EFFECTIVENESS OF LUSEOGLIFLOZIN OVER CONVENTIONAL THERAPY ON LIVER FIBROSIS, DISEASE ACTIVITY AND METABOLIC ATTRIBUTES IN PATIENTS WITH METABOLIC ASSOCIATED FATTY LIVER DISEASE AND TYPE II DIABETES MELLITUS: AN OPEN-LABEL RANDOMIZED CONTROLLED TRIAL

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MOHAMAD SYAMIL MAZRI

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LIST OF SYMBOLS, ABBREVIATIONS, AND ACRONYMS

ALT	Alanine transaminase
ANOVA	Analysis of variance
AST	Aspartate transaminase
BMI	body mass index
BNP	B-type natriuretic peptide
CKD	Chronic kidney disease
CONSORT	Consolidated Standards of Reporting Trials
CPG	Clinical Practice Guidelines
DPP-4	Dipeptidyl peptidase-IV
FBC	Full blood count
Fib-4 index	Fibrosis-4 index score
GGT	Gamma-glutamyl transferase
HbA1C	Hemoglobin A1C
HCC	Hepatocellular carcinoma
HUSM	Hospital Universiti Sains Malaysia
kg	Kilogram
m	meter
MAFLD	Metabolic associated fatty liver disease
mg	milligram
SD	Standard deviation
SGLT2	Sodium glucose co-transporter 2
T2DM	Type 2 Diabetes Mellitus

ABSTRAK

Latar Belakang

Metabolic-associated fallty liver disease (MAFLD) atau penyakit hati berlemak berkait dengan metabolik (lemak dalam hati) merupakan terma baharu yang digunakan untuk mengklasifikasi penyakit hati yang disebabkan oleh faktor metabolisme badan yang merangkumi obesiti, diabetes mellitus jenis 2 (T2DM) dan sindrom metabolik. Sehingga kini, masih tiada rawatan khusus dan berkesan untuk mengubati penyakit ini. Kajian ini dijalankan untuk mengenalpasti keberkesanan luseogliflozin, sejenis anti-Sodium Glucose co-transporter 2 (SGLT2) untuk rawatan fibrosis hati, kadar kolesterol dan parameter metabolik bagi pesakit MAFLD dan T2DM.

Kaedah

Kajian prospektif, label-terbuka ini adalah satu kajian rawak terkawal melibatkan pesakit yang menghidap MAFLD dan T2DM. Pesakit dibahagikan kepada 2 kumpulan secara rawak. Kumpulan kontrol menerima rawatan farmakologi standard tanpa melibatkan sebarang ubat anti-SGLT2. Kumpulan aktif pula menerima tambahan ubat oral luseogliflozin 5mg sekali sehari bersama dengan rawatan standard sedia ada bagi tempoh kajian selama 3 bulan. Bacaan Indeks Fibrosis-4 (Fib-4 index) untuk mengkaji tahap fibrotik hati beserta dengan kajian darah berkaitan enzim hati, HbA1c, dan kandungan kolesterol akan dilakukan pada permulaan kajian dan selepas 3 bulan.

Keputusan

Seramai 58 pesakit terlibat dalam kajian ini dan dapat membuktikan perbezaan signifikan dalam mengurangkan indeks Fib-4 bagi pesakit dalam kumpulan aktif yang mengambil

luseogliflozin, berbanding kumpulan kontrol. Indeks Fib-4 menurun daripada 2.24 ± 0.77 kepada 1.57 ± 0.75, p <0.001 bagi kumpulan aktif. Sementara itu, bacaan indeks Fib-4 dalam kumpulan kontrol meningkat daripada 2.41 ± 1.58 kepada 2.86 ± 2.26, p <0.001. Luseogliflozin juga menambah baik bacaan HbA1c (-1.22 ± 1.36%, p<0.001), AST (-11.59 ± 13.41U/L, p = 0.003), kolesterol (-0.4 ± 0.78mmol/L, p=0.002), dan trigliserida (-0.19 ± 0.63mmol/L, p= 0.024) jika dibandingkan antara pada permulaan kajian dan selepas 3 bulan kajian.

Kesimpulan

Jika dibandingkan dengan kumpulan kontrol, tambahan ubat luseogliflozin memberi kesan positif untuk fibrotik hati, kawalan glukosa, dan kadar lemak dalam darah bagi pesakit MAFLD dengan T2DM.

ABSTRACT

Background

Metabolic Associated Fatty Liver Disease (MAFLD) is a new term that describes a spectrum of liver disease with its causative metabolic factors comprising obesity, type 2 diabetes mellitus (T2DM), and metabolic syndrome. To this day, there is no definitive treatment for this condition. This study aimed to ascertain the effectiveness of luseogliflozin, a sodium-glucose co-transporter 2 (SGLT2) inhibitor on liver fibrosis and metabolic attributes in patients with MAFLD and T2DM.

Method

This is a prospective, open-label, randomized controlled study involving patients diagnosed with MAFLD and T2DM. Subjects were randomly allocated into two groups, either the control group who received standard pharmacological T2DM treatment without any SGLT2 inhibitor or active group who received oral luseogliflozin 5mg daily for 3 months. Measurement of Fibrosis-4 Index score (Fib-4) to determine fibrosis and blood tests including liver enzymes, HbA1C and cholesterol level to determine liver and metabolic attributes were recorded at baseline and 3 months post intervention.

Results

There were 58 patients randomized in this study and the results showed statistically significant differences in the reduction of Fib-4 index between the two groups after 3 months. Fib-4 index in active group reduced from 2.24 ± 0.77 to 1.57 ± 0.75 vs control group from 2.41 ± 1.58 to 2.86 ± 2.26, $p < 0.001$. Between group analysis for Fib-4 index shown significant difference

between the two group. Luseogliflozin was also observed to improved HbA1C (-1.22 ± 1.36%, $p<0.001$), AST (-11.59 ± 13.41U/L, $p = 0.003$), total Cholesterol (-0.4 ± 0.78mmol/L, $p=0.002$), and triglyceride (-0.19 ± 0.63mmol/L, $p= 0.024$) levels after 3 months of study as compared to baseline.

Conclusion

Compared to controls, addition of luseogliflozin had shown to be effective in improving liver fibrosis, liver disease activity, glycemic control, and lipid parameters in MAFLD with T2DM.

CHAPTER 1: INTRODUCTION

1.1 BACKGROUND

Metabolic Associated Fatty Liver Disease (MAFLD) is a new term developed to describe a spectrum of liver disease with its causative metabolic factors comprising obesity, type 2 diabetes mellitus (T2DM) and metabolic syndrome. It encompasses a wide spectrum of disease severity which does not only involve the liver-related complications such as liver cirrhosis and hepatocellular carcinoma, is also a key player in the development of cardiovascular-related diseases, chronic kidney disease (CKD), osteoporosis, mental health, cognitive disorder and even extrahepatic malignancies. The worryingly growing disease burden due to MAFLD in the lack of definitive and effective treatment may cause significant public health issues. In recent years, with the improvement of living standards in Asia, toehe prevalence of MAFLD has been reported to be as high as 30% among Asian populations[1, 2]. There is significant evidence showing patients with MAFLD may develop hepatic steatosis in >30%, hepatic fibrosis in approximately 25%, liver cirrhosis in 10-20%, and hepatocellular carcinoma in 4% of cases[2].

T2DM is a condition where co-occurrence of insulin resistance and inability of the pancreatic islet's beta cells to produce enough insulin to overcome the deficit[3]. With insulin resistance and development of obesity, it leads to changes in lipid metabolism and increase fat deposition, thus escalated the free fatty acids in the circulation. These free fatty acids will be accumulated within the liver and cause excess liver fat content[4].

As one of the contributing factors to MAFLD, managing T2DM using antidiabetic agents can also decelerate the progression of the disease[5]. Luseogliflozin is a type of antidiabetic agent, which is a Sodium Glucose Co-transporter 2 (SGLT2) inhibitor. This medication's mechanism

of action is through inhibition of the reabsorption of glucose at the proximal tubule of nephron, thus promoting urinary glucose excretion (glucosuria) and urinary calorie loss[6]. Besides improving glycemic control in T2DM patients, SGLT2 inhibitors were used for their effect on cardiovascular outcomes, renal protective properties, and lowering body cholesterol levels[7, 8]. Luseogliflozin, as part of SGLT2 inhibitors, can be given as monotherapy for treatment of T2DM or in combination with other antidiabetic agents to improve glycemic control. Researchers and clinicians are looking for various treatment for MAFLD and initial trials of SGLT2 inhibitors for treatment of liver fibrosis has been promising[9, 10]. However, luseogliflozin has not been studied in depth with regards to its effect in MAFLD patients with T2DM. If proven to be effective, the use of luseogliflozin would be beneficial in treatment of T2DM patients with multiple co-morbidities like MAFLD, CKD, and heart failure. Few side effects of luseogliflozin includes: hypoglycemia, gastrointestinal disturbance, dehydration and urinary tract infection[6].

1.2 STUDY RATIONALE

In recent years, diagnosis of MAFLD among Malaysians had been increasing, mainly contributed by high prevalence of obesity and obesity-related diseases[11]. The high and increasing number of MAFLD will lead to increase in incidence of hepatocellular carcinoma (HCC), decompensated cirrhosis, and liver related mortality from the condition. It carries a high burden of disease and hence the needs to integrate MAFLD into the national healthcare plan for noncommunicable diseases. This will ensure a precise assessment, referral pathways and subsequent management can be carried out on patients with the condition.

In order to tackle this growing issue, apart from lifestyle modifications, a suitable treatment modalities need to be established to prevent progression of disease and avoiding severe complications. Previous studies using SGLT2 inhibitors in MAFLD patients had shown promising results[10, 12, 13]. As of now, Luseogliflozin was previously studied in MAFLD patients with T2DM in a single arm trial. In this study, we would like to compare the effectiveness of Luseogliflozin in MAFLD patients against those who were conservatively treated for T2DM.

1.3 LITERATURE REVIEW

MAFLD: Diagnostic Criteria

The diagnosis of MAFLD is established based on histopathology finding (liver biopsy), liver imaging, or blood parameters showing liver's fat deposition biomarkers. On top of these markers are another three factors, namely overweight/obesity, diabetes mellitus, and evidence of metabolic dysregulations[14]. The consensus made by international experts in the latest update of the disease and the diagnostic criteria of MAFLD is depicted in figure 1.

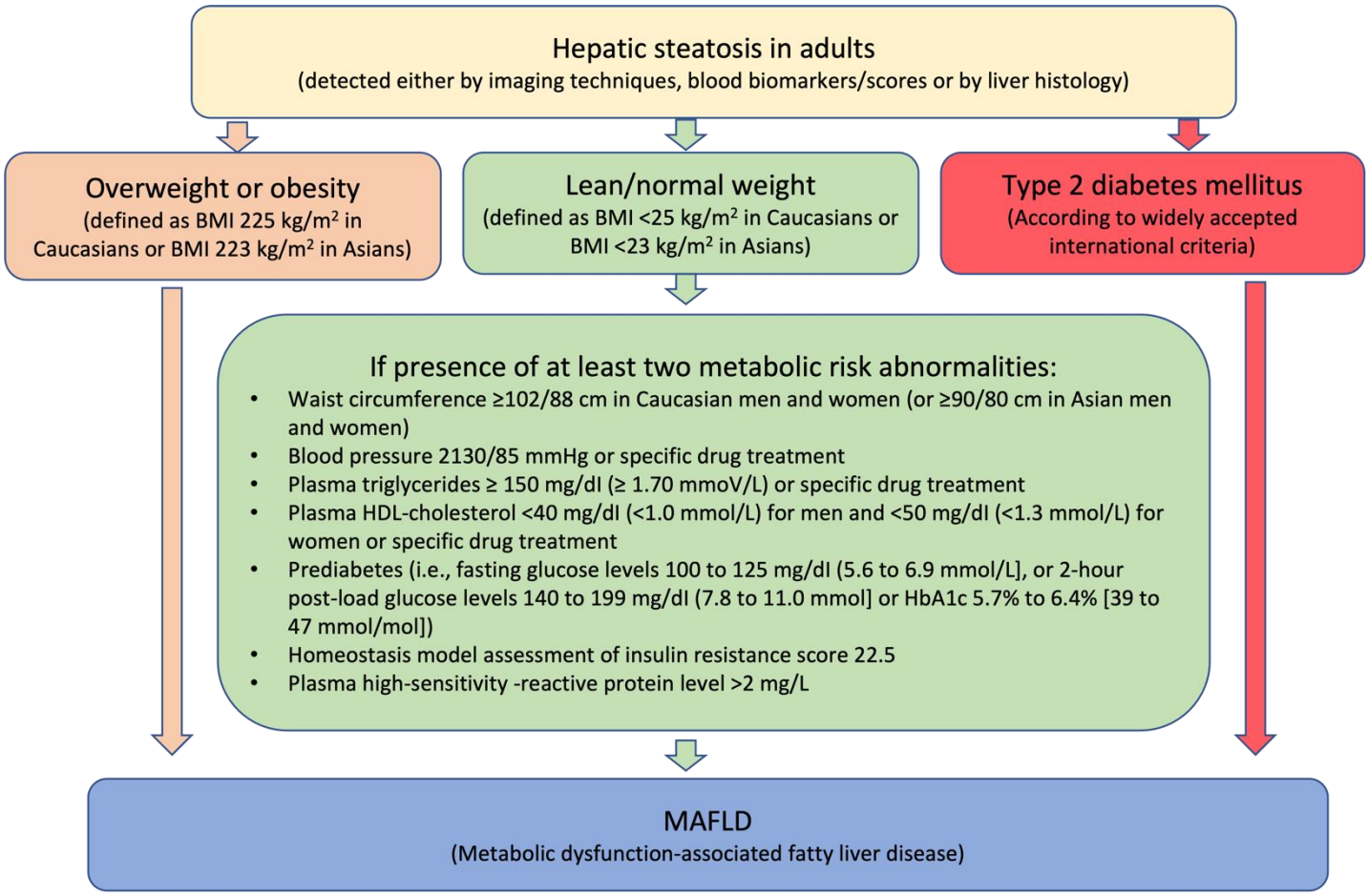


Figure 1: Flowchart of diagnostic criteria for MAFLD[15].

Jeong et al. had highlighted the non-invasive options as alternatives in diagnosing MAFLD using imaging modalities and blood tests, which were significantly correlated with liver biopsy findings[16]. Raj et al. described a clinical scoring method using blood parameters, particularly liver biochemistries such as Aspartate Transaminase (AST), Alanine Transaminase (ALT), and gamma-glutamyl transferase (GGT) to assess liver fibrosis such as Fibrosis-4 Index Score (FIB-4) and NAFLD fibrosis score[17].

There are several scoring systems being used to classify hepatic fibrosis especially in liver imaging. The commonly used hepatic fibrosis staging system is the METAVIR system[18]. METAVIR characterized liver fibrosis into 0 to 4 scales, further interpreted into stage F0, no fibrosis; F1, portal fibrosis without septa; F2, portal fibrosis and few septa; F3, numerous septa without fibrosis; and F4, cirrhosis. Significant fibrosis is considered when the fibrosis score is ≥ F2 or higher, whereas advanced fibrosis is considered when the fibrosis score is ≥ F3 or higher [19].

Fib-4 index was validated to correlate with stages of liver fibrosis. Fib-4 score of <1.45 translated to no or mild liver fibrosis, stage 0 -1. Score of 1.45 – 3.25 considered to have significant fibrosis, stage 2. Fib-4 score of > 3.25 correlated to advanced fibrosis, stage 3-4[20].

Fib-4 index	**Degree of fibrosis**	**Fibrosis stage**
<1.45	No or mild fibrosis	Stage F0 - F1
1.45 – 3.25	Significant fibrosis	Stage F2
>3.25	Advance fibrosis	Stage F3 - F4

The use of Fib-4 Index will provides noninvasive, simple, and easily accessible method to monitor patients on treatment for this liver fibrosis. According to Shah et al. Fib-4 Index was validated and proven to be superior to other noninvasive markers of fibrosis in patients with MAFLD, as compared to other markers namely NAFLD fibrosis score, Goteborg University Cirrhosis Index, AST/ALT ratio, AST to platelet ratio, cirrhosis discriminant score and BARD (BMI, AST:ALT, diabetes) score[21].

SGLT2 inhibitor for MAFLD treatment

In the latest Clinical Practice Guidelines (CPG) Management of Type 2 Diabetes Mellitus (6th Edition) released by Malaysia's Ministry of Health in 2020, stated wide range of antidiabetics used to treat diabetes mellitus, from metformin to Insulin. It was highlighted in the CPG that SGLT2 inhibitors reduced glucose reabsorption by increasing the urinary glucose excretion and the risk of hypoglycemia is very low, as similar to placebo. In MAFLD with type II diabetes mellitus, it is recommended for all patients above 40 years old to be treated with statins regardless of baseline LDL-Cholesterol levels[22].

Multiple studies and meta-analysis were carried out to determine the effectiveness of SGLT2 inhibitors in treatment of liver fibrosis. Li B. et al. did a meta-analysis study comprising of eleven randomized controlled trials which used Canagliflozin in T2DM patients with fatty liver disease. The results showed that Canagliflozin significantly reduced ALT, AST, and GGT levels and hence provide protective effect on fatty liver in T2DM patients[13].

A systemic review was done by Raj H. et al., which shows that SGLT2 inhibitor usage leads to a significantly reduced hepatic fat fraction (evaluated using MRI-derived proton density fat

fraction and ultrasounds evidence), reduced in FIB-4 index, reduced fasting plasma glucose, and glycosylated hemoglobin (HbA1c) levels, and body mass index (BMI) reduction[17].

Luseogliflozin: Use in current practice

Luseogliflozin is marketed with two different formulations: the minimum dose starts with 2.5mg daily and the maximum dose is 5mg daily. The efficacy of this medication is dependent on renal function; hence it is not recommended for use in patients with creatinine clearance less than 60ml/min. No dosage adjustment necessary for patient with mild or moderate hepatic impairment.

Seino et al. had proven that luseoglifozin significantly improved glycemic control in a large RCT [23]. Its efficacy was also proven to preserve renal function in T2DM patients based on study by Ito et al [6, 8]. The use of luseogliflozin was investigated in Management of Diabetic Patients with Chronic Heart Failure and Preserved Left Ventricular Ejection Fraction (MUSCAT-HF) study. The study was designed to assess reduction in heart failure biomarker, B-type natriuretic peptide (BNP) in heart failure patients taking luseogliflozin. The primary outcome of the study revealed that although BNP concentrations reduces after 12 weeks of treatment, it was not statistically significant as compared to other agent. Analysis from the study however showed that luseogliflozin reduce cardiac load within 4 weeks of its initiation, and significantly reduce systolic blood pressure[7]. It was further investigated and proven to be effective in reducing intravascular volume, which consequently may improve heart failure prognosis[24].

Luseogliflozin was also studied for treatment of fatty liver disease in a prospective single arm trial by Sumida et al and a pilot study by Shibuya et al[25, 26]. Hepatic fat content and fibrosis markers were used, which include Fib-4 index and were measured after 24 weeks of therapy

with luseogliflozin. The study showed that significant reduction in hepatic fat content based on MRI imaging evaluation post treatment. Improvement in liver biomarkers were also seen in this single arm trial. They concluded that luseogliflozin can be a novel promising agent for the treatment of T2DM patients with MAFLD[26]. A randomized control trial is necessary to validate this initial findings from the pilot study.

CHAPTER 2: STUDY OBJECTIVES

2.1 GENERAL OBJECTIVES

To compare the efficacy between conventional plus luseogliflozin therapy versus the conventional therapy on liver fibrosis and liver biochemical parameters among Metabolic Associated Fatty Liver Disease (MAFLD) patients with type II diabetes mellitus.

2.2 SPECIFIC OBJECTIVES

To compare at baseline and at 3 months of therapy between the active group and the conventional therapy group among MAFLD patients with type II diabetes mellitus:

a. The degree of hepatic fibrosis using Fibrosis-4 Index Score (Fib-4).
b. The difference of HbA1C.
c. The difference of Aspartate Transaminase (AST), Alanine Transaminase (ALT), triglyceride and total cholesterol levels.

2.3 RESEARCH HYPOTHESIS

NULL HYPOTHESES

There is no significant difference on the Fib-4 index calculated between the MAFLD patients with type II diabetes mellitus receiving luseogliflozin or the conventional therapy groups at baseline and 3 months of therapy.

There is no significant difference on the level of HBA1C between the MAFLD patients with type II diabetes mellitus receiving luseogliflozin or conventional therapy groups at baseline and 3 months of therapy.

There is no significant difference on the Aspartate Transaminase (AST), Alanine Transaminase (ALT), triglyceride (TAG) and total cholesterol levels between the MAFLD patients with type II diabetes mellitus receiving luseogliflozin or conventional therapy groups at baseline and 3 months of therapy.

ALTERNATIVE HYPOTHESES

Patients of MAFLD with type II diabetes mellitus who received luseogliflozin display improvement in the calculated Fib-4 index at baseline and 3 months of therapy compared to the patients who received conventional therapy.

Patients of MAFLD with type II diabetes mellitus who received luseogliflozin has a lower level of HBA1C at baseline and 3 months of therapy compared to the patients receiving conventional therapy.

Patients of MAFLD with type II diabetes mellitus who received luseogliflozin has a lower level of Aspartate Transaminase (AST), Alanine Transaminase (ALT), triglyceride (TAG) and total cholesterol levels at baseline and 3 months of therapy compared to the patients receiving conventional therapy.

CHAPTER 3: STUDY PROTOCOL & ETHICAL APPROVAL

3.1 STUDY PROTOCOL

Study Design

Prospective, open-label, randomized control trial

Study Site

Gastroenterology Clinic, Hospital Universiti Sains Malaysia (HUSM) Kubang Kerian, Kelantan.

Study Population

Reference Population

Patients on treatment for for type 2 diabetes mellitus with MAFLD in Malaysia.

Source Population

Patients on treatment for type 2 diabetes mellitus with MAFLD under follow up with Gastroenterology Clinic, Hospital Universiti Sains Malaysia (HUSM) Kubang Kerian, Kelantan.

Sampling Frame

Patients on treatment for type 2 diabetes mellitus with MAFLD under follow up with Gastroenterology Clinic, Hospital Universiti Sains Malaysia (HUSM) Kubang Kerian, Kelantan from the 1st January 2022 to 31st December 2022.

Research Criteria

Inclusion Criteria:

a) Age 18 years old or more.

b) AST ≥ 34 U/L or ALT ≥ 41 U/L or AST / ALT ratio > 0.8.

c) MAFLD patients who fulfilled either one of the diagnostic criteria as listed below:

- Liver histology - ≥5% of fat containing hepatocytes assessed by light microscopy as reported by pathologist.
- Radiological imaging – based on MRI/ CT-scan/ Ultrasound (include shear-wave elastography and fibroscan) of the liver reported by radiologist as fatty liver/hepatic steatosis.
- Blood biomarkers – Includes basic evaluation of AST/ALT/platelet levels with calculations of validated liver fibrosis scoring which includes Fib-4 Index, AST to platelet ratio index (APRI) and satisfy diagnostic criteria of liver fibrosis.

d) Type II diabetes mellitus patients and maintained their regular diet intake and usual daily activities.

Exclusion Criteria:

a) Severely ill patients (patients who have severe illness requiring urgent admission or critical care).
b) Patients who have fourfold raise of the transaminase values (AST or ALT) will be excluded as the raised values may indicate acute infection or inflammation which warrant further investigation.
c) Patients with decompensated liver disease (patients who have underlying chronic liver disease and its complications which include portal hypertension, hepatocellular insufficiency, jaundice, spontaneous bacterial peritonitis, hepatorenal syndrome and coagulopathy).
d) Pregnant or lactating patients (child-bearing age patients who have irregular menses or period of amenorrhea will be subjected for pregnancy test prior to patient selection).
e) Patients who change their regular daily diet intake or usual daily activities or sudden weight loss.

Withdrawal Criteria

a) Patients who request to withdraw.
b) Patients who suddenly became too ill to be involved or unable to proceed with the study.

Operational Terms

Liver fibrosis is defined as non-physiological scarring process of the liver as response to liver injury. Stages of liver fibrosis - Stage 0: No fibrosis. Stage 1: Mild/ non-significant fibrosis. Stage 2: significant fibrosis. Stage 3: Advance fibrosis. Stage 4: Liver cirrhosis.

Conventional therapy is defined as standard treatment received by patient with diabetes mellitus, according to the degree of disease control and other co-morbidities. Conventional T2DM treatment includes biguanide (Metformin) with the addition of sulfonylurea (Gliclazide, glibenclamide) or subcutaneous insulin therapy. Other groups of oral anti-diabetic agents such as thiazolidinediones and Dipeptidyl peptidase-IV (DPP-4) inhibitors were continued if patient already started on the medication prior to study enrollment.

Body mass index (BMI) is a measurement of person's weight with respect to their height and it correlates with body fat. The WHO defines an adult who has a BMI between 25 and 29.9 as overweight - an adult who has a BMI of 30 or higher is considered obese - a BMI below 18.5 is considered underweight, and between 18.5 to 24.9 a healthy weight.

Fibrosis-4 Index score (Fib-4 Index) is a scoring used to measure degree of liver fibrosis based on patient's age and these blood parametres: AST, ALT and platelet levels. The calculation for Fib-4 Index as follow:

Fib-4 index: Age (years) x AST /(platelets (10^9/l) x (ALT (U/l))$^{1/2}$

Fib-4 index was validated to correlate with stages of liver fibrosis. Fib-4 score of <1.45 translated to no or mild liver fibrosis, stage 0 -1. Score of 1.45 – 3.25 considered to have significant fibrosis, stage 2. Fib-4 score of > 3.25 correlated to advanced fibrosis, stage 3-4.

Alanine Transaminase (ALT) is a cytosolic enzyme that is found in the liver.

Aspartate Transaminase (AST) is present as cytosolic and mitochondrial isoenzymes and is found in the liver, cardiac muscle, skeletal muscle, kidneys, brain, pancreas, lungs, leucocytes, and red cells. ALT and AST are markers of hepatocellular injury. In liver fibrosis, ALT and AST levels would raise and has been frequently use as parameters' to measure severity of liver injury.

Total Cholesterol measures the total amount of Cholesterol in human body.

Triglyceride is a component of body cholesterol. Increment in total Cholesterol and triglyceride are associated with cardiovascular, liver, and metabolic complications.

Sample size estimation

Significance level, $\alpha = 0.05$

Power, 90%= 0.9

We based our assumption that our treatment with Luseogliflozin for T2DM patients with MAFLD would be superior to conventional therapy thus, the study would have 90% power to detect a difference in mean of Fib-4 index by 0.47[27] . From the calculation using PS software, we would need to study 27 experimental subjects and 27 control subjects to be able to reject the null hypothesis.

The total sample required is 54, and with the additional 10% for dropout rate, this study required 30 patients in each group, giving a total of 60 patients.

Sampling method and subject recruitment

Sampling was by convenience sampling based on time frame and target number of subjects. Subjects were enrolled during our review in clinic during their appointments. They were explained thoroughly on the study including the risks and benefits. An informed and written consent were obtained during the acquaintance. They were advised to stick to their daily stable diet intake and usual daily activities; and to avoid sudden dietary change or sudden weight loss. Subjects were given a copy of the consent for their keepsake. Concealment of the allocation sequence will be adhered to minimize bias to treatment.

Research Tool

None.

Study Protocol & Method

58 subjects were enrolled in this study from the 1st of January 2022 to the 31st of December 2022. Patients aged 18 years old or more who were diagnosed with metabolic associated fatty liver disease (MAFLD) with stable Type II diabetes mellitus were selected. Liver function tests of these patients were reviewed and those with mild to moderately raised transaminases; AST ≥ 34 U/L or ALT ≥ 41 U/L or AST / ALT ratio > 0.8 were included in this study. Informed and written consent from each patient were collected and these subjects were advised to commence their usual daily activities and were required to maintain their stable diet intake.

Subjects with decompensated liver disease (patients who have underlying chronic liver disease and its complications which include portal hypertension, hepatocellular insufficiency, jaundice, spontaneous bacterial peritonitis, hepatorenal syndrome and coagulopathy) and those with a fourfold raise of the transaminases value were excluded as this might indicate ongoing infection or inflammation. Pregnant or lactating patients, subjects with sudden weight loss and those who had modified their stable daily dietary intake and daily activities were also not included in this study.

Demographic data including age, gender, weight, height and BMI were evaluated. Subjects were explained regarding the study which involves compliance to the medications given, and to be present for reassessment and blood taking. Blood investigations that were acquired during patients' follow up are full blood count (FBC), hemoglobin A1C (HbA1C), aspartate aminotransferase (AST), alanine aminotransferase (ALT), triglycerides and total Cholesterol. Fib-4 index will be derived from this blood parameters.

Subject were randomized using a computer-generated randomization software using block size 4 into 2 groups; active and control group, each consisting of 29 subjects. The allocation within each block was random as determined by the computer random number generator. This technique was chosen to ensure similar numbers of patients in each group at any point during the study. Study investigators, research coordinators, and patients are not blinded to the treatment allocation during the study.

The active group was assigned to the interventional arm of study and thus receiving luseogliflozin 5mg once daily (OD) for 3 months on top of their current T2DM treatment, while the other group was assigned as the control group. This control group of patients continue their current anti-diabetic medications which does not include any SGLT2 inhibitor medication. Conventional T2DM treatment includes biguanide (Metformin) with the addition of sulfonylurea (Gliclazide, glibenclamide) or subcutaneous insulin therapy. Other groups of oral anti-diabetic agents such as thiazolidinediones and Dipeptidyl peptidase-IV (DPP-4) inhibitors were continued if patient already started on the medication prior to study enrollment. Both antihypertensive and statins medications which already in their prescriptions were not omitted during the study. Patients were reinforced to stick to their daily stable diet intake and avoiding changes in their diet regime. In addition, they were also advised to maintain their usual daily activities.

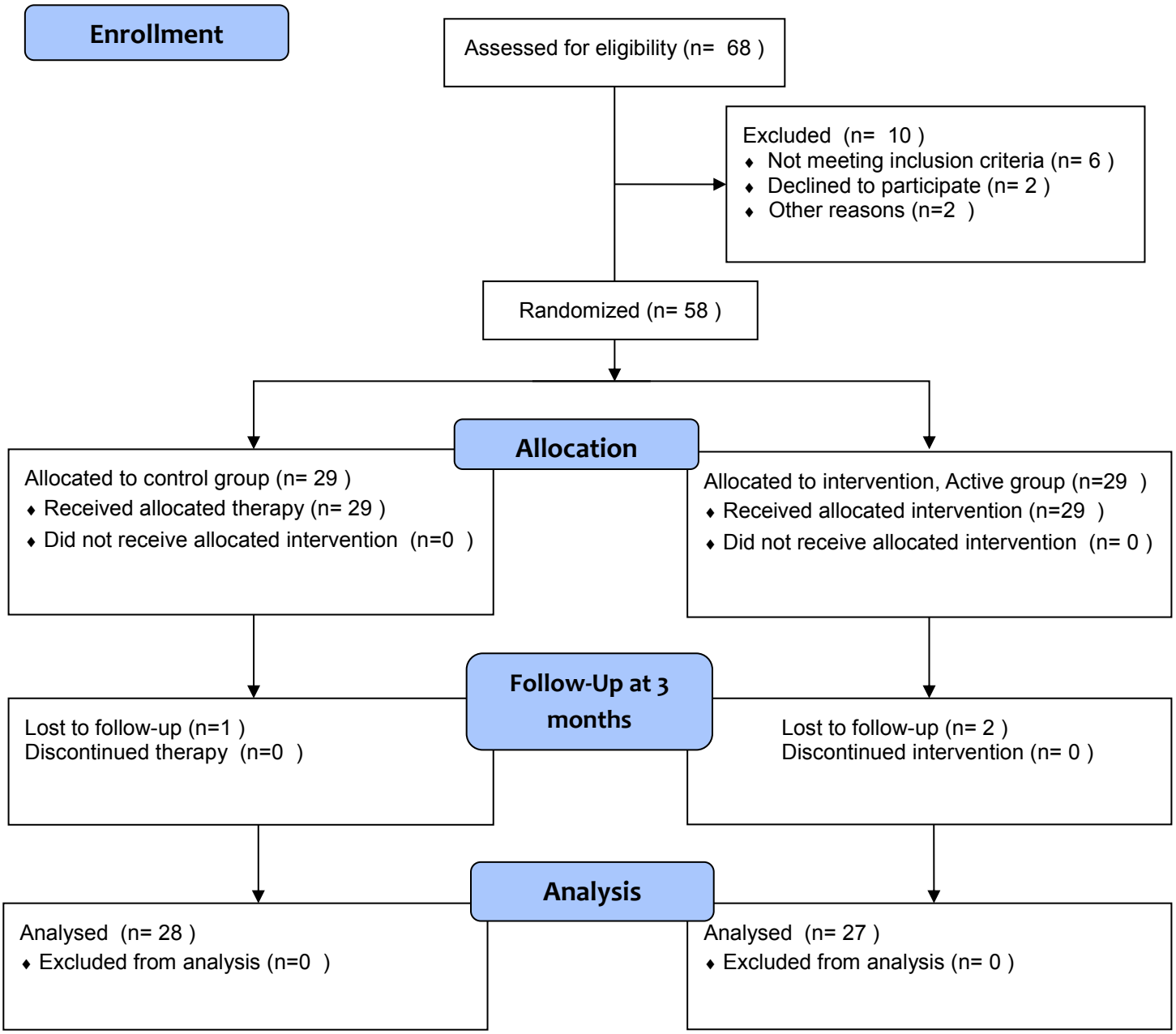
Assessments of both groups of patients were performed prior to the initiation of treatment and after three months of therapy. Biochemical tests which include FBC, HbA1C, AST, ALT, triglycerides and total cholesterol were repeated during patients' review in clinic. Fib-4 index was then calculated from these parameters.

Fib-4 index: Age (years) x AST /(platelets (10^9/l) x (ALT (U/l))$^{1/2}$

Data were retrieved and documented in the datasheet, with patients' information labelled with a serial number to maintain privacy and confidentiality. The data collected were kept and secured for analysis. In this intention-to-treat analysis, from the 58 patients randomized for this study, 1 subject from control group and 2 subjects from active group were lost to follow up. Data from 58 patients enrolled were then analyzed for this study.

Study flow diagram *(Prepared according to CONSORT 2010 Guidelines)*

Study Flow Diagram



Data Analysis

Data analysis was performed using SPSS version 28 for MAC.

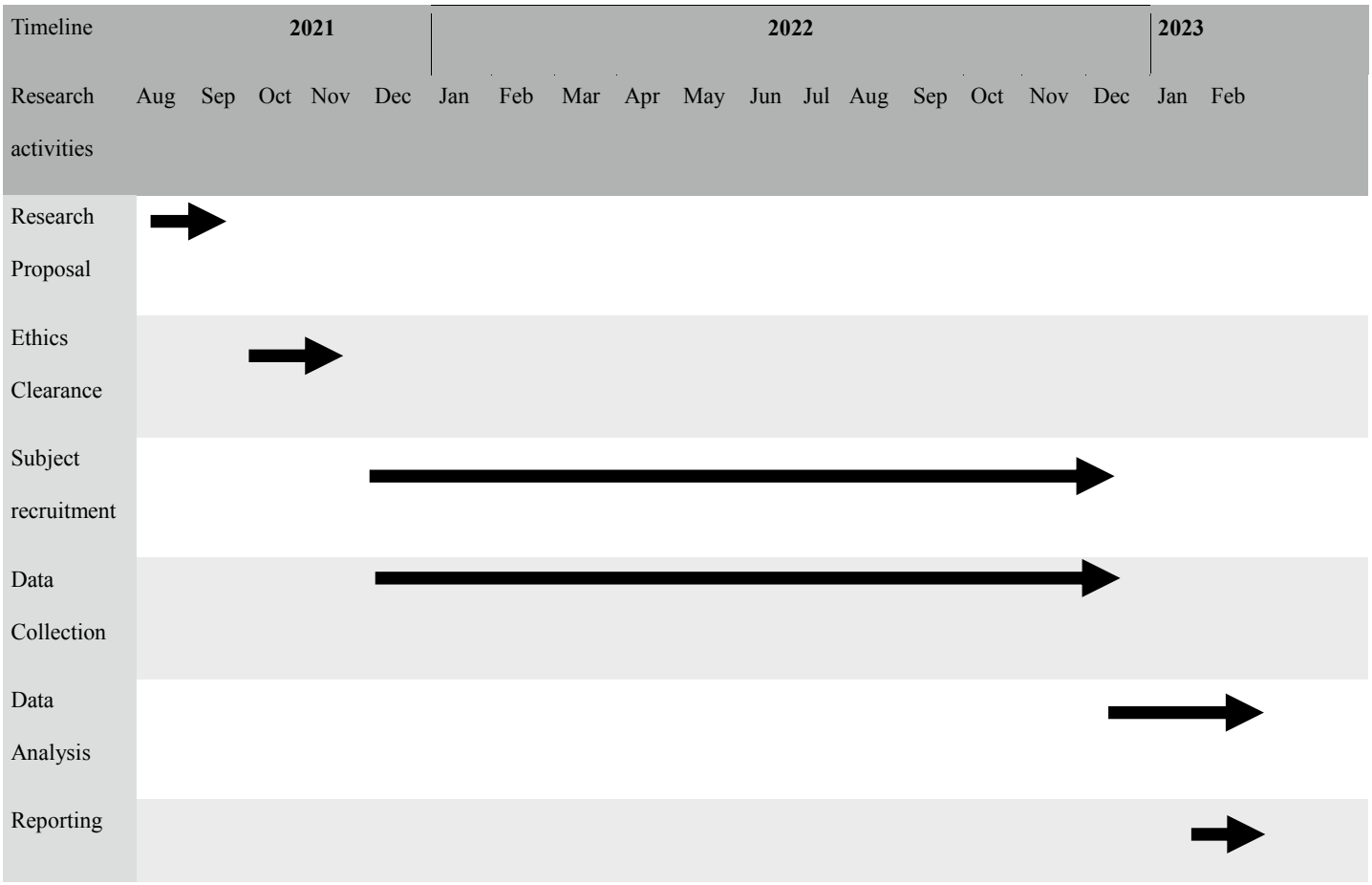
Baseline characteristics of the two study groups were summarized with means and standard deviations for continuous variables - age, weight, height, and BMI. Categorical variable of gender was presented as frequency and percentage.

Independent sample T-test and one-way analysis of variance (ANOVA) was used to assess differences between the two study groups for continuous numerical data of Fib-4 index, HBA1C, ALT, AST, total Cholesterol, and triglyceride. These data are presented by means and standard deviations.

Intended Statistical Analysis

OBJECTIVE	Parameters	Staistical analysis
Objective 1	Fib-4 Index (AST, ALT, Age, Platelet level)	Independent sample T-test One-way ANOVA
Objective 2	HbA1c	Independent sample T-test One-way ANOVA
Objective 3	AST, ALT, total cholesterol, triglyceride	Independent sample T-test One-way ANOVA

Gantt chart


Timeline
2021
2022
2023
Research activities
Aug Sep Oct Nov Dec Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec Jan Feb
Research Proposal
Ethics Clearance
Subject recruitment
Data Collection
Data Analysis
Reporting

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