Int J Clin Exp Med 2022;15(9):282-292
www.ijcem.com /ISSN:1940-5901/IJCEM0135781

Original Article

Decreased dose and shorter duration of obeticholic acid in nonalcoholic steatohepatitis patient: a randomized control trial

Shahinul Alam, Natasha Tarannum, Saequa Habib

Department of Hepatology, Bangabandhu Sheikh Mujib Medical University, Dhaka 1000, Bangladesh

Received May 28, 2021; Accepted January 25, 2022; Epub September 15, 2022; Published September 30, 2022

Abstract: Background and aims: Non-alcoholic fatty liver diseases (NAFLD) have a spectrum of conditions where the progressive form is nonalcoholic steatohepatitis (NASH). However, to date, there is a lack of approved therapies for NASH. In this study, we aimed to explore the impact of smaller doses and shorter duration of obeticholic acid on NASH. Methods: In this, open-label randomized control trial, 36 adult non/diabetic patients with NAFLD activity score (NAS) ≥5 were included. Patients were assigned to obeticholic acid 10 mg twice daily with lifestyle modification (group-OCAL) and only lifestyle modification (group-L) after 1:1 randomization for 24 weeks. The primary outcome was measured with NAS improvement ≥2 without worsening of fibrosis. Results: A total of 31 NASH patients were evaluated as per standard protocol at the end of the study. Among them, 15 belonged to the OCAL group, and 16 belonged to the L group. Baseline epidemiological, metabolic, anthropometric, biochemical, and histological observations were similar in both groups. After 24 weeks NAS improved in the OCAL group from 5.53±0.60 to 3.40±1.1 (P<0.001) and in the L group from 5.31±0.50 to 4.44±1.20 (P=0.011). In the OCAL group, fibrosis improved from 1.40±0.80 to 0.67±0.60, (P=0.001). NASH ≥2 improvement without worsening fibrosis in the OCAL group was 13 (68%) and 6 (32%) in the L group. ALT, AST, and GGT were significantly improved in the OCL group. Histological improvement was irrespective of weight reduction and status of diabetes. Conclusion: Obeticholic acid of a decreased dose of 20 mg daily for a shorter duration of 24 weeks improved NASH and fibrosis, independent of weight reduction and diabetes.

Keywords: Obeticholic acid, NAFLD, NASH, fibrosis; steatosis, ballooning, lobular inflammation, NAFLD

Introduction

Nonalcoholic fatty liver disease (NAFLD) is a complex manifestation of hepato-metabolic disease demonstrating steatohepatitis, fibrosis, cirrhosis, and carcinoma in the liver [1]. In the Asia pacific region, NAFLD is one of the foremost common liver diseases [2-4]. A couple of years back, a study conducted in Asian countries reported that two-thirds of the population was suffering from NAFLD [5]. Because of its close association to the progression of many liver diseases and several other serious cardiometabolic abnormalities, (eg. type-2 diabetes, coronary heart condition, etc), NAFLD has become a vital public health concern [6]. It is reported that 20% of NAFLD patients develop nonalcoholic steatohepatitis (NASH) whereas ten to fifteen percent develop cirrhosis of the liver [7]. Pooled data from a previous study demonstrated that ~21% of patients with NASH conjointly suffer from some degree of fibrosis beyond steatosis [8-10]. Thus, the treatment of NASH, requires a multifaceted approach. Most hepatologists attempt to manage NASH by lifestyle changes (eg. weight reduction, exercise, etc.) with customary therapeutic interventions to regulate concomitant diseases (eg. hyperlipemia, cardiovascular disease, type-2 polygenic disease, and so on) [11].

Thus, pharmacotherapies or interventions that are investigated include- insulin sensitizer; thiazolidinediones, lipid-lowering agents (statins and fibrates), cytoprotective agents (UDCA), and anti-oxidants (Vitamin E, vitamin C) [10, 12-16]. A number of these interventions improve some features of the liver injury related

to NASH. Nevertheless, most of those approaches necessitate dietary changes and lifestyle modification to achieve targeted weight reduction and improve insulin sensitivity. However, the long-term effects of those interventions are debatable as several patients are unable to take care of correct dietary and lifestyle changes, underlying the requirement for novel pharmacotherapies.

Studies into the pathologic process of NAFLD have yielded many potential targets for novel pharmacotherapies together with Farnesoid X receptors (FXRs) [17-19]. Consequently, many pre/clinical trials conducted specialize in FXR-ligand searches, and have developed a unique FXR-ligand, obeticholic acid [20, 21]. Later studies, showed improvement of histopathology and biochemical parameters of the liver when administered higher dose obeticholic acid (>20 mg/daily) for >48 weeks [22-24]. In addition to the NAFLD activity score (NAS), the outcome was also measured by blood sugar, ALT, AST, GGT, lipid profile, etc [25, 26].

However, none of those trials have targeted lower dose obeticholic acid (20 mg) for a shorter duration of 24 weeks in NASH. Thus, with this background, we aimed to conduct this trial to test the effectiveness of obeticholic acid of 20 mg for 24 weeks in NASH patients.

Materials and methods

Inclusion criteria

This open-labeled randomized control trial was conducted at the Department of Hepatology, Bangabandhu Sheik Mujib Medical University from January 2019 to September 2020. Thirty-six adult patients who had NAS ≥5 histopathology were enrolled for the trial.

Exclusion criteria

Patients with a previous history of alcohol intake, history of intake hepatotoxic drugs (i.e. tamoxifen, anticonvulsant, amiodarone, methotrexate), history of taking drugs that have shown benefit for NAFLD in previous studies (i.e. vitamin E, pioglitazone), chronic liver disease due to any cause (HBV, HCV, Wilson's disease, drug elicited liver injury, etc.), pregnancy and patients with co-morbid conditions (COPD, chronic nephropathy, symptom internal organ failure and ischaemic cardiovascular disease) were excluded from the study.

Patients

In this study, 18 patients were selected in the treatment group (OCAL) and 18 patients were selected for the lifestyle change (L) group by 1:1 randomization. Before inclusion for study full rationalization concerning the aims, objectives of the study, and necessity of the investigations were provided. Written consent was provided from the patient. His/her blood sample was drawn and diagnostic investigations (CBC, FBS, 2HABF, ALT, AST, PT with INR, GGT, TSH, fasting blood glucose profile, HBsAg, and anti-HCV) were performed at the Department of Biochemistry, Department of Microbiology, and Department of Virology, BSMMU.

Histopathological analysis

Histopathological analysis was done by a single knowledgeable faculty of the Pathology Department of BSMMU, who wasn't conscious of clinical and biochemical reports. The specimen was stained with hematoxylin and eosin and the NASH score was made per Kleiner et al. (2005) Masson's trichrome stain was done to assess the extent of fibrosis [27].

Dose selection and treatment period

Obeticholic acid total of 20 mg per day was given in 2 separate doses of 10 mg in conjunction with lifestyle changes for the OCAL group and lifestyle changes were suggested for the L group for the total duration of 24 weeks.

Patient lifestyle during trial

Lifestyle modification was suggested for each team of patients. Patients were inspired to perform moderate exercise, including walking half-hour each day. Dietary advice to avoid saturated fat, excessive sugar, soft drinks, fast food, and refined carbohydrates was given to both groups of patients according to the diet chart of NAFLD because saturated fat increases lipotoxicity, and excess sugar-containing food stimulates de novo lipogenesis which increases hepatic fat.

Treatment of comorbid conditions

Diabetic patients were treated with lifestyle modification and if required with oral sulphonylureas-gliclazide, glimepiride; metformin, or with Insulin. Patients with dyslipidemia were treated

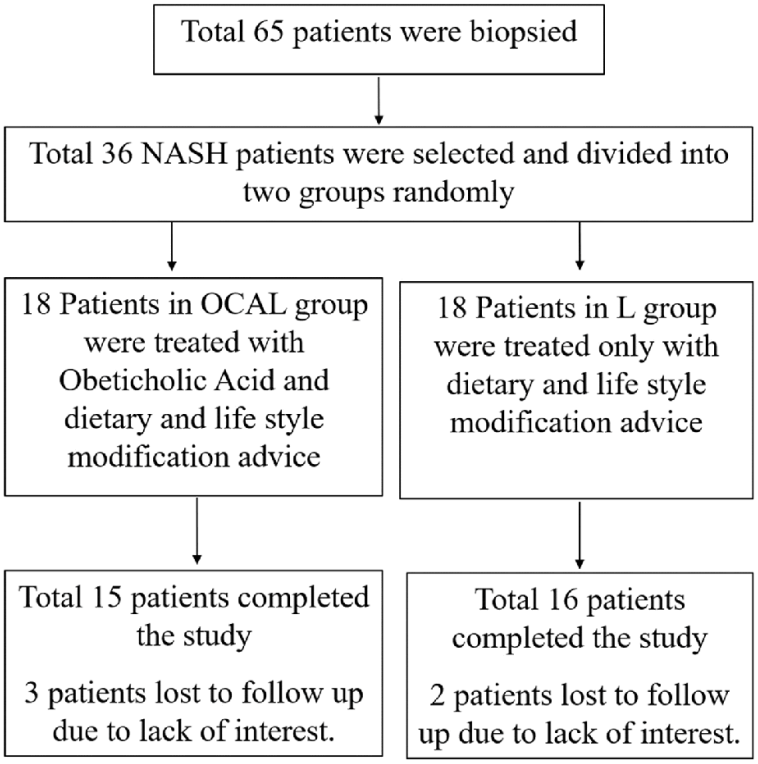

Figure 1. Flow chart of patient selection for the study.

with a statin. Hypertensive patients were treated with antihypertensive drugs.

Follow up

To avoid confounding influence a close liaison was maintained between all groups of patients. Permanent addresses, present addresses including the phone number of all patients were kept. A telephone survey was done to assess patients' compliance with treatment. All patients were advised to contact their liaison immediately if any alarming physical symptoms occurred. Each patient was followed monthly for three months and after three months.

Each follow-up was provided at the Department of Hepatology between 10:00 am to 2:00 pm and consisted of clinical examination, blood pressure (BP), body mass index (BMI) determination. A follow-up questionnaire was filled in during each visit. BMI was calculated by using the following formula: weight in kg/square of height in meter. An alcohol consumption questionnaire was administered each visit and study compliance was strictly monitored. FBS, 2HABF, and fasting lipid profiles for the diabetic and dyslipidaemic patients were assessed according to need. All the side effects were recorded at each visit. As liver biopsy is an invasive procedure, 5 patients were noncompliant. They dropped from study due to their lack of interest in 2nd biopsy. Total 31 NASH patients (15 in the OCAL group and 16 in the L group) were considered for final analysis as per protocol (**Figure 1**).

After 24 weeks, liver biopsy was repeated for both the treatment and control group. The primary parameters that were compared between the first and last visit were SBP, DBP, WC, BMI, ALT, AST, GGT, Cholesterol, TG, HDL, LDL, FBS, 2HABF, NAS (including its components such as steatosis, ballooning, and lobular inflammation) and fibrosis score.

Primary outcome and secondary outcome

The primary outcome was histological scoring, defined as NAS improvement ≥2 without worsening of fibrosis. The secondary outcome was a biochemical improvement after 24 weeks.

Statistical analysis

Quantitative data were presented as mean ± SD & qualitative data were presented in percentage. All data were analyzed by statistical software package SPSS (version-21). Qualitative data were analyzed by Chi-square test and quantitative data were analyzed by student's t-test. Pretreatment and end treatment data within the group were compared by paired t-test. The univariate and multivariate logistic regression analyses were done to find out the best predictor of patients' responses. A statistically significant result was considered when the *P-value* was less than 0.05.

Ethical consideration

Prior to commencement of the study ethical approval for the study was given from the Institutional Review Board (IRB) of Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh. Approval for the paper was

given by the 177th IRB, BSMMU meeting held on 16 March 2019 (No. BSMMU/2019/8873). We performed this study in accordance to the Declaration of Helsinki. To maintain confidentiality, each of the study subjects was given a special ID number which was followed during each and every step of the study procedure. All the research data were coded and stored in a locked cabinet. Only research personnel were allowed to access the data.

Results

Baseline characteristics of patients

A total of 31 NASH patients were evaluated as per protocols at the end of the study. Among them, 15 patients belonged to the OCAL group and 16 patients to the L group. The mean age of patients was 38.73±8.85 years in the OCAL group and 40.31±8.82 years in the L group. Eighteen patients were female and 13 patients were male. In the OCAL group, 8 (53.3%) patients were female and in the L group, 10 (62.5%) patients were female. In the OCAL group, 7 (46.7%) patients were male, and in the L group, 6 (37.5%) patients were male.

According to Asian criteria, BMI ≥25 kg/m^2 was considered obese. In the OCAL group, 11 (73.3%) patients were obese and 13 (81.3%) patients in the L group were obese. The difference was not statistically significant (P>0.05) between the two groups. Five (33.3%) patients in the OCAL group and 6 (37.5%) patients in the L group had hypertension. In total 15 patients were diabetic, 8 (53%) in the OCAL group and 7 (44%) in the L group (P=0.337). In the OCAL group mean fasting blood sugar was 6.38±2.7 mmol/L and 6.06±2.21 mmol/L in the L group. Mean 2HABF was 9.39±3.95 mmol/L in the OCAL group and 9.09±3.57 mmol/L in the L group. There was no significant difference in baseline FBS and 2HABF between the two groups.

Metabolic syndromes were found in 9 (60%) patients in the OCAL group and 11 (68%) patients in the L group. According to Asian criteria, 5 (33.3%) patients in the OCAL group and 10 (62.5%) patients in the L group had increased waist circumference. Mean BMI was 27.32±4.13 kg/m^2 in the OCAL group and 26.55±2.88 kg/m^2 in the L group. Mean waist circumference was 90.03±10.90 cm in the OCAL group and 93.28±8.70 cm in the L group. There was no significant anthropometric difference between the two groups.

The baseline lipid profile including total cholesterol, LDL, HDL, and TG did not differ significantly between the 2 groups at baseline. Mean AST was 59.00±20.3 U/L in the OCAL group and 45.50±34.2 U/L in the L group. Mean ALT was 89.27±41.1.0 U/L in the OCAL group and 64.25±30.5 U/L in the L group. Mean AST/ALT ratio was 0.71±0.22 in the OCAL group and 0.74±0.42 in the L group. Mean GGT was 46.40±18.20 U/L in the OCAL group and 43.31±24.20 U/L in the L group. The liver enzymes were similar in the 2 groups at baseline. The mean NAFLD activity score (NAS) was 5.53±0.64 in the OCAL group and 5.31±0.48 in the L group. The mean Fibrosis score was 1.53±0.64 in the OCAL group and 1.50±0.97 in the L group (**Table 1**). There was no significant difference of NASH and fibrosis scores between the two groups. Steatosis, lobular inflammation, and ballooning were also similar in the 2 groups at baseline. So baseline anthropometric, biochemical, and histological characters were similar in the two groups.

Comparison between pretreatment and post-treatment histological response in OCAL and L group

After 24 weeks of treatment, obeticholic acid with life style modification caused a significant reduction of histological activity and fibrosis. NAS improved in the OCAL group from 5.53± 0.60 to 3.40±1.10 (P<0.001) and in the L group from 5.31±0.50 to 4.44±1.20 (*P*=0.011). In the OCAL group steatosis improved from 2.20±0.60 to 1.33±0.80 (P=0.004), lobular inflammation from 1.73±0.50 to 1.00±0.40 (P=0.001) and hepatocyte ballooning from 1.53±0.50 to 1.07±0.50 (P=0.004). In the OCAL group, steatosis, lobular inflammation, and hepatocyte ballooning improvement were all statistically significant (**Figure 2**).

In the OCAL group, fibrosis improved from 1.40±0.80 to 0.67±0.60, which was statistically significant (P=0.001) (**Figure 3**).

On the other hand, in the L group, NAS and steatosis had statistically significant improvement (P=0.011 and 0.007). But there was no improvement of lobular inflammation, hepato-

Table 1. Baseline characteristics of patients (n=31)

Variables	OCAL group (*n*=15)	L group (*n*=16)	*P* Value
Age (years)	38.73±8.85	40.31±8.82	0.651^{ns}
Sex: Male/Female n (%)	7/8 (46.7%/53.3%)	6/10 (37.5%/62.5%)	0.619^{ns}
Obese (yes/no)	11/64 (73.3%/26.7%)	13/3 (81.3%/18.8%)	0.313^{ns}
WC increased	5/10 (33.3%/66.7%)	10/6 (62.5%/37.5%)	0.111^{ns}
Diabetes (yes/no)	8/7 (53.3%/46.7%)	7/9 (43.8%/56.3%)	0.337^{ns}
Hypertension (yes/no)	5/10 (33.3%/66.7%)	6/10 (37.5%/62.5%)	0.299^{ns}
BMI (kg/m^2)	27.32±4.13	26.55±2.88	0.549^{ns}
WC (cm)	90.03±10.90	93.69±8.70	0.363^{ns}
FBS (mmol/L)	6.38±2.7	6.06±2.21	0.713^{ns}
2HABF (mmol/L)	9.39±3.95	9.09±3.57	0.829^{ns}
Cholesterol (mg/dl)	207.73±37.98	209.31±73.25	0.941^{ns}
LDL (mg/dl)	130.29±38.78	123.5±65.74	0.742^{ns}
HDL (mg/dl)	36.47±8.54	33.13±7.27	0.249^{ns}
TG (mg/dl)	257.80±129.70	256.13±124.80	0.971^{ns}
AST (U/L)	59.00±20.30	45.50±34.20	0.195^{ns}
ALT (U/L)	89.27±41.10	64.25±30.50	0.0.63^{ns}
AST/ALT ratio	0.71±0.22	0.74±0.42	0.195^{ns}
GGT (U/L)	46.40±18.20	43.31±24.20	0.693^{ns}
NAS	5.53±0.64	5.31±0.48	0.284^{ns}
Fibrosis score	1.53±0.64	1.50±0.97	0.911^{ns}

Yes/no, ns= not significant, s= significant. WC= Waist circumference, BMI= Body mass index, ALT= Alanine transaminases, AST= Aspartate transaminases, GGT= Gamma glutamyl transferase, FBS= Fasting blood sugar, LDL= Low density lipoprotein, HDL= High density lipoprotein. Comparison was done by Independent t test and Chai square test.

cyte ballooning and fibrosis score in the L group (**Table 2**).

NAS ≥2 improvement without worsening of fibrosis was considered as significant histological improvement (histological responder). In the OCAL group, 13 (68%) patients and 6 (32%) patients in the L group were histological responders (**Figure 4**).

Biochemical changes after intervention

Mean fasting blood sugar improved from 6.38±2.70 mmol/L to 5.48±1.28 mmol/L (P=0.138) in the OCAL group and 6.06±2.21 mmol/L to 5.56±1.24 mmol/L (P=0.085) in the L group. Mean 2HABF improvement was 9.38±3.95 mmol/L to 7.86±2.57 mmol/L (P=0.012) in the OCAL group and 9.09±3.57 mmol/L to 8.10±1.79 mmol/L (P=0.205) in the L group. In the OCAL group, mean total cholesterol improvement was from 207.73±37 mg/dl to 168.80±38 (*P*=0.001) and 209.31±73 mg/dl to 179.14±35 mg/dl (P=0.224) in the L group. Mean LDL improvement was from 130.29±38 mg/dl to 100.93±38 (P=0.001) in the OCAL group and 123.5±65 mg/dl to 103.71±27 mg/dl (P=0.304) in the L group. Mean HDL increased from 36.47±8.54 mg/dl to 38.67±5.90 (P=0.136) in the OCAL group and decreased from 33.13±7.20 mg/dl to 31.57±6.50 mg/dl (P=0.900) in the L group. In the OCAL group, mean TG improvement was from 257.80±129 mg/dl to 156.53±50 (P=0.008) and 256.13±12 mg/dl to 168.57±35 mg/dl (P=0.023) in the L group. In the OCAL group, Cholesterol, LDL and TG improved significantly, but not in the L group.

AST improvement was from 59.00±20.3 U/L to 32.20±11 U/L (P=0.001) in the OCAL group and 45.50±34 U/L to 31.13±8.9 U/L (P=0.126) in the L group. ALT improvement was from 89.27±41.1 U/L to 38.20±16 U/L (P=0.001) in the OCAL group and 64.25±30 U/L to 33.56±12 U/L (P=0.001) in the L group. Mean ALT and AST improvement in the OCAL group was significant (P=0.001 and 0.001). In the L group, ALT had significant improvement, while AST had no significant improvement. AST/ALT ratio improvement was from 0.71±0.22 to 0.87±0.10 (P=0.019) in the OCAL group and 0.74±0.42 to 0.98±023 (P=0.050) in the L group. AST/ALT ratio improvement was statistically significant in the OCAL group and had no significant improvement in the L group. GGT improvement was from 46.40±18.20 U/L to 34.80±13 U/L (P=0.003) in the OCAL group and from 43.31±24 U/L to 38.13±29 U/L (P=0.231) in the L group. GGT improvement was statistically significant in the OCAL group (**Table 3**).

Comparison between histological responders and non-responders' group of patients

NAS improvement ≥2 without worsening of fibrosis was considered as responding histo-

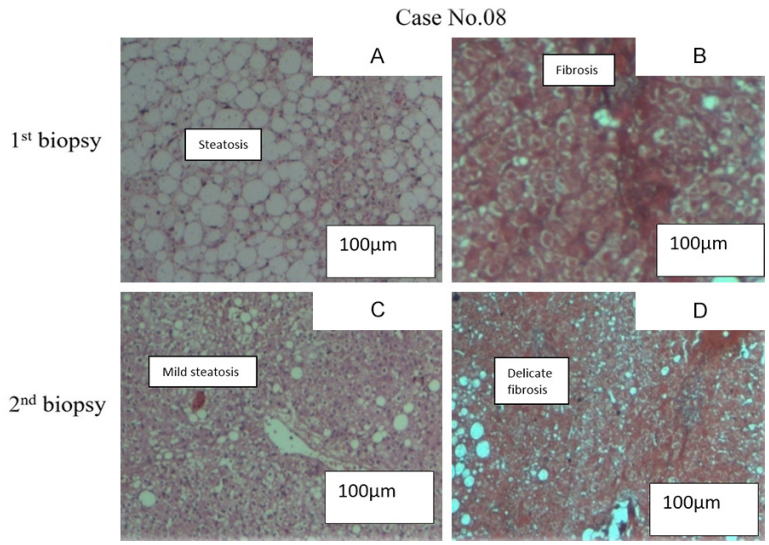

Figure 2. Histological improvement between index and end of study liver biopsy. The upper panel showed 1st biopsy (A: Haematoxylin-eosin ×10) (B: Masson's trichrome ×10) and the lower panel showed 2nd biopsy (C: Haematoxylin-eosin ×10) (D: Masson's trichrome ×10) of the same patient (Case No. 08). NAS score improvement from 6 to 4 and fibrosis score improvement from 2 to 1.

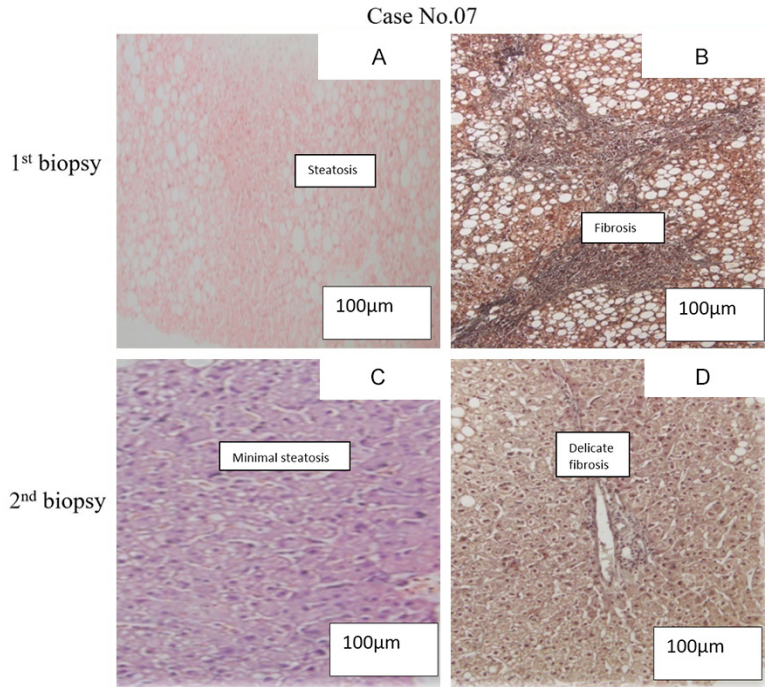

Figure 3. Histological and fibrosis improvement between index and end of study liver biopsy. The upper panel showed 1st biopsy (A: Haematoxylin-eosin ×10) (B: Masson's trichrome ×10) and the lower panel showed 2nd biopsy (C: Haematoxylin-eosin ×10) (D: Masson's trichrome ×10) of the same patient (Case No. 07). NAS improvement from 6 to 3 and fibrosis score improvement from 3 to 1.

logically. Accordingly, total 19 patients were responders and 12 patients were non-responders. Among the responders 13 (68%) patients were in the OCAL group and 6 (32%) patients in the L group. Among the non-responders 2 (17%) patients were in the OCAL group and 10 (83%) patients in the L group. The difference of response between the OCAL group and the L group was statistically significant (P=0.004).

Out of 31 patients, 20 patients had lost weight. Among them, 13 patients (65%) had histological improvement and 7 patients (35%) had no significant histological improvement. A total of 11 patients were diabetic among 31 patients. Four (27%) patients were in the OCAL group and 7 (44%) patients were in the L group. Among diabetic patients, 5 (45.45%) patients were histological responders and 6 (54.55%) patients were histological non-responders. Among non-diabetic patients, 14 (70%) patients were histological responders and 6 (30%) patients were non-responders. There was no significant difference in histological response between diabetic and non-diabetic patients (P= 0.191). Thus, pointing out the presence of diabetes had no significant interference with the histological response.

The mean difference of age versus female and male ratio was not significant between responders and non-responders. Mean BMI was 26.52± 2.1 kg/m^2 in responders and 27.6±5.0 kg/m^2 in non-responders. Mean waist circumference was 89.03±8.3 cm in responders and 95.96±10.7 cm in non-responders. Waist circumference was statistically borderline significant between responders and non-responders (P=0.052). Mean fasting

Table 2. Histopathological changes after intervention

Variables	OCAL group (*n*=15)			L group (*n*=16)		
	Before Intervention	After Intervention	*P-value*	Before Intervention	After Intervention	*P-value*
NAFLD activity score (NAS)	5.53±0.60	3.40±1.10	0.001	5.31±0.50	4.44±1.20	0.011
Steatosis	2.20±0.60	1.33±0.80	0.004	2.19±0.50	1.63±0.6	0.007
Lobular Inflammation	1.73±0.50	1.00±0.40	0.001	1.69±0.50	1.44±0.50	0.164
Hepatocytes ballooning	1.53±0.50	1.07±0.50	0.004	1.69±0.60	1.38±0.50	0.173
Fibrosis score	1.40±0.80	0.67±0.60	0.001	1.63±0.80	1.31±0.60	0.173

In the OCAL group, all segments of NAS and fibrosis improved significantly, in the L group NAS and steatosis improved significantly. But there was no improvement of lobular inflammation, hepatocyte ballooning and fibrosis score in L group (**Table 2**) (Paired sample t tests).

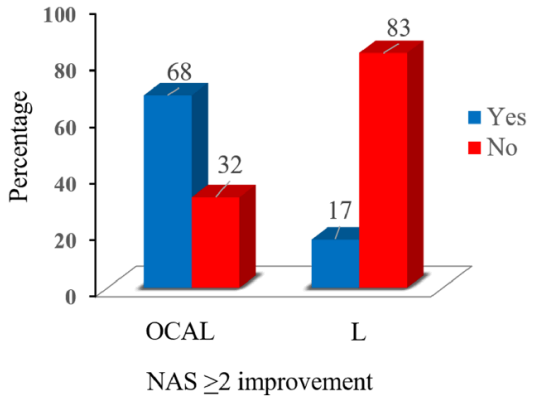

Figure 4. Distribution of NAS improvement ≥2 without worsening fibrosis in the OCAL and L the group. NAS ≥2 improvement without worsening of fibrosis was considered as the histological responder. In the OCAL group, 13 (68%) patients and 6 (32%) patients in the L group were histological responders.

blood sugar was 6.1±2.5 mmol/L in responders and 6.3±2.5 mmol/L in non-responders. Mean 2HABF was 9.1±3.8 mmol/L in responders and 9.4±3.8 mmol/L in non-responders. Thus, the baseline blood sugar did not differ in the two groups (**Table 4**).

Predictors of patient's response

Univariate analysis showed only treatment category as a significant factor of patient's response (P=0.009). Other factor such as BMI and T2DM improvement could not predict patient response significantly. Multivariate regression analysis showed that, only treatment category significantly predicted patient's response (P=0.009). Both univariate and multivariate analysis revealed only 'Treatment category' as a predictor of patient response (**Table 5**). So, response to obeticholic acid was independent of BMI improvement and diabetes.

Probable side effects

Treatment was generally well-tolerated. A total of 6 patients in the OCAL group and 4 patients in the L group had fatigue in the first follow-up. There was an improvement of this symptom in the subsequent follow-ups. Only 2 patients in the OCAL group and none in the L group developed itching. Both the patients were treated with antihistamine and they were relieved of their symptoms in the subsequent follow-up. One patient in each group had complaints of abdominal pain and constipation. They were given advice regarding fiber containing diet, and the symptoms improved. There was no statistically significant difference in side effects between the two groups. There was no sufficient side effect in the OCAL group which necessitated dose reduction of Obeticholic acid or cessation of the drug.

Discussion

We evaluated the effectiveness of a selective FXR ligand (obeticholic acid) in the Bangladeshi population with biopsy-proven NASH with or without type 2 diabetes mellitus. This is the first report, to our knowledge, which showed a lower dose of 20 mg and a shorter duration of 24 weeks of obeticholic acid improves NAS and fibrosis irrespective of glycemic conditions and weight loss in an open-labeled randomized control trial. Many more beneficial outcomes were also noted in several biochemical parameters as such a significant decrease in 2HABF, Cholesterol, LDL, TG, AST, ALT, GGT, except

Table 3. Comparison of variables before and after intervention

Variables	OCAL group (*n*=15)			L group (*n*=16)		
	Before intervention	After intervention	*P* Value	Before intervention	After intervention	*P* Value
FBS (mmol/L)	6.38±2.70	5.48±1.28	0.138^{ns}	6.06±2.21	5.56±1.24	0.085^{ns}
2HABF (mmol/L)	9.38±3.95	7.86±2.57	0.012^{s}	9.09±3.57	8.10±1.79	0.205^{ns}
Cholesterol (mg/dl)	207.73±37	168.80±38	0.001^{s}	209.31±73	179.14±35	0.224^{ns}
LDL (mg/dl)	130.29±38	100.93±38	0.001^{s}	123.5±65	103.71±27	0.304^{ns}
HDL (mg/dl)	36.47±8.54	38.67±5.90	0.136^{ns}	33.13±7.20	31.57±6.50	0.900^{ns}
TG (mg/dl)	257.80±129	156.53±50	0.008^{s}	256.13±12	168.57±35	0.023^{s}
AST (U/L)	59.00±20.3	32.20±11	0.001^{s}	45.50±34.	31.13±8.9	0.126^{ns}
ALT (U/L)	89.27±41.1	38.20±16	0.001^{s}	64.25±30.	33.56±12	0.001^{s}
AST/ALT ratio	0.71±0.22	0.87±0.10	0.019^{s}	0.74±0.42	0.98±023	0.050^{ns}
GGT (U/L)	46.40±18.2	34.80±13	0.003^{s}	43.31±24.	38.13±29	0.231^{ns}
NASH	5.53±0.64	3.40±1.12	0.001^{s}	5.31±0.48	4.44±1.12	0.011^{s}
Fibrosis score	1.53±0.64	0.67±0.62	0.001^{s}	1.50±0.97	1.31±0.61	0.173^{ns}

ns= not significant, s= significant. ALT= Alanine transaminases, AST= Aspartate transaminases, GGT= Gamma glutamyl transferase, FBS= Fasting blood sugar, 2HABF= Blood sugar 2 hours after breakfast, LDL= Low density lipoprotein, HDL= High density lipoprotein, NAS= NAFLD activity score. Paired t test.

Table 4. Comparison of baseline factors between responders and non-responders

Baseline factors	Responders (*n*=19)	Non-responders (*n*=12)	*P* Value
Category of patients (Treatment/control)	13/6 (68%/32%)	2/10 (17%/83%)	0.004^{s}
Age (in years)	40.00±6.8	38.58±10.5	0.653^{ns}
Sex (Male/female)	7/12 (37%/63%)	6/6 (50.0%/50.0%)	0.486^{ns}
Obesity (yes/no)	2/17 (10.5%/89.5%)	3/9 (25%/75%)	0.302^{ns}
WC increased (yes/no)	7/12 (37%/63%)	8/4 (67%/32%)	0.113^{s}
Diabetes mellitus (yes/no)	5/14 (26%/74%)	6/6 (50%/50%)	0.191^{s}
Hypertension (yes/no)	4/15 (21%/79%)	5/7 (42%/58%)	0.232^{ns}
BMI (kg/m^2)	26.52±2.11	27.6±5.01	0.429^{ns}
WC (cm)	89.03±8.3	95.96±10.7	0.052^{ns}
FBS (mmol/L)	6.1±2.5	6.3±2.5	0.882^{ns}
2HABF (mmol/L)	9.1±3.8	9.4±3.8	0.764^{ns}
Cholesterol (mg/dl)	203.7±53.7	216.2±65.9	0.570^{ns}
LDL (mg/dl)	128.7±55.1	123.7±51.9	0.818^{ns}
HDL (mg/dl)	36.26±8.2	32.33±7.2	0.185^{ns}
TG (mg/dl)	244.05±112.3	277.33±145.9	0.480^{ns}
AST (U/L)	57.79±32.8	42.92±18.3	0.163^{ns}
ALT (U/L)	80.37±38.7	70.00±35.5	0.464^{ns}
GGT (U/L)	41.47±13.1	50.08±29.9	0.278^{ns}
NAS score	5.53±0.6	5.25±0.5	0.189^{ns}
Fibrosis score	1.53±0.8	1.50±0.8	0.932^{ns}

Yes/no, ns= not significant, s= significant. WC= Waist circumference, BMI= Body mass index, ALT= Alanine transaminases, AST= Aspartate transaminases, GGT= Gamma-glutamyl transferase, FBS= Fasting blood sugar, 2HABF= Blood sugar 2 hours after breakfast, LDL= Low-density lipoprotein, HDL= High-density lipoprotein, NAS= NAFLD activity score. Independent t-test and Chai square test.

FBS, and a modest increase in HDL cholesterol.

In this study, we observed NAS ≥2 improvement without worsening fibrosis in 68% of

Table 5. Predictors of patient's response

Predictors	Univariate analysis				Multivariate analysis			
	OR	95% CI		*P-value*	OR	95% CI		*P-value*
		Lower	Upper			Lower	Upper	
Category of patients (treatment)	0.077	0.011	0.51	0.009[s]	0.074	0.01	0.52	0.009[s]
DM improvement	1.75	0.21	14.86	0.498[ns]	1.73	0.20	0.52	0.625[ns]
BMI improvement	1.5	0.71	3.18	0.260[ns]	0.62	0.30	1.30	0.205[ns]

BMI, Body mass index; T2DM, Type 2 Diabetes mellitus.

patients in the OCAL group with significant improvement of steatosis, hepatocyte ballooning, and lobular inflammation after 24 weeks of treatment with obeticholic acid. This study also demonstrated that there was a significant improvement in fibrosis, which is a hopeful treatment strategy to halt the progression of NASH to cirrhosis. This result is encouraging with the six-month duration of the study. This study is a novel one in this regard.

It is known that obeticholic acid exerts function through FXRs receptor activation, and FXRs activation reduces bile acid synthesis by inhibiting the conversion of cholesterol to bile acids, a major mechanism of cholesterol disposal. Thus, blocking the conversion of cholesterol to bile acids could increase serum cholesterol concentrations, which might account for the changes in serum cholesterol concentrations during obeticholic acid treatment. In contrast, in this study, we observed a decrease in serum cholesterol concentrations with obeticholic acid treatment, suggesting other pathways that decrease serum cholesterol concentrations during obeticholic acid treatment. FXR activation might also increase the expression of hepatic scavenger receptors (SRB1), which accelerates reverse cholesterol transport by increasing the clearance of HDL. Clinically FXRs receptor activation-induces beneficial outcomes, which could be explained as the amelioration of hepatic hyperlipidemia, glucose intolerance, and insulin resistance, which protects against cholestasis-induced liver injury, and induces hepatocyte regeneration [28].

This open-label RCT, for the first time, investigated the effects of 24 weeks of treatment with obeticholic acid on liver tissue morphology in NAFLD patients. In this study, patients were treated with 20 mg of obeticholic acid in two divided doses for 24 weeks before the second biopsy. In contrast to us, in a FLINT trial, 25 mg of obeticholic acid was given daily for 72 weeks before the end of the trial assessment [19, 21, 22]. The difference between the FLINT trial and the current study was the dosage and duration of treatment with obeticholic acid. Similarity with that trial is the improvement of all three components (steatosis, hepatocytes ballooning, and lobular inflammation) of NAS without worsening of fibrosis. We chose the a lower-dose obeticholic acid because of ideas of racial and genetic differences in populations among different countries.

In the OCAL group, fibrosis score ≥1 improved in 53% of patients, whereas, in the L group, improved in 31% of patients. This finding was consistent with Newuschwander-Tetri et al. [21], where obeticholic acid caused an aggressive effect in reduction of NAS (P=0.001), with a significant reduction of fibrosis score (P= 0.004). The antidiabetic drug, gliptins though previously reported for reducing NAS but not for improving fibrosis [13]. Therefore, obeticholic acid is expected to be a promising drug for NASH patients with advanced fibrosis. Here, we observed no significant difference in histological response between diabetic and non-diabetic patients (P=0.191). A previous study demonstrated that NAFLD has a variable natural history of steatosis, thus, obeticholic acid has the potential to improve NASH with advanced fibrosis, particularly in patients who have diabetes [29].

In addition, clinical trials of thiazolidinediones such as pioglitazone, and vitamin E suggested that they are both effective to some degree in improving NAS in NAFLD patients. However, the usage of thiazolidinediones is restricted by adverse effects such as weight gain, fluid retention, increased fracture risk (especially in older women), and bladder cancer. Also, the long-term safety of vitamin E has not been established and it might increase the risk of prostate cancer. Thus, it seems likely that, obeticholic acid is a good choice of drug for NASH patients

with a variable natural history of steatosis irrespective of diabetes and obesity.

Furthermore, controversy is persistent for maintenance of weight reduction by lifestyle modification with diet and exercise, weight reduction is the established mode of management of NASH. In this series of multivariate regression analyses, we explored that obeticholic acid superseded the beneficial effect of weight reduction as a confounder. An important strength of this study is that all participants underwent paired liver biopsies. Liver biopsy has the advantage of providing important information relating to the degree of liver damage, as well as comparing histological changes before and after therapy.

Histopathological reports were performed by a single pathologist. For better accuracy reports should be made by consensus of the expert pathologist. Clinical adverse events addressed in this study were generally mild in both groups except fatigue and itching. The limitations of this study are a single-center small-scale study, so the variability of findings in different ethnicities is not reflected by this study. Thus, new studies with larger samples and multi-center are needed to confirm our results.

In conclusion, this study revealed that obeticholic acid 20 mg for 24-week improves NAS, including its all components- steatosis, lobular inflammation, hepatocyte ballooning, and fibrosis. This histological improvement was independent of weight reduction, BMI improvement, and the status of diabetes. Obeticholic acid can be considered with a smaller dose and shorter duration for treatment of both diabetic and non-diabetic NASH patients.

Acknowledgements

Obeticholic acid used in this study was supplied as complimentary by Everest Pharmaceuticals Ltd. Dhaka, Bangladesh.

This study was partially funded by Bangabandhu Sheikh Mujib Medical University.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Shahinul Alam, Department of Hepatology, Bangabandhu Sheikh Mujib Medical University, Dhaka 1000, Bangladesh. Tel: +8801973007173; E-mail: shahinul67@yahoo.com

References

[1] Byrne CD and Targher G. NAFLD: a multisystem disease. J Hepatol 2015; 62 Suppl: S47-64.
[2] Anstee QM, Targher G and Day CP. Progression of NAFLD to diabetes mellitus, cardiovascular disease or cirrhosis. Nat Rev Gastroenterol Hepatol 2013; 10: 330-44.
[3] Farrell GC. Non-alcoholic steatohepatitis: what is it, and why is it important in the Asia-Pacific region? J Gastroenterol Hepatol 2003; 18: 124-38.
[4] Mittal S, El-Serag HB, Sada YH, Kanwal F, Duan Z, Temple S, May SB, Kramer JR, Richardson PA and Davila JA. Hepatocellular carcinoma in the absence of cirrhosis in united states veterans is associated with nonalcoholic fatty liver disease. Clin Gastroenterol Hepatol 2016; 14: 124-31.
[5] Alam S, Fahim SM, Chowdhury MAB, Hassan MZ, Azam G, Mustafa G, Ahsan M and Ahmad N. Prevalence and risk factors of non-alcoholic fatty liver disease in Bangladesh. JGH Open 2018; 2: 39-46.
[6] Marchesini G, Bugianesi E, Forlani G, Cerrelli F, Lenzi M, Manini R, Natale S, Vanni E, Villanova N, Melchionda N and Rizzetto M. Nonalcoholic fatty liver, steatohepatitis, and the metabolic syndrome. Hepatology 2003; 37: 917-23.
[7] Pasumarthy L and Srour J. Nonalcoholic steatohepatitis: a review of the literature and updates in management. South Med J 2010; 103: 547-50.
[8] Argo CK and Caldwell SH. Epidemiology and natural history of non-alcoholic steatohepatitis. Clin Liver Dis 2009; 13: 511-31.
[9] Chalasani N, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, Charlton M and Sanyal AJ. The diagnosis and management of non-alcoholic fatty liver disease: practice Guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. Hepatology 2012; 55: 2005-23.
[10] Chitturi S, Farrell GC, Hashimoto E, Saibara T, Lau GK and Sollano JD; Asia-Pacific Working Party on NAFLD. Non-alcoholic fatty liver disease in the Asia-Pacific region: definitions and overview of proposed guidelines. J Gastroenterol Hepatol 2007; 22: 778-87.
[11] Hardy T, Anstee QM, and Day CP, Nonalcoholic fatty liver disease: new treatments. Curr Opin Gastroenterol 2015; 31: 175-83.
[12] Sanyal AJ, Chalasani N, Kowdley KV, McCullough A, Diehl AM, Bass NM, Neuschwander-Tetri BA, Lavine JE, Tonascia J, Unalp A, Van

Natta M, Clark J, Brunt EM, Kleiner DE, Hoofnagle JH and Robuck PR; NASH CRN. Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. N Engl J Med 2010; 362: 1675-85.

[13] Rizzo MR, Barbieri M, Marfella R and Paolisso G. Reduction of oxidative stress and inflammation by blunting daily acute glucose fluctuations in patients with type 2 diabetes: role of dipeptidyl peptidase-IV inhibition. Diabetes Care 2012; 35: 2076-82.

[14] Miller ER 3rd, Pastor-Barriuso R, Dalal D, Riemersma RA, Appel LJ and Guallar E. Meta-analysis: high-dosage vitamin E supplementation may increase all-cause mortality. Ann Intern Med 2005; 142: 37-46.

[15] Madan K, Bhardwaj P, Thareja S, Gupta SD and Saraya A. Oxidant stress and antioxidant status among patients with nonalcoholic fatty liver disease (NAFLD). J Clin Gastroenterol 2006; 40: 930-5.

[16] Blaslov K, Bulum T, Zibar K and Duvnjak L. Incretin based therapies: a novel treatment approach for non-alcoholic fatty liver disease. World J Gastroenterol 2014; 20: 7356-65.

[17] Takaki A, Kawai D and Yamamoto K. Molecular mechanisms and new treatment strategies for non-alcoholic steatohepatitis (NASH). Int J Mol Sci 2014; 15: 7352-79.

[18] Schuppan D, Gorrell MD, Klein T, Mark M and Afdhal NH. The challenge of developing novel pharmacological therapies for non-alcoholic steatohepatitis. Liver Int 2010; 30: 795-808.

[19] Adorini L, Pruzanski M and Shapiro D. Farnesoid X receptor targeting to treat nonalcoholic steatohepatitis. Drug Discov Today 2012; 17: 988-97.

[20] Markham A and Keam SJ. Obeticholic acid: first global approval. Drugs 2016; 76: 1221-6.

[21] Neuschwander-Tetri BA, Loomba R, Sanyal AJ, Lavine JE, Van Natta ML, Abdelmalek MF, Chalasani N, Dasarathy S, Diehl AM, Hameed B, Kowdley KV, McCullough A, Terrault N, Clark JM, Tonascia J, Brunt EM, Kleiner DE and Doo E; NASH Clinical Research Network. Farnesoid X nuclear receptor ligand obeticholic acid for non-cirrhotic, non-alcoholic steatohepatitis (FLINT): a multicentre, randomised, placebo-controlled trial. Lancet 2015; 385: 956-65.

[22] Abenavoli L, Falalyeyeva T, Boccuto L, Tsyryuk O and Kobyliak N. Obeticholic acid: a new era in the treatment of nonalcoholic fatty liver disease. Pharmaceuticals (Basel) 2018; 11: 104.

[23] Younossi ZM, Ratziu V, Loomba R, Rinella M, Anstee QM, Goodman Z, Bedossa P, Geier A, Beckebaum S, Newsome PN, Sheridan D, Sheikh MY, Trotter J, Knapple W, Lawitz E, Abdelmalek MF, Kowdley KV, Montano-Loza AJ, Boursier J, Mathurin P, Bugianesi E, Mazzella G, Olveira A, Cortez-Pinto H, Graupera I, Orr D, Gluud LL, Dufour JF, Shapiro D, Campagna J, Zaru L, MacConell L, Shringarpure R, Harrison S and Sanyal AJ; REGENERATE Study Investigators. Obeticholic acid for the treatment of non-alcoholic steatohepatitis: interim analysis from a multicentre, randomised, placebo-controlled phase 3 trial. Lancet 2019; 394: 2184-2196.

[24] Nevens F, Andreone P, Mazzella G, Strasser SI, Bowlus C, Invernizzi P, Drenth JP, Pockros PJ, Regula J, Beuers U, Trauner M, Jones DE, Floreani A, Hohenester S, Luketic V, Shiffman M, van Erpecum KJ, Vargas V, Vincent C, Hirschfield GM, Shah H, Hansen B, Lindor KD, Marschall HU, Kowdley KV, Hooshmand-Rad R, Marmon T, Sheeron S, Pencek R, MacConell L, Pruzanski M and Shapiro D; POISE Study Group. A placebo-controlled trial of obeticholic acid in primary biliary cholangitis. N Engl J Med 2016; 375: 631-43.

[25] Mundi MS, Velapati S, Patel J, Kellogg TA, Abu Dayyeh BK and Hurt RT. Evolution of NAFLD and its management. Nutr Clin Pract 2020; 35: 72-84.

[26] Papatheodoridi M and Cholongitas E. Diagnosis of non-alcoholic fatty liver disease (NAFLD): current concepts. Curr Pharm Des 2018; 24: 4574-4586.

[27] Kleiner DE, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, Ferrell LD, Liu YC, Torbenson MS, Unalp-Arida A, Yeh M, McCullough AJ and Sanyal AJ; Nonalcoholic Steatohepatitis Clinical Research Network. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology 2005; 41: 1313-21.

[28] Pathak P, Liu H, Boehme S, Xie C, Krausz KW, Gonzalez F and Chiang JYL. Farnesoid X receptor induces Takeda G-protein receptor 5 cross-talk to regulate bile acid synthesis and hepatic metabolism. J Biol Chem 2017; 292: 11055-11069.

[29] McPherson S, Hardy T, Henderson E, Burt AD, Day CP and Anstee QM. Evidence of NAFLD progression from steatosis to fibrosing-steatohepatitis using paired biopsies: implications for prognosis and clinical management. J Hepatol 2015; 62: 1148-55.