be followed up tightly.

9.0 RECRUITMENT OF SUBJECT/PARTICIPANT & INFORMED CONSENT SEEKING

All RA patients will be invited face-to-face during their routine medical follow-up in the medical clinic. Advertisement poster and flyer will be placed in the clinic to attract participation. Research information will be provided to the patients. If they are eligible, informed consent will be obtained. In obtaining and documenting informed consent, the research team will comply with the applicable regulatory requirement(s), and adhere to GCP and to the ethical principles that have their origin in the Declaration of Helsinki. The research team will not coerce or unduly influence a subject to participate in the trial.

10.0 ASSENT FORM REQUIREMENT

Not applicable

11.0 RANDOMIZATION, MATCHING AND BLINDING

(a) Randomization

Step 1: Generation of an unpredictable allocation sequence

To generate a random allocation sequence, a computer-generated list of random numbers will be used. Simple randomization, where a 1:1 allocation ratio (1 supplement group: 1 control group) will be applied.

Step 2: Concealment of this sequence from the investigators enrolling participants

The allocation sequence will be concealed from the investigator enrolling and assessing participants in sequentially numbered, opaque, sealed and stapled envelopes. To prevent subversion of the allocation sequence, the name and date of birth of the participant will be written on the envelope.

Step 3: Implementation

Determination of whether a patient would be supplemented by multigrain powder (S) or control (C) will be made by reference to a statistical series based on random sampling numbers drawn up by statistician; the details of the series are unknown to any of the investigators or to the coordinator. After acceptance of a patient by the panel, the appropriate numbered envelope will be opened at the clinical trial ward; the number indications inside will be notified if the patient will to be S or a C group, and this information will be then given to the medical officer in charge of the trial.

(b) Matching

Not applicable.

(c) Blinding

Participants will be notified whether they have been randomly assigned into either the S or C group since this is an open-labelled trial. However, only data collectors, coordinator and medical officer in charge of the trial are aware of the allocated arm. Investigators, data analyst and outcome adjudicator will be kept blinded to the allocation.

12.0 SUITABILITY OF STUDY AREA/STUDY LOCATION

The established Clinical Trial Unit, located in the HUSM will be used to conduct the clinical trial. The mentioned clinical trial unit is the referred clinical trial hub in southern region of Malaysia. The clinical trial unit is equipped with 3 beds for patients' observation, medication supply room, seminar room, prayer room, centrifugation room, case report files room, diagnostic lab and restroom. All staffs in the clinical trial unit is good clinical practice (GCP) certified.

13.0 VALIDITY OF RESEARCH INSTRUMENTS

Key indicators of the quality of a measuring instrument are the reliability and validity of the measures. Validity is the extent to which the interpretations of the results of a test are warranted, which depends on the particular use the test is intended to serve. In this study, the validity of the research instruments will be measured via self-report and secondary data sources. Validity evidence from established instruments is maintained when the instrument is used in the same

form and follow the same administration procedures. Overall, the validity of the research instruments will be assessed as below:

- Calibrated and reliable research instruments (refer methodology)
- Hypothetical or abstract concepts (constructs), such as severity of disease, supplement efficacy, supplement safety, burden of illness, patient satisfaction, quality of life, quality of provider–patient communication, and adherence to supplement regimens, will be measured

14.0 METHOD OF INTERVENTION

Intervention group

Participants in the Intervention group will be supplemented with high fiber multigrain powder (in a sachet) for up to 12 weeks. They are required to consume the supplement following the preparation regimen (see Attachment Supplement Labelling), equivalent to 15.6g fiber/day. Intervention group will be attended to the clinical trial ward to receive and replenish the supplement at baseline, week 4 and week 8. Safety, tolerability and compliance will be assessed. After week 12, blood will be taken for the evaluation of efficacy studies. Patients in the intervention group are taking standard medication as prescribed beforehand.

Control group only receive standard medication as prescribed beforehand. They are required to attend to the clinical trial ward during baseline and after 12 weeks.

(a) Subjects

Eligible RA patients will be recruited and taking part in this randomized, open trial between May 2019 and March 2020. The study will be conducted in accordance with the 4th edition Malaysian Good Clinical Practice (GCP) and the Helsinki Declaration. Written informed consent will be obtained from each patient after being fully informed of the study details. Patients will be reviewed at monthly intervals for a period of 12 weeks. The interviewing rheumatologist will be conducting a global assessment of disease activity. Blood will be collected for full blood count and routine biochemistry. After week-12, all the procedures undertaken at baseline will be repeated.

Assessment at baseline and after 12 weeks:

- Disease Severity Score (DAS-28)
- Health Assessment Questionnaire-Disability Index (HAQ-DI)
- Visual Analogue Scale (VAS)
- Full blood count, liver function test, kidney function test, Erythrocyte Sedimentation Rate (ESR), lipid profile
- Serum inflammatory molecules (IL-6, IL-1β and TNF-α), histidine-C-reactive protein (hs-CRP), matrix metalloproteinase-3 (MMP-3)
- Nutritional status (body weight, standing height, BMI changes, body composition, dietary habits)
- Peripheral antioxidant status and oxidative stress [Total antioxidant capacity (TAC), antioxidant capacity; malondialdehyde (MDA) concentration lipid oxidation; protein carbonyl, protein oxidation; superoxide dismutase (SOD), enzymatic activity; hydrogen peroxide, free radical; and Comet assay, DNA damage.

(b) Randomization and assignment

(i) Randomization

Step 1: Generation of an unpredictable allocation sequence

To generate a random allocation sequence, a computer-generated list of random numbers will be used. Simple randomization, where a 1:1 allocation ratio (1 supplement group: 1 control group) will be applied.

Step 2: Concealment of this sequence from the investigators enrolling participants

The allocation sequence will be concealed from the investigator enrolling and assessing participants in sequentially numbered, opaque, sealed and stapled envelopes. To prevent subversion of the allocation sequence, the name and date of birth of the participant will be written on the envelope.

Step 3: Implementation

Determination of whether a patient would be supplemented by multigrain powder (S) or control (C) will be made by reference to a statistical series based on random sampling numbers drawn up by statistician; the details of the series are unknown to any of the investigators or to the coordinator. After acceptance of a patient by the panel, the appropriate numbered envelope will

be opened at the clinical trial ward; the number indications inside will be notified if the patient will to be S or a C group, and this information will be then given to the medical officer in charge of the trial.

(ii) Matching

Not applicable.

(iii) Blinding

Participants will be notified whether they have been randomly assigned into either the S or C group since this is an open-labelled trial. However, only data collectors, coordinator and medical officer in charge of the trial are aware of the allocated arm. Investigators, data analyst and outcome adjudicator will be kept blinded to the allocation.

(c) Study Outcomes

The patient outcome measures will be assessed at two time points: baseline and end of treatment period (week-12).

(i) Primary clinical outcomes

The primary efficacy criterion is clinical remission as assessed by changes in the DAS-28 score from baseline (Day 0) to end of treatment (week-12).

1. Disease Severity Score (DAS-28)

Assessment of number of tender (t28) and swollen (s28) joints out of 28 (10 proximal interphalangeal, 10 metacarpophalangeal, 2 wrists, 2 elbows, 2 shoulders and 2 knees) will be conducted by a rheumatologist and the validated DAS-28 form will be filled to calculate disease activity index according to the following formula:

$$DAS\text{-}28 = 0.56 \surd t28 + 0.28 \surd s28 + 0.70 \ln(ESR) + 0.014 VAS$$ (Harrison et al., 2008)

Where ESR = erythrocyte sedimentation rate

VAS = Visual Analogue Scale

DAS-28 is recommended by the European League Against Rheumatism (EULAR) as the only gold standard to measure the disease activity in patients with RA (Pincus, 2005).

2. Health Assessment Questionnaire-Disability Index (HAQ-DI)

The Health assessment questionnaire disability index (HAQ-DI) is a questionnaire for the assessment of RA. The questionnaire is a patient reported outcome (PRO) which is usually self-administered by the patient.

The following categories will assessed by using the HAQ-DI:

- dressing and grooming
- arising
- eating
- walking
- hygiene
- reach
- grip
- common daily activities

The patients report the amount of difficulty they have in performing some of these activities. Each question asks on a scale ranging from 0 to 3 if the categories can be performed without any difficulty (scale 0) up to cannot be done at all (scale 3). HAQ has been conducted by the Stanford Arthritis, Rheumatism, and Aging Medical Information System (ARAMIS) more than 200,000 times in routine and research contexts, and is also recommended by the ACR to measure physical functionality. Test-retest correlations confirming reproducibility were ranged from 0.87 to 0.99, and correlations between interview and questionnaire formats were ranged from 0.85 to 0.95 (Bruce & Fries, 2003)

3. Visual Analogue Scale (pain severity)

The patients will evaluate their own pain severity on a 100 mm VAS (from 0 mm = no pain to 100 mm = extreme pain) with 5 mm intervals. The standard VAS is a 10 cm scale with a border on each side. To the left of the “0” mark appears the indication “No pain at all”, and to the right of the “10” mark “Pain as bad as it could be”. The visual analog pain scale has proven a great advance in the assessment of pain.

(ii) Secondary outcomes

Twelve-hour fasting blood samples will be collected by venipuncture at baseline and week-12. 20 ml blood samples will be taken according to a standard protocol and immediately centrifuged. Then, the samples will be stored at -80°C until further analysis at the USM reference laboratory.

- General health outcomes

- Full blood count
- Lipid profile
- Erythrocyte Sedimentation Rate (ESR)
- Liver function test
- Kidney function test
- C-reactive protein (CRP)

- Serum inflammatory molecules

Serum inflammatory molecules (IL-6, IL-1β and tumor necrosis factor-α), histidine-C-reactive protein (hs-CRP), and matrix metalloproteinase-3 (MMP-3) will be determined by human high sensitivity ELISA kits.

The only blood tests that are routinely used to assess disease activity in RA are the ESR and CRP (Peabody et al., 2013). Study found that IL-6 plays a key role in the development of RA disease (Yoshida & Tanaka, 2014). A transient synthesis of IL-6 contributes to host defense against infectious agents and tissue injuries by inducing acute phase reactions and immunological and hematopoietic responses. Patients with RA have excessive amounts of IL-1. IL-1 including IL-1β produce the biological effects attributed to this cytokine (Kay & Calabrese, 2004). Tumor necrosis factor-α (TNF-α) as a key molecule in the control of inflammatory production. Individuals with RA have high levels of TNF-α in the synovial fluid and it plays an important role in inflammation and joint destruction that are hallmarks of RA (Vasanthi et al., 2007). Histidine-C-reactive protein (hs-CRP) was detected before the onset of RA and also indicated the activity of the disease during the course of the disease (Nielen et al., 2004). Study showed elevated MMP-3 serum levels reflect disease activity in RA patients and can be used as a specific marker for joint damage (Fadda et al., 2016)

(iii) Tertiary outcomes

- Nutritional status (body weight, standing height, BMI changes, body composition, dietary habits)

- Weight and height (SECA, Hamburg, Germany) will be determined at the beginning and the end of the study in a fasting status without shoes and minimal clothing by a trained nutritionist. BMI will be calculated using the height and weight measurements. Body composition (fat mass, fat free mass, total body water) will be measured using TANITA InnerScan.
- Nutrients intakes will be estimated using dietary history questionnaire (DHQ) at baseline and at the end of the trial for 3 days. The subjects will be reminded not to change their usual diets and physical activities throughout the study, and any changes in their medications will be avoided if possible.

- Peripheral antioxidant status and oxidative stress [Total antioxidant capacity (TAC), antioxidant capacity; malondialdehyde (MDA) concentration lipid oxidation; protein carbonyl, protein oxidation; superoxide dismutase (SOD), enzymatic activity; hydrogen peroxide, free radical; and Comet assay, DNA damage] will be measured using commercial kits.

Comet assay is an adaptable and potential tool for estimating the extent of DNA damage and repair efficacy in various clinical conditions including rheumatoid arthritis as this disease prone to free radical damage and decreased efficacy of DNA repair mechanism (Gunasekarana et al., 2015)

(d) Compliance to the study protocol

Compliance with the supplement intake will be assessed by counting number of the sachets used at every follow up visit. For control group, standard medication will be remained unchanged, if possible.

(e) Promotion of the Trial Adherence

Trial adherences will be promoted via continuous encouragement and tight follow up with the respected patients through face-to-face dialogue and phone conversation. The study will apply maximum adherence by using intact interviewer for each subject. Continuous financial remuneration and refreshment will be given to subject at each appointment and follow up sessions. Reimbursement of transportation mileage and remuneration will be provided to each subject.

15.0 CONTROL GROUP

Control group will be the existing medium to severe-stage RA patients, who are currently receiving his/her standard or preceding medical treatment/regimen. They will be reminded not to alter their habitual dietary intake during the clinical trial period.

16.0 POTENTIAL RISK TO SUBJECT/PARTICIPANT

16.1 Known potential risks

(a) The new intervention may not work, or it may not be better than the standard treatment.

(b) Patients may expose to side effects, mainly abdominal discomfort for supplements.

(c) Patients will be randomly assigned into either supplement (active) or standard care (control) group.

(d) Patients will be requested to undergo more frequent testing (for blood withdraw specifically) and doctor visit.

16.2 Known potential benefits

(a) Assess to complementary medicine that isn't tested yet. This complementary medicine may be more effective or alleviate side effects than the treatments that are currently available.

(b) Patient will play a more active role in their own health care. This can lead to a more positive outlook and better quality of life.

(c) Regular and careful attention will be provided to the subject. Researchers may provide the patient with medical care and more frequent health check-ups as part of their treatment.

(d) Patients are given opportunity to contribute to research that may help others get a better treatment for their health problems in the future.

17.0 DIRECT & INDIRECT BENEFIT TO SUBJECT/PARTICIPANT

17.1 Direct benefit

(a) Patients will be given opportunity to have better access to health care

(b) The trial is expected capable to increase the quality of life of patients.

(c) Patients are expected to have knowledge gain about their health problem.

17.2 Indirect benefit

(a) Patients will be motivated to feel diversion from routine, the opportunity to meet with other people and to feel useful and helpful.

(b) All patients will be paid in the form of reimbursement/remuneration.

18.0 DATA/SPECIMEN HANDLING

(a) Data handling

The planned clinical trial data management activities are:

• Data acquisition/collection

- Data collection is the responsibility of the clinical trial staff at the site under the supervision of the PI. The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.
- The use of case report form (CRF) as data reporting document will allow efficient and complete data collection, processing, analysis and reporting. Paper CRF, together with electronic CRF (eCRF) will be applied, together with an instruction manual. One CRF book will be prepared for each subject enrolled in the study. All investigator will ensure the accuracy, completeness, legibility, and timeliness of the data reported on the CRFs and in all required reports. Only data outlined in the trial protocol will be collected. Only designated members of the research staff should be allowed to record and/or correct data in the CRFs.

• Data abstraction/extraction

- Data will be taken from the source documents (e.g.: medical record) and entered onto the CRFs by study personnel. Clinical data (including AEs, concomitant medications, and expected adverse reactions data) and clinical laboratory data will be entered into the data capture system. The data system includes password protection and internal quality checks.

• Data entry, data processing/coding

- Data entry may take a form of direct computer entry by a person transferring data from paper-based CRFs into a computer database. Data entry allows the process of entering information into a computer for further processing. Coding may be used to represent data category.

• Data storage

- No records will be destroyed without the written consent of the sponsor, if applicable. It is the responsibility of the sponsor to inform the investigator when these documents no longer need to be retained. Study documents should be retained for 7 years after the completion of the trial.

• Data quality control and assurance

- Quality control will be applied to each stage of data handling to ensure that all data are reliable and have been processed correctly. Ongoing and concurrent review of subject data will be conducted, in conjunction with the verification of all data collected and abstracted.
- Quality assurance will be implemented via planned and systematic check. Verification is compulsory to make sure that the trial is performed as per the plan. Checking will be conducted to ensure that staff is compliant with internal and external regulations/guidelines.

(b) Blood collection and storage

One registered medical officer and one staff nurse will be assigned to take 20 ml blood from all patients. Lithium heparinized blood tube, EDTA blood tube and serum separating tube (SST) will be used. On-site processing of serum and plasma will be carried out at centrifugation room. Storage temperature will be set at -80^0C. Aliquot freezing will be applied. Samples will be frozen once, and thawed once by the researcher.

19.0 DURATION OF PARTICIPANT INVOLVEMENT

The subjects will be involved in the trial for up to a 12 weeks period.

20.0 WITHDRAWAL CRITERIA

The investigator may terminate participation in the study if:

(a) The participant will be terminated if they meet an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation.
(b) Any clinical adverse event (AE), serious adverse events (SAEs), laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant.
(c) The participant will be terminated if they take any probiotic during the study time. However, the use of antibiotic is allowed if it is needed.

All withdrawals and dropouts of enrolled subjects from the trial will be reported and explained on the CRFs. Any terminated/withdrawal will be follow up if possible.

21.0 PROPOSED DATA ANALYSIS

Data analysis, in the form of intention-to-treat will be performed at the end of the study. All statistical analyses will be implemented using version 24.0 of the Statistical Package for Social Sciences (SPSS Inc., Chicago, IL) software. An alpha level of 0.05 will be used for all statistical tests. The following statistical methods has been proposed:

(a) For descriptive statistics, categorical and continuous data will be presented as percentages, means with standard deviations, median and range.
(b) For inferential tests $p<0.05$ will be used to indicate for statistical significance (Type I error) (two-tailed).
(c) Assumptions will be checked for normality tests, and transformation will be applied as corrective procedures.
(d) Analysis of the primary, secondary and tertiary outcomes will be measured using Pearson's correlation, multiple regression, repeated measures mixed models, logistic regression, and analysis of covariance.

22.0 INCENTIVE, COMPENSATION &/OR REIMBURSEMENT

Trial subjects will be reimbursed for mileage spent to attend to the clinical trial unit. A token of appreciation, food and also beverages will be provided to each subjects at every monthly follow up.

23.0 DECLARATION OF CONFLICT OF INTEREST

Both the principle investigators and co-investigators declare that there is no conflict of interest. To achieve study integrity, all investigators will ensure that the trial will be conducted according to good clinical practice in three key areas: data collection, training of data collectors, and data monitoring. The use of these protocols will increase the rigor of the clinical trial and assist in maintaining study validity.

24.0 HANDLING PRIVACY & CONFIDENTIALITY ISSUE

All forms are anonymous and will be entered into SPSS software. Only research team members can access the data. Data will be presented as grouped data and will not identify the responders individually. The data system includes password protection and internal quality checks. All patients will be given individual feedback at the end of the study, in the form of laboratory results and assessment results.

25.0 COMMUNITY SENSITIVITIES & BENEFITS

This study hopes to benefit the RA patients by offering a complementary dietary supplementation regimen to alleviate the disease progression. All patients will be given individual feedback at the end of the study, in the form of laboratory outcomes and assessment results.

26.0 PI & CO-INVESTIGATOR QUALIFICATION (INCLUDE IN GCP CERTIFICATE)

Principle investigator : Dr. Lee Lai Kuan (USM, PhD, clinical nutritionist)

Co-investigators : Dr. Wan Syamimee Wan Ghazali (USM, MD, Rheumatologist)
Dr. Siti Mardhiana Mohamad (USM, MD, MRes, PhD, Immunologist)
Dr. Siti Mastura Mohd Sopian (USM, MD, MMed, Family Medicine Specialist)
Associate Professor Dr. Foo Keng Yuen (USM, Chemical Engineer)
Dr. Thamarai Supramaniam (USM, Medical Officer)
Farzana Athirah bt Abdul Latif (USM, PhD candidate)

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