Empagliflozin Improves Liver Steatosis and Fibrosis in Patients with Non-Alcoholic Fatty Liver Disease and Type 2 Diabetes: A Randomized, Double-Blind, Placebo-Controlled Clinical Trial

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ABSTRACT

Introduction: To evaluate the efficacy of empagliflozin compared to pioglitazone in patients with non-alcoholic fatty liver disease (NAFLD) and type 2 diabetes (T2DM).

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Methods: In this prospective randomized, double-blind, placebo-controlled trial, we assigned 106 patients with NAFLD and T2DM to receive empagliflozin 10 mg ($n = 35$), pioglitazone 30 mg ($n = 34$), or placebo ($n = 37$) for 24 weeks. Liver fat content and liver stiffness were measured using fibroscans. Body composition assessment was performed by dual-energy x-ray absorptiometry (DEXA) scans. The primary end point was change from baseline in liver steatosis, using the controlled attenuation parameter (CAP) score.

Results: A borderline significant decrease in CAP score was observed with empagliflozin compared to placebo, mean difference: -29.6 dB/m (-39.5 to -19.6) versus -16.4 dB/m (-25.0 to -7.8), respectively; $p = 0.05$. Using multivariate analysis, we observed a significant reduction in the placebo-corrected change in liver stiffness measurement (LSM) with empagliflozin compared to pioglitazone: -0.77 kPa (-1.45, -0.09), $p = 0.02$, versus 0.01 kPa (95% CI -0.70, 0.71, $p = 0.98$), p for comparison = 0.03. Changes in serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT), fasting insulin, homeostatic model assessment for insulin resistance (HOMA-IR), HOMA2-IR, fibrosis-4 index (FIB4 index), NAFLD fibrosis score, aspartate aminotransferase to platelet ratio index (APRI), android/gynecoid ratio (A/G ratio), and skeletal muscle index (SMI) were comparable between the two treatment groups, while significant

reductions of the body weight and visceral fat area were observed only in the empagliflozin group ($p < 0.001$ and $p = 0.01$, respectively) and both were increased in the placebo and pioglitazone groups. There were no serious adverse events in either group.

Conclusion: Treatment for 24 weeks with empagliflozin, in contrast to pioglitazone, was associated with improvement of liver steatosis and fibrosis in patients with NAFLD and T2DM. In addition, body weight and abdominal fat area were decreased in the empagliflozin group.

Trial Registration: Iranian Registry of Clinical Trials (IRCT), IRCT20190122042450N3.

Keywords: Body composition; Empagliflozin; Fibroscan; Liver fibrosis; Non-alcoholic fatty liver disease (NAFLD); Nonalcoholic steato-hepatitis (NASH); Pioglitazone; Steatosis

Key Summary Points
The prevalence of non-alcoholic fatty liver disease (NAFLD) has been increased worldwide
Hepatic fibrosis is an important factor in morbidity and mortality due to liver failure, cardiovascular events, and metabolic disorders
Currently no approved treatment is available
This study aimed to explore the effect of empagliflozin versus pioglitazone and placebo on liver steatosis and fibrosis in patients with T2DM and NAFLD
Compared to placebo, empagliflozine improves the CAP score and liver stiffness measurement (LSM) in patients with non-alcoholic fatty liver disease and type 2 diabetes mellitus
Empagliflozin decreases LSM more effectively than pioglitazone
Body weight and abdominal fat area decrease with empagliflozin

DIGITAL FEATURES

This article is published with digital features, including a summary slide, to facilitate understanding of the article. To view digital features for this article go to https://doi.org/10.6084/m9.figshare.13622393.

INTRODUCTION

The prevalence of non-alcoholic fatty liver disease (NAFLD) is parallel to obesity, which has been increasing worldwide for the past 30 years [1]. According to one meta-analysis, the prevalence of ultrasound-determined fatty liver in patients with type 2 diabetes mellitus (T2DM) has been reported from 29.6 to 87.1% [2]. In recent years, NAFLD has become doubly important because of the growing incidence of obesity and T2DM. In susceptible individuals, NAFLD can progress to cirrhosis, end-stage liver disease and hepatocellular carcinoma (HCC). It is predicted that NAFLD will become the main cause of mortality due to liver disease in the next 20 years and will be an important cause for liver transplantation in the next few years [3]. Although its potential for progression to cirrhosis and HCC has been recognized for decades, recent findings suggest that NAFLD is a major cause of cryptogenic cirrhosis [4].

NAFLD is a metabolic disorder caused by a complex interaction among genetic, hormonal, and nutrient factors [5]. Obesity and metabolic syndrome are the most important risk factors for NAFLD, with T2DM and hypertension being associated with further disease progression [6, 7]. An important pathogenic mechanism of both NAFLD and T2DM is insulin resistance. T2DM also worsens liver steatosis leading to NASH, fibrosis, and cirrhosis, and ultimately to increased risk of developing HCC [7–9]. On the other hand, NAFLD patients have an increased risk of diabetes [10]. Moreover, advanced hepatic fibrosis is known as an independent factor in predicting mortality [11, 12] and is considered an independent risk factor for cardiovascular events associated with reduced life expectancy [13, 14]. Some data suggested

NAFLD is associated with cardiac arrhythmia and venous thrombosis [15].

As NAFLD and non-alcoholic stateohepatitis (NASH) are closely related to macro-cardiovascular events and are associated with reduced life expectancy, early and appropriate therapeutic intervention is essential [16]. However, despite the role of incretin hormones in NAFLD pathogenesis [17] and various interventions targeting the gut-pancreas-liver axis, in NASH treatment no approved treatment is currently available [17, 18].

Sodium-glucose cotransporter 2 (SGLT2) inhibitors prevent glucose reabsorption in renal proximal tubules, leading to increased urinary glucose excretion and a decrease in the blood glucose and insulin levels [19, 20]. This class of medications reduces macrovascular events [19, 20] and also has beneficial effects on liver function, in both clinical trials and animal models [21–23]. Therefore, SGLT2 inhibitors could prove to be useful in the treatment of patients with T2DM with NAFLD. On the other hand, thiazolidinediones that target and decrease insulin resistance, adipose tissue dysfunction, and inflammation are accepted to be useful in the treatment of these patients [24], and studies using pioglitazone have shown improvements in insulin resistance as well as in laboratory and histology indices of liver pathology in patients with fatty liver [25–28].

This study aimed to explore the effect of empagliflozin versus placebo on liver steatosis and fibrosis in patients with T2DM and NAFLD. The results would be compared to those in a similar group of patients treated with pioglitazone, an agent that has shown efficacy in some patients with NAFLD and T2DM.

METHODS

Study Design

This was a 24-week, prospective, randomized, double-blind, placebo-controlled clinical trial, using 1:1:1 allocation to the treatment arms using a computer-generated randomization sequence. It was in accordance with the principles of the Declaration of Helsinki and was approved by the Ethics Committee of Iran University of Medical Sciences (ethics code. R.IUMS.FMD.REC.1398.463). The study was also registered in the Iranian Registry of Clinical Trials (IRCT), registration number: IRCT20190122042450N3). All the participants signed written informed consent before enrolment. Data management was performed by the Institute of Endocrinology and Metabolism monitoring committee, which was blinded to the study arms. Abidi Pharmaceuticals supplied empagliflozin, pioglitazone, and placebo and had no other role in the study.

Patients

Patients with T2DM, aged 20 and 65 years, with a hemoglobin A1c (HbA1c) of 7–10% were eligible to enroll. Inclusion criteria were established T2DM and NAFLD with controlled attenuation parameter (CAP) $\geq$ 238 dB/m in transient hepatic elastography [29]. Exclusion criteria included: type 1 diabetes; active or chronic hepatitis; cirrhosis and biliary disease; heart failure defined as New York Heart Association (NYHA) class III and IV; renal dysfunction estimated glomerular filtration (eGFR) < 45 ml/min/1.73 m^2); history of alcohol consumption > 20 g per day in women and 30 g per day in men; taking medications associated with fatty liver nonsteroidal anti-inflammatory drugs (NSAIDs) (amiodarone, tamoxifen, sodium valproate, corticosteroids, methotrexate); taking other fatty liver-related therapies such as vitamin E and trial medications (empagliflozin and pioglitazone); using supplements including vitamin C, zinc, selenium, or antioxidant agents over the last months; history of cardiovascular events within the past 3 months; pregnancy and breastfeeding; active cancer or history of cancer treatment over the past 2 years; untreated thyroid disorder; body mass index (BMI) $\geq$ 40 kg/m^2.

Randomization and Masking

Eligible subjects were randomly assigned to the three study arms using a random block method to receive empagliflozin 10 mg once daily,

pioglitazone 30 mg once daily, or placebo once daily for 24 weeks. Both the investigators and patients were blinded to the study arms, and measurement of end points was performed by clinicians and technicians who were not aware of the randomization groups and treatment arms. Also, all of the pills were concealed and did not have a visible name or information.

Procedures

All baseline measurements were performed within 1 week of enrollment. Fibroscan, dual-energy x-ray absorptiometry (DEXA) scan, and the biochemical variables were re-measured at week 24 with the same device and method and by the same physician who was blinded to the study arms. Liver fibroscan was performed by FibroScan® 502 Touch equipped with both M and XL probes. Controlled attenuation parameter (CAP) and liver stiffness measurement (LSM) were determined, as described [29]. CAP was deemed valid only in matched LSM values to ensure the accuracy of the measurements. According to the usual definition, all the following criteria had to be met to consider LSM as reliable: ten valid measurements, LSM success rate 60%, and LSM interquartile range/median (IQR/M) 0.30 [30–32]. Participants with CAP score > 238 dB/m were enrolled in the study [29, 33, 34]. Fibrosis was also determined using the METAVIR score according to a previous study [35]. In addition, a DEXA scan (Hologic Discovery DXA system) was performed in all participants. Full-body DXA in supine position was performed for analysis of lean and fat masses. We calculated two indices using the appendicular lean mass (ALM) (kg): ALM divided by height squared (kg/m^2); ALM divided by weight and multiplied by 100 ($kg/kg \times 100$). Skeletal muscle index (SMI) ($ALM/height^2$) was categorized to low risk (%): men < 7 and women < 5.4; high risk: (%) men ≥ 7 and women ≥ 5.4. SMI (ALM/weight) was categorized as low risk (%): men ≤ 29 and women ≤ 25; high risk (%): men > 29 and women > 25 [36, 37].

Lipid profile, fasting blood sugar (FBS), serum aspartate aminotransferase (AST), and alanine aminotransferase (ALT), serum creatinine (Pars biochemical kits using the photometric method), HbA1c (SEBIA using the capillary method), fasting insulin [Monobind kit, code: 5825-300, immunoenzymometric assay ("IEMA" method], and complete blood count (Sysmex cell counter device using the electric resistance-light scattering method) were measured at baseline and the end of the study. We also measured viral hepatitis markers (hepatitis B via SURASE B-96 kit and hepatitis C via NANBASE C 96 Kit), ANA (EUROIMMUN kit using the immunofluorescence method), and thyroid function (ELISA, Pishtaz Medical Co.).

NAFLD fibrosis risk score was calculated by the following formula (38): NAFLD fibrosis score = $- 1.675 + (0.037 \times$ age [years]) + $(0.094 \times$ BMI [kg/m^2]) + ($1.13 \times$ IFG/diabetes [yes = 1, no = 0]) + ($0.99 \times$ AST/ALT ratio) $- (0.013 \times$ platelet count [$\times 10^9$/l]) $- (0.66 \times$ albumin [g/dl]). NAFLD fibrosis score was categorized as low risk (%): $< - 1.455$, intermediate risk (%): $- 1.455$–0.675, and high risk (%): > 0.675. Furthermore, the FIB-4 (fibrosis 4) index [39] was calculated using the following formula: FIB-4 index = [age $\times$ AST (units/l)]/(platelet count ($\times 10^9$/l) $\times$ [ALT (units/l)]$^{1/2}$. The FIB4 index was categorized as low risk (%): < 1.3, intermediate risk (%): 1.3–2.67, and high risk (%): > 2.67. Also, we calculated the APRI (aspartate aminotransferase to platelet ratio index) [40] by the following formula: APRI = [(AST/AST upper limit of normal)/platelet ($\times 10^9$/l)] $\times$ 100 and categorized it as low risk (%): < 0.5, intermediate risk (%): 0.5–1.5 and high risk (%): > 1.5.

Insulin resistance was estimated by calculating HOMA2-IR, using the HOMA Calculator and HOMA-IR by the following formula: FBS (mg/dl) $\times$ fasting serum insulin (μIU/ml)/405. [41].

Follow-Up

Participants were asked to perform moderate-intensity physical activity based on a metabolic equivalent task (METS) at least 3 times a week, and they were encouraged to follow the recommendation at least 45 min without

interruption during the study period. Participants were given standard dietary advice as well.

Participants were followed monthly by phone calls to assess their adherence to the treatment protocol and presence of possible adverse events including genital or urinary tract infections, hypoglycemia, lower extremity edema, shortness of breath, nausea, angioedema, or drug intolerance and other adverse events. All patients had an in-clinic visit 3 months after enrollment and at the end of the study.

Outcomes

The primary end point was the change in CAP score from baseline to 24 weeks of treatment. The key secondary end point was the change in LSM from baseline to 24 weeks of treatment. Other secondary end points were the changes in liver enzymes (AST, ALT), fasting insulin, HOMA2 IR, VAT (visceral adipose tissue), and body composition parameters as well as non-invasive measurement scores for hepatic fibrosis.

Statistical Analysis

We calculated that a sample of 75 patients (total with 25 per group) would be required to detect a difference of 28.2 dB/m (9% from base) in the CAP score, with 80% power at a significance level of 0.05 [42, 43]. Assuming up to a 30% drop out, the sample size was 105 participants [35].

All analyses for the efficacy parameters were performed in the intention-to-treat population. Baseline characteristics of the participants were summarized as means $\pm$ standard deviation (SD) for continuous variables and percentages for categorical variables. For continuous variables, paired t test was used to compare before/after measurements, and comparison of differences in each group was performed using analysis variance (ANOVA). Also, for continuous variants, only measured at baseline, ANOVA was used. Comparisons of discrete covariates between the groups and the before/after

measurements were done using the chi-squared test. All p-values presented are two-tailed, and differences were considered statistically significant at $p < 0.05$. Finally, the regression models with repeated measures were fitted to assess the effects of the covariates on the outcomes.

RESULTS

Study Population

A total of 186 individuals were screened according to the inclusion/exclusion criteria, and 106 individuals who met the eligibility criteria were randomized to receive empagliflozin ($n = 35$), pioglitazone ($n = 35$), or placebo ($n = 36$). Of all randomized patients, 78 completed the trial (empagliflozin group, $n = 25$; pioglitazone group, $n = 27$, and placebo group, $n = 26$). The patient enrollment flow diagram and the reasons for drop-out are shown in Fig. 1.

Patient Characteristics

At baseline, the three groups were matched regarding to demographic and anthropometric characteristics. The duration of T2DM (about 6.5 years), statin consumption, and biochemical indices were comparable among the three groups (Table 1).

In the empagliflozin group, weight and BMI decreased significantly ($p < 0.001$ for both), while in the pioglitazone group, both body weight and BMI had increased significantly by the end of the trial ($p = 0.007$ and $p = 0.005$, respectively). In the placebo group, there was no significant change in these parameters.

Liver Enzymes and Insulin Resistance State

After 24 weeks, AST levels decreased significantly in the empagliflozin group ($p = 0.02$), and both AST and ALT levels decreased in the pioglitazone group ($p = 0.03$ and $p = 0.01$, respectively).
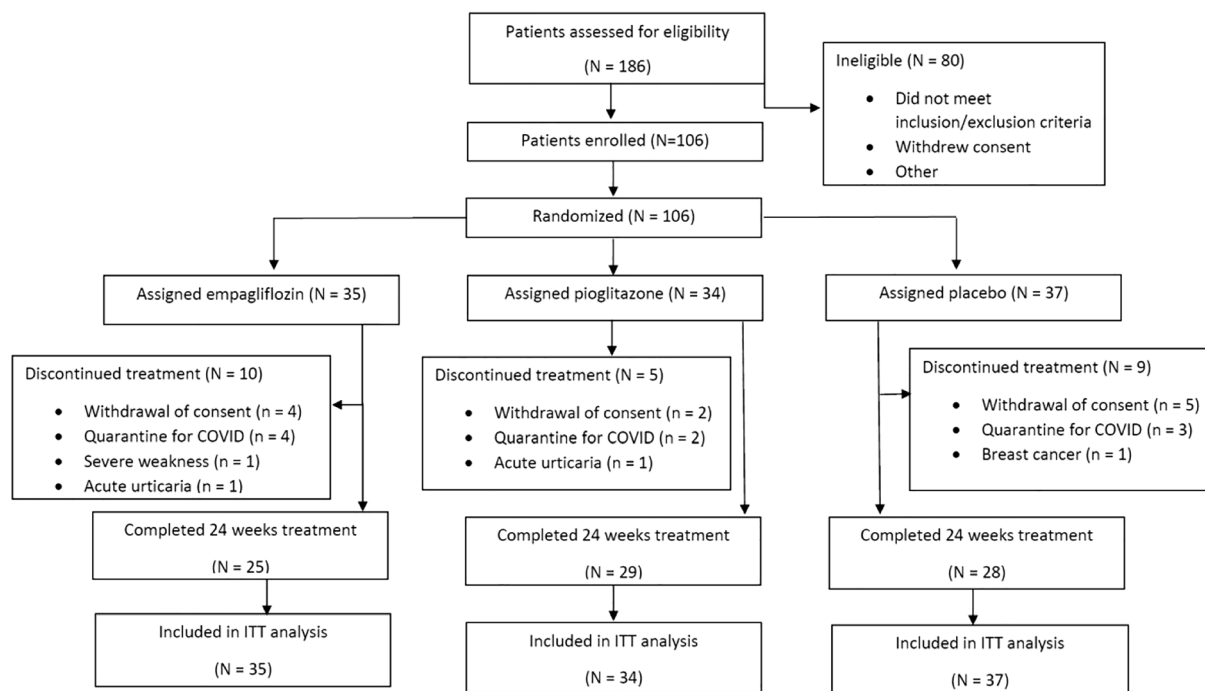
Fig. 1 Patient enrollment flow diagram. *ITT* intention to treat

In addition, by the end of the trial, there was a significant decrease (p = 0.008) in fasting insulin levels in the pioglitazone group (14.7 ± 6.2 mIU/l to 12.2 ± 3.8 mIU/l) with no significant change in the empagliflozin or placebo groups. HOMA2 IR was significantly decreased (2.1 ± 0.9 to 1.7 ± 0.5; p = 0.01) in the pioglitazone group, and the small change in the empagliflozin group did not reach statistical significance (2.4 ± 1.2 to 2.2 ± 1.1; p = 0.14). The same pattern was observed for HOMA-IR, namely that in the pioglitazone group, HOMA-IR decreased significantly from 6.0 ± 2.5 to 4.3 ± 1.7 ($p < 0.001$) and the smaller decrease in the empagliflozin group did not reach significance (6.3 ± 4.4 to 5.7 ± 3.7; p = 0.27). Changes in AST, ALT, fasting insulin, HOMA-IR, and HOMA2-IR were comparable between the pioglitazone and empagliflozin groups (p = 0.43, 0.81, 0.36, 0.10, and 0.48, respectively, for between groups).

Metabolic Profile

After 24 weeks, the HbA1C level decreased significantly in both the empagliflozin and pioglitazone groups (p = 0.001 and < 0.001, respectively); however, the reduction was greater in the pioglitazone group (p = 0.01). Also, there was a significant decrease in FBS levels in the pioglitazone group ($p < 0.001$). At the end of the trial, there was no significant change in low-density lipoprotein (LDL) and triglyceride (TG) levels in any of the groups, but there was a significant increase in the high-density lipoprotein (HDL) level in the pioglitazone group (p = 0.002).

Liver Steatosis (CAP) and Fibrosis (LSM)

The CAP score was not significantly different between the three groups at baseline (p = 0.11). It decreased from 317.37 ± 28.46 dB/m to 287.80 ± 31.14 dB/m ($p < 0.001$) in the empagliflozin group, from 308.76 ± 30.59 dB/m to 280.91 ± 34.52 ($p < 0.001$) in the pioglitazone group, and from 313.14 ± 30.40 dB/m to

Table 1 Characteristics of the study participants at baseline and at 24 weeks

	Placebo group (n = 37)			EMPA group (n = 35)			PIO group (n = 34)			Between-group p at baseline	Between-group p at Week 24
	Baseline	Week 24	Within-group p	Baseline	Week 24	Within-group p	Baseline	Week 24	Within-group p		
Age (years)	51.8 ± 7.8			50.5 ± 8.4			52.5 ± 7.9			0.58	–
Gender (male)	14 (37.8%)			15 (42.9%)			17 (50.0%)			0.58	–
DM duration#	7.0 ± 5.3			5.5 ± 4.0			7.3 ± 5.2			0.27	–
Statin use	35 (94.6%)			34 (97.1%)			29 (85.3%)			0.16	–
Weight (kg)	80.3 ± 12.8	80.0 ± 13.0	0.29	82.2 ± 12.3	79.5 ± 11.9	< 0.001	79.7 ± 10.6	81.4 ± 10.9	0.007	0.67	0.78
BMI (kg/m²)	30.2 ± 4.4	30.1 ± 4.7	0.38	30.9 ± 3.3	29.9 ± 3.1	< 0.001	29.4 ± 3.7	30.1 ± 3.9	0.005	0.27	0.97
FBS (mg/dl)	156.3 ± 34.0	154.9 ± 35.5	0.80	149.6 ± 42.2	150.9 ± 33.9	0.84	169.4 ± 48.3	146.2 ± 53.3	< 0.001	0.13	0.68
HbA1C (%)	7.96 ± 0.62	7.95 ± 0.96	0.92	8.08 ± 0.92	7.66 ± 0.86	0.001	8.23 ± 0.79	7.25 ± 0.94	< 0.001	0.35	0.007
Chol (mg/dl)	153.4 ± 40.0	157.0 ± 40.6	0.47	146.1 ± 27.8	144.1 ± 23.9	0.61	150.1 ± 29.1	153.3 ± 38.8	0.58	0.64	0.28
LDL (mg/dl)	91.3 ± 24.1	92.2 ± 27.4	0.79	85.0 ± 15.1	81.3 ± 14.2	0.17	86.9 ± 22.1	83.1 ± 21.0	0.23	0.42	0.07
HDL (mg/dl)	47.6 ± 9.4	49.7 ± 8.4	0.03	45.6 ± 9.1	48.3 ± 9.3	0.05	46.9 ± 8.3	52.1 ± 11.4	0.002	0.64	0.25
TG (mg/dl)	183.5 ± 127.7	166.8 ± 69.1	0.34	183.2 ± 149.3	162.5 ± 129.0	0.10	196.1 ± 98.0	176.4 ± 129.5	0.27	0.89	0.86
ALT (IU/l)	32.1 ± 17.3	28.5 ± 19.3	0.14	31.1 ± 16.9	27.0 ± 12.7	0.06	28.8 ± 16.6	24.1 ± 11.8	0.01	0.69	0.45
AST (IU/l)	24.2 ± 10.7	23.1 ± 9.6	0.30	26.0 ± 16.5	20.6 ± 8.1	0.02	23.4 ± 9.6	20.1 ± 6.9	0.03	0.67	0.27
Insulin (mIU/l)	17.4 ± 10.9	15.0 ± 10.5	0.05	16.5 ± 8.2	15.2 ± 8.1	0.22	14.7 ± 6.2	12.2 ± 3.8	0.008	0.42	0.22
HOMA-IR	6.5 ± 3.4	5.6 ± 3.5	0.14	6.3 ± 4.4	5.7 ± 3.7	0.27	6.0 ± 2.5	4.3 ± 1.7	< 0.001	0.83	0.11
HOMA2-IR	2.4 ± 1.3	2.1 ± 1.3	0.06	2.4 ± 1.2	2.2 ± 1.1	0.14	2.1 ± 0.9	1.7 ± 0.5	0.01	0.42	0.19

Data are mean ± SD for normally distributed parameters or n (%). p value and statistically significant p values ($p < 0.05$) are in bold, # DM duration: years, BMI: body mass index; Chol: cholesterol

296.73 ± 40.13 in the placebo group ($p < 0.001$) (Table 2).

Compared to the placebo group as the reference, there was a borderline significant decrease in the CAP score in the empagliflozin group ($p = 0.05$), while no significant change was observed in the pioglitazone group ($p = 0.08$). The distribution of CAP scores at baseline and at the end of the study among the participants is shown in Fig. 2a.

Liver fibrosis, was significantly decreased after 24 weeks in the empagliflozin group (LSM: 6.83 ± 2.44 to 6.01 ± 1.65 kPa; $p = 0.005$), while the change in fibrosis score in the pioglitazone group and placebo groups was not significant: (6.48 ± 1.67 to 6.42 ± 2.14 kPa; $p = 0.80$) and (7.49 ± 2.65 to 7.17 ± 2.67 kPa; $p = 0.27$), respectively (Table 2).

In univariate regression analysis, changes from baseline in HOMA2-IR ($p = 0.06$), BMI ($p = 0.21$), and HbA1c ($p = 0.33$) were not associated with the degree of liver fibrosis at the end of the study. In multivariate analysis, after adjusting for baseline covariates, we found a significant difference between the empagliflozin and placebo groups in relation to liver fibrosis at the end of the study: -0.77 ($p = 0.02$); however, there was no significant difference between the pioglitazone and placebo groups: 0.01 ($p = 0.98$).

Additionally we found that a lower HOMA2-IR at baseline was associated with a lower fibrosis score, while BMI, age, gender, and HbA1c did not have any association with liver fibrosis. In this model, one unit lower HOMA2-IR at baseline was associated with a 0.34 lower fibrosis score ($p = 0.02$) (Table 3). The distribution of fibrosis scores at baseline and at the end of the study is shown in Fig. 2b.

Non-Invasive Scoring Systems for Assessment of NAFLD

There were no significant changes in the non-invasive scoring for liver fibrosis, including the NAFLD fibrosis score and FIB-4 index. However, there was a numerical decrease in the number of individuals in the empagliflozin group who

Table 2 Change in hepatic steatosis (CAP score) and fibrosis (LSM)

	Placebo ($n = 37$)			Empagliflozin ($n = 35$)			Pioglitazone ($n = 34$)		
	Baseline	Week 24	p	Baseline	Week 24	p	Baseline	Week 24	p
CAP score (dB/m)	313.14 ± 30.40	296.73 ± 40.13	< 0.001	317.37 ± 28.46	287.80 ± 31.14	< 0.001	308.76 ± 30.59	280.91 ± 34.52	< 0.001
LSM (KPa)	7.49 ± 2.65	7.17 ± 2.67	0.27	6.83 ± 2.44	6.01 ± 1.65	0.005	6.48 ± 1.67	6.42 ± 2.14	0.80

Data are mean $\pm$ SD. p value within each group that compared baseline data with end of trial results; statistically significant p values ($p < 0.05$) are in bold
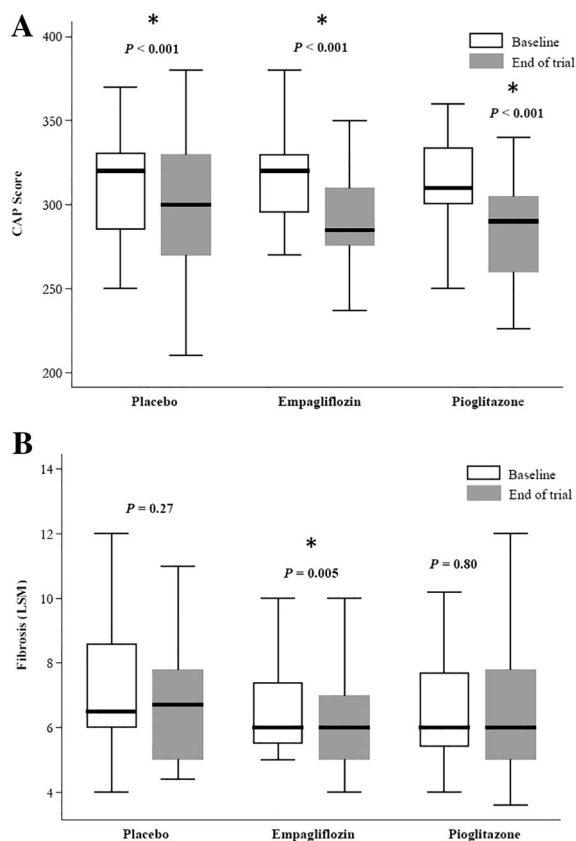
Fig. 2 a Distribution of CAP score stratified by the study groups. White boxes show the CAP score at baseline, and black ones indicate the final results after the intervention. *$p < 0.05$ compared with baseline and significant results. **b** Distribution of liver stiffness measurement stratified by the study groups. White boxes show liver stiffness measurement at baseline, and black ones indicate final results after the intervention.*$p < 0.05$ compared with baseline and significant results

were classified in the intermediate risk category of the FIB-4 index (25.7–20%) (see Appendix).

In evaluating the APRI parameter, there was a numerical decrease in the number of individuals at the intermediate risk (11.8–5.9%) in the pioglitazone group, but this change was not statistically significant (p = 0.31). In the empagliflozin group, a decrease in the number of intermediate risk individuals and their conversion to low risk individuals (20–5.7%) resulted in a statistically significant difference (p = 0.02) (see Appendix).

Changes in Body Composition

After 24 weeks, there was a significant increase in truncal fat mass area in the pioglitazone and placebo groups ($p < 0.001$ in both), while in the empagliflozin group the modest increase was not statistically significant (p = 0.12) (Table 4). Although, there was no significant difference among the three groups regarding changes in the VAT (p = 0.57), the VAT area increased significantly in the pioglitazone and placebo groups (p = 0.006 and 0.005, respectively). The VAT area did not change in the empagliflozin group from the baseline (p = 0.85). There was a significant difference in the change in VAT area and truncal fat mass in the empagliflozin group compared to the pioglitazone group (p = 0.01 and < 0.001, respectively).

There was a significant decrease in the skeletal muscle index (SMI) when ALM was adjusted for height^2 in all groups. Given that the height was constant throughout the study, this finding suggests that ALM significantly decreased in all groups; however, changes were not significant among the the groups (p = 0.82). Furthermore, we found a significant decrease in SMI per weight (ALM/weight) in all groups with no significant difference among groups (p = 0.80) (Table 4).

A statistically significant decrease was observed in the pioglitazone and empagliflozin groups in their android/gynoid (A/G) ratio and AFR (android fat ratio), while no significant change was observed in the placebo group and when compared among the three groups (Table 4).

Adverse Events

One patient in each of the groups had an episode of mild hypoglycemia. Other adverse events included two cases of urticaria (one each in the empagliflozin and pioglitazone groups), a case of nocturia and polyuria in the empagliflozin group, and one case of severe weakness and fatigue leading to discontinuation of the medication in the empagliflozin group. Also, one case of diabetic foot ulcer was seen in the placebo and in the pioglitazone groups. There

Table 3 Multivariate analysis of fibrosis (LSM)

		Coefficient	Multivariate p value	95% Confidence interval		Comparing empagliflozin with pioglitazone p value
				Lower	Upper	
Groups	Empagliflozin	− 0.77	**0.02**	− 1.45	− 0.09	**0.03**
	Pioglitazone	0.01	0.98	− 0.70	0.71	
Variates	HOMA2-IR	0.34	**0.02**	0.04	0.64	
	BMI	0.07	0.07	− 0.01	0.15	
	Age	0.10	0.61	− 0.03	0.05	
	Gender	0.32	0.28	− 0.27	0.91	
	HbA1C	0.04	0.82	− 0.32	0.40	

Statistically significant p values ($p < 0.05$) are in bold

was one case of breast cancer in the placebo group; this was discovered during an annual screening program, and the participant was excluded from continuing in the study.

DISCUSSION

In this randomized, double-blind, placebo-controlled trial, we found that treatment with 10 mg/day of empagliflozin for 24 weeks improved hepatic steatosis and fibrosis in patients with T2DM and NAFLD.

CAP Score and Liver Function

Visceral adiposity and liver fat accumulation predispose individuals with NAFLD to extrahepatic disorders including cardiovascular diseases, chronic kidney disease, T2DM, and colorectal cancer [44–46]. Liver biopsy remains the gold standard in assessment of NAFLD. However, limited studies have used this method to evaluate the efficacy of SGLT2 inhibitors on NAFLD progression [47]. Biopsy-proven improvement of liver histology has been reported in patients treated with empagliflozin [48]. A limitation of this study was the lack of a true placebo arm along with its open-label design. Recent studies have shown that fibroscans can effectively evaluate the liver steatosis

percentage and its grade compared to liver histology as the gold standard. [33, 35]. In addition, a large-scale prospective study demonstrated the accuracy of CAP for the diagnosis of NAFLD [35].

Here, we assessed the effect of empagliflozin and pioglitazone by measuring CAP as an index of hepatic steatosis [35]. Compared to placebo, empagliflozin patients had a marginally significant improvement in hepatic steatosis, while no significant change was observed with pioglitazone.

Previous studies have examined the effects of various SGLT2 inhibitors on patients with T2DM and NAFLD [18, 43, 49–52]. They showed improvement in at least one liver enzyme level [31, 43, 49, 50, 52] and in hepatic fat content [43, 49, 52]. An absolute reduction in liver fat content has been reported in patients with NAFLD treated with empagliflozin in the E-LIFT Trial. In this study, 4% reduction in absolute liver fat was associated with improvement of steatosis [49]. However, they used MRI-PDFF, which is expensive and time-consuming [53]. A study from Japan reported that ipragliflozin (a SGLT-2 inhibitor) reduced liver fat in patients with T2DM and NAFLD. However, the authors used the fatty liver index for the assessment of liver fat [54]. Moreover, similar results have been described in patients treated with luseogliflozin using the liver-to-spleen (L/S)

Table 4 Body composition assessment

	Placebo group (n = 37)			EMPA group (n = 35)			PIO group (n = 34)			Between-group p at baseline	Between-group p at week 24
	Baseline	Week 24	Within group p	Baseline	Week 24	Within group p	Baseline	Week 24	Within group p		
Truncal fat mass*	15,362.9 ± 3858.0	16,499.9 ± 4209.7	**< 0.001**	15,483.9 ± 2976.3	15,790.3 ± 3099.3	0.12	15,232.7 ± 2701.6	17,346.8 ± 3771.9	**< 0.001**	0.94	0.22
VAT area	174.6 ± 52.5	186.2 ± 62.9	**0.005**	178.9 ± 40.7	178.3 ± 46.6	0.85	178.0 ± 37.1	191.7 ± 47.9	**0.006**	0.90	0.57
SMI (ALM/height^2)	7.43 ± 1.09	7.08 ± 1.11	**< 0.001**	7.76 ± 1.42	7.08 ± 1.11	**< 0.001**	7.76 ± 1.42	7.18 ± 1.31	**< 0.001**	0.41	0.82
Low*	3 (8.1)	5 (13.5)	0.15	1 (2.9)	3 (8.6)	0.15	2 (5.9)	7 (20.6)	0.15	0.77	0.36
High*	34 (91.9)	32 (86.5)		34 (97.1)	32 (91.4)		32 (94.1)	27 (79.4)			
SMI (ALM/weight)	24.8 ± 3.3	23.8 ± 3.5	**< 0.001**	25.2 ± 4.4	24.1 ± 4.3	**< 0.001**	25.4 ± 2.9	23.5 ± 3.4	**< 0.001**	0.78	0.80
Low*	28 (75.7)	30 (81.1)	0.31	25 (71.4)	29 (82.9)	**0.04**	27 (79.4)	32 (94.1)	**0.04**	0.74	0.26
High*	9 (24.3)	7 (18.9)		10 (28.6)	6 (17.1)		7 (20.6)	2 (5.9)			
A/G ratio	0.70 ± 0.16	0.68 ± 0.16	0.12	0.72 ± 0.17	0.69 ± 0.15	**< 0.001**	0.75 ± 0.17	0.69 ± 0.17	**0.004**	0.43	0.98
AFR	0.095 ± 0.014	0.094 ± 0.013	0.48	0.098 ± 0.013	0.095 ± 0.012	**0.002**	0.100 ± 0.012	0.096 ± 0.012	0.01	0.27	0.81
Tertile-1*	14 (37.8)	13 (35.1)	0.52	9 (25.7)	10 (28.6)	0.10	9 (26.5)	9 (26.5)	0.05	0.22	0.91
Tertile-2*	11 (29.7)	11 (29.7)		9 (25.7)	12 (34.3)		5 (14.7)	10 (29.4)			
Tertile-3*	12 (32.4)	13 (35.1)		17 (48.6)	13 (37.1)		20 (58.8)	15 (44.1)			

Data are mean ± SD for normally distributed parameters and *others are numbers or n (%). Statistically significant p values (p < 0.05) are in bold. All of the definitions are in the text

EMPA empagliflozin, PIO pioglitazone, VAT visceral adipose tissue, SMI skeletal muscle index, AFR android fat ratio

attenuation ratio to estimate liver fat. However, this method is not accurate for quantifying liver fat content [52]. Similar to our study, Shimizu et al. reported improvement of the CAP score with dapagliflozin vs. placebo [43]. Moreover, ipragliflozin was compared with pioglitazone, and the results showed a reduction of L/S on computed tomography (CT) scan but no significant difference was observed between ipragliflozin and pioglitazone [18].

We also demonstrated improvement in liver enzymes with pioglitazone and reduction of AST in patients treated with empagliflozin. This finding has been demonstrated by others who found a significant decrease in serum ALT levels (p = 0.006) after ipragliflozin treatment [54]. Another study showed that the use of ipragliflozin in patients with T2DM and NAFLD improved serum AST and gamma-glutamyl transferase (GGT) levels [44]. Furthermore, canagliflozin improved the serum ALT level in patients with T2DM [55], and improvement in liver enzymes was reported in the E-LIFT [49] and PIVENS studies [56]. However, similar to our results, a study comparing ipragliflozin with pioglitazone reported no significant difference in the reduction of AST and ALT levels [18].

Liver Stiffness Measurement

In this study, a liver fibroscan was used to evaluate fibrosis. It has been shown that LSM reliably estimates liver fibrosis [57]. We detected a significant decrease in liver fibrosis after 24 weeks of treatment with empagliflozin (p = 0.005), while no change was observed with use of pioglitazone. Another study showed improvement of LSM in patients with T2DM who were treated with dapagliflozin, although the improvement was significant in the subgroup with significant liver fibrosis at baseline [43]. Also, an umbrella review indicated SGLT2 inhibitors effectively decrease liver steatosis, but not liver fibrosis, in diabetic patients with NAFLD [58]. However, one recent trial in non-diabetic patients with NAFLD showed improvement of the CAP score and LSM with empagliflozin vs. placebo [59].

The PIVENS trial compared the effects of a 2-year treatment with low-dose pioglitazone and vitamin E versus placebo in patients with T2DM [56]. The results showed that pioglitazone improved all histological features except for fibrosis and reduced the stage of NASH more than the placebo group [56]. By activating PPARγ, glitazones sensitize adipose tissue to insulin, thereby stimulating uptake and storage of fatty acids [60]. Also, by increasing adiponectin and reducing pre-inflammatory adipokines, they lead to decreased gluconeogenesis and the influx of fatty acids and improve insulin sensitivity [61]. Use of this class of medications improves adipose tissue function and leads to improvement in hepatic steatosis [25].

In a placebo-controlled study, Cusi et al. reported that patients with T2DM and NASH who were treated with pioglitazone showed improvement in their hepatic steatosis as well as in inflammation and ballooning [25]. There was also improvement in fibrosis and insulin sensitivity in the liver, skeletal muscle, and adipose tissue; these changes were maintained for 36 months after treatment [25]. Belfort et al. in a 6-month clinical trial in patients with pre-diabetes or T2DM with proven NASH by liver histology reported that pioglitazone caused a significant improvement in liver steatosis and inflammation; furthermore, the NAFLD activity score (NAS) improved in 73% of patients who were treated with pioglitazone compared with 24% in the placebo group ($p < 0.001$) [28].

The mechanisms leading to a beneficial effect of empagliflozin on liver fibrosis are not well understood. Inhibition of proinflammatory cytokines such as IL-6, TNF-α, and MCP-1 might be one possible explanation. [62]. Thus, inhibition of inflammation in the liver may contribute to inhibition of hepatic fibrosis in patients with NAFLD who are treated with empagliflozin.

We found that empagliflozin improved liver fibrosis compared with pioglitazone or placebo (p = 0.03). In addition, our study provides evidence on relationship between HOMA2-IR, an index of insulin resistance, and liver stiffness, while HbA1c, BMI, age, and gender were not associated with liver fibrosis. Fujii et al. showed that HOMA-IR and age are independent

predictors of advanced fibrosis in patients with NAFLD but without T2DM [63]. In contrast, a cross-sectional study in children with obesity and diabetes mellitus showed that HOMA-IR is not an independent predictor of liver fibrosis assessed by LSM [64].

On the other hand, Watt et al. presented evidence of a relationship between glycemic control and liver stiffness [65]. Also, in a cohort study Tanaka et al. reported that HbA1c is significantly associated with liver fibrosis as assessed by the FIB4 index [66]. Furthermore, a prospective study found that although HOMA IR and BMI were independent factors associated with significant liver fibrosis, the HbA1c level was not a significant factor when compared between subjects with and without significant liver fibrosis (liver stiffness > 7.0 kPa) [67].

Insulin Resistance

We also found a significant decrease in the fasting insulin level in the pioglitazone group, while no change was observed in the empagliflozin or placebo groups. Suppression of insulin resistance and hepatic steatosis are reported with empagliflozin in mice. This might be due to the anti-inflammatory effect of empagliflozin on fat tissue and liver [68]. However, in our study, a significant decrease in insulin resistance as estimated by the HOMA-IR and HOMA2-IR was seen only in the pioglitazone group ($p < 0.001$ and $p = 0.01$, respectively); the modest decreases in the empagliflozin and placebo groups were not statistically significant. In this regard, Shimizu et al. found a significant decrease in insulin resistance measured as HOMA-IR in the dapagliflozin-treated group in a randomized, active controlled, placebo-based open-label trial in patients with T2DM and NAFLD at 24 weeks [43]. However, while the effect of pioglitazone on insulin sensitivity is widely accepted, Ito et al. did not detect any significant difference between the effect of pioglitazone and ipragliflozin on insulin sensitivity [18].

Non-Invasive Scoring Systems

In our study there were no significant changes in non-invasive scores for measurement of liver fibrosis, namely, the FIB4 index and APRI. The FIB-4 index is a simple and valuable marker of liver fibrosis. While some studies did not find a significant change from baseline in liver fibrosis [43, 69, 70], others reported SGLT2 inhibitors effectively decrease the FIB-4 index. [18, 71]. In another study, the APRI index decreased significantly with pioglitazone [72]. Moreover, empagliflozin has been reported to lower the APRI index [73]. However, the FIB-4 index did not decrease significantly in the last two studies [72, 73]. On the other hand, Ito et al. reported a reduction of the FIB4 index with either ipragliflozin or pioglitazone [18]. The reasons for these discrepancies are uncertain. Patients' clinical characteristics and different durations of follow-up could partly explain different outcomes. Moreover, in our study, a higher percentage of the subjects was in the low-risk group. Hence, it might be difficult to show an improvement in this group of patients after 24 weeks.

Body Composition

Considering the importance of fat distribution, we also determined the body composition using DXA. This method involves less interference from body fluids than the bioimpedance method. In addition, DXA is a suitable method for measuring the lean mass. Therefore, the SMI, which is based on the lean mass of the extremities, could be a good indicator for the estimation of true skeletal muscle mass [36, 37].

Similar to previous studies [74–77], we found a significant increase in the truncal fat and VAT area with pioglitazone; however, the changes in the empagliflozin group were not significant (see Appendix). In addition, we found a significant decrease in SMI at 24 weeks in all three groups with no difference among the groups. The same reduction for total lean mass was reported in other studies using SGLT2 inhibitors [73, 78, 79]. On the other hand, we found that BMI and weight decreased significantly in the empagliflozin group ($p < 0.001$) while it

increased significantly in the pioglitazone group (p = 0.005 and 0.007, respectively). It can be hypothesized that decrements in SMI and the loss of skeletal muscle may be caused by increased protein catabolism secondary to calorie loss through glycosuria, and this may lead to sarcopenia, especially in elderly patients [78]. The reduction in muscle mass could itself be part of fatty liver disease [77]. Sasaki, et al. reported that treatment with luseogliflozin induced favorable changes in body composition and metabolism of moderately obese Japanese patients with T2DM accompanied by body fat reduction [80]. Previous studies showed that SGLT2 inhibitor-associated weight loss is mainly attributed to the reduction of fat mass rather than the lean body mass [81, 82].

Although this was the first study comparing the effect of empagliflozin with pioglitazone in patients with T2DM and NAFLD, our study has some limitations. We did not perform liver biopsy as the gold standard to evaluate the status of NAFLD. There was also a relatively large number of drop-outs for various reasons (including COVID-19) in all three groups during the study, which may have decreased the statistical power. However, to address this problem, we repeated the analysis after inclusion of the existing drop-outs and missing data, which showed similar results. Moreover, a longer duration of treatment might be necessary to ascertain effects. The strength of the study includes a reasonable male-to-female ratio of those completing the study (39–52% males) and the fact that the trial was a double-blind, placebo-controlled study. Finally, all the non-invasive measurements were carried out by the same individual blinded to the study arm of the participants.

CONCLUSION

In conclusion, this is the first study to investigate the effects of empagliflozin versus pioglitazone on hepatic steatosis and fibrosis in patients with NAFLD and T2DM. We demonstrated that empagliflozin improves steatosis and fibrosis. Further studies are needed to explore the mechanism of action of SGLT2i(s) on NAFLD.

This study was registered with the Iranian Registry of Clinical Trials (IRCT), registration number: IRCT20190122042450N3.

Data Availability. The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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