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ORIGINAL ARTICLE

First-in-Asian double-blind randomized trial to assess the efficacy and safety of insulin sensitizer in nonalcoholic steatohepatitis patients

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Abstract

Background The efficacy and safety of insulin sensitizer in Asians with non-alcoholic steatohepatitis (NASH) remain elusive.

Aims The double-blind, randomized, placebo-controlled trial was conducted aiming to investigate the efficacy and safety of pioglitazone in NASH patients.

Methods A total of 90 NASH patients (66 males, age = 44.1 ± 12.7 years) were prospectively randomized into oral pioglitazone 30 mg/day (Arm A) or placebo (Arm B) for 24 weeks. The primary endpoint was the efficacy of pioglitazone in reducing inflammation and liver fat at end-of-treatment (EOT). NASH resolution/improvement without fibrosis worsening was also evaluated.

Results At EOT, there was a significantly decline of alanine aminotransferase (86.9 ± 34.3 to 45.7 ± 35.8 IU/L, $p = 0.003$) level in Arm A patients. In intention-to-treat analysis among 66 patients who completed paired biopsies, The NAFLD activity score (NAS) of 30 Arm A patients significantly decreased from 4.27 ± 1.14 at baseline to 2.53 ± 1.63 at EOT ($p < 0.0001$), whereas there was no significant change in patients of Arm B (3.94 ± 1.41 vs 3.94 ± 1.51, $p = 1.0$). NASH improvement without worsening of fibrosis was achieved in 46.7% (14/30) patients in Arm A, compared to 11.1% (4/36) patients in Arm B ($p = 0.002$). Liver fat content reduced (20.2 ± 9.0 to 14.3 ± 6.9%, $p < 0.0001$) on MRI–PDFF in Arm A compared to their counterparts. No significant difference of adverse events occurred between groups.

Conclusions A 24-week pioglitazone treatment was well-tolerated and effective in improving liver histology and reducing liver steatosis in Asian NASH patients. (ClinicalTrials.gov number: NCT01068444)

Keywords Non-alcoholic steatohepatitis · Insulin sensitizer · Insulin resistance · Steatosis · Liver inflammation · Fibrosis · Magnetic resonance imaging–proton density fat fraction · Asians · Clinical trial · Safety

Abbreviations

NAFLD	Non-alcoholic fatty liver disease
BMI	Body mass index
MetS	Metabolic syndrome
T2DM	Type 2 diabetes mellitus
NASH	Non-alcoholic steatohepatitis
IR	Insulin resistance
HCC	Hepatocellular carcinoma
PPARγ	Peroxisome proliferator-activated receptor-gamma
TZD	Thiazolidinediones
FPG	Fasting plasma glucose
TC	Total cholesterol
HDL-C	High-density lipoprotein cholesterol
LDL-C	Low-density lipoprotein cholesterol
TG	Triglycerides
UA	Uric acid
hs-CRP	High-sensitive C-reactive protein
AST	Aspartate aminotransferase
ALT	Alanine aminotransferase
ULN	Upper limit of normal
EOF	End-of-follow-up
EOT	End-of-treatment

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AE	Adverse events
DMC	Data Monitoring Committee
HOMA-IR	The homeostasis model assessment method
NAS	NAFLD activity score
ITT	Intention-to-treat
FIB-4	Fibrosis-4
MRI–PDFF	Magnetic resonance imaging–proton density fat fraction

Introduction

Non-alcoholic fatty liver disease (NAFLD) is currently the most common liver disease worldwide [1]. The clinical spectrum ranges from isolated intrahepatic triglyceride accumulation to necroinflammation of hepatocytes [2]. The scenario of a higher overall mortality due to cardiovascular events as compared with controls has made it a critical global health issue. The epidemic has particularly been rapidly progressing in the past decades in Asia–Pacific in parallel to the rapid Westernization in the region [3]. However, the relative lower BMI in Asians is not protective from metabolic insults. Moreover, Asian people are more prone to metabolic syndrome (MetS), type 2 DM (T2DM) and NAFLD than other races [4].

Non-alcoholic steatohepatitis, defined by the presence of necroinflammation and ballooning on histopathology, is an extreme form of NAFLD. NASH is generically a hepatic manifestation of MetS and has a close link with other metabolic disorders, such as obesity, dyslipidemia, hypertension and DM [5, 6]. The insulin resistance (IR)-based metabolic liver disease carries a progressive potential for fibrosis/cirrhosis development and/or hepatocellular carcinoma (HCC). The risk becomes critical especially in patients with older age, obesity and diabetes. Lifestyle modifications, namely weight loss, exercise and diet control, are the current recommended management for NASH patients, yet most patients do not achieve or maintain dietary goals and weight loss. In addition, there is a pressing need of therapeutic exploration for the patients with advanced fibrosis or cirrhosis [7]. Currently, there is no approved medicine for NASH, and therapies to arrest or reverse disease progression are urgently needed. However, the therapeutic intervention of NASH has not been established and no drug has been approved for efficacy at present [8].

Pioglitazone, an agonist of peroxisome proliferator-activated receptor-gamma (PPARγ), belongs to thiazolidinediones (TZD) and anti-diabetes drug which decreases IR. It also increases peripheral tissue glucose disposal and decreases hepatic gluconeogenesis. Previous study by Belfort et al. demonstrated that PPARγ as well as diet control could improve glycemic control, decrease hepatic necroinflammation, decrease hepatic fat distribution and increase intrahepatic insulin sensitivity [9]. Aithal et al. further extended pioglitazone therapy over a 12-month period in nondiabetic NASH patients and demonstrated it improved metabolic and histologic parameters, most notably liver injury and fibrosis [10]. Meanwhile, PPARγ could also prevent the development of alcohol-induced steatohepatitis, improve hepatic necroinflammatory activity and decrease lipid deposition. The therapeutic efficacy and the safety of PPARγ agonist in Asian NASH patients in a well-designed manner deserve investigation.

Consequently, we conducted the first Asian double-blind, randomized, placebo-controlled, phase II study aiming to assess the efficacy and safety of pioglitazone in NASH patients. The primary outcome measurements were significant improvement of liver inflammation and significant reduction of liver fat content. NASH resolution/improvement without fibrosis worsening were assessed as the secondary endpoint. We also aimed to assess the safety and the changes of associated metabolic profiles in the current study.

Materials and methods

Study design

This prospective, multi-centre, double-blind, randomized study was an investigator-initiated phase II trial. It was conducted in one medical centre and 2 regional core hospitals in Taiwan since April 2009 (ClinicalTrials.gov number: NCT01068444). The Institutional Review Board of the Kaohsiung Medical University Hospital approved the study and the trial was conducted in compliance with the Declaration of Helsinki and the Good Clinical Practice guidelines of the International Conference on Harmonization. Written informed consent for interview, anthropomorphic measurements, blood sampling, liver biopsies and medical record review were obtained from patients prior to enrollment. All subjects underwent a 12-h overnight fast before blood tests, which included fasting plasma glucose (FPG), insulin, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglycerides, uric acid (UA), high-sensitive C-reactive protein (hs-CRP), aspartate aminotransferase [11] and alanine aminotransferase (ALT) levels. In addition, anthropometric data, which included blood pressure, waist circumference and body weight and height, were measured using standardized techniques. For those without known DM in their past history, they first received a 75-g oral glucose tolerance test (OGTT) and then 2-h post load plasma glucose level was measured.

Patient selection

Inclusion criteria

Eligible patients were treatment-naive Taiwanese patients, aged 18–70 years, who satisfied all of the following inclusion criteria were eligible to participate: (1) had undergone a liver biopsy within 6 months before entry, the results of which were consistent with NASH, i.e. a combination of steatosis (> 5% steatosis), hepatocellular injury and/or inflammation and ballooning; (2) ALT level between 1.3 and 5 upper limit of normal (ULN) for two occasions during 6 months before screening; (3) ethanol consumption of < 20 g/day; (4) Negative urine or blood pregnancy test (for female of childbearing potential) documented one day prior to the screening process; (5) Compensated liver disease; (6) HbA1C ≦ 8.0 during screening.

Exclusion criteria

Patients were excluded from the study if any of the following criteria existed: (1) laboratory or histologic findings highly suggestive of liver diseases of other etiologies, such as autoimmune hepatitis, primary biliary cirrhosis, biliary obstruction, hemochromatosis, alpha-1-antitrypsin deficiency or Wilson's disease; (2) ALT or AST levels greater than five times ULN; (3) abnormal total bilirubin or albumin level, prolonged prothrombin time, or platelet count below the lower limit of normal; (4) history or other evidence of bleeding from oesophageal varices or other conditions consistent with decompensated liver disease or cirrhosis (Child–Pugh class B or C) or overt hepatic failure; (5) treatment with any drugs known to cause hepatic steatosis (i.e., corticosteroids, high-dose estrogens, methotrexate, amiodarone, calcium channel blockers, spironolactone, sulfasalazine, naproxen, or oxacillin) within 6 months prior to the study; (6) treatment with drugs known to modify IR (i.e., vitamin E, sodium glucose transporter-2 inhibitor, glucagon-like peptide 1 agonist, insulin sensitizer or modulators); (7) serum creatinine level > 1.5 times the upper limit of normal at screening and calculated creatinine clearance as calculated by Cockcroft and Gault < 60 mL/min during screening; (8) history of ischaemic heart disease during screening; (9) any evidence met New York Heart Association (NYHA) Functional Class 1 or more cardiac status during screening; (10) history of metformin or insulin use within 6 months prior to screening or type 1 diabetes; (11) seropositive of HBsAg, anti-HCV or anti-HIV during screening; (12) psychiatric condition, previous liver transplantation, or evidence of HCC.

Procedures

During the screening period, patients underwent a liver biopsy or provided a liver biopsy tissue specimen obtained within the 6 months before screening. Patients were interviewed by the licensed dietitians and/or hepatologists for standard lifestyle modification instructions before randomization. Patients were randomly assigned in a 1:1 ratio to receive pioglitazone 30 mg/day for 6 months (Arm A) or matching placebo (Arm B) administered orally once daily with or without food. The treatment duration was 24 weeks and patients in each arm received 3 months of follow-up period (EOF) after end-of-treatment (EOT) (Fig. 1).

Patient randomization was performed using an interactive web response system (Bracket, San Francisco, CA). Patients and all personnel directly involved in the conduct of the study were blinded to treatment assignment. Study drugs were supplied as bottles of masked capsules and were dispensed by the study pharmacist in a blinded fashion to the

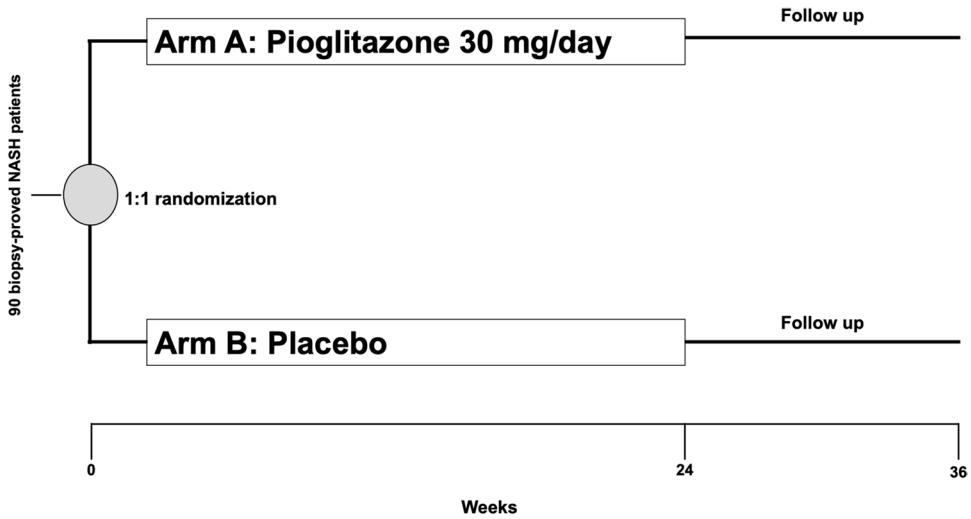

Fig. 1 Flowchart of the study

patients. Data were collected by investigators and managed by an independent biostatistician blinded to clinical profiles.

Safety and study oversight

Safety was assessed by clinical laboratory tests, physical examinations, measurement of vital signs and by the documentation of adverse events (AEs). Safety data were analysed from the first dose of study drug up to 4 weeks after the last dose of study drug. An independent data monitoring committee (DMC) reviewed the progress and provided oversight of the study. DMC also reviewed all clinical events, either liver-related or non-liver-related. All investigators had access to the data and assumed responsibility for the integrity and completeness of the reported data.

Laboratory analyses

FPG, TC, HDL-C, LDL-C, TG, UA, AST and ALT levels were measured on a multichannel autoanalyzer (Hitachi Inc, Tokyo, Japan). All assays were performed in duplicates. Fasting serum insulin levels were measured by radioimmunoassay (Diagnostic Products Co., Los Angeles, CA).

MetS was defined based on the updated National Cholesterol Education Program Adult Treatment Panel III criteria, modified by the criteria of obesity proposed for Asians by the Steering Committee of the Regional Office for the Western Pacific Region of WHO as presenting at least three of the following components: (1) waist circumferences > 90 cm in male or > 80 cm in women; (2) TG > 150 mg/dL; (3) HDL-C < 40 mg/dL in male or < 50 mg/dL in women; (4) blood pressure > 130/85 mmHg or current use of antihypertensive medications; or (5) FPG > 100 mg/dL or on oral antidiabetic agents or insulin. IR was calculated on the basis of FPG and insulin levels, according to the homeostasis model assessment method [12]. The formulas for the HOMA-IR = FPG (mg/dL) × fasting insulin level (μU/mL)/405.

Histological analyses

For each patient, a liver biopsy specimen of at least 2 cm in length was taken and fixed in 10% formalin buffer. Biopsy samples were stained with haematoxylin–eosin and the results were then reviewed by one independent certified liver pathologist (Dr. Huang SF) blinded to each patient. Histological diagnosis of NASH was based on the NAFLD activity score (NAS) of 4 or higher, with 1 or higher for each items (steatosis, ballooning, and lobular inflammation) defined by the NASH Clinical Research Network [13].

The extent of hepatic steatosis was graded according to the area occupied by that fatty hepatocytes on light microscopy; none (0–5%), mild (5–33%), moderate (33–66%) and severe (> 66%).

The histological efficacy of the paired biopsies was assessed by intention-to-treat (ITT) analysis with the following items: (1) NASH improvement: at least 2-point reduction in NAS (at least 1-point reduction in either lobular inflammation or hepatocellular ballooning) without worsening of fibrosis; (2) NASH resolution: disappearance of ballooning and disappearance or persistence of minimal, lobular inflammation that do not qualify for the diagnosis of NASH. The fibrosis stage was at least one stage reduction or without worsening of fibrosis (14). We further applied Fibrosis-4 (FIB-4) Score to assess the continuous changes of fibrosis between baseline and EOT. FIB-4 Score = (Age × AST)/(Platelets × $\sqrt{\text{ALT}}$) [15].

Imaging analysis

Longitudinal changes in liver fat from baseline to EOT were assessed using magnetic resonance imaging–proton density fat fraction (MRI–PDFF). The imaging studies were conducted by current standard of procedures. The results and assessment were performed by experienced central readers blinded to clinical and histologic data.

Statistical analyses

Frequency was compared between groups using the χ^2 test, with the Yates correction, or Fisher's exact test. Results are expressed as mean values ± standard deviation (SD) and were compared between groups using analysis of variance and the Student's *t* test, or nonparametric Mann–Whitney *U* test when appropriate. All statistical analyses were based on two-sided hypothesis tests with a significance level of $p < 0.05$. All the parameters of response and the side effects will be adequately recorded as description and frequencies. Quality control procedures, database processing and analyses were performed using the SPSS 12.0 statistical package (SPSS Inc., Chicago, IL, USA).

Results

Patient characteristics

A total of 90 eligible Taiwanese NASH patients were recruited into the study from April 2009 to August 2019. The demographic and baseline characteristics are shown in Table 1. The 90 patients included 43 patients receiving 24 weeks of pioglitazone (Arm A), and 47 patients receiving placebo (Arm B), respectively. Two patients of Arm A and 1 patient of Arm B withdrew from the study due to personal considerations. There were 66 males (73.3%) and the mean age was 44.1 ± 12.7 years. Their mean BMI was 28.9 ± 3.9 kg/m^2. There were 29 patients (32.2%) of obesity

Table 1 Demographic and baseline characteristics of the patients

	Total ($N=90$)	Pioglitazone ($n=43$)	Placebo ($n=47$)	p
Age (years)	43.9 ± 12.7	43.9 ± 13.7	43.8 ± 11.9	0.96
Male, *n* (%)	66 (73.3)	27 (62.8)	39 (83.0)	0.03
BMI (kg/m^2)	28.9 ± 3.9	28.4 ± 2.8	29.4 ± 4.6	0.02
Diabetes, *n* (%)	21 (23.3)	11 (25.6)	10 (21.3)	0.63
Hypertension, *n* (%)	35 (46.5)	20 (46.5)	15 (31.9)	0.16
Leukocyte count (mm^3)	7.1 ± 1.7	7.2 ± 1.9	7.1 ± 1.5	0.69
Haemoglobin (g/dL)	15.0 ± 1.3	14.8 ± 1.4	15.1 ± 1.2	0.36
Platelet count (mm^3)	249.7 ± 54.4	255.0 ± 60.4	244.9 ± 48.3	0.39
AST (U/L)	52.0 ± 23.5	50.6 ± 21.3	53.3 ± 25.4	0.58
ALT (U/L)	90.1 ± 39.0	90.0 ± 39.4	90.3 ± 39.0	0.97
GGT (IU/L)	68.8 ± 61.3	76.8 ± 76.1	61.4 ± 43.2	0.25
Creatinine (mg/dL)	0.9 ± 0.2	0.9 ± 0.2	0.9 ± 0.2	0.64
FIB-4	1.1 ± 0.6	1.0 ± 0.7	1.1 ± 0.6	0.75
FPG (mg/dL)	103.8 ± 16.2	98.3 ± 11.9	108.8 ± 18.0	0.002
Insulin (μU/mL)	10.4 ± 8.1	9.2 ± 7.5	11.5 ± 8.6	0.17
HOMA-IR	2.82 ± 2.74	2.3 ± 2.3	3.3 ± 3.0	0.10
HbA1c (%)	6.1 ± 0.7	6.0 ± 0.6	6.1 ± 0.7	0.30
Cholesterol (mg/dL)	215.1 ± 33.7	217.7 ± 36.2	212.7 ± 31.4	0.49
Triglyceride (mg/dL)	165.6 ± 116.0	154.8 ± 85.4	175.6 ± 138.4	0.39
HDL-C (mg/dL)	44.0 ± 10.6	45.1 ± 12.5	43.0 ± 8.5	0.36
LDL-C (mg/dL)	141.1 ± 35.2	142.8 ± 34.9	139.5 ± 35.7	0.66
Free fatty acid (mmol/L)	0.70 ± 0.24	0.73 ± 0.21	0.67 ± 0.25	0.28
Uric acid (mg/dL)	6.8 ± 1.4	6.8 ± 1.3	6.8 ± 1.5	0.96
hs-CRP (mg/dL)	0.31 ± 0.36	0.28 ± 0.29	0.34 ± 0.41	0.40
MetS, *n* (%)	38 (42.2)	22 (51.2)	30 (62.8)	0.22
FIB-4	1.1 ± 0.6	1.0 ± 0.7	1.1 ± 0.6	0.75
MRI–PDFF (%)	21.2 ± 8.4	20.6 ± 9.3	21.6 ± 7.5	0.60
NAS (0–8)	4.3 ± 1.3	4.3 ± 1.1	4.2 ± 1.4	0.74
Steatosis	2.4 ± 0.8	2.4 ± 0.9	2.4 ± 0.8	0.95
Lobular inflammation	1.1 ± 0.5	1.0 ± 0.6	1.1 ± 0.5	0.60
Ballooning	0.8 ± 0.7	0.9 ± 0.7	0.7 ± 0.7	0.36
NAS fibrosis stage, 0/1/2/3/4	39/36/4/10/1	18/18/1/5/1	21/18/3/5/0	0.84

(BMI ≥ 30 kg/m^2), whereas 10 patients (11.1%) were of normal BMI (< 25 kg/m^2). T2DM, dyslipidemia and MetS were found in 21 (23.3%), 56 (62.2%) and 52 (57.8%) of the patients, respectively. The mean fat content on MRI–PDFF was 21.2 ± 8.4%, whereas the mean NAS was 4.3 ± 1.3. According to NAS fibrosis scores, there were 39 (43.3%) patients of F0, 36 patients (40%) of F1, 4 patients (4.4%) of F2 and 11 patients (12.2%) of F3 or F4, respectively.

Biochemical efficacy

There was significantly decline in all patients from baseline to EOT in terms of AST (52.0 ± 23.5 to 41.4 ± 27.0 U/L, $p < 0.001$), ALT (90.1 ± 39.0 to 63.9 ± 45.6 U/L, $p < 0.001$), Alk-P (124.3 ± 139.6 to 113.9 ± 114.3 IU/L, $p = 0.02$), rGT (68.8 ± 61.3 to 54.4 ± 56.2 U/L, $p = 0.003$) and hs-CRP (0.31 ± 0.36 to 0.25 ± 0.33 mg/dL, $p = 0.01$) levels. The pre-treatment mean ALT level was 90.0 ± 39.4 IU/L in 41 Arm A patients, and it significantly decreased to 45.7 ± 35.8 IU/L at EOT ($p = 0.003$). The significant decreases of other biochemical tests in Arm A were also observed in AST (50.6 ± 21.3 to 32.6 ± 17.7 U/L, $p = 0.004$), HbA1c (6.0 ± 0.6 to 5.8 ± 0.4, $p = 0.003$), FPG (98.3 ± 11.9 to 94.0 ± 12.2 mg/dL, $p = 0.02$), UA (6.8 ± 1.3 to 6.1 ± 1.6 mg/dL, $p = 0.007$) and hs-CRP (0.28 ± 0.29 to 0.14 ± 0.08 mg/dL, $p = 0.004$) levels (Fig. 2). The HOMA-IR substantially decreased from baseline to EOT (2.3 ± 2.3 to 1.8 ± 1.1) in Arm A, whereas it increased from 3.3 ± 3.0 of baseline to 4.3 ± 7.0 of EOT in Arm B. By contrast, there were no significant changes of AST (53.3 ± 25.4 to 49.2 ± 31.5 U/L), ALT (90.3 ± 39.0 IU/L to 79.8 ± 48.0 IU/L) and hs-CRP (0.34 ± 0.41 to 0.35 ± 0.43 mg/dL) levels in 46 patients of Arm B.

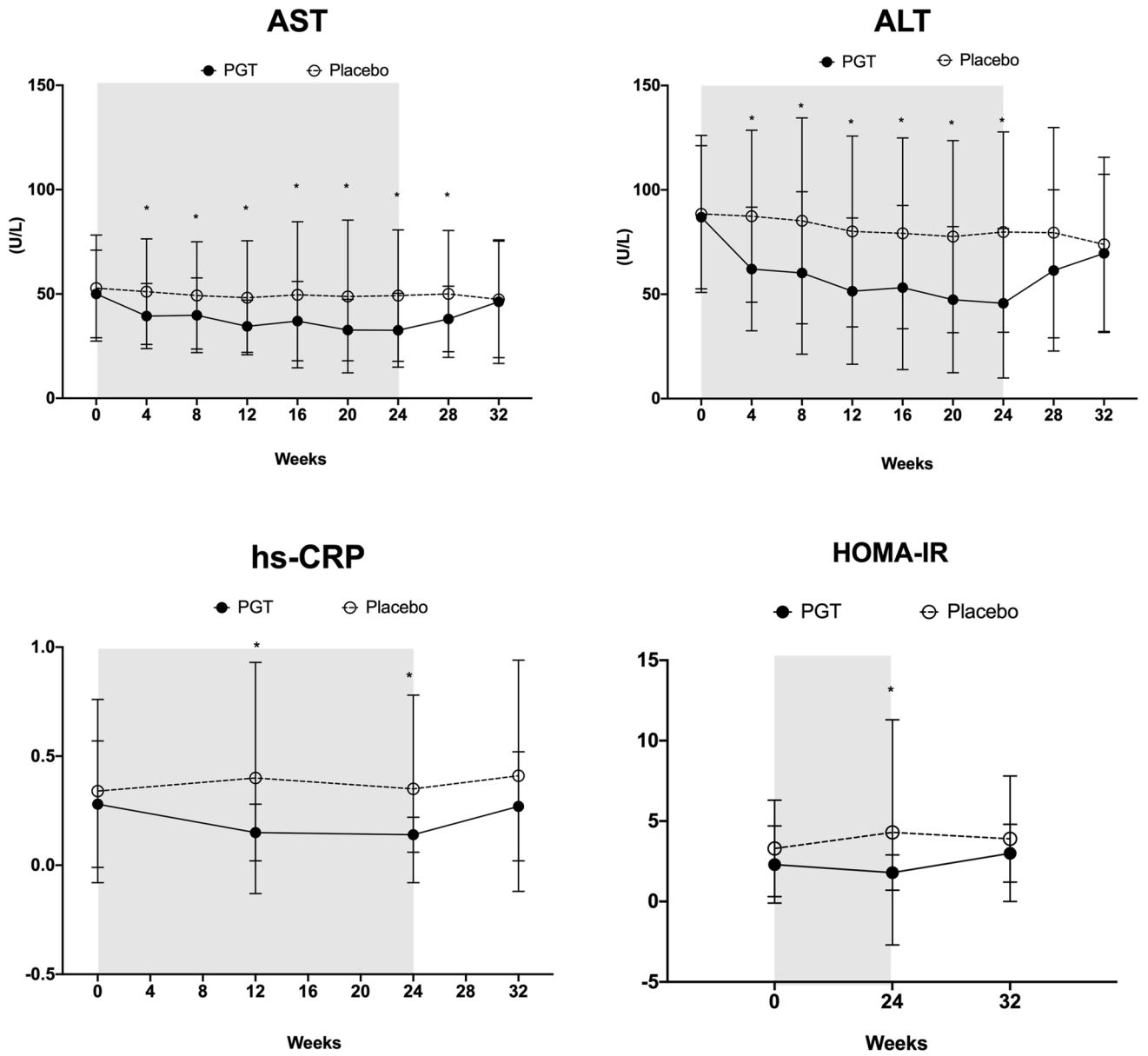

Fig. 2 The sequential biochemical changes between pioglitazone (PGT) Arm and placebo Arm. *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *hs-CRP* high-sensitive C-reactive protein, *HOMA-IR* the homeostasis model assessment-insulin resistance

Regarding the changes of lipid profiles, there was no significant change in terms of TC, HDL-C, LDL-C and TG levels in all patients. No significant change of lipid profiles was observed between arms from baseline to EOT.

Histologic efficacy

Figure 3 demonstrated the histopathologic changes between baseline and EOT among 66 patients who received paired biopsies (30 patients of Arm A and 36 patients of Arm B). In ITT analysis, the patients of moderate and severe steatosis at EOT among 30 patients in Arm A were 7 (23.3%), and 5 (16.7%), respectively. The percentage was significantly lower than 83% patients carrying moderate (5 patients) to severe (20 patients) steatosis at baseline ($p = 0.0002$). There was no significant difference of steatosis changes on paired biopsies in Arm B.

The NAS of Arm A patients significantly decreased from 4.27 ± 1.14 at baseline to 2.53 ± 1.63 at EOT ($p < 0.0001$), whereas there was no significant change in patients of Arm B (3.94 ± 1.41 vs 3.94 ± 1.51, $p = 1.0$). The items of steatosis and lobular inflammation in Arm A decreased significantly from 2.5 ± 0.78 and 0.97 ± 0.56 to 1.27 ± 1.08 ($p < 0.0001$), and 0.63 ± 0.49 ($p = 0.002$),

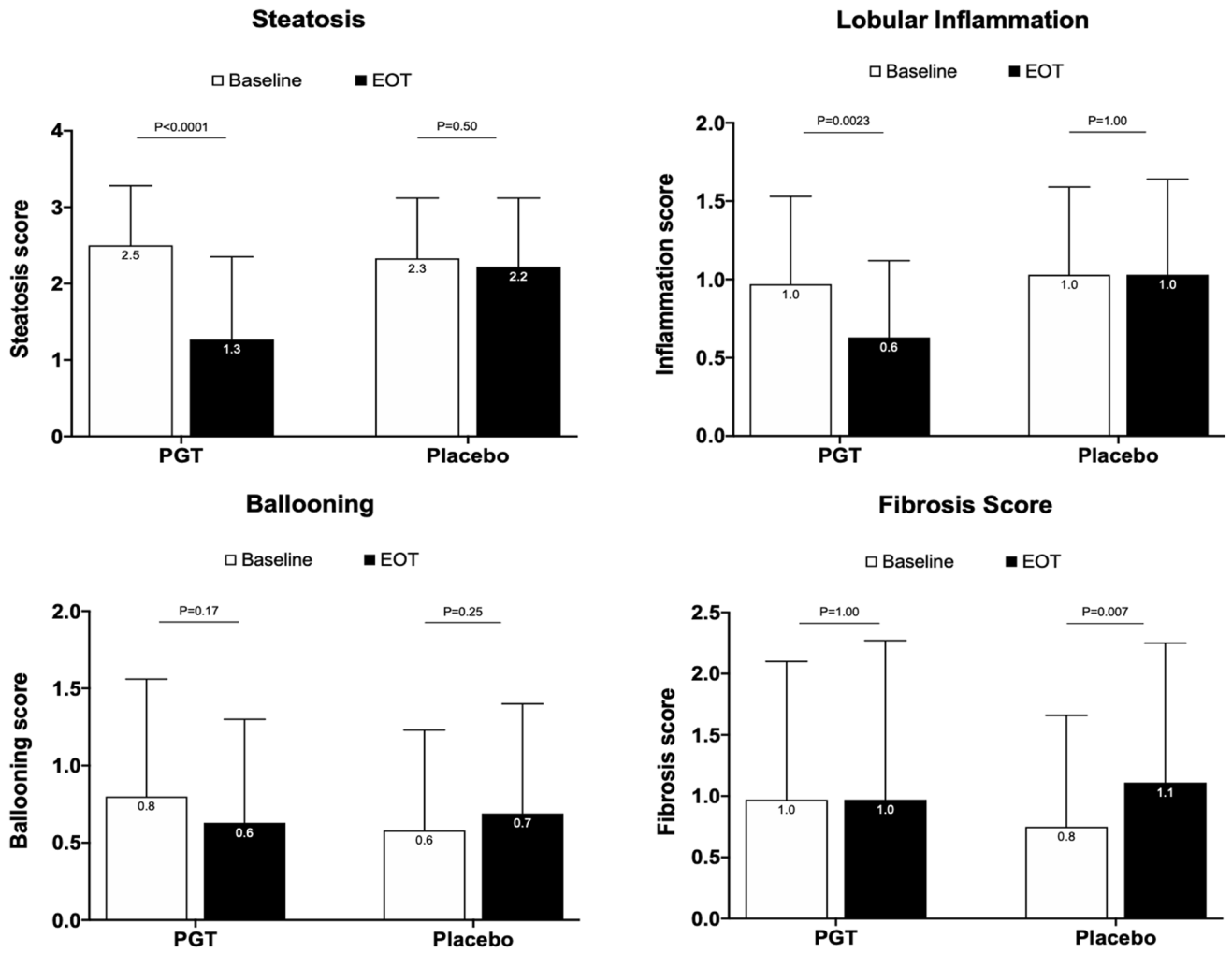

Fig. 3 The histopathologic changes between paired biopsies according to pioglitazone (PGT) Arm and placebo Arm

Table 2 The paired histologic features between pioglitazone and placebo arms

	Pioglitazone ($n=30$)			Placebo ($n=36$)		
	BL	EOT	p	BL	EOT	p
NAS	4.27 ± 1.14	2.53 ± 1.63	< 0.0001	3.94 ± 1.41	3.94 ± 1.51	1.0
Steatosis	2.50 ± 0.78	1.27 ± 1.08	< 0.0001	2.33 ± 0.79	2.22 ± 0.90	0.5
Lobular inflammation	0.97 ± 0.56	0.63 ± 0.49	0.002	1.03 ± 0.56	1.03 ± 0.61	1.0
Ballooning	0.80 ± 0.76	0.63 ± 0.67	0.17	0.58 ± 0.65	0.69 ± 0.71	0.25
NAS fibrosis stage (0/1/2/3/4)	12/13/0/4/1	14/11/0/2/3	< 0.0001	17/14/2/3/0	11/18/1/4/2	0.0003
NAS fibrosis Score	0.97 ± 1.13	0.97 ± 1.30	1.00	0.75 ± 0.91	1.11 ± 1.14	0.007
Ballooning reduced ≥ 1 score	7 (23.33)			4 (11.11)		0.19
Fibrosis reduced ≥ 1 score	2 (6.67)			2 (5.56)		1.0

respectively. By contrast, there was no significant change of ballooning in Arm A (0.80 ± 0.76 to 0.63 ± 0.67, $p=0.17$) and in Arm B (0.58 ± 0.65 to 0.69 ± 0.71, $p=0.25$), respectively. There were seven (23.3%) patients of Arm A with ballooning improved for 1 stage and more, which was not significantly different from Arm B (11.1%) ($p=0.19$) (Table 2).

There was no significant change of fibrosis from baseline to EOT in Arm A according to NAS fibrosis score (0.97 ± 1.13 to 0.97 ± 1.30, $p=1.0$). In Arm B, there was

an increase of fibrosis from 0.75 ± 0.91 of baseline to 1.11 ± 1.14 of EOT ($p = 0.007$). Further analysis showed that two (6.7%) patients of Arm A had their fibrosis stage progress for 1 stage and more, which was significantly lower than 33.3% (12/36) patients of Arm B ($p = 0.02$). Those patients who had fibrosis improvement for 1 stage and more were 2 (6.7%) of Arm A and 2 (5.7%) of Arm B, respectively. There were no significant changes of FIB-4 score in Arm A (from 1.09 ± 0.76 at baseline to 1.09 ± 0.76 at EOT, $p = 0.47$) and in Arm B (from 0.98 ± 0.54 at baseline to 1.0 ± 0.52 at EOT, $p = 0.69$), respectively.

NASH improvement without worsening of fibrosis was achieved in 46.7% (14/30) patients in Arm A, which was significantly higher than 11.1% (4/36) patients in Arm B ($p = 0.002$). NASH resolution was in 26.7% (8/30) patients in Arm A, which was substantially higher than 11.1% (4/36) patients in Arm B ($p = 0.103$) (Fig. 4).

Liver fat on MRI–PDFF

The liver fat content demonstrated a significantly decline from 21.0 ± 8.3% to 17.3 ± 7.5% ($p < 0.001$) on MRI–PDFF imaging. There were 78 patients completed paired assessment on MRI–PDFF, including 31 patients of Arm A and 46 patients of Arm B. There was a significant decrease of fat content (20.2 ± 9.0 to 14.3 ± 6.9%, $p < 0.0001$) in Arm A, whereas the change of fat content was not significant in Arm B patients (21.7 ± 7.6 to 20.1 ± 7.0%, $p = 0.16$) (Fig. 5).

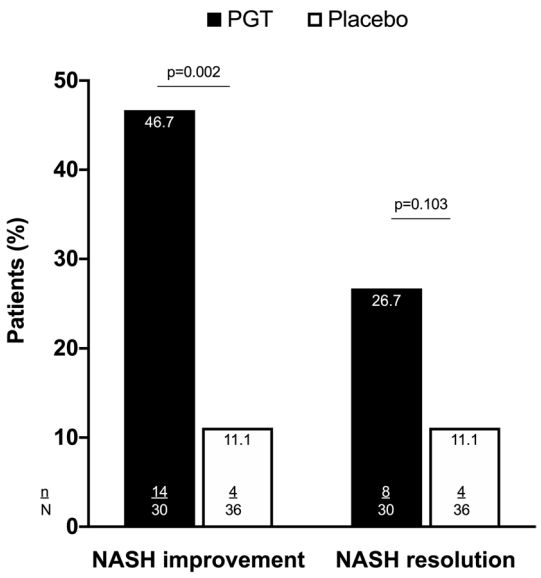

Fig. 4 Patients who achieved NASH improvement and NASH between pioglitazone (PGT) Arm and placebo Arm

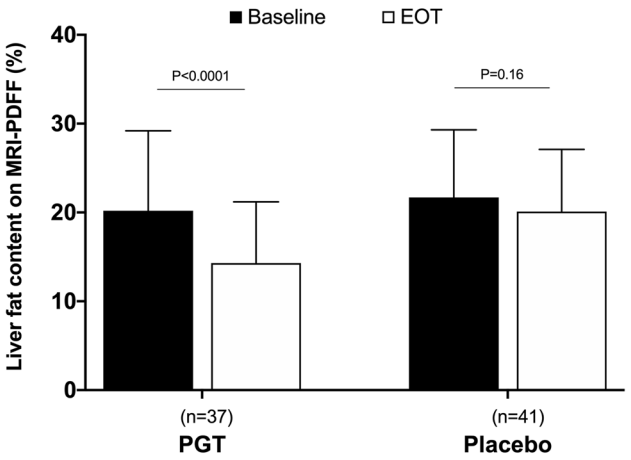

Fig. 5 Liver fat content on MRI–PDFF between pioglitazone (PGT) Arm and placebo Arm. *MRI–PDFF* magnetic resonance imaging–proton density fat fraction

Safety

There were 69 adverse events (AE) that occurred during treatment duration, including 34 (79.1%) patients in Arm A and 35 (74.5%) patients in Arm B, respectively. No significant difference of AE development between groups ($p = 0.63$) except that those patients in Arm A had a higher incidence of insomnia and anxiety than Arm B (11.6% vs 0%, $p = 0.02$) (Table 3). Serious AE (SAE) of colon diverticulitis leading to hospitalization occurred in 1 patient of Arm B at week 4 of dosing, and he recovered completely a few days after conservative management. No patient experienced body weight gain > 10% in both arms.

Discussion

NASH is a progressive liver disease globally and can lead to cirrhosis and HCC, especially in patients with older age, obesity--- and T2DM [6]. IR is the key player of NASH independent of obesity or visceral adiposity, even in the absence of DM [2]. Therefore, the therapeutic exploration targeting on amelioration of IR was the initial effort for NASH treatment. The current study, to our knowledge, was the first one in Asia aiming to assess the treatment efficacy and safety of insulin sensitizer. Our results demonstrated that there were significant improvements of transaminase and hs-CRP levels as well as other metabolic profile in patients receiving 24 weeks of TZD. Liver steatosis improvement was significantly identified both on histologic and MRI–PDFF in TZD-treated arm. In addition, TZD-treated patients had significant NAS reduction in histology. They also carried a significantly higher chance of NASH improvement without fibrosis worsening (46.7%) and NASH resolution (26.7%) compared to

Table 3 Safety profiles

	Total ($n=90$)	Pioglitazone 30 mg/day ($n=43$)	Placebo ($n=47$)	p value
All AEs	69 (76.7)	34 (79.1)	35 (74.5)	0.63
Upper respiratory infection	47 (52.2)	25 (58.1)	22 (46.8)	0.28
Neuromuscular symptoms	16 (17.8)	6 (14.0)	10 (21.3)	0.36
Lower leg edema	2 (2.2)	1 (2.3)	1 (2.1)	1.00
Elevated ALT level (< 2 ULN)	2 (2.2)	0 (0.0)	2 (4.3)	0.17
Gastrointestinal symptoms	19 (21.1)	12 (27.9)	7 (14.9)	0.13
Insomnia/anxiety	5 (5.6)	5 (11.6)	0 (0.0)	0.02
Headache	6 (6.7)	5 (11.6)	1 (2.1)	0.10
Cardiovascular symptoms	2 (2.2)	1 (2.3)	1 (2.1)	1.00
Constipation	4 (4.4)	3 (7.0)	1 (2.1)	0.35
Fatigue	6 (6.7)	0 (0.0)	6 (12.8)	0.03
Skin symptoms	8 (8.9)	5 (11.6)	3 (6.4)	0.47
Ophthalmic symptoms	4 (4.4)	2 (4.7)	2 (4.3)	1.00
Herpes zoster infection	3 (3.3)	2 (4.7)	1 (2.1)	0.60
Hyperglycemia	4 (4.4)	1 (2.3)	3 (6.4)	0.62
Weight gain > 10%	0	0	0	0
SAE	1 (1.1)	0 (0.0)	1 (2.1)	1.00
Discontinuation of treatment due to AE	3 (3.3)	2 (4.7)	1 (2.1)	0.60

AE adverse event, *SAE* serious adverse event

their counterparts. Although TZD-treated patients had a higher incidence of insomnia and anxiety, it was well-tolerated without treatment discontinuation or occurrence of significant BW gain in TZD-treated arm.

PPAR-γ is a ligand-activated nuclear receptor that forms a heterodimer with retinoid X receptor alpha and regulates gene transcription, mitigating IR in peripheral tissues. Pioglitazone, a PPARγ agonist, has been used as an antidiabetes drug aiming to decreases IR [16, 17]. The effects of increasing skeletal muscle glucose disposal and decreasing hepatic gluconeogenesis have made it the initial therapeutic exploration for NASH in the past. Previous study by Belfort et al. compared diet alone with the combination of diet and pioglitazone for 24 weeks in 55 documented NASH patients. It demonstrated that pioglitazone-treated group had a significant improvement in transaminase levels, steatosis, intrahepatic IR and necroinflammation [9]. In another multicenter placebo-controlled trial, PIVENS study by Sanyal et al. 80 patients with NASH were treated for 96 weeks with pioglitazone 30 mg daily [18]. Pioglitazone arm was associated with highly significant reductions in transaminase level, steatosis and inflammation, as well as IR improvements. It also led to the resolution of steatohepatitis in a significant proportion of subjects. Recent study including 101 NASH patients with prediabetes or T2DM showed that extending pioglitazone 45 mg daily to 3 years significantly decreased transaminase levels, improved liver and peripheral insulin sensitivity, reduced steatosis and improved liver histology [19]. Our results echoed the main findings of previous studies showing pioglitazone effectively improved liver biochemical profile, steatosis and necroinflammation. Of note was that we demonstrated there was a significant decrease of fat content in TZD-treated arm on both histologic and MRI–PDFF manifestations. It addressed previous study showing TZD improves IR and liver steatosis by an adiponectin-mediated effect on insulin sensitivity and hepatic fatty acid metabolism [20]. The significant reduction of hs-CRP and UA levels, the major surrogate biomarkers for proinflammation and inflammation, may imply the decrease of metabolic risks in the aspect. However, our study demonstrated that IR reduction during TZD treatment, reflected by HOMA-IR, showed a substantial decrease pattern from baseline to EOT. However, it then resumed to baseline state at EOF. Our results thus raised a doubt regarding the efficacy of 24 weeks TZD treatment in the significant reduction of IR, at least in Taiwanese. The doubt may be partly attributed to a lower BMI, a lower baseline IR, a lower NAS and/or the single ethnicity of the current study. Further collaborative study across different regions and demographic varieties will be needed to clarify the important issue.

Fibrosis is the major determining factor associated with outcomes of NASH patients. Regression and/or improvement of fibrosis could subsequently lead to NASH improvement and resolution. Previous study by Cusi et al. demonstrated that pioglitazone 45 mg daily for 3 years was effective to achieve regression of fibrosis stage for 1 and more in 39%

TZD-treated patients, whereas ballooning improvement was observed in 51% TZD-treated patients [19]. Our results demonstrated that patients of TZD-treated arm had a higher chance to achieve NASH improvement without worsening of fibrosis and it was beneficial for NASH resolution. The two features have recently been regarded as the histologic endpoints of major trials [21, 22]. However, the incidence of NASH resolution in TZD-treated patients (26.7%) of our study was significantly lower than 47–51% of the previous studies [18, 19]. It might be attributed to the lower fibrosis severity of our patients. Meanwhile, TZD-treated arm of the current study did not achieve the significant amelioration of hepatocyte ballooning and fibrosis improvement on histology. Further assessment with continuous non-invasive FIB-4 score also disclosed the failure to significantly fibrosis reduction in TZD-treated patients. The somewhat discordant results may be attributed to the difference between studies in terms of baseline necroinflammation, fibrosis stage, ethnicity, BMI, dosing and treatment duration. It also raised the issue indicating the optimal treatment duration and dosing across demographic and metabolic factors.

In addition to viral hepatitis infection, the importance of NAFLD/NASH has progressively been concerned in recent decades in Asia [3, 23]. Sharma et al. performed a randomized controlled trial in India by comparing TZD and pentoxifylline (anti-TNF-α) for 24 weeks in generally low-BMI NASH patients [24]. They demonstrated that both pentoxifylline and TZD were effective in improving transaminases, IR and adiponectin levels significantly. Our study provided concordant results showing that pioglitazone effectively improved transaminase, UA and hs-CRP levels besides reduced necroinflammation and steatosis. Our study further demonstrated the significant reduction of liver fat by MRI–PDFF. It may imply that pioglitazone could be a therapeutic approach at least in Asians. Generally, Asians have a higher visceral fat and carry a higher risk of metabolic abnormalities than other ethnicities and are more susceptible to NASH and disease progression dependent on the same BMI [7, 25, 26]. Of note is that a high percentage (15–21%) of Asia–Pacific NAFLD subjects have been found to be lean or non-obese [27]. The Asian carbohydrate-rich diet may be transformed into triglycerides and accumulates in liver, which may activate carbohydrate responsive element-binding protein. The process may further lead to liver steatosis and/or NASH in the end [4, 28]. All of the racial characteristics could contribute to the disparate association between NAFLD/NASH and BMI in Asian population [29].

On the other hand, the safety profile is a concern because weight gain and heart failure exacerbation have been listed as the major AE in previous Western studies [9, 10, 18, 19]. The current study showed that the safety profile was acceptable and pioglitazone was well-tolerated without significant adverse events reported previously. Therefore, the long-term outcomes in both cardiovascular events and liver-related events deserve further investigation in this first-in-Asia randomized controlled trial. The optimal dosing and treatment duration also need investigation. The link between disease course and outcomes across genetic predispositions and BMI also awaits exploration.

There were some limitations of the current study. First, certain lifestyle, environmental, genetic and ethnic factors may contribute in NASH development. Our study did not recruit the genetic factor, environmental factor and lifestyle patterns into analysis since NASH is generally a complex dynamic scenario interacted by the major factors. Recently, several genetic predispositions have been demonstrated to be associated with the development, disease course and disease outcomes in chronic liver diseases. The role of the genetic variants in the treatment efficacy of TZD deserves further investigation. Second, our patients had a lower baseline NAS and fibrosis score, which may raise the concern of definite NASH diagnosis on histology. It may imply that the current diagnostic criteria may not cover the whole spectrum of NASH histopathologically. Third, despite the randomized, double-blind design, there were significant difference between groups in terms of gender, BMIand FPG level, which might affect the outcome of the results. Last, NASH is usually associated with many metabolic disorders and the patients may have many drugs for disease control in a long-term fashion. The drug–drug interaction between TZD and other drugs might lead to potential therapeutic impact on the results.

In conclusion, the first-in-Asia randomized controlled trial demonstrated that 24 weeks pioglitazone treatment was effective in reducing liver and metabolic biochemistry in NASH patients. Histologic improvement was also significantly observed in terms of steatosis reduction, inflammation and NASH improvement without worsening of fibrosis. Pioglitazone was safe and well-tolerated. Further studies of TZD in Asian NASH patients to assess long-term clinical benefit and/or in combination with other potential agents are needed.

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Availability of data and material Authors can confirm that all relevant data are included in the article. We agree with the policy in the journal.

Code availability Not applicable.

Declarations

Conflict of interest Jee-Fu Huang: Consultant of Roche, BMS, Gilead, Merck, Sysmex, Pharmaessential, Polaris, and Instylla. Speaker for Abbvie, BMS, Gilead, Merck, Sysmex, and Roche. Chia-Yen Dai: Consultant of Abbvie and Roche; Speaker for Abbvie, Gilead, and Roche. Chung-Feng Huang: Speaker for Abbvie, BMS, Bayer, Gilead, Merck, and Roche. Ming-Lung Yu: Research grant from Abbott, BMS, Merck, and Gilead; Consultant of Abbvie, Abbott, Ascletis, BMS, Merck, Gilead, and Roche; Speaker for Abbvie, Abbott, BMS, Merck, Gilead, and IPSEN. Wan-Long Chuang: Consultant of Gilead, AbbVie, BMS, and PharmaEssentia; Speaker for Gilead, AbbVie, BMS, and PharmaEssentia.

Ethics approval The Institutional Review Board of the Kaohsiung Medical University Hospital approved the study.

Consent to participate The trial was conducted in compliance with the Declaration of Helsinki and the Good Clinical Practice guidelines of the International Conference on Harmonization. Written informed consent for interview, anthropomorphic measurements, blood sampling, liver biopsies and medical record review were obtained from patients.

Consent for publication All authors contributed to the interpretation of the data and reviewed and approved the manuscript.

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