MINISTRY OF HIGHER EDUCATION

SINGLE DISCIPLINARY PROJECT

APPLICATION FORM
FUNDAMENTAL RESEARCH GRANT SCHEME (FRGS)
Skim Geran Penyelidikan Fundamental
(Pindaan 1/2012)

JABATAN PENDIDIKAN TINGGI
KEMENTERIAN PENGAJIAN TINGGI

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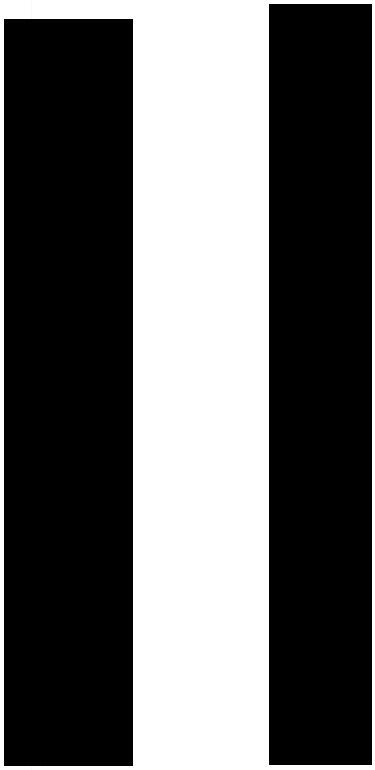

Title	Grant Name	Role	Progress (%)	Status	Duration	Start Date	End Date
Big Data Detection of Cognitive Frailty	LRGS	Member	34	In Progress	5 years 9 months	01/12/2019	31/08/2025
Unravelling the Secrets Behind the Links Between Obesity, Sarcopaenia and Falls in Older Persons (OSFOP)	FRGS	Member	39	In Progress	3 years 9 months	01/09/2019	31/05/2023

C(x). Academic publications that have been published by the project leader in the last five years

Title	Name of Journal	Year
Impact of Knee Pain on Fear of Falling, Changes in Instrumental Activities of Daily Living, and Falls Among Malaysians Age 55 Years and Above	Frontiers in Public Health	2020
Falls, frailty, and metabolic syndrome in urban dwellers aged 55 years and over in the Malaysian elders longitudinal research (MELoR) study - a cross-sectional Study	Postgraduate in Medicine	2020
Perceptions of Family Physicians about Falls Risk Screening, Falls Risk Assessment, and Referral Practices for Falls Prevention in Malaysia	Topic in Geriatrics	2019
Could Obesity be Linked to Falls in Older Adults?	Biomedical Journal of Science & Technical Research	2019
Ethnic differences in the Prevalence, Socioeconomic and Health Related Risk Factors of Knee Pain and Osteoarthritis Symptoms in Older Malaysians	Plos One	2019
Anticholinergic medications and long-term risk of hospitalization with falls: EPIC-Norfolk study	Drugs & Aging	2019
Factors Determining the Increased Risk of Falls in Individuals with Knee Pain in the Malaysian Elders Longitudinal Research (MELoR) Study	Frontiers in Medicine	2019
Reliability and Validity of the Short Falls Efficacy Scale International in English, Mandarin, and Bahasa Malaysia in Malaysia.	The International Journal of Aging and Human Development	2018
Vitamin D Deficiency is Associated with Ethnicity and Knee Pain in a Multi-ethnic South East Asian Nation: Results from the Malaysian Elders Longitudinal Research (MELoR).	International Journal of Rheumatic Diseases	2018
. Individually-Tailored Multifactorial Intervention to Reduce Falls in the Malaysian Falls Assessment and Intervention Trial (MyFAIT): A Randomized Controlled Trial,	PloS One	2018
Effect of Modified Otago Exercises on Postural Balance, Fear of Falling, and Fall Risk in Older Fallers With Knee Osteoarthritis and Impaired Gait and Balance: A Secondary Analysis	PM&R	2017
INFLUENCE OF HIP AND KNEE OSTEOARTHRITIS ON DYNAMIC POSTURAL CONTROL PARAMETERS AMONG OLDER FALLERS	Journal Medical Rehabilitation	2017
The Modified Otago Exercises Prevent Grip Strength Deterioration Among Older Fallers in the Malaysian Falls Assessment and Intervention Trial (MyFAIT)	Journal of GERIATRIC Physical Therapy	2017
The Mediating Role of Psychological Symptoms on Falls Risk Among Older Adults with Osteoarthritis	Clinical Intervention in Aging	2017
Validation of the CASP-19 Quality of Life Measure in Three Languages in Malaysia.	Journal of Tropical Psychology	2017
Vitamin D Deficiency Is Associated With Ethnicity And Knee Pain Severity In A Multi-ethnic South East Asian Nation	Age and Ageing	2017

C(xi). Executive Summary of Research Proposal

(Please include the problem statement, objectives, research methodology, expected output/outcomes/implication, and significance of output from the research project)

Diabetes Mellitus (DM) accentuates pain, morphological change and physical deterioration in Osteoarthritis (OA). However, diabetes clinics primarily address glycaemic, blood pressure and lipid control, as well as screening for microvascular complications of retinopathy, peripheral neuropathy and nephropathy partially due to the absence of convenient tests for OA. The complex pathophysiological processes underlying DM-related OA also remains poorly understood. There is, therefore, an urgent need for the identification of novel biomarkers for early DM-related knee OA utilizing enhanced techniques from which the intricate cellular and biological processes could be unraveled. A total of 150 participants in the age 50 years old and above and diagnosed with Diabetes Mellitus will be recruited into this study. They will be divided into 3 groups according to knee OA status; 50 participants as non-OA,

50 participants at early OA, and remaining 50 participants at late stage of OA. They will be matched according to age and sex. Metabolomic analysis will be conducted using urine sample to compare the metabolomic profile between these three group and will be correlated with the MR imaging quantification and other serum biochemical markers found in ELISA assay. Findings from this study will improve early detection of OA in individuals with DM in which reduce the need for exposure to ionizing radiation from X-rays. In addition, identified novel putative biomarkers will help in monitoring the effectiveness of OA treatment especially in development of Disease-modifying osteoarthritis drugs (DMOADs) which lead to precision medicine.

C(xii). Detail Planning

(a) Research background

1. Problem Statement

Diabetes Mellitus (DM) accentuates pain, morphological change and physical deterioration in Osteoarthritis (OA). However, diabetes clinics primarily address glycaemic, blood pressure and lipid control, as well as screening for microvascular complications of retinopathy, peripheral neuropathy and nephropathy partially due to the absence of convenient tests for OA. The complex pathophysiological processes underlying DM-related OA also remains poorly understood. There is, therefore. an urgent need for the identification of novel biomarkers for early DM-related OA utilizing enhanced techniques from which the intricate cellular and biological processes could be unraveled.

2. Hypothesis

This study hypothesizes that urine metabolomic profile in individuals with DM at early OA stages is unique from those without OA and at the late stage of OA. Next, urinary metabolites in individuals with DM correlate with blood inflammatory markers and MR imaging quantification and lastly, metabolites found in urine sample in individuals with DM has high discriminatory power of the different stages of OA.

3. Research Questions

- What are the metabolites found in Individuals with DM, without OA, with early OA and with late OA?
- How do urinary metabolites in Individuals with DM correlate with blood inflammatory markers and MR imaging?
- What is the discriminatory ability of the metabolites for different stages of OA?

4. Literature Reviews

The Increasing Prevalence of Diabetes Mellitus among Older Malaysian
Diabetes mellitus (DM) affect more than 470 million people worldwide.(Lin et al., 2020) According to the National Health and Morbidity Survey (NHMS) in year 2019 by the (Institute of Public Health, Malaysia), 18.3% Malaysian adults (aged 18 years and above) suffered from diabetes with 49% being undiagnosed. This indicates that nearly one in five Malaysian has diabetes with Indians having the highest prevalence of DM (24.9% in 2011 and 19.9% in 2006), followed by Malays (16.9% in 2011 and 11.9% in 2006), and Chinese (13.8% in 2011 and 11.4% in 2006). There is also a staggering increase observed in the prevalence of DM among older people. The prevalence of DM has quadrupled in 13 years, from 16% in year 1996 with a spike to 43.4% in year 2019 (Health, 2020)

Early Osteoarthritis (OA)
OA is the most common type of joint problem and affects mainly the knee and hip joints. It has also been reported to be one of the significant contributors for the global disability burden which account for 19.3% of Disability Adjusted Life Years (DALYs) and Years Lived with Disability (YLDs) worldwide (Safiri et al., 2020). In Malaysia, a population-based study among 1226 participants demonstrated that around 33.3% of Malaysian older adults were reported to have some kind of joint pain particularly in the knee (Mat et al., 2019). Late diagnosis of OA and inapparent of symptoms in subclinical stage of OA hampered the early management of OA. Intervention is invariably delayed until severe, and maybe even irreversible, structural damage has occurred. Thus, it is crucial to detect OA as early as possible. Advance technology on imaging such Magnetic resonance imaging (MRI) is a useful imaging biomarker for assessing early phase of knee OA. The Framingham osteoarthritis study suggested that the prevalence of any abnormality was seen in 89% of participants without radiographic abnormalities. These findings suggest that MRI can detect features suggestive of knee osteoarthritis that cannot be visualized on conventional radiographs. In addition, biochemical markers are also frequently used to assess disease activity precisely and quantitatively. There are reports regarding the availability of serum biomarkers to evaluate synovitis at an early phase and to predict OA progression. Interleukin-6 (IL-6) and keratan sulfate levels increase in the early phase of knee OA accompanied by knee pain. However, there is still paucity in identification of novel biomarkers that can help in diagnosing early OA in patients with specific OA phenotype.

Preliminary results (unpublished data)
Harmonized data from three cohort studies from the Malaysian older people MELoR, TUA and PEACE with sample size of 6095 showed that older persons with DM had 20% increased odds of having some sort of joint pain with odds ratio; 1.20 (1.06-1.37). This preliminary finding could pave a way for future studies on the understanding the nature of diversified OA phenotype such a 'Diabetes-induced-OA' and in the long run contribute to precision medicine. The cross-sectional analysis among participants from the MELoR cohort showed that there is a significant association between diabetes and falls which was partially mediated by presence of knee pain (Figure 1). This is suggesting that individuals with diabetes are more likely to fall due to joint pain namely in the knee. This finding is important in directing population-based strategies in addressing falls and joint pain within a population with increasing diabetes incidence.

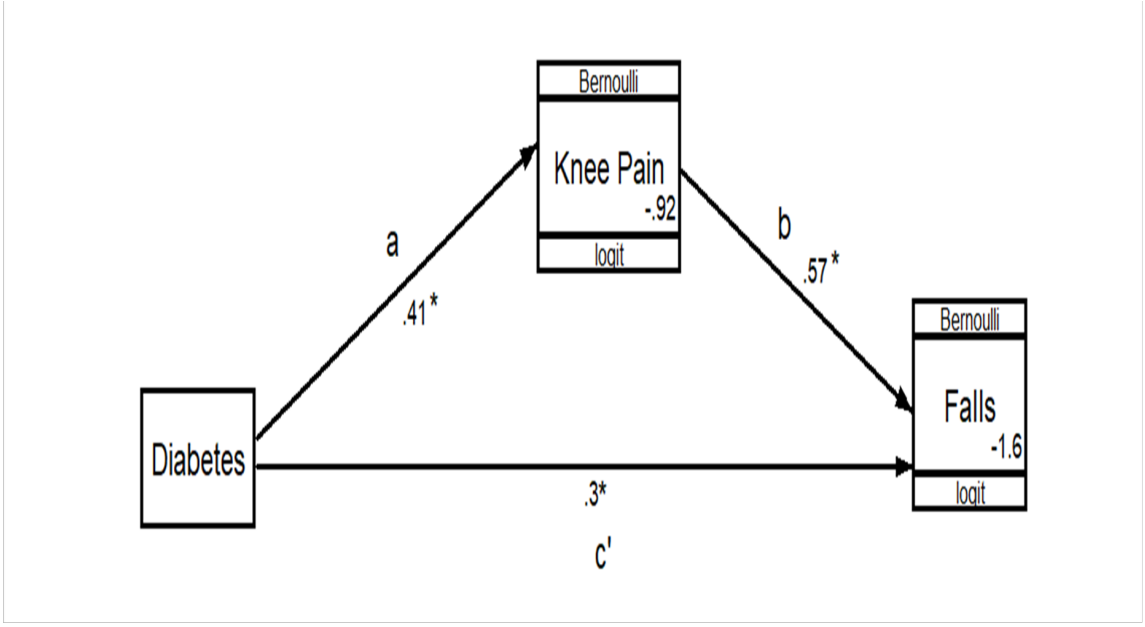

Figure 1: Standardized regression coefficient in GSEM model, *p<0.05:

Path *a*: β coefficient, SE (95% CI) = 0.413, 0.123 (0.172-0.655)
Path *b*: β coefficient, SE (95% CI) = 0.569, 0.1415 (0.292-0.846)
Path *c'*: β coefficient, SE (95% CI) = 0.295, 0.1398 (0.021-0.569)

Diabetes Mellitus as predictor for OA

Presence of diabetes is believed to accelerate the progression of OA and further complicate the management of OA. A new OA phenotype that link these two diseases has been highlighted as 'Diabetes-induced-Osteoarthritis' previously . (Berenbaum, 2011). Berenbaum (2011) proposed that diabetes led to OA through inflammaging process. This premise was however yet to be proven. From epidemiological studies, the prevalence of OA among people with DM and DM among people with OA were 29.5±1.2% and 14.4±0.1% respectively. Moreover, OA and DM were significantly associated in which the overall risk of OA in people with DM was 1.46 (1.08 to 1.96) and that of DM in people with OA was 1.41 (1.21 to 1.65)(Louati, Vidal, Berenbaum, & Sellam, 2015).In contrast, , a recent meta-analysis on 295 100 participants, it was suggested that diabetes is not the significant risk factor for OA but emphasized the role of obesity as the confounding factor (Khor, Ma, Hong, Hui, & Leung, 2020). This indicates that the existing evidence on the independent associations between DM and OA is conflicting and remains unclear. Nevertheless, as reported, these studies were mainly from western population and further research among Asian population is warranted.
<IMAGE818597>

Potential Pathophysiological DM-related-OA.

Inflammation and oxidative stress have been suggested as the cause for the progression of OA in people with DM (Courties & Sellam, 2016). Further cartilage degeneration and joint inflammation causing enrichment of advanced glycation end products (AGE) and matrix stiffening preventing optimal cushioning of the joint is aggravated by diabetes. This process leads to a worsening in OA symptoms resulting in physical inactivity and weight gain. As a consequence, a vicious cycle that maintains the metabolic dysregulation and increases joint symptoms. In addition, pain may also arise from the frequent complications of diabetic polyneuropathy (Schwarz, Mrosewski, Silawal, & Schulze-Tanzil, 2018; W. Zhang et al., 2017)

The traditional approach in identifying the biomarkers such ELISA assay is however limiting the ability to understand DM-related-OA pathophysiology as a whole (Sakamoto et al., 2018) due to the tremendous heterogeneity in the disease process. The cytokines identified are rather unspecific to OA as it is also commonly found in other kind of inflammation. Moreover, multiple tests on potential biomarkers are required as no single biomarker can reflect the breadth of temporal and pathological processes associated with OA.

Recent developments in the field of metabolomics therefore provide a new array of tools to fingerprint or to profile in which allows a large number of small-molecule metabolites from body fluids or tissues to be detected quantitatively in a single step. A numbers of studies has been conducted utilizing this approach in people with OA and DM separately. As an example, in the study by Zhai G et al. (2010) proposed that the serum ratio of branched-chain amino acids to histidine is a potential biomarker for with knee OA (Tootsi et al., 2020; Zhai et al., 2010). While, others proposed metabolites such as hydroxybutyrate, pyruvate, creatine/creatinine, glycerol, glycolate, hippurate, histidine (in early OA), histamine (in latter OA), trigonelline, aconitic acid, isocitric acid, and citric acid to be increased in urine samples among people with OA (Qingmeng Zhang, Li, Zhang, Yang, & Chen, 2015). While, people with DM is found to have increased in Valine, alanine, γ-aminobuthyrate, betaine, citric acid, trimethylamine-N-oxide in their urine (Tam et al., 2017).

Nevertheless, there are still gaps in the knowledge, as in none of the available studies , the the characteristic of individuals with both DM and OA have been profiled to understand the mechanism that led DM to OA. Understanding how components of this systemic metabolic disarrangement interact may help find a shortcut to detect a metabolic target early in OA pathophysiology. This notion is

however not new, it has also been proposed by Zhai G in year 2019 (Figure 2). In addition, there is also a need of including imaging modalities in such studies to fill up the loopholes on understanding how biomarkers are related to the morphological changes in early OA. This information is important in understanding and improving early OA diagnosis and management.

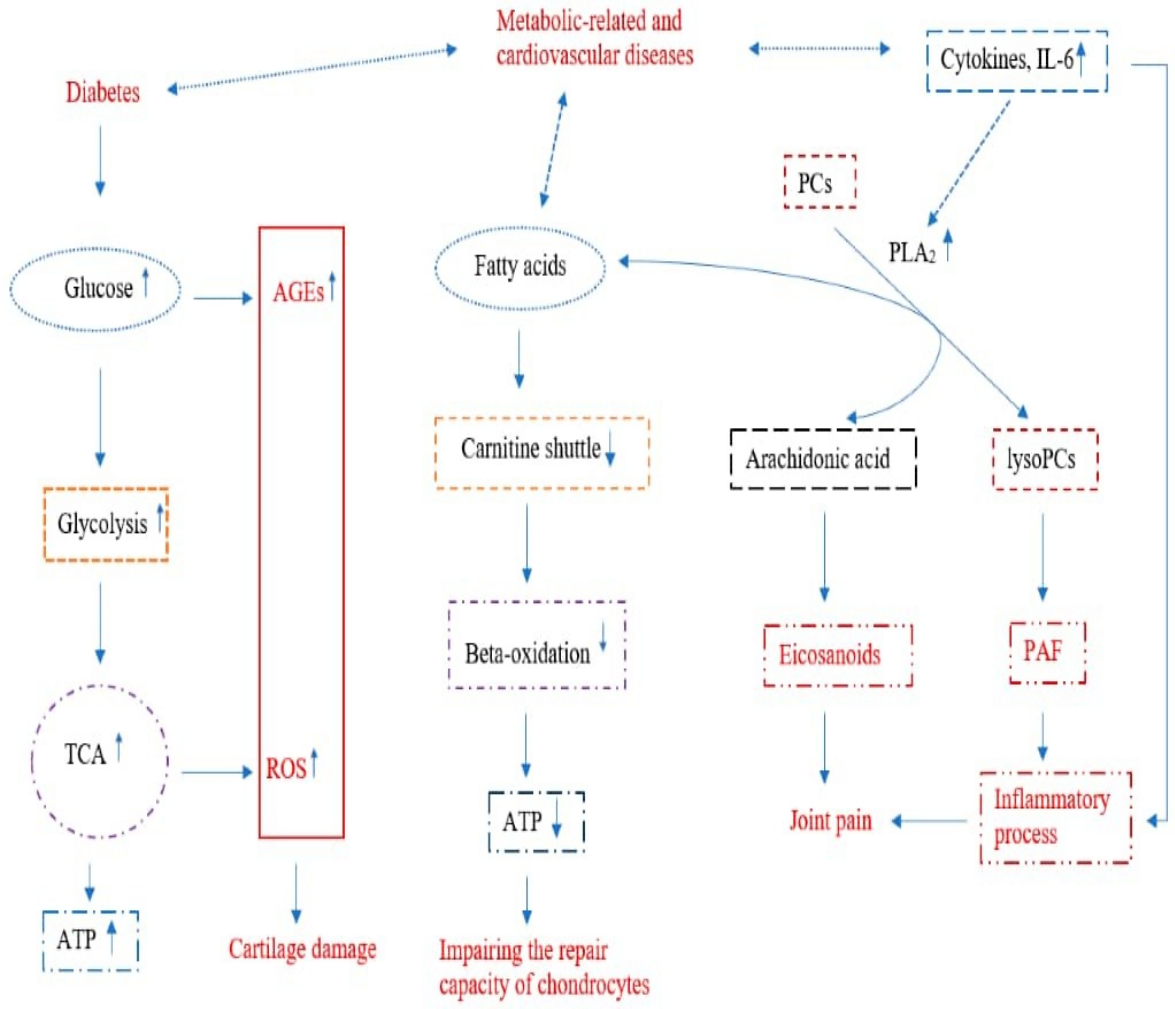

Figure 2: Chart of the metabolic alterations in osteoarthritis (OA) reviewed in this article. Arrows with dash line indicates possible relationships. TCA—tricarboxylic acid cycle; ATP—adenosine triphosphate; PC—phosphatidylcholine; lysoPC—lysophosphatidylcholine; PAF—platelet activating factor; PLA2—phospholipase A2; IL-6—interleukin 6. (Zhai G, 2019)

Early detection of OA in Diabetic Patient

In Malaysia, Diabetes clinics primarily address glycaemic, blood pressure and lipid control, as well as screening for microvascular complications of retinopathy, peripheral neuropathy and nephropathy. While OA patient with DM has been reported to have higher pain intensity, poorer physical performance, and lower QoL as compared to those without DM, there is no screening test available for risk of OA due to the absence of convenient test. Thus it is crucial to improve early detection of OA method in this high risk population. Early detection may help tailor early treatment such life style intervention and weight management which might be beneficial in slowing OA progression in reducing pain, and maintaining or improving function especially in population with this specific OA phenotype (Diabetes-related-Osteoarthritis).

5. Relevance to Government Policy (if any)

Considering the increase in ageing population, prevalence of DM and OA, this study findings may provide important insights of the pathophysiological between DM and early OA. Early detection of OA will then facilitate tailored OA prevention and management strategies that will support the Shared Prosperity Vision 2030. In addition, in line with one of the 30 National STIE niche areas for 10 socio-economic drivers (Ministry of Health Malaysia, 2019) which is precision medicine, the novel biomarkers may help in developing targeted antibody therapy. Early detection of OA through urine biomarkers will reduce the need for exposure to ionizing radiation from X-rays.

(b) References

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(c) Objective(s) of the Research

General objective:
1- To identify novel putative biomarkers for early OA by metabolome analysis in individuals with Diabetes Mellitus

Specific objective:
1- To compare urine metabolomic profiles among non-OA, early OA and late OA in individuals with Diabetes Mellitus
2- To correlate urine metabolites with serum level inflammatory markers, and morphological changes as quantified by MRI
3- To study the discriminatory power of the identified urine metabolites on different stages of OA in individuals with Diabetes Mellitus

(d) Methodology:

1. Description of Methodology

Study design
This is a cross-sectional comparative study of individuals with DM at different stages of OA

Participants
Participants attending Diabetic clinic in PPUKM and potential participants via phone interview will be recruited into this study.

Inclusion criteria
Individuals in the age of 55 years or over, have body mass index (BMI) in range 21-25, diagnosed with type 2 diabetes mellitus.

Exclusion criteria
The exclusion criteria were clinically diagnosed dementia (International Classification of Diseases, 10th Edition (ICD-10) definition), severe physical disabilities, major psychiatric illnesses, psychosis or brain damage, or contraindication to MRI, other type of arthritis; rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis. post-meniscectomy OA, femoro-patellar OA, Genu varum/valgum, diagnosed with type 1 diabetes and had joint prosthesis.

Cognitive Assessments
Participants' attention and concentration will be assessed using the coloured trails test or equivalent. Further detailed cognitive performance will also be conducted using a locally validated tool such as the MoCA or VCAT.

Anthropometric

Height and body weight will be measured using a height stadiometer (SECA™ 220, Hamburg, Germany) and calibrated weighing scale (SECA™ 769, Hamburg Germany) respectively. Body Mass Index (BMI) will be calculated using the formula: weight[kg] / height[m2]. The waist and hip circumferences are measured in centimetres with participants standing position using a tape measure. The waist circumference is obtained with the tape measure was placed at approximately midpoint between the lower margin of the last palpable rib and the superior border of the iliac crest while the hip circumference is measured at the widest part of the buttocks

Definition of Osteoarthritis

All participants will be assessed for presence of lower extremity knee OA (i.e. either one or both sides) during the 1st visit for confirmation. Participants will be defined as having self-reported knee OA if they answered 'yes' to the following question, "Have you ever been told by a doctor that you have or have had knee osteoarthritis?". Radiographic OA will be classified using Kellgren-Lawrence grading. The clinical diagnosis of knee OA will also be made by a rheumatologist blinded from clinical data based on the American College of Rheumatology criteria, using both history and physical examination findings. Clinical diagnosis of knee OA required the presence of knee arthralgia plus any three of: (i) age over 50 years; (ii) morning stiffness; (iii) crepitus on active motion on at least one knee, (iv) body tenderness on at least one knee, (iv) body enlargement in at least one knee, (v) no palpable warmth of synovia in both knees.

Severity of OA symptoms

If OA will be identified using any of the three diagnostic methods, the severity of OA symptoms (subscale and categorized) will be determined using the Knee Injury and Osteoarthritis Outcome Score KOOS. Three language versions (English, Malay and Chinese) of the KOOS questionnaire will be provided to cater for the different ethnic groups of Malaysia. In addition, VAS pain assessment will also be conducted. Pain will alse be measured using DN4 and PainDetect. Homunculus will be added in which the investigator will be able to tick the other symptomatic sites to measure the burden of OA.

Classification criteria for early OA and late OA of the knee.

Early knee OA will be defined based on the classification criteria proposed by Luyten. The classification criteria for early knee OA are as follows: A) patient-based questionnaires, the knee injury and osteoarthritis outcome scores (KOOS) (two of the following needed to score "positive" (i.e.,≤85%): pain (9 items), symptoms (7 items), activities of daily living (ADL) (short version, 7 items), and knee-related quality of life (QOL) (4 items)); B) clinical examination (at least one of the following needed to present: joint line tenderness or crepitus of the knee); C) radiographs, KL grade zero and one at the standing, extension, and weight-bearing positions. Based on the above criteria, subjects with KL grade 0/1 will be classified into non-OA and early knee OA. Those with KL-grade 3-4 will be classified as late OA.

Definition of Diabetes

Type 2 diabetes will be defined as fasting hyperglycaemia (serum fasting glucose > 6.66 mmol/l), HbA1c > 48 mmol/mol (6.1%) or self-reported physician diagnosed diabetes. Serum fasting glucose and HbA1c concentrations will be measured using standardized laboratory methods. In addition, self-reported diabetes by participants will be verified by the medications taken. Microcirculation complications (retinopathy, peripheral nerves, nephropathy) will also be collected.

Urine metabolites profiling

Urine samples will be collected early in the morning in 60-mL urine collection bottles for metabolomic analysis. Urine samples will be then centrifuged for 3000 rpm for 10 min and transferred into multiple aliquots of 1.5-mL sterile microcentrifuge tubes. The microcentrifuge tubes will be sealed tightly and stored at −80 ◦C until being used for metabolomic analysis. The preparation of urine samples for metabolomic analysis by 1H-NMR will be performed manually. After being thawed at room temperature, 400 µL of the urine sample will then mix with 200 µL of 0.3 M sodium phosphate buffer (1 mM TSP (sodium trimethylsilyl [2,3,3,3-2H4]propionate) and 20% D2O) (pH 7.4) in 1.5 mL microcentrifuge tubes, and centrifuge at 9600 rpm for five minutes at 4 ◦C. A total of 550 µL of the sample will be transferred into a 5-mm NMR tube. The TSP will act as internal chemical shift reference, and D2O as lock signal for the NMR spectrometer.

NMR Acquisition

The NMR spectra will be acquired using a Bruker Ascend 600 MHz NMR Spectrometer (Bruker Biospin, Rheinstetten, Germany). The NMR experiments will be obtained at the temperature of 26.85 ◦C. Before measurement, each sample will be loaded into the prop and the temperature was calibrated and kept constant for 3 to 5 min. In order to observe the dynamic range of metabolites concentrations efficiently, the water signal will be suppressed by running 1D nuclear Overhauser enhancement spectrometers (NOESY)-presat experiments. Standard one-dimensional (1D) 1H-NMR spectra will be acquired using a single 90◦ pulse length experiment with water presaturation using a relaxation delay of 2 s. Each NMR spectrum baseline correction, phasing, and chemical shift calibration will be done. Then, all spectrums will be binned to 0.04 ppm wide segments between 0.00 and 10.0 ppm. All pre-processing steps will be completed using Chenomx NMR Suite Professional software version 8.3 (Chenomx Inc., Edmonton, AB, Canada). The spectrum regions of water (δ = 4.8 ppm) and urea (δ = 5.8 ppm) will be removed from the analysis for all groups in order to prevent baseline effects of variability in the suppression of the water resonance and the nonquantitative contribution of urea. Each NMR variable will be normalized to the total area under peak curves in order to allow a spectrum-to-spectrum comparison.

Urinary Metabolites Identification

Urinary metabolites will be assigned by comparing spectrum obtained from NMR with the chemical shifts and peak shapes of standard compounds from internal database of reference spectra in Chenomx Profiler software version 8.3 (Chenomx Inc., Edmonton, AB, Canada). These metabolites will be then compared with NMR spectral data, i.e., those available in Human Metabolome Database (http://www.hmdb.ca) and along with the existing NMR-based metabolomic literature.

ELISA assay of serum biomarkers

Presence of inflammations will be assessed through level of inflammatory markers in blood routine test and multiplex ELISA test. Fasting blood samples will be collected in the morning after a 12-h fast for routine screening blood tests during baseline assessment. Data on C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and plasma viscosity (PV) will be collected through blood routine test. Multiplex ELISA assay will be conducted using selected biomarkers for Diabetes-related-Osteoarthritis such cytokines (interleukin-1β [IL-1β]), tumor necrosis factor-α (TNF-α), radical oxygen species, AGEs, and prostaglandins.

MRI imaging of the knee

MRI acquisition

Magnetic Resonance examinations will be performed on a clinical 3.0 Tesla Signa® HDx MR Systems (GE Healthcare, Milwaukee, Wisconsin, USA) equipped with a commercial circumferential knee coil for the knees and body coil for the thighs. Standard knee protocol will be used which includes Axial 3D fast imaging employing steady-state acquisition (3D FIESTA), Sagittal 3D FIESTA and Coronal proton density-weighted images. Axial 3D FIESTA with imaging parameters of minimum full TE, 32 mm field of view (FOV), 384 x 256 matrix, 2mm/0 (slice thickness/interslice gap) covering all articular cartilage plates in the knee, scan time of 1min 51s. Sagittal 3D FIESTA images with minimum full TE, 22mm FOV, 384 x 256 matrix, 2mm/0 (slice thickness/ interslice gap) will be obtained over a scan time of 3mins 20s. Coronal proton density-weighted image with 3640ms TR, 41ms TE, 32mm FOV, 512 x 256 matrix, 3mm/1mm (slice thickness/interslice gap) will also obtain, scan time of 3mins 17s. This will follow by the thigh protocol, in which coronal localizer images will be used to delineate the distal femoral epiphyses and acquisition was started 10cm proximal to the distal femoral epiphysis and extended proximally. Axial fat suppressed T2-weighted with 2000ms TR, 100ms TE, 35mm FOV, 512 x 256 matrix, 5mm/0 (slice thickness/interslice gap) will be obtained, scan time of 2mins 32s.

MRI Assessment

Two board –certified radiologists will be evaluated the MR images, with at least 5 years of musculoskeletal imaging expertise. Both will be blinded to the clinical information. All images will be scored using the Whole-Organ Magnetic Resonance Imaging Score (WORMS) by consensus. The WORMS grading will require 10-15 extra minutes of reporting. WORMS is a semi-quantitative assessment of knee OA features published by Peterfy et al. in 2004.(Peterfy et al., 2004) It has been an accepted standard scoring method widely used in epidemiologic studies and clinical trials over the years[10-13]. WORMS uses a sub-regional division of the knee compartments and covers the following features: cartilage, subchondral bone marrow lesions, subchondral cysts, osteophytes, bone attrition, meniscal status, and a combined effusion-synovitis score, as well as collateral and cruciate ligaments. Periarticular features are also evaluated, such as meniscal and popliteal cysts, periarticular bursitis, and loose bodies. As adapted from Peterfy et al, the knee was divided into 15 regions subdivided by anatomical landmarks in the fully extended knee. Both knees of each participant will be graded. Osteoarthritis features will be scored according to the WORMS index. As our study population will consist of older participants, we will apply a more stringent WORMS-based threshold to exclude marginal changes. For cartilage and osteophytes, which had a wider 8-point score, a grade of >2 is considered abnormal. Grade of >1 is considered abnormal for other features (bone marrow abnormality, bone cysts, and bone attrition). For meniscal lesions, ligamentous lesions, joint effusion, and muscle atrophy, a grade of > 0 is considered abnormal.

Functional Performance

This will be assessed using the timed-up and go test (TUG), gait speed and handgrip strength. The participant will be instructed to rise from a standard chair with arms, walk at their normal speed using their usual walking-aid and regular footwear, to a marker at three metres away from the front legs of the chair, turn round and walk back to the chair and sit back down again. The TUG time is considered the time between the participant's back leaving and touching the back of the chair. The handgrip strength test will be performed using a calibrated handgrip dynamometer (Jamar Plus+, Sammons Preston, Illinois, USA) to test the maximum isometric strength of the hand. Participants are required to hold the dynamometer with elbow flexed at 90 degrees held by the side of the body, while seated.

Three measurements are obtained from the dominant hand. The average grip strength measured in kilograms are considered in subsequent analyses.The prayer sign test for assessing the cheiroarthropathy will aldo be conducted.

Physical Activity level

Physical activity will be assessed with the Physical Activity Scale for the Elderly (PASE). It is a validated 12-item self-administered document that is designed to measure the amount of physical activity undertaken by individuals over the age of 65. The PASE assesses the types of activities typically chosen by older adults (walking, recreational activities, exercise, housework, yard work, and caring for others. It uses frequency, duration, and intensity level of activity over the previous week to assign a score, ranging from 0 to 793, with higher scores indicating greater physical activity. This tool has been recommended to be used in assessing physical activity in older people due to high validity (Sattler et al., 2020). In addition IPAQ questionnaire will be using to assess physical activity level.

Psychological status and Quality of Life

Depression, anxiety and stress will be evaluated using depression, anxiety, and stress scale (DASS21) on four-point Likert scales with the score of zero indicating "does not apply to me at all" and the score of three indicating "applies to me all or most of the time". Each domain score will be obtained by summing up the scores of the seven individual items relevant to each domain which will be then multiplied by two to obtain a total maximum score of 42 for each domain. A higher score will indicate higher level of anxiety, stress or depression. The total scores of each domain will be used for subsequent analysis. Validated translated versions of the DASS-21 is available in Bahasa Malaysia and Traditional Chinese. Quality of life will be assessed with the locally validated 12-item Control, Autonomy, Self-realization and Pleasure questionnaire (CASP-12).

The sample size calculations were performed using the G*Power 3.1 software (Dusseldorf, Germany) based on our hypothesis in which early OA ahs significant lower pain intensity as it is patient-driven outcome. A sample-size of 50 Early OA and 50 late OA will provide 80% power to detect significant differences in pain intensity between early OA and late OA, assuming 50% of the OA

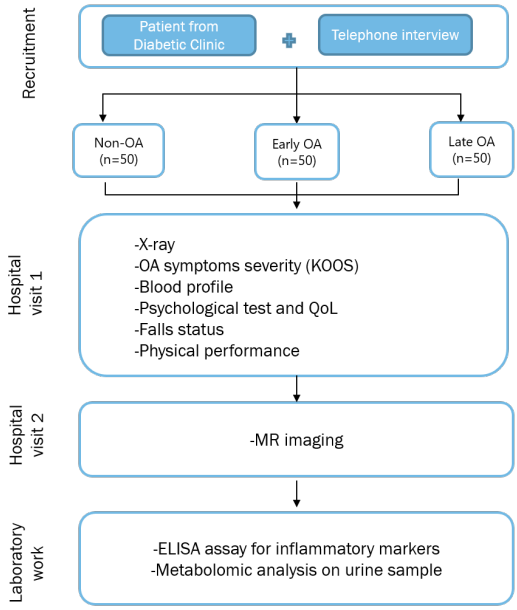
population fulfil criteria. Thus, total of 150 participants will be recruited.

T tests - Means: Difference between two Independent means (two groups)
Analysis: Means: Difference between two independent means
Input:
Effect size d = 0.8
α err prob = 0.05
Power (1-β err prob) = 0.80
Allocation ratio N2/N1 = 1
Output: Noncentrality parameter δ = 4.00
Critical t = 2.154
Df = 98
Sample size group 1 = 50
Sample size group 2 = 50
Total sample size = 100
Actual power = 0.96

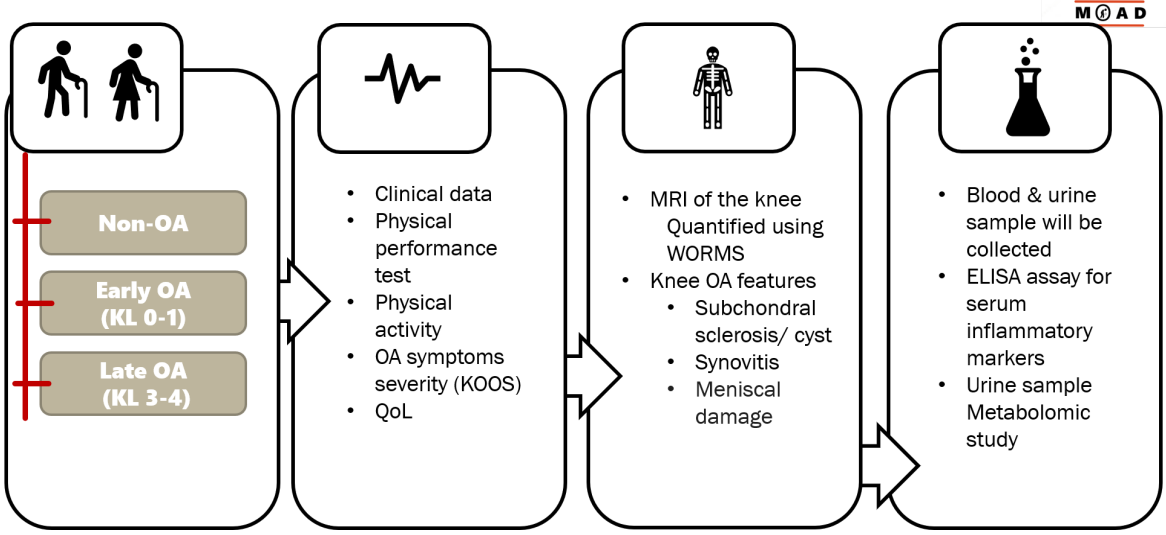
Statistical Data Analysis

Data analysis will be conducted using the Statistical Package in Social Science 23.0 software (IBM Ltd., USA) and SIMCA-P+ software version 13.0 (Umetrics, Umeå, Sweden) for multivariate analysis. The bucketed spectral data will be converted to Microsoft Excel format and imported into in which all spectral data will be transformed into Log-10 mode, mean-centered with UV scaling. Normal distribution will be determined for continuous variables using histograms and the Komolgorov-Smirnov test. The relationship between the various measurement will be compared using the Pearson's correlation coefficient or Spearman's test for parametric and non-parametric data respectively. Kappa agreement between the different tests for metabolites, inflammatory markers and MRI findings will then be determined. Comparisons between groups will be conducted using student t-test and the Mann-Whitney U test accordingly for continuous data and the Fisher's exact test for categorical data. Subsequently, linear or logistic regression analysis will be employed to adjust for confounders and to correlates between metabolomic profiling with serum biomarkers and quantitative MR imaging. To study the discriminatory power, Receiver-Operating Characteristic Curves (ROC) curve will be developed for each metabolites on early OA.

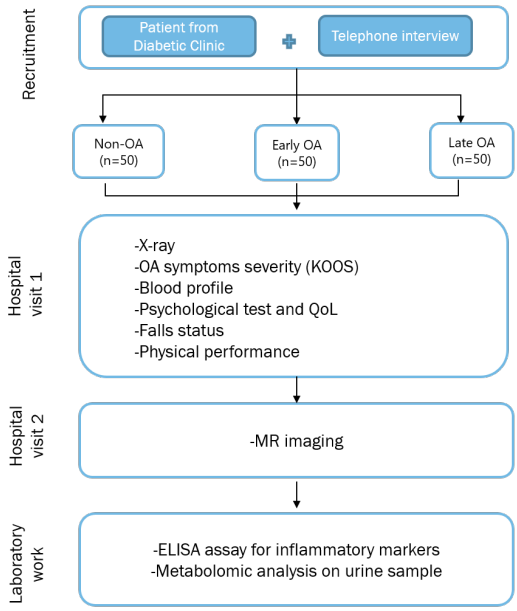
FLOWCHART FOR ACTIVITIES



METHODS



FLOWCHART FOR ACTIVITIES



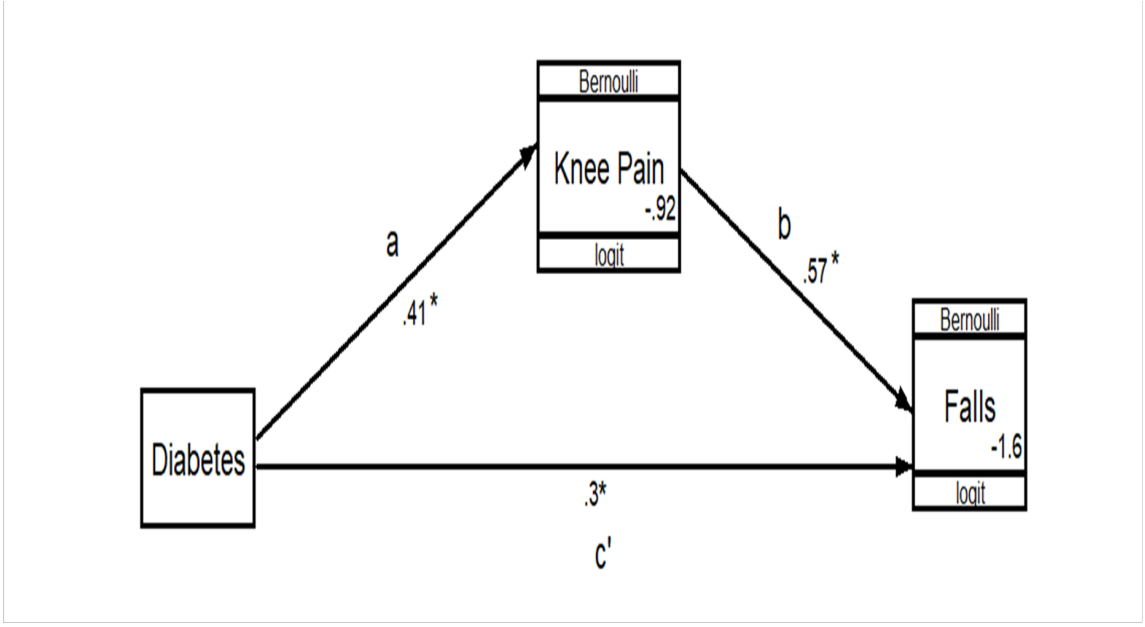
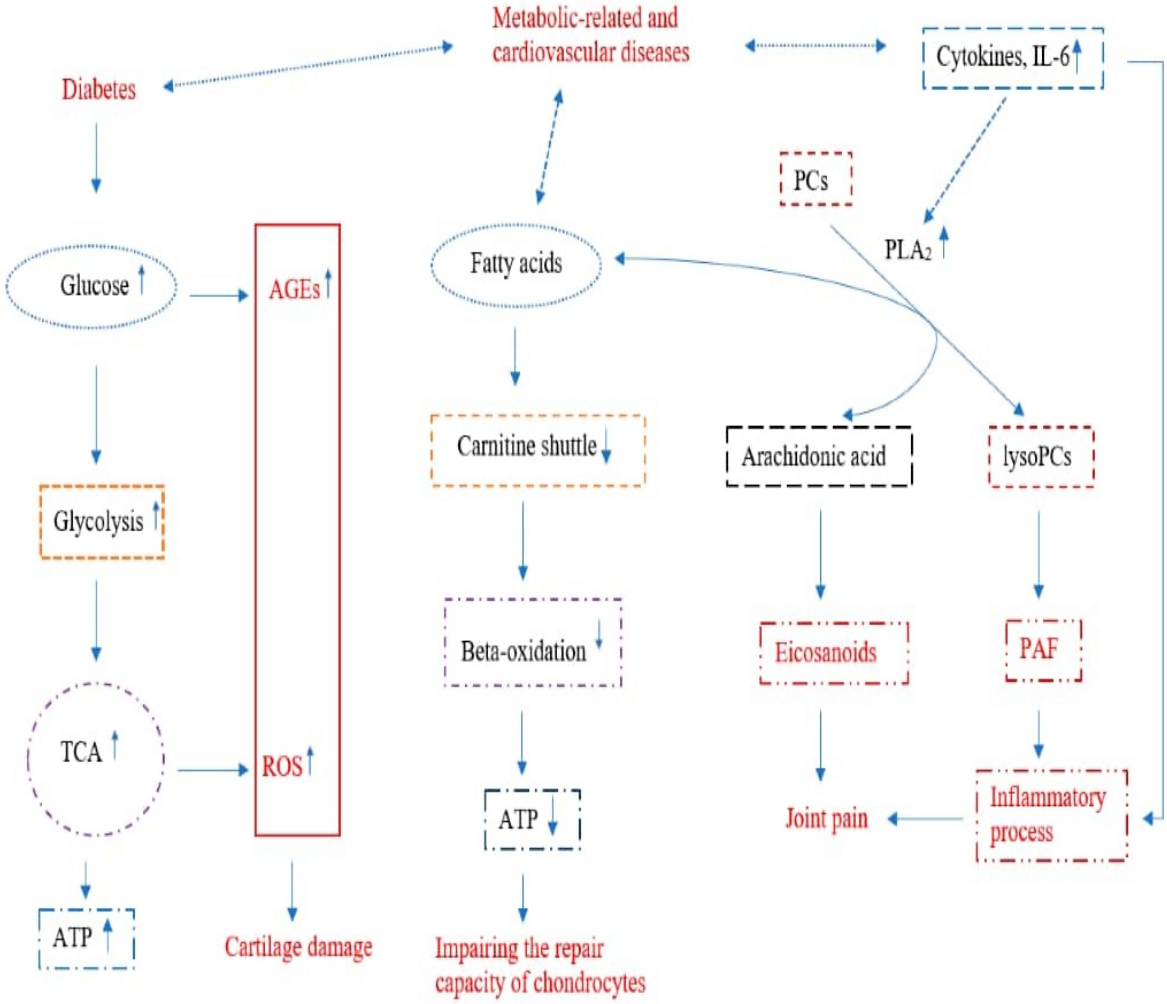


Figure 1: Standardized regression coefficient in GSEM model, *p<0.05:

Path *a*: β coefficient, SE (95% CI) = 0.413, 0.123 (0.172-0.655)
Path *b*: β coefficient, SE (95% CI) = 0.569, 0.1415 (0.292-0.846)
Path *c'*: β coefficient, SE (95% CI) = 0.295, 0.1398 (0.021-0.569)



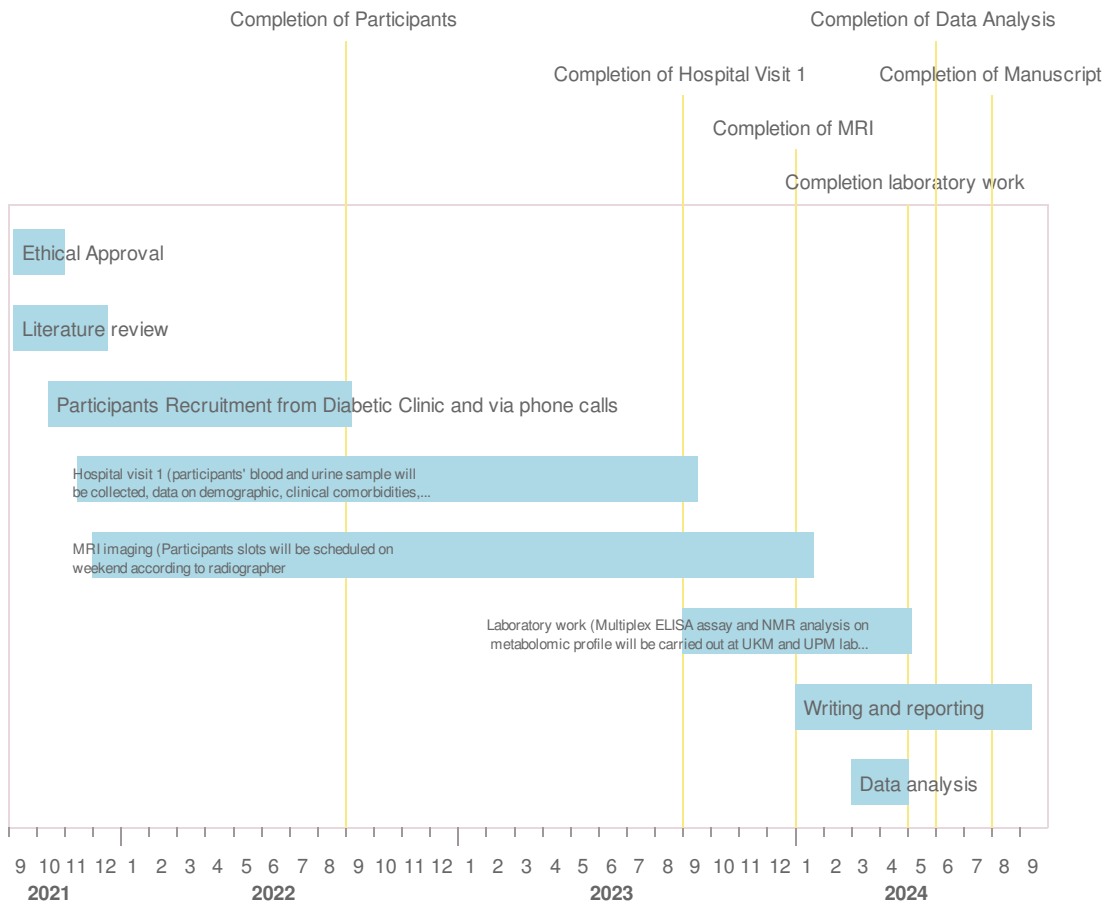
2. Flow Chart of Research Activities	'flow chart 15.3.pdf'
3. Research Activities	

Activity	Start Date	End Date
Ethical Approval	07/09/2021	31/10/2021
Literature review	07/09/2021	15/12/2021
Participants Recruitment from Diabetic Clinic and via phone calls	15/10/2021	31/08/2022
Hospital visit 1 (participants' blood and urine sample will be collected, data on demographic, clinical comorbidities, symptoms severity will be collected and their physical performance will be tested, X-ray on knee will be obtained for all participants)	15/11/2021	31/08/2023
MRI imaging (Participants slots will be scheduled on weekend according to radiographer availability)	01/12/2021	31/12/2023
Laboratory work (Multiplex ELISA assay and NMR analysis on metabolomic profile will be carried out at UKM and UPM lab respectively)	01/09/2023	30/04/2024
Writing and reporting	01/01/2024	06/09/2024
Data analysis	01/03/2024	01/05/2024

4. Milestones

Description	Date	Cumulative Project Completion Percentage(%)
Completion of Participants recruitment	31/08/2022	40
Completion of Hospital Visit 1	31/08/2023	50
Completion of MRI	31/12/2023	70
Completion laboratory work	30/04/2024	80
Completion of Data Analysis	31/05/2024	85
Completion of Manuscript Writing	31/07/2024	100

Gantt Chart of Research Activities with Milestones



(e) Expected Results/Benefit

1. Novel theories/New findings/Knowledge

This study will identified metabolome profile that is biologically more relevant than standard ELISA test. Correlations between metabolites, ELISA findings, clinical symptoms with MRI will shed some light on understanding the early OA stages especially in those with DM which has complex OA pathophysiology as compared to normal OA. The discriminatory power of each metabolites will provide important insight on the novel putative biomarkers to detect early OA which is usually symptomless in Diabetic patient which in turn improve the current treatment of OA by treating at the early stage.

2. Impact Statement on Quintuple Helix (please delineate/describe expected research deliverables on Society, Academia, Government, Industry and Environment)

Diabetes and Osteoarthritis both lead to increased healthcare, societal and psychological burden. In addition to answering the research questions relating to the study hypotheses, the following outputs will be achieved. We will disseminate our results in national and international conferences to engage with the global scientific community, and participate in knowledge exchange and transfer activities. The enriched data already collected will be further enhanced by this study and will generate novel scientific outputs with regards to publication of papers in high impact journals in relevant fields. This interdisciplinary research project will provide the foundation for Malaysian research capacity building in the field of diabetes and OA. The cohort data accompanied by stored blood and urine samples will allow future opportunities for clinical and non-clinical PhDs in the fields of cardiovascular epidemiology, molecular basic science in diabetes, metabolic syndrome, and nutritional epidemiology.

3. Research Publications (Each proposal must produce at least two (2) papers in indexed journals, one of which should be in Web of Science (WoS))

Indexing Body	**Indexed Journal**	
Number of Publication	**Name of Journal**	
WoS	2	BMJ Open (IF 2.469 Q2) PloS One (IF 2.740 Q2) Scientific Report (IF 3.998 Q1) Osteoarthritis and Cartilage (IF 4.798 Q1)
SCOPUS		
ERA		
MyCITE		
	Total 2	

4. Specific or Potential Applications of the Research Findings

The are several potential application of the research findings particularly in early detection of OA in DM patient. Identification of novel putative biomarkers are crucial in developing point of care diagnostic kits for early OA detection specifically in those with DM in which this population has poorer outcome. This could inform clinician for early treatment of OA in individuals with DM. In addition, identified novel putative biomarkers will help in monitoring the effectiveness of OA treatment especially in development of Disease-modifying osteoarthritis drugs (DMOADs). This point of care tools are also important where the main laboratory is not easily accessible, especially in remote areas.

Total Number of Applications: 1

5. Number of PhD and Masters (by research) Students

Total Number of PhD (by research) Student(s):
1

Total Number of Masters (by research) Student(s):

Remark (if any):

Student must be Malaysian and enroll for full time research mode.

6. Intellectual Properties (IPs)

Patent on Urine Metabolites as Novel Putative Biomarkers of Early OA in Individuals with Diabetes Mellitus

Total Number of IP: 1

Access to Equipment & Material(s)

Type	**Description**	**Owner**	**Location**	**Address**
Centrifuge	Centrifuge	Fakulti Sains Kesihatan	UKM kampus KL	
-80 Freezer	For serum and urine sample	Fakulti Sains Kesihatan	UKM Kampus KL	
Stadiometer	SECA	HCARE	HCARE	UKM Kampus KL
Weighing scale	SECA	HCARE	HCARE	UKM Kampus KL
JAMAR Hand Dynamometer	For grip strength test	HCARE	HCARE	UKM Kampus KL

E. Budget

Budget Type	Description	Year 1	Year 2	Year 3	Grand Total
11000 - Allowance					
GRA Master (Max RM2,000.00/person for 2 years)					**0**
Sub-Total		0	0	0	0
GRA Ph.D (Max RM2,500.00/person for 3 years)	1 GRA (PhD) x RM2500 x 32 months	30000	30000	20000	**80000**
Sub-Total		30000	30000	20000	80000
Vot-Total		**30000**	**30000**	**20000**	**80000**
					Recommended Sub-Total:RM80000
21000 - Travelling and Transportation					
Local					**0**
Sub-Total		0	0	0	0
Overseas	airfare for International conferences (OARSI World congress)			1000	**1000**
Sub-Total		0	0	1000	1000
Field work					**0**
Sub-Total		0	0	0	0
Vot-Total		**0**	**0**	**1000**	**1000**
					Recommended Sub-Total:RM1000
24000 - Rental					**0**
Vot-Total		**0**	**0**	**0**	**0**
					Recommended Sub-Total:RM0
27000 - Research Materials and Supplies	NMR tube		1000 NMR t…		**1000**
	NMR solvent and reagent		5000		**5000**
	Urine bottle tube RM25*8	200 urine b…			**200**
	Eppendorf tube	40 tube ep..			**40**
	Multiplex ELISA assay (RM7k+10k)		8500 _Biom…	8500 _Biom…	**17000**
	EDTA tube and syringe (RM74*3)	250 EDTA.…			**250**
Vot-Total		**490**	**14500**	**8500**	**23490**
					Recommended Sub-Total:RM23490

Ulasan CRIM 17/9/2021: Keluarkan butiran "COVID safety equipment, gloves, PPE, apron" kerana tiada dalam proposal asal yang diluluskan. Penyelidik perlu mendapatkan kelulusan CRIM/KPT terlebih dahulu untuk menambahkan butiran perbelanjaan yang baharu selepas pindaan proposal ini diluluskan.
Ulasan CRIM 19/9/2021: Ok, pindaan telah dilakukan.

28000 - Maintenance and Minor Repair Services					**0**
Vot-Total		**0**	**0**	**0**	**0**

Recommended Sub-Total:RM0

29000 - Professional Services					
Services/Consultancy	MRI (RM200*150)+ Radiographer honorarium (RM50*150)	20000 Whats…	17500		**37500**
	NMR run sample(RM104*150)			15600 Whats…	**15600**
	Token of appreciations for participants	1000			**1000**
	XRays (RM50*150)	5000 X-Ray…	2500		**7500**
Sub-Total		**26000**	**20000**	**15600**	**61600**
Short term course	International conferences (OARSI World congress) fees			400	**400**
Sub-Total		**0**	**0**	**400**	**400**
Journal Page Charges (WoS / SCOPUS / ERA / MyCITE)	Plos One (RM5000), BMJ Open (RM 5000)	1500 117830..	Author…	1500 Journa…	**3000**
Sub-Total		**1500**	**0**	**1500**	**3000**
Vot-Total		**27500**	**20000**	**17500**	**65000**

Recommended Sub-Total:RM65000

Ulasan CRIM 17/9/2021: Butiran "International conferences (OARSI World congress) fees" perlu dimasukkan kerana terdapat perancangan perbelanjaan perjalanan menghadiri persidangan ini dalam vot 21000.
Ulasan CRIM 19/9/2021: Ok, pindaan telah dilakukan.

35000 - Accessories and Equipment					**0**
Vot-Total		**0**	**0**	**0**	**0**

Recommended Sub-Total:RM0

Grand Total		**57990**	**64500**	**47000**	**(100.00%) 169490**

F(i). Patent Search (describe how your research output shall produce an innovative idea or technology that has the potential to be a solution for stakeholders (community, industry, government etc.) and offers a unique proposition)

To identify if the researcher is able to coherently present a compelling argument for his/her proposal in light of the IP landscape and factors identified in the (Yes/No) Section. The answer would reflect an understanding of the applicant's research advantage and limitations and the prospect of moving the completed research beyond this stage of funding.

To date none of the available patent have been filed for metabolomics profiling among diabetic Malaysian with early OA

Simplified Patent Search Report (MyGRANTS) 2021.pdf

F(ii). Research Collaborator

☑ Industrial Linkages (Please identify any industry or end-user group involved in the project, and describe its role/contribution to the project)

ACT4Health Services and Consultancy Sdn Bhd has shown interest to collaborate in this study. ACT4Health will provide consultation services on medical and community health aspect. They will also helping out in patient recruitment.

industrial partner_ACT4Health.pdf

☑ Agency/Organisation (Please identify all agencies/organisations collaborating in the project, and describe their role/contribution to the project)

Prof Francis Berenbaum, Head of the Department of Rheumatology, Saint-Antoine hospital, Team leader INSERM CDR « Age-related joint diseases and metabolic diseases », PARIS, FRANCE will be the international collaborators for this study. He is the renowned expert in the field of DM-related-OA and has reviewed this proposal.

Dr Hisayo Yokoyama is an Associate Prof. of Research Center for Urban Health and Sports from Osaka University Japan, she is clinical diabetologist, she will provide important insight on the pathophysiology of Diabetes Mellitus.

Hisayo LoI-merged.pdf

F(iii). Risk Assessment (Please describe factors that may cause delays in, or prevent implementation of, the project as proposed above; estimate also the degree of risk)

Please consider an appropriate approach to working in the current conditions (pandemic, travel ban, social distancing etc.)

Pandemic and travel ban has medium-to-high risk that may cause delays in recruitment. Nevertheless, since older Malaysia are expected to get their vaccination jab starting April and will be completed by August 2021, there should be sufficient time for recruitment.

Risk	Low	Medium	High
1. Technical	☑	☐	☐
2. Timing	☐	☑	☐
3. Budget	☑	☐	☐

G. Declaration

- [x] All details provided in this application are accurate. KPT has the right to reject or to cancel the offer without prior notice if there is any inaccurate information given.
- [x] This proposal is not currently considered for any other research grant(s).
- [x] The exact proposed work has not been funded through other research grant(s).
- [ ] Necessary approvals from relevant bodies (i.e.: Ethics Committee) are granted before related research activities commence.
- [ ] I have an on-going Fundamental Research Grant Scheme (FRGS) project.
- [x] This proposed research is my original work, is not copied from my MSc. or Ph.D. thesis or any other work (published or unpublished), and has not been submitted for grant application either at KPT or elsewhere.

Name: Sumaiyah Binti Mat

Signature:

Date: 15/02/2021

Approved By:

Signature:

RMC
Date:

Appendix	
Flow Chart	flow chart 15.3.pdf