THE EFFECT OF BRISK WALKING EXERCISE ON IMMUNE FUNCTION, INTESTINAL FATTY ACID BINDING PROTEIN, FATIGUE AND SEVERITY SCORES IN PATIENTS WITH IRRITABLE BOWEL SYNDROME :

A RANDOMISED CONTROLLED TRIAL

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Dissertation Submitted in Partial Fulfillment Of The Requirements For The Degree Of Master Of Medicine (Internal Medicine)

UNIVERSITI SAINS MALAYSIA

2020

ACKNOWLEDGEMENT

In the name of Allah, most gracious and most merciful.

Praise upon Allah for His grace, I manage to complete this dissertation.

Firstly, I would like to express my gratitude to my family for the utmost support and stand firm beside me, share with me the tears, joy, and sacrifice together for this study.

Also, I would like to thank my supervisor, Professor Dr Lee Yeong Yeh, for his trust, and continuous support throughout my study. His immense knowledge and patience help me to complete my thesis on time. I could not imagine having a better mentor and supervisor for my research

Besides my advisor, I would like to convey my acknowledgement to the rest of my thesis committee, Dr Marilyn Ong, Dr Ayu, Siti Rubiatul, and Sport Science Lab staff for their ultimate support, assistance, and encouragement. The opportunity to be in the Lab and research facilities were precious and meaningful for me.

I would like to thank my Head of Department in Hospital Raja Permaisuri Bainun, Ipoh, Dr G.R. Letchumanan, who is also my mentor for his understanding, sharing the same aspiration and supporting me throughout completing this thesis.

Also, I would like to thank Dr Mohamad Fadli Kharie from Public Health Department in the Ministry of Health to assist me for the analytical advice.

Lastly, I would like to express the warmest gratitude to my fellow friends and everyone who has contributed, supported and encourage me directly and indirectly throughout this period. May Allah bless every one of you.

Raja Abdul Wafy bin Raja Muhammad Rooshdi

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LIST OF ABBREVIATION

%BF : Body fat percentage

ANOVA: Analysis of Variance

App: Application

BMI: Body Mass Index

C&S: Culture and Sensitivity

EDTA: Ethhylenediamene Tetraacetic Acid

ELISA: Enzyme-linked Immunoassay

FBC: Full Blood Count

FEME: Full and Microscopic Examination

GPS: Global Positioning System

HR: Heart Rate

HRR: Heart Rate Reserve

IBS: Irritable Bowel Syndrome

IBS-SSS: Irritable Bowel Syndrome Symptom Severity Scale

iFABP: Intestinal Fatty Acid Binding Protein

IL: Interleukin

IPAQ: International Physical Activity Questionnaire

METS: Metabolic Equivalents

MFSI-SF: Multiimensional Fatigue Symptom Inventory – Short Form

$PARQ^{+}$: Physical Activity Readiness Questionnaire +

RCT: Randomized controlled trial

SD: Standard Deviation

SE: Standard Error

TBW: Total Body Water

THR: Target Heart Rate

TNF-α: Tumour necrosis factor α

WHO: World Health Organizations

ABSTRAK

Latar belakang: Sindrom Usus Rengsa (IBS) merupakan penyakit saluran pencernaan usus yang banyak di dapati, namun ianya sukar dirawat kerana tiada rawatan khusus. Kajian menunjukkan gejala keletihan meningkat di kalangan pesakit IBS, dan ianya disebabkan oleh proses keradangan di dalam otot. Aktiviti fizikal telah terbukti meningkatkan proses anti-keradangan yang mana boleh memberi kaedah rawatan terbaru untuk merawati IBS.

Kaedah: Ini merupakan kajian intervensi terkawal secara rawak, yang melibatkan subjek berumur antara 18 tahun sehingga 70 tahun, yang telah didiagnos menghidap penyakit sindrom usus rengsa (IBS) sehingga bulan Ogos 2019 di Hospital Universiti Sains Malaysia. Semua subjek akan dipecahkan kepada dua kumpulan, iaitu kumpulan kawalan dan intervensi. Kumpulan intervensi akan menjalani senaman berintensiti sederhana selama 4 minggu. Beberapa parameter sebagai tanda ukur akan diambil sebanyak dua kali iaitu sebelum dan selepas tempoh intervensi 4 minggu.

Keputusan: Sebanyak 44 subjek telah dianalisis. Terdapat persamaan yang signifikan di antara IL-6 dalam kumpulan intervensi berbanding dengan kumpulan kawalan di kalangan pesakit IBS ($P < 0.001$; 95% CI 14.70 – 27.80). Tiada faktor yang signifikan dicatat pada TNF-α ($P = 0.274$; 95% CI -20.57 – 70.61) dan penanda aras kecederaan usus (iFABP) ($P = 0.288$; 95% CI -71.04 – 21.65). Bagi perubahan IBS-SSS, tiada perbezaan yang signifikan di antara kedua kumpulan di kalangan pesakit IBS ($P=0.157$; 95% CI -6.09 – 36.37). Begitu juga tiada perbezaan yang signifikan

dijumpai terhadap kadar kelesuan menggunakan skor MFSI-SF di antara kedua kumpulan groups (P=0.212; 95% CI -1.46 – 6.41).

Kesimpulan: Penanda keradangan seperti IL-6, TNF-α, dan iFABP mempunyai peranan di dalam menentukan kadar kecederaan dan kerosakan sel. Namun, terdapat beberapa kekangan bukti yang menunjukkan bahawa aktiviti fizikan mempunya peranan dalam mengurangkan keradangan ini. Tidak terdapat perbezaan ketara yang ditunjukkan dalam kelesuan di antara kumpulan intervensi dan kawalan di kalangan pesakit IBS.

Kata kunci: *Sindrom usus rengsa, keradangan, aktiviti fizikal, fungsi sistem imun, IL-6, TNF-α, iFABP*

ABSTRACT

Background: Irritable bowel syndrome (IBS) is the most prevalent functional gastrointestinal disorder, which is challenging to treat as no specific treatment are available. It has been shown that fatigability increases in physically active IBS patients; it is associated with the pro-inflammatory process, which takes place in the muscles. Physical activity has proven to increase the anti-inflammatory process and maybe a potential in IBS treatment.

Method: This is a randomized controlled intervention study, involving IBS subjects of more than 18 years old and less than 70 years old whom meet the ROME III criteria were recruited between June 2019 till August 2019 from Hospital Universiti Sains Malaysia, Kubang Kerian, Kelantan. Subjects subsequently assigned into two groups, control and exercise groups where the exercise group underwent moderate intensity exercises for four weeks. Measurements were taken twice, which is before and after the completion of the four-week intervention period.

Results: A total of 44 participants were analyzed. There was a significant difference between two randomization factors in IL-6 between exercise group compared with control group among IBS patients ($P < 0.001$; 95% CI 14.70 – 27.80). There was no significant difference observed among two groups for TNF-α ($P = 0.274$; 95% CI -20.57 – 70.61) as well as intestinal cell damage markers (iFABP) ($P = 0.288$; 95% CI -71.04 – 21.65). In the IBS-SSS comparison, there was no significant difference noted between two randomization factors ($P=0.157$; 95% CI -6.09 – 36.37). Similarly, no

significant difference was found with regards to fatiguability using MFSI-SF score among two groups (P=0.212; 95% CI -1.46 – 6.41).

Conclusion: Inflammatory markers such as IL-6, TNF-α, and iFABP have some role in determining cellular inflammation and injury. Still, there is a lack of accuracy in terms of the evidence showing that physical activity has some role in reducing these inflammatory markers. No significant difference was demonstrated in fatigability between the exercise and control groups among IBS patients.

Keyword: *Irritable bowel syndrome, chronic inflammation, physical activity, fatiguability, immune function, IL-6, TNF*-α, *IFABP*

CHAPTER 1: INTRODUCTION

Irritable bowel syndrome (IBS) is a common functional gastrointestinal disorder that affects one-fifth of the population globally (Rasquin et al., 2006; Quigley et al.,2012). IBS is characterized by disordered gut-brain axis such as recurring abdominal pain or discomfort, including altered defecation and absent of structural abnormalities (Brandt et al., 2009; Talley et al., 1991). IBS has a significant impact on the community quality of life as well as an economic burden towards the healthcare system.

Prevalence of this IBS in Malaysia being reported ranging from 6.8% to 15.8% (Tan et al.,2003; Rajendra and Alahuddin, 2004). One survey found 10.9% Malay ethnic subject demonstrated IBS in north-east Malaysia (Lee YY et al., 2012).

ROME III Criteria is used for the diagnosis of IBS, using symptoms-based criteria (Khan and Chang, 2010) and validation study shows patients with IBS symptoms and colonoscopy findings correlations reported sensitivity of 68.8% and specificity of 79.5% (Ford et al., 2013).

The exact mechanism of IBS is still unknown. However, there are multifactorial causes and risk factors for IBS that includes food allergy, infectious pathogens, smoking, physiological and physical stress (Klem et al.,2017; Nam et al., 2010). Low-grade inflammation over intestinal-mucosal layer, with activation of T-lymphocyte and pro-inflammatory cytokines, has been reported in IBS patients (Sundin et al.,2014; Martin-Vinas & Quigley, 2016; Ohman and Simren, 2010; El-Salhy 2015). Despite infection already cleared, the initial episode of acute gastroenteritis often results in

relapsing IBS patients. It leads to a significant reduction of health quality of life due to systemic manifestation of symptoms such as chronic fatigue syndrome and musculoskeletal pain (Peyton and Greene, 2014; Wang and Zhao, 2016; Undseth et al., 2016; Janssens et al., 2015). A recent report in post major flood disaster found that 17% of flood victims developed IBS, and those developed IBS shows disturbed gut microbiota (dysbiosis) (Yusof et al., 2017). In the post-flood study, they were more Pleisomonas and Trabulsiella reported in IBS, with effect size of 3.0 and these organisms were environmentally derived, presumably from flood waters during index exposure.

Physically active IBS patients reported a reduction in fatigue and symptoms of IBS (Johannesson et al., 2011). Previous studies utilizing walking as a treatment intervention for IBS shown positive results on quality of life and reducing IBS severity scores but were limited (Ng et al., 2007; Bilski et al., 2014). In the previous randomized controlled trial (RCT), the study utilized intervention through a phone call with a physiotherapist for advice regarding the duration of physical activity per month. The experiment using relayed verbal input to IBS patients in the randomized control trial (RCT), and it must be adequately monitored for the intensity and duration of the exercise to investigate the effectiveness of the intervention. High exercise duration and strength have been shown to influence human functional outcomes as well negatively impact cardiac function and causes systemic stress (Stewart et al., 2016).

Van Wijck et al., (2011) has been investigated dose-response relationship between exercise and intestinal function. Increased exercise intensity causes intestinal hypoperfusion, and it can lead to intestinal dysfunction when prolonged exposure.

Splanchnic hypoperfusion, which occurs in many pathophysiological conditions has caused intestinal barrier dysfunction and subsequently lead to multiorgan dysfunction (Swank and Deitch, 1996). Critically ill patients who underwent major surgery, trauma, or shock compromised their intestinal barriers by hypoxia, lack of nutrient supply, and ischemia that potentially worsen the systemic body inflammation (De Souza and Greene, 2005). Regular to moderate-intensity exercise has been shown to generally improve the quality of life in IBS patients including condition such as depression and fibromyalgia which were commonly detected in IBS patients (Whitehead et al., 2002). Exercise as little as four weeks duration significantly improved pain symptoms of IBS patients (Colwell et al., 1998).

To date, treatment of IBS was non-specific. It comprised of lifestyle adjustments, dietary and pharmacological interventions (Khan and Chang, 2010). Exercise or physical activity seems to be effective in managing IBS symptoms by its immunomodulatory effect. Moderate and habitual exercise has anti-inflammatory and can be protective against chronic inflammatory diseases (Gleeson, 2007; Gleeson et al., 2011). However, the mechanism of this protective properties still unclear. Relapsed IBS patients were found to have low-grade inflammation with increases in circulating interleukins (IL-6, IL-8, IL-10, IL-1β) and tumor necrosis factor (TNF) (Ohman and Simren, 2010). It is still unclear the increase of these anti-inflammatory cytokines (IL-6, IL-10) expressions is a result of the rise of pro-inflammatory cytokines (TNF-α, IL-8, IL-1β). The increase in plasma concentration of IL-6 with the response to exercise due to its release from contracting muscles have been shown to inhibit TNF-α induced insulin resistance (Pedersen et al., 2016). The release of anti-inflammatory cytokines may have a role in preventing chronic inflammatory disease

such as IBS. Also, low-grade inflammation and impaired intestinal integrity were observed in IBS warrants further investigation in exercise state. Intestinal cellular damage will release intestinal fatty acid binding protein (iFABP) to the blood circulation and its plasma concentration increases (March et al., 2017). Thus, we conduct at trial to investigate the effect of brisk walking exercise on immune function, intestinal fatty acid binding protein, fatigue and severity scores in irritable bowel syndrome patients.

CHAPTER 2: OBJECTIVE OF STUDY

2.1 General Objective

To investigate the effect of brisk walking exercise on immune function, intestinal fatty acid binding protein, fatigue and severity scores in patients with irritable bowel syndrome.

2.2 Specific Objectives

1. To compare the mean changes of immune function (blood immune cells, plasma IL-6, and TNF-α) between exercise and control group among IBS patients
2. To compare the mean changes of intestinal cell damage marker (i-FABP) between exercise and control group among IBS patients
3. To compare the changes of mean IBS-SSS between exercise and control group among IBS patients
4. To compare the changes of mean MFSI-SF score on fatiguability between exercise and control group among IBS patients

CHAPTER 3: STUDY PROTOCOL

Research title: The Effect of Brisk Walking Exercise on Immune Function, Intestinal Fatty Acid Binding Protein, Fatigue and Severity Scores in Patients with Irritable Bowel Syndrome: A Randomised Controlled Trial

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The Effect of Brisk Walking Exercise on Immune Function, Intestinal Fatty Acid Binding Protein, Fatigue and Severity Scores in Patients with Irritable Bowel Syndrome: A Randomised Controlled Trial

INTRODUCTION

Irritable bowel syndrome (IBS) is a common functional disorder that affect the intestines characterised by multiple symptoms which includes abdominal pain, cramping, diarrhoea and constipation (Longstreth et al., 2009; Holtmann, 2016). Risk factors for IBS includes food allergy, infectious pathogens, smoking, psychological and physical stress. (Klem et al., 2017; Nam et al., 2010). In Malaysia, the prevalence of IBS has been reported to be ranging from 6.8% to 15.8% (Tan et al., 2003; Rajendra & Alahuddin, 2004). A survey found 10.9% Malay ethnic subjects demonstrated IBS (Lee et al., 2012). Most recent report after a major flood disaster found that 17% of

flood victims developed IBS, and those who developed IBS, there was disturbed gut microbiota (dysbiosis) (Yusof et al 2017). In this post-flood study, there were more Plesiomonas and Trabulsiella reported in IBS, with effect size of 3.0, and these organisms were environmentally derived, presumably from flood waters during index exposure. Despite infection has been cleared, initial episode of acute gastroenteritis often results in relapsing IBS in the gastrointestinal tract and other organs, of which can cause significant reduction in quality of life due to systemic symptoms associated with IBS such as chronic fatigue syndrome and musculoskeletal pain (Peyton & Greene, 2014; Wang & Zhao, 2016; Undseth et al., 2016; Janssens, et al., 2015). Low-grade intestinal mucosal inflammation, with T-lymphocyte activation and pro-inflammatory cytokines, has been reported in IBS patients (Sundin et al., 2014; Martin-Viñas, & Quigley, 2016; Ohman & Simren, 2010; El-Salhy, 2015). Plasma intestinal fatty acid binding protein recently used as a marker for intestinal damage may be further warranted in IBS patients due to the low-grade inflammation damage to barrier (March et al., 2017; Sinagra et al., 2016; Liu et al., 2014). IBS patients have been encouraged to increase their physical activity level by walking at least 20 min or approximately 1.6 km per day (Chey et al., 2015). However, the effectiveness of the type and duration of exercise has yet to be established for treating IBS symptoms, although the general guideline for overall well-being of an adult has been recommended (Lacy et al., 2015 WHO, 2011). Most exercises for IBS in previous studies have been self-paced and performed at patient's convenience (Schumann et al., 2016; Rutten et al., 2017; Shahabi et al., 2016). Hence, there is a need to establish the effectiveness of exercise intensity and duration as an alternative method to treat IBS.

Assumption and Limitation of the Study

1. We assumed that the IBS patients will adhere to the exercise procedures regardless of exercise motivation level, personality and other external influences on their desire to complete the exercises.
2. We assumed that the mobile devices have been calibrated according to devices' settings (GPS) for recording exercise data that will be used to ascertain the compliance of the IBS patients to exercise.
3. We assumed the IBS patients in the control group resume their typical lifestyle and avoid physical activities.

IBS is defined as recurring abdominal pain or discomfort including altered defecation and absent of structural abnormalities (Brandt et al., 2009; Talley et al., 1991). The diagnosis of IBS is performed using symptoms-based criteria and were limited to IBS patients with red flags (Khan & Chang, 2010). There was a lack of research on the biomarkers of IBS to demonstrate the effectiveness of regular exercise used to manage recurrent IBS. Exercise has been shown to increase quality of life and reduce symptoms of IBS (Johannesson et al., 2011; Daley et al., 2008). Although exercise has been shown to reduce overall symptoms in IBS patients, the underlying mechanism of exercise in the management of relapsing IBS is unclear (Johannesson et al., 2011). The mechanism of exercise in treating IBS, particularly moderate and progressive type of exercise is unclear. Increased levels of immunemarkers such as interleukins (IL-6, IL-8, IL-10, IL-1β) and tumor necrosis factor (TNF) also suggests that low grade inflammation has a role in the development of IBS thus causing a relapsed IBS (Ohman & Simren, 2010). Most recently, plasma i-FABP has been used as a marker of intestinal cellular damage or injury (March et al., 2017). Hence, using an intestinal

injury biomarker may be beneficial in understanding the role of exercise in managing relapsed IBS. Also, physical activity may improve dysbiosis, restoring and promoting beneficial microbes in patients with IBS.

Literature review

Irritable bowel syndrome (IBS) can cause a general feeling of fatigue akin to chronic fatigue syndrome and musculoskeletal pain (Peyton & Greene, 2014; Wang & Zhao, 2016; Undseth et al., 2016). However, physically active IBS patients reported a reduction in fatigue and symptoms of IBS (Johannesson et al., 2011). Previous studies utilizing walking as a treatment intervention for IBS has shown positive effects on quality of life and reducing IBS scores but were limited (Ng et al., 2007; Bilski et al., 2014). In a previous randomised controlled trial (RCT), the study utilized intervention through phone call with a physiotherapist for advice regarding physical activity once or twice a month (Johannesson et al., 2011). Although the physical activity advice was relayed to IBS patients verbally in that RCT study, intervention such as this must be properly monitored for exercise intensity and duration to investigate the effectiveness of the intervention. Exercise duration and intensity have been shown to influence human functional outcomes. High intensity and prolonged exercise duration can negatively impact cardiac function and cause systemic stress (Stewart et al., 2016).

Dose-response relationship between exercise and intestinal function has been well investigated (van Wijck et al., 2011). Increased exercise intensity causes intestinal hypoperfusion and when prolonged can cause intestinal dysfunction. Splanchnic hypoperfusion, which often occurs in many pathophysiological conditions has caused intestinal barrier dysfunction and subsequently lead to multiple-organ dysfunction (Swank & Deitch, 1996). Critically ill patients such has those who had undergo major surgery, trauma or shock had their intestinal barriers compromised due to lack of blood flow to the splanchnic regions. As a results from lack of blood oxygen and nutrient

supply, intestinal injury that occur could potentially worsen the body's systemic inflammation (De-Souza & Greene, 2005). These effects can be seen in elite triathletes, marathon and ultra-endurance runners who experienced gastrointestinal bleeding, bowel ischemia, diarrhea and nausea among others (Peters et al., 1999).

Although the impact of physical exercise can be both harmful and beneficial to the gastrointestinal tract, regular moderate intensity exercise has been shown to generally improve the quality of life in IBS patients including condition such as depression and fibromyalgia which were commonly diagnosed in IBS patients (Whitehead et al., 2002). One study offered 40 minutes initial exercise consultations aimed at motivating IBS patients to take up exercise as well as sustaining their motivation, self-efficacy for exercise and overcoming barriers to becoming physically active (Daley et al., 2008). Exercise in as little as 4 weeks duration significantly improved pain symptoms of IBS patients (Colwell et al., 1998). Regular light-to-moderate intensity physical exercise intervention at least 12 weeks have also been recommended in previous studies (Daley et al, 2008; Maxton et al., 1992). However, maintaining exercise adherence is challenging with approximately 50% of people who begins a new exercise programme tends to drop-out within 6 months (Dishman, 1991). With the advent of the fourth industrial revolution (Industry 4.0.), new technologies have become greatly accessible for individuals to improve their way of life. Benefits include performing exercises at a convenient time without the need for a paid fitness coach and going to the gym. Customized exercise work out routines may be instructed via an artificial intelligence fitness coach smartphone application soon (Daws, 2018). Currently, there are various free smartphone applications with features for recording workout pace and duration.

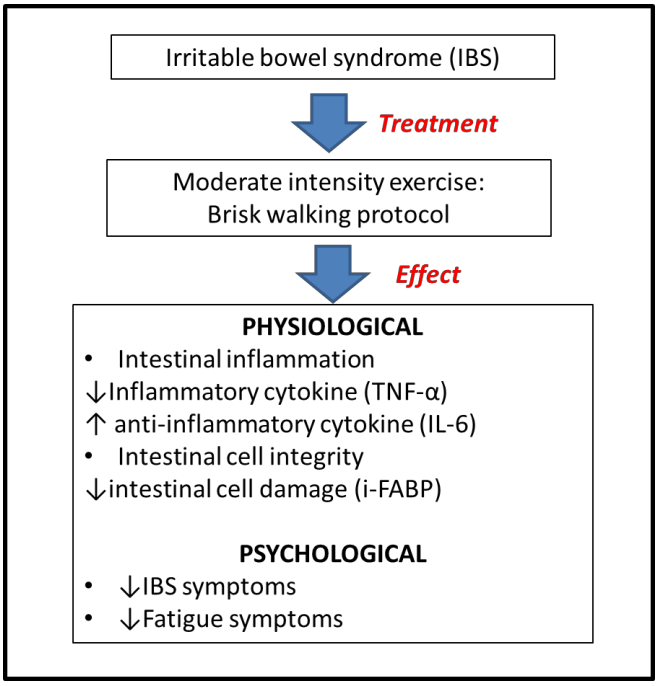
Treatment of IBS included combined lifestyle adjustments, dietary and pharmacological interventions (Khan & Chang, 2010). Physical activity or exercise seems to be effective, sometimes more than pharmacological agents that are used to treat symptoms. A study has shown that pharmacological bulking agents and loperamide alone were ineffective while antidepressants were moderate in efficacy (Henningsen et al., 2007). The therapeutic properties of progressive exercise have been said to focus on improving overall bodily function and fitness rather than alleviating the specific IBS symptoms (Henningsen et al., 2007). This statement was echoed from an earlier study, in which the effects of exercise on IBS showed a reduction of severe somatic symptoms, as demonstrated by a lower level of fatigue (Lustyk et al., 2001). A reduced feeling of incomplete evacuation after bowel movements and increased intestinal gas clearance were also reported following physical activities (Lustyk et al., 2001; Villoria et al., 2006; Dainese et al., 2004). Conversely, as mentioned previously, intense exercise can induce intestinal hypoperfusion injury (van Wijck et al., 2011) that may aggravate the symptoms of IBS patients, leading to intestinal dysfunction.

In addition, exercise has an immunomodulatory effect. Moderate, habitual exercise is anti- inflammatory and can be protective against chronic inflammatory diseases (Gleeson, 2007; Gleeson et al., 2011). The mechanism of the protective properties of exercise on IBS has been unclear. IBS patients who experienced episodes of relapse was found to have low grade inflammation with increases in circulating interleukins (IL-6, IL-8, IL-10, IL-1β) and tumor necrosis factor (TNF) (Ohman and Simren, 2010). It is not clear if the increase of these anti-inflammatory cytokine (IL-6, IL-10) expressions is a result from the increase of pro- inflammatory cytokines (TNF-α, IL-

8, IL-1 β). The increase in plasma concentration of IL-6 with response to exercise due to its release from the contracting muscles have been shown to inhibit TNF-α induced insulin resistance (Pedersen et al., 2016). Therefore, the release of anti-inflammatory cytokines may have a role in preventing chronic inflammatory disease such as IBS. Furthermore, low-grade inflammation and impaired intestinal integrity observed in IBS patients warrants investigation in exercise settings. During intestinal damage, intestinal fatty acid binding protein (i-FABP) is released into the blood circulation and its plasma concentration increases. Anti- and pro-inflammatory cytokine markers will be measured together with i-FABP marker measurements to understand their immune regulatory response to the impaired intestinal integrity in IBS.

Hence, moderate intensity exercise for 4-12 weeks seems to be an appropriate modality to manage IBS in conjunction with their usual symptomatic treatment (includes anti-spasmodic e.g. mebeverine, alverine citrate, anti-diarrheal (e.g. loperamide) or laxatives (e.g. psyllium, lactulose). Based on the global exercise recommendation guideline for 18-64 years of age (WHO, 2011), we postulate that moderate intensity walking for 30 minutes, 5 times a week will improve IBS symptoms in 4 weeks. We chose 4 weeks exercise intervention to encourage exercise participation and to maintain short-term exercise adherence to exercise in IBS patients who may be previously sedentary. However further investigation on the immunomodulatory effect of exercise in reducing low grade inflammation and prevent intestinal injury is warranted.

Conceptual framework



Research Question(s)

1. Does brisk walking exercise show any difference(s) on immune function (blood immune cells, IL-6 and TNF-α) among IBS patients?
2. Does brisk walking exercise show any difference in intestinal cells damage marker (i-FABP) among IBS patients?
3. Does brisk walking exercise show any difference (s) in the IBS severity scoring systems (IBS-SSS) among IBS patients?
4. Does the brisk walking exercise show any differences in the fatigability score (MFSI-SF) among IBS patients?
5. Which parameters has highest mean rating among IBS patients?

GENERAL OBJECTIVE

To investigate the effect of brisk walking exercise on immune function, intestinal fatty acid binding protein, fatigue and severity scores in patients with irritable bowel syndrome.

SPECIFIC OBJECTIVES

1. To compare the mean changes of immune function (blood immune cells, plasma IL-6, and TNF-α) between exercise and control group among IBS patients
2. To compare the mean changes of intestinal cell damage marker (i-FABP) between exercise and control group among IBS patients
3. To compare the changes of mean IBS-SSS between exercise and control group among IBS patients
4. To compare the changes of mean MFSI-SF score on fatigability between exercise and control group among IBS patients

Research design

This study is a randomised, controlled interventional trial. Participants will be randomly assigned into two groups: control and exercise groups. Measurements will be taken twice which is before and after 4 weeks of intervention period.

Study area

Hospital Universiti Sains Malaysia and the Exercise and Sports Science Laboratory, Universiti Sains Malaysia both located at Kubang Kerian, Kelantan.

Study population

Participants will be recruited among IBS patients attending the Hospital Universiti Sains Malaysia, Kubang Kerian, Kelantan (HUSM) for follow-up review. The sample size is calculated by using G*Power software version 3.9.1.2 based on a clinically significant improvement in the IBS-SSS using an effect size of 0.4, the power kept at 80% with a confidence interval of 95%, calculated sample size was 20 (Johannesson et al., 2015). A reduction of 50 in the IBS-SSS is adequate to detect a clinical improvement (Francis et al., 1997). Considering a 20% drop out rate, 24 participants are required in each group. Hence, a total of 48 participants will be recruited.

SUBJECT CRITERIA

Inclusion criteria for participation

1. Age 18 to 70 years (gender and age-matched)
2. IBS diagnosed with ROME III criteria on the initial visit, regardless of subtypes
3. Follow-up IBS patients (post-pharmacological treatment)
4. Sedentary (IPAQ category 1/Low)
5. Passed PARQ+ (Answered 'NO' to all general health questions)

Exclusion criteria for participation

1. Pregnant
2. Having organic gastrointestinal disorder – Ulcerative colitis, Crohn's or celiac disease.
3. Taking painkillers, intestinal relaxants, antibiotics, probiotics, antidepressants, anticholinergic or anti-diarrheal medications
4. Taking herbal supplements or other medications with potential effect on fatigue
5. Having cardiopulmonary disease
6. Hypertensive
7. Having musculoskeletal injuries which limit physical activity
8. Had bowel surgery
9. Diabetic
10. BMI> 30 kg/m2

Withdrawal criteria

1. Exercise intervention participant did not complete 80% of the exercise programme
2. Participants' requests to withdraw at any point of time without obligation

Medical Screening

Following approval from Human Research Ethics Committee Universiti Sains Malaysia, IBS follow-up patients will be approached and briefed regarding the current study objectives and procedures. Should they agree to participate in this study, they will be given an informed consent form to be signed and International Physical Activity Questionnaire (IPAQ) to be completed. Then, they will be screened by a medical doctor from HUSM for their eligibility. Judicious medical screening (clinical pathway for IBS) may include the following:

1. Cardiopulmonary test
2. Full blood count
3. Pregnancy test
4. Thyroid function test
5. Upper and or lower gastrointestinal endoscopy, if red flags are present
6. Stools test (FEME, C&S, parasites)
7. Hydrogen and methane breath test (glucose, fructose, lactose)
8. Fecal calprotectin

Sample size estimation

Participants will be recruited among IBS patients attending the Hospital Universiti Sains Malaysia, Kubang Kerian, Kelantan (HUSM) for follow-up review. The sample size is calculated by using G*Power software version 3.1.9.2 based on a clinically significant improvement in the primary measures, IBS-SSS, iFABP, IL-6, TNF-α using an effect size of 0.4, the power kept at 80% with a confidence interval of 95%, calculated sample size was 20 (Johannesson et al., 2015). A reduction of 50 in the IBS-SSS is adequate to detect a clinical improvement (Francis et al., 1997). Considering a 20% drop out rate, 24 participants are required in each group. Hence, a total of 48 participants will be recruited.

i. Variable IBS-SSS

t tests – Means: Difference between two dependent means (matched pairs)

Analysis: A priori: Compute required sample size

Input:	Tail(s)	=	Two
	Effect size dz	=	0.46
	α err prob	=	0.05
	Power (1-β err prob)	=	0.80
Output:	Noncentrality parameter δ	=	2.9092954
	Critical t	=	2.0226909
	Df	=	39
	Total sample size	=	40
	Actual power	=	0.8097619

ii. Variable: iFABP

t tests - Means: Difference between two dependent means (matched pairs)

Analysis: A priori: Compute required sample size

Input:	Tail(s)	=	Two
	Effect size dz	=	4.0909091
	α err prob	=	0.05
	Power (1-β err prob)	=	0.95
Output:	Noncentrality parameter δ	=	8.1818182
	Critical t	=	3.1824463
	Df	=	3
	Total sample size	=	4
	Actual power	=	0.9987607

ii. Variable: IL-6

t tests - Means: Difference between two dependent means (matched pairs)

Analysis: A priori: Compute required sample size

Input:	Tail(s)	=	Two
	Effect size dz	=	8.4200000
	α err prob	=	0.05
	Power (1-β err prob)	=	0.95
Output:	Noncentrality parameter δ	=	14.5838678
	Critical t	=	4.3026527
	Df	=	2
	Total sample size	=	3
	Actual power	=	0.9999702

iii. Variable: TNF-α

t tests - Means: Difference between two dependent means (matched pairs)

Analysis: A priori: Compute required sample size

Input:	Tail(s)	=	Two
	Effect size dz	=	46.9090909
	α err prob	=	0.05
	Power (1-β err prob)	=	0.95
Output:	Noncentrality parameter δ	=	11.9668965
	Critical t	=	4.3026527
	Df	=	2
	Total sample size	=	3
	Actual power	=	0.9991174

Sampling method and subject recruitment

After consent and screening, participants will be selected by block randomisation equally into either control group (n=24) or exercise treatment intervention group (n=24). Randomisation will be done by a researcher not involved in administering questionnaires and intervention procedures. A block size of 8 is chosen with possible combination of 4 C (control) and 4 T (treatment) participants, calculated as 6 blocks (CCCCTTTT, TTTTCCCC, CCTTCCTT, TTCCTTCC, CTCTCTCT, TCTCTCTC). Then, the blocks will be randomly chosen by drawing a number from a box to determine the selection of all 48 participants. Participants in the exercise group will undergo a 4-week brisk walking exercise programme while those in the control group will be requested to maintain their lifestyle.

RESEARCH TOOL

1. Body weight and height will be measured with a stadiometer incorporated weighing machine (707, Seca corporation, USA) (Ibrahim et al., 2017)
2. Body fat percentage (%BF) will be measured using a bioelectrical impedance analyser (TBF-410, TANITA, Japan) (Ibrahim et al., 2017)
3. Heart rates will be measured using a heart rate monitor and transmitter (V800, Polar, Finland) with intraclass correlation (ICC) >0.999 for supine measurement (Giles, et al., 2016)
4. Runkeeper mobile phone application (alternatively Pace Control) has been reported as the most commonly used application for monitoring physical activity (Turner-McGrievy et al., 2013). With the integration of accelerometer, gyroscope and Global Positioning System (GPS) in most of the current mobile phone devices, brisk-walking intensity showed high accuracy (90.1%) using a machine learning algorithm (K-Nearest Neighbour) for physical activity classification (Wu et al., 2012). Therefore, it is ecologically valid for participant to carry only one device that was commonly carried at present.
5. Blood analysis will be done using high-sensivity enzyme-linked immunoassay method commercially available kit (Biosource Int., USA), with inter-and intra-assay coefficients variation of <7% (Tsukui et al., 2000).
6. To use the Physical Readiness Questionnaire for Everyone (PAR-Q+) and Multidimensional Fatigue Symptoms Inventory-Short Form (MFSI-SF), a translation and cultural adaptation from the English to Malay version will be conducted based on the Principles of Good Practice and a pilot test of the questionnaires will be done on 5-8 relevant persons for cognitive debriefing as

described by Wild et al. (2005). This Principle of Good Practice guideline will include; preparation, forward translation, reconciliation, back translation, back translation review, harmonization, cognitive debriefing, review of cognitive debriefing results and finalization, proofreading and final report.

Operational definition

1. **Brisk walking** refers to low-moderate intensity of heart rate reserve (HRR) of 40-59 % target exercise intensity which correspond approximately to metabolic equivalents (METS) of 4-6 and is performed for 30 minutes per day, 5 days per week.
2. **Heart rate reserve (HRR)** is the target exercise heart rate (intensity) calculated as the difference between predicted maximum heart rate and resting heart rate before exercise. The HRR formula is given as follows: HRR = predicted HRmax – HRrest

DATA COLLECTION METHOD

After screening, participants having met all the inclusion criteria without any exclusion criteria will be recruited (N=48). Participants will be requested to abstain from caffeine intake throughout the study. Pre-intervention and post-intervention (after 4 weeks of exercise) measurements will be conducted.

Physiological data

Height, weight, BMI, body fat percentage (%BF), body fat mass, muscle mass, total body water (TBW), will be measured using an integrated bioelectrical impedance and height/weight scale. Resting blood pressure and resting heart rate will also be measured.

Blood sampling

Eight (8) ml of blood sample from the antecubital vein will be collected by an investigator trained in venipuncture. The blood will be drawn into an EDTA tube for blood count (FBC) analysis with a haematology analyser. The remaining of the blood will be centrifuged and its plasma obtained to analyse for iFABP, IL- 6 and TNF-α by enzyme-linked immunoassay (ELISA) method. A -80 freezer will be used to store the plasma samples before analysis.