Hepatic Medicine: Evidence and Research

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ORIGINAL RESEARCH

Effect of sitagliptin on hepatic histological activity and fibrosis of nonalcoholic steatohepatitis patients: a 1-year randomized control trial

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Background/purpose: Dipeptidyl peptidase 4 (DPP-4) expression is directly associated with hepatic lipogenesis and liver injury in nonalcoholic steatohepatitis (NASH). This study has been designed to elucidate the histological improvement of NASH with the DPP-4 inhibitor sitagliptin.

Materials and methods: In this open-label randomized control trial, paired liver biopsy was taken from 40 NASH patients. Sitagliptin 100 mg was given once daily to the SL group and no sitagliptin was given to the L group for 1 year. Patients from both groups were encouraged to exercise moderately and advised to avoid saturated fat, excessive sugar, soft drinks, fast food, and refined carbohydrates to reduce weight.

Results: Steatosis improved in the SL group (from 2.3±0.6 to 1.2±0.8; *P*=0.000) and the L group (from 2.1±0.6 to 1.6±0.9; *P*=0.008), ballooning decreased from 1.8±0.6 to 1.3±06 (*P*=0.002) in the SL group, but not in the L group. Nonalcoholic fatty liver disease activity score (NAS) attenuated in both groups: the SL group (from 5.8±0.9 to 3.9±1.4; *P*=0.000) and the L group (from 5.3±0.6 to 4.6±1.2; *P*=0.009). NAS improvement was much higher in the SL group (1.9±1.4) than in the L group (0.7±1.1) (*P*=0.006), with NAS improving by ≥2 in 13 patients from the SL group and five patients from the L group (*P*=0.01). Improvement was irrespective of diabetes. Regression analysis explored that sitagliptin had odds of 6.38 and weight reduction had odds of 4.51 for NAS reduction.

Conclusion: Sitagliptin 100 mg once daily for 1 year ameliorates NAS by improving steatosis and ballooning, irrespective of diabetes. Sitagliptin has stronger efficacy than that of weight reduction.

Keywords: fatty liver, NASH, sitagliptin, NAS, fibrosis, steatosis, ballooning, histological activity

Introduction

Nonalcoholic fatty liver disease (NAFLD) is considered as the hepatic manifestation of a metabolic syndrome and is currently the most common cause of liver disease in many developed countries worldwide.[1] NAFLD is a relatively common co-morbidity in patients with type 2 diabetes mellitus and is a leading cause of chronic liver disease. It is also known that, for patients with type 2 diabetes mellitus, the risk of developing cirrhosis or hepatocellular carcinoma is doubled. Furthermore, the risk of dying from liver cirrhosis was doubled in a cohort of patients with type 2 diabetes mellitus relative to the general population.[2] Liver fat accumulation may range from simple triglyceride accumulation (steatosis), nonalcoholic steatohepatitis (NASH), cirrhosis, and even hepatocellular carcinoma.[3] NASH, the progressive form of NAFLD, is

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characterized by hepatocellular damage, inflammation, and liver fibrosis that can progress to cirrhosis.[4] It is reported that almost 10–20% of individuals with NAFLD have NASH and 10–15% of individuals with NASH progress to cirrhosis.[5] The prevalence of NASH is 42.4% among NAFLD patients in Bangladesh, which is much higher than Western countries.[6] NASH probably causes ~80% of cases of cryptogenic cirrhosis, which accounts for 10–20% of all cirrhosis and progresses to advanced fibrosis in 30–37% of patients.[7] The risk of developing decompensated cirrhosis is 5–10% and that for hepatocellular carcinoma is 1–2%.[8]

There is currently no FDA-approved treatment available for NASH. Most hepatologists attempt to manage NASH by lifestyle changes, such as weight reduction with exercise, as well as standard therapeutic interventions to control concomitant disease, eg hyperlipidemia, hypertension, and type 2 diabetes mellitus. Enzyme dipeptidyl peptidase 4 (DPP-4) has widespread organ distribution throughout the body and exerts pleiotropic effects via its peptidase activity. The liver expresses DPP-4 to a high degree, and recent accumulating data suggested that DPP-4 is involved in the development of various chronic liver diseases such as hepatitis C virus (HCV) infection, nonalcoholic fatty liver disease, and hepatocellular carcinoma, even before development of diabetes. Hepatic DPP-4 expression in NAFLD may be directly associated with hepatic lipogenesis and liver injury. Sitagliptin, an oral antihyperglycemic agent that competitively inhibits DPP-4, inactivates the hormones glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) released in response to meals. By blocking GLP-1 and GIP breakdown, sitagliptin increases insulin secretion and suppresses glucagon, which lowers blood glucose levels. Improvement in hyperglycemia and hyperinsulinemia results in the downregulation of sterol regulatory element binding protein-1c (SREBP-1c), and the blockage of fatty acid synthase leads to improvement in liver fat and NASH.[9,10] Recently, the DPP-4 inhibitor has been reported to improve hepatic steatosis in mice and humans.[11] An animal study as well as an uncontrolled pilot study on humans explored that sitagliptin improved features of liver histology in NASH patients.[12] However, there have been few trials of sitagliptin on human NASH to date. None of the studies had been done in patients without diabetes, although NASH may exist with prediabetes and even before diabetes.

We have designed this randomized control trial (RCT) aiming to elucidate the histological improvement of NASH patients with or without diabetes with sitagliptin 100 mg every day for 1 year.

Materials and methods

Study design and patient population

This was an open-label RCT. The duration of the study was from August 2015 to June 2017. Patients aged 18–65 years in whom the NAFLD activity score (NAS) was ≥5 in liver histology, irrespective of the presence of diabetes, were selected as the sample of our study.

Exclusion criteria

Those with significant alcohol intake, a history of taking drugs that may cause a fatty liver, or a history of taking drugs that have shown benefit in previous NASH studies, eg using angiotensin II receptor blockers, estrogens, amiodarone, steroids, tamoxifen, lipid lowering agents, and other antidiabetic medications (thiazolidinediones, metformin, sulfonylureas, alpha-gucosidase inhibitors), were not eligible for this study. Chronic viral hepatitis (hepatitis B virus [HBV] and HCV), pregnancy, and patients with hemochromatosis, Wilson's disease, autoimmune hepatitis, primary biliary cirrhosis, sclerosing cholangitis, biliary obstruction, alpha-1 antitrypsin deficiency, ischemic cardiac or cerebrovascular disease, impaired renal function, or malignancies were excluded.

Randomization and allocation

The patients were randomized into two groups in a 1:1 ratio using computer-generated numbers: the SL group (sitagliptin plus lifestyle modification) and the L group (only lifestyle modification). Sitagliptin 100 mg was given once daily in the SL group of patients, and no sitagliptin was given in the L group patients for 1 year. Both patients and researcher were aware of allocation. Lifestyle modification was advised for both groups of patients, but none was in any strict protocol for diet and exercise, and the result was evaluated by weight reduction only. The patient was encouraged toward moderate exercise, that is walking 30 min a day. Dietary advice to avoid saturated fat, excessive sugar containing diet, soft drinks, fast food. and refined carbohydrate were given to both groups of patients according to the diet chart of NAFLD. Diabetic patients were treated with lifestyle modification and, if required, with oral sulfonylureas – gliclazide, glimepiride, or with insulin. Hypertensive patients were treated by antihypertensive drugs, except angiotensin converting enzyme inhibitor, angiotensin receptor blocker, and calcium channel blocker (diltiazem), due to their beneficial effect on steatohepatitis and fibrosis. A total of 48 patients were selected for randomization; 24 in group 1/SL and 24 in group 2/L were followed for the next 1 year. Four patients of

both groups were lost from the study due to lack of interest of doing liver biopsy the end of the study. So, a total of 40 patients, 20 in each group, were considered for the protocol analysis (Figure 1).

Primary and secondary outcomes

The primary outcomes were measured by the changes in steatosis, lobular inflammation, ballooning, fibrosis, and NAS in paired liver biopsy. The secondary outcomes were the changes in body mass index (BMI), weight reduction, liver enzymes, lipid profile, and HOMA IR. Safety was accessed via regular monitoring of treatment-emergent adverse events and laboratory tests.

Study schedule and surveillance parameters

After screening, the included patients were followed for 12 months. Patients were followed monthly for an initial 3 months and then every 3 months for the next 9 months. Each visit consisted of a clinical examination, blood pressure (BP), and BMI determinations. The parameters compared between first and last visits were systolic blood pressure, diastolic blood pressure, waist circumference, body mass index (BMI), alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (GGT), homeostatic model assessment insulin resistance, cholesterol, triglycerides (TG), high density lipoprotein (HDL), low density lipoprotein (LDL), fasting blood sugar (FBS), and blood sugar 2 h after breakfast (2HABF). The estimate of insulin resistance was calculated using the HOMA-IR index, with the following formula: insulin resistance=fasting plasma insulin×fasting plasma glucose/22.5. FBS, 2HABF, and lipid profile for diabetic and dyslipidemic patients were monitored according to need. A follow-up questionnaire was filled out during each visit. An alcohol consumption questionnaire was administered at each visit, and study compliance was strictly monitored. Additionally, the 1st visit comprised recording of the result of the index liver biopsy, while the last visit ended with the 2nd liver biopsy, performed at a maximum 2 weeks after the end of treatment. NAS (including its components, such as steatosis, ballooning, and lobular inflammation) and fibrosis scores were compared.

Biochemical analysis

The University biochemistry laboratory was used for estimation of FBS, 2HABF, ALT, AST, GGT, bilirubin (B), total cholesterol (TC), TG, LDL-C, and HDL-C on fresh serum using an autoanalyzer. Serum samples obtained after an overnight fast of at least 12 h and immediately frozen at –20°C were used to determine the levels of immunoreactive insulin (IRI) by a chemiluminescence immunoassay. We have determined IRI by the homeostasis model assessment, HOMA-IR.

Histopathology analysis

Pre- and post-treatment biopsies were performed within 1 month of the study. All liver biopsies were done with full resuscitation facilities, and samples were fixed with 10%

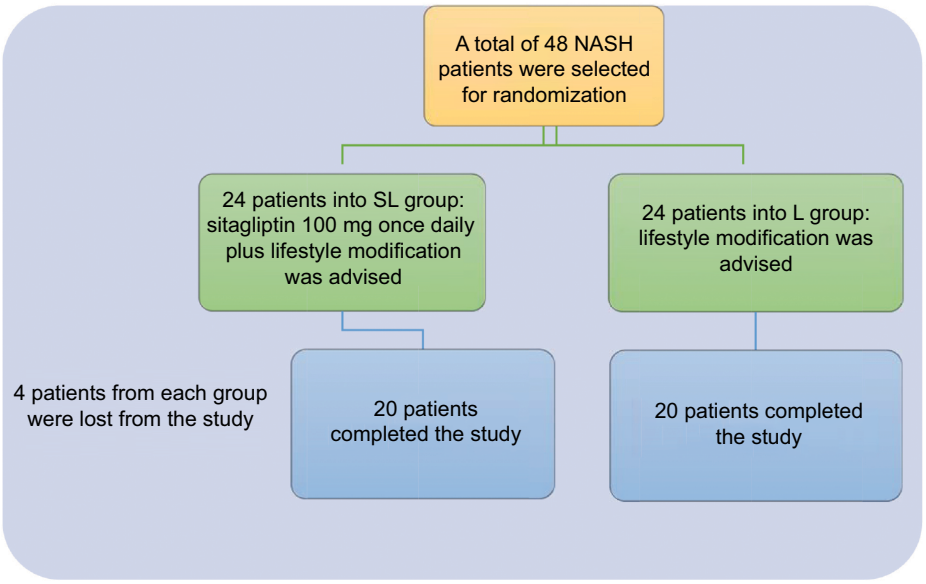

Figure 1 Flow chart for patient selection for the study.
Abbreviation: NASH, nonalcoholic steatohepatitis.

formalin and stained with hematoxylin–eosin and Masson's trichrome. Biopsies were evaluated by consensus of three experienced pathologists and concluded by the team leader who was working as a faculty member of the university, not aware about the allocation of treatment or about the clinical and biochemical parameters of any patient, using the scoring system validated by Kleiner et al.[13] This histological scoring system quantifies steatosis (0–3), lobular inflammation (0–3), and ballooning (0–2) and the resulting NAS ranged between 0 and 8. Scores greater or equal to 5 are largely diagnostic for NASH. Fibrotic changes are evaluated separately from NAS, ranging from 0 (no fibrosis) to 4 (cirrhosis).

Statistical analysis

Quantitative data were presented as mean±SD, and qualitative data were presented as a percentage. All data were analyzed by SPSS (version 20). Qualitative data were analyzed by the Chi-square test, and quantitative data were analyzed by Student's *t*-test. Pretreatment and end of treatment data within group were compared by the paired *t*-test. All quantitative and qualitative data were analyzed between responders and non-responders. The univariate and multivariate binary logistic regression analyses were done to find out the best predictor of patient's response. A statistically significant result was considered when the *P*-value was <0.05.

Ethical consideration

Ethical clearance for the study was obtained from the Institutional Review Board (IRB) of the Bangabandhu Sheikh Mujib Medical University (BSMMU) prior to the commencement of this study. Approval of the paper was decided by the 92th IRB, BSMMU meeting held on August 2, 2015 (No. BSMMU/2015/12651). The study was conducted in compliance with good clinical practice and the principles of the Declaration of Helsinki. All patients gave written informed consent to participate in the study. Prior to commencement of the study, the aim and objectives of the study, along with the procedure, risk, and benefit of the study, were explained to the patients. It was assured that all information and records would be kept confidential.

Results

Baseline characteristics of patients

A total of 40 NASH patients were analyzed as per protocol; 20 in the SL group and 20 in the L group. Mean age of patients was 41.7±9.1 years in the SL group and 35.5±6.9 years in the L group. The female predominated in both groups. Diabetes (11/6) and hypertension (10/6) were equally prevalent in both the groups. Mean BMI, serum glutamic pyruvic transaminase, serum glutamic oxaloacetic transaminase, GGT, FBS, HOMA IR, serum cholesterol, HDL, and triglyceride were similar in both groups. Mean serum LDL was 147.6±33.1 mg/dL in the SL group and 110.7±45.8 mg/dL in the L group. So, incidentally SL groups were older and had a higher LDL level than the L group (Table 1).

End of study results

In the SL group, ALT was reduced from 71.6±41.6 U/L to 33.1±16.0 U/L (*P*=0.001). AST was reduced from 49.3±25.3 U/L to 31.4±11.8 U/L (*P*=0.003). GGT was reduced from 61.8±38.6 U/L to 36.6±21.6 U/L (*P*=0.003); on the other hand, there was no significant reduction of GGT value in the L group (from 45.4±25.3 U/L to 46.6±46.9 U/L; *P*=0.874). ALT and AST reduction in the L group was significant (from 53.4±25.5 to 32.2±11.1 U/L; *P*=0.002 and from 35.1±15.8 to 28.3±10.5 U/L; *P*=0.043). Improvement in FBS was significant in the SL group (from 7.3±3.9 mmol/L to 5.7±1.6 mmol/L; *P*=0.032), whereas the improvement was not satisfactory in the L group. Reduction of serum cholesterol and LDL was significant (*P*=0.000) in the SL group but not in the L group. Reduction of serum triglyceride level was insignificant for both groups.

Steatosis significantly improved in the SL group (from 2.3±0.6 to 1.2±0.8; *P*=0.000), improvement was

Table 1 Baseline characteristics of the sitagliptin and control group

Variable	SL group (n=20)	L group (n=20)	*P*
Age, years	41.7±9.1	35.5±6.9	0.02
Sex (male:female)	4:16	8:12	0.17
Diabetes (present/absent)	11/9	6/14	0.11
Hypertension (present/absent)	10/10	6/14	0.20
BMI, kg/m^2	27.6±5.1	25.3±2.8	0.08
Waist circumference, cm	96.8±10.0	91.5±6.7	0.06
ALT, U/L	71.6±41.6	53.4±25.5	0.11
AST, U/L	49.3±25.3	35.1±15.8	0.05
ALP, U/L	77.8±22.0	78.2±28.0	0.97
GGT, U/L	61.8±38.6	45.4±25.3	0.12
FBS, mmol/L	7.3±3.9	5.4±1.5	0.05
Blood sugar 2 h after breakfast, mmol/L	10.4±4.7	8.3±2.6	0.09
Insulin resistance index (HOMA IR)	2.8±2.2	2.5±1.5	0.58
Serum cholesterol, mg/dL	229.7±46.3	198.2±58.9	0.07
HDL, mg/dL	38.9±10.6	34.0±10.7	0.16
LDL, mg/dL	147.6±33.1	110.7±45.8	0.00
Triglyceride, mg/dL	185.4±81.4	269.2±204.9	0.10

Note: SL, sitagliptin plus lifestyle modification; L, only lifestyle modification.
Abbreviations: BMI, body mass index; ALT, alanine aminotransferase; AST, aspartate-aminotransferase; ALP, alkaline phosphatase; GGT, gamma-glutamyl transpeptidase; FBS, fasting blood sugar; HOMA IR, homeostatic model assessment insulin resistance; HDL, high density lipoprotein; LDL, low density lipoprotein.

also significant in the L group (from 2.1±0.6 to 1.6±0.9; *P*=0.008). Ballooning was also improved (from 1.8±0.6 to 1.3±0.6; *P*=0.002) in the SL group but not in the L group (from 1.6±0.5 to 1.5±0.5; *P*=0.748). Lobular inflammation was not improved in any of the groups in this study. NAS improvement was significant in the SL group (from 5.8±0.9 to 3.9±1.4; *P*=0.000), and L group (from 5.3±0.6 to 4.6±1.2; *P*=0.009) whereas fibrosis improvement was not significant in either group (Table 2 and Figure 2). Although overall weight reduction was significant (Figure 3), it was not significant within two groups. Weight was reduced in 21 (52.5%) cases, but with 7 and 10% weight reductions in three (7.5%) and two (5%) cases, respectively, only. Out of those 21 who reduced weight, 14 (77.8%) ameliorated NAS≥2 (*P*=0.004), but reduction of fibrosis ≥1 was insignificant (*P*=0.698). A weight reduction of 7% also reduced NAS≥2, and a 10% weight reduction ameliorated both NAS≥2 and fibrosis≥1.

Comparative analysis of anthropometric, biochemical, and histological changes between the sitagliptin and control groups

A difference of NAS improvement was significant in the SL group (1.9±1.4) compared to that of the L group (0.7±1.1) (*P*=0.006). On the other hand, there was no significant difference of improvement in fibrosis between the SL and L groups (*P*=0.580). Improvement in GGT in the SL group (25.0±32.4) and the L group (−1.2±33.4) was significant (*P*=0.018), whereas there was no significant improvement in ALT and AST between the SL and L groups. Reduction in serum cholesterol was significant between the SL group and the L group (*P*=0.040); on the contrary, reductions in LDL and triglyceride were not significant between the SL and L groups (*P*=0.061 and *P*=0.157, respectively). Differences in improvements of other parameters, such as body weight, BMI, and waist circumference, were not significant between the SL and L groups (Table 3).

Factors associated with NAS improvement

In univariate analysis, NAS improved ≥2 in 13 patients in the SL group and five patients in the L group (*P*=0.01). After treatment, weight reduction was 2.1±2.6 kg. Fibrosis improvement was 0.3±1.2. In those with NAS<2 improvement; weight reduction was 0.1±2.7 kg after 1 year. Fibrosis improvement was 0.1±1.1. Age, sex, presence of diabetes, hypertension, metabolic syndrome, HOMA IR, baseline ALT, AST, GGT, NAS, and BMI had no significant influence on improvement of NAS. Weight reduction was significantly higher in the NAS improvement group (2.1±2.6 and 0.1±2.7; *P*=0.02). ALT and AST change did not correlate with NAS improvement, rather GGT significantly correlated with

Table 2 Anthropometric, biochemical, and histological changes after 1 year

Variable	Total (n=40)		*P*	Sitagliptin (SL) (n=20)		*P*	Control (L) (n=20)		*P*
	Baseline	After 12 months		Baseline	After 12 months		Baseline	After 12 months	
ALT, U/L	62.3±35.3	32.6±13.6	0.000	71.6±41.6	33.1±16.0	0.001	53.4±25.5	32.2±11.1	0.002
AST, U/L	42.0±21.9	29.8±11.1	0.000	49.3±25.3	31.4±11.8	0.003	35.1±15.8	28.3±10.5	0.043
Alkaline phosphatase, U/L	78.0±28.4	77.8±27.1	0.969	77.8±22.0	75.3±23.3	0.786	78.2±28.0	80.8±32.2	0.954
GGT, U/L	53.3±33.6	41.7±36.8	0.047	61.8±38.6	36.6±21.6	0.003	45.4±25.3	46.6±46.9	0.874
BMI, kg/m^2	26.5±4.2	26.1±4.4	0.019	27.6±5.1	27.2±5.4	0.097	25.3±2.8	24.9±2.9	0.114
Weight, kg	64.4±10.6	63.4±11.1	0.025	65.6±12.6	64.6±13.3	0.169	63.2±8.4	62.2±8.5	0.063
FBS, mmol/L	6.5±3.2	5.7±1.4	0.071	7.3±3.9	5.7±1.6	0.032	5.4±1.5	5.6±1.3	0.707
HOMA IR	2.8±1.9	1.9±1.0	0.001	2.8±2.2	1.5±0.9	0.003	2.5±1.5	1.9±1.0	0.115
Serum cholesterol mg/dL	218.2±54.5	180.5±56.7	0.003	229.7±46.3	170.0±34.8	0.000	198.2±58.9	192.8±74.1	0.538
LDL mg/dL	136.6±39.6	95.7±28.6	0.000	147.6±33.1	95.6±28.6	0.000	110.7±45.8	95.8±29.7	0.016
HDL mg/dL	36.8±11.1	38.7±8.7	0.285	38.9±10.6	41.5±8.9	0.080	34.0±10.7	35.4±7.5	0.794
Triglyceride mg/dL	232.1±163.1	229.4±240.1	0.915	185.4±81.4	149.2±75.4	0.096	269.2±204.9	323.8±324.7	0.466
Steatosis	2.2±0.6	1.4±0.8	0.000	2.3±0.6	1.2±0.8	0.000	2.1±0.6	1.6±0.9	0.008
Ballooning	1.7±0.5	1.4±0.6	0.014	1.8±0.6	1.3±06	0.002	1.6±0.5	1.5±0.5	0.748
Lobular inflammation	1.7±0.5	1.4±0.5	0.070	1.7±0.5	1.5±0.5	0.096	1.6±0.5	1.5±0.5	0.428
NAS	5.5±0.8	4.2±1.3	0.000	5.8±0.9	3.9±1.4	0.000	5.3±0.6	4.6±1.2	0.009
Fibrosis	1.7±0.8	1.6±0.8	0.403	1.8±0.6	1.7±0.9	0.853	1.7±0.9	1.5±0.7	0.309

Note: SL, sitagliptin plus lifestyle modification; L, only lifestyle modification.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate-aminotransferase; GGT, gamma-glutamyl transpeptidase; BMI, body mass index; FBS, fasting blood sugar; HOMA IR, homeostatic model assessment insulin resistance; LDL, low density lipoprotein; HDL, high density lipoprotein; NAS, nonalcoholic fatty liver disease activity score.

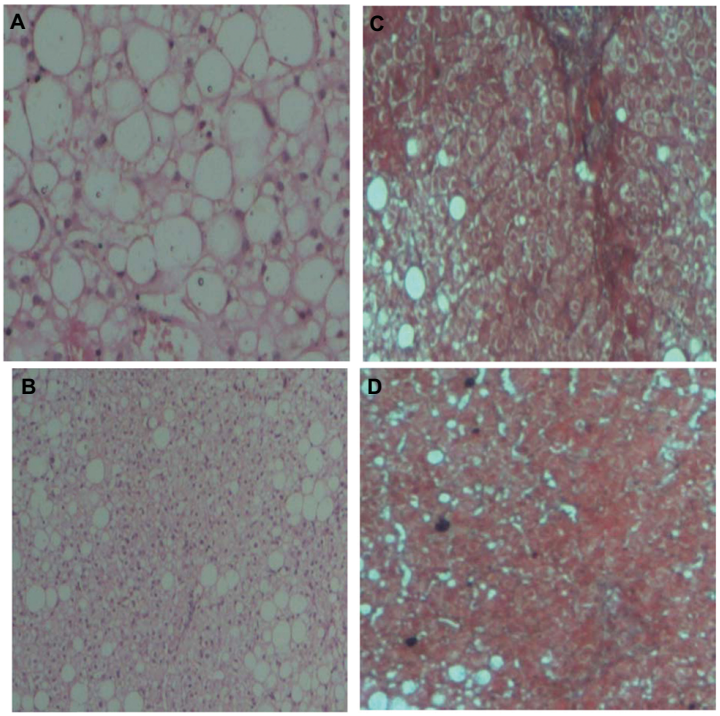

Figure 2 Histological improvement between index and end of study liver biopsy.
Notes: Upper panel revealed the first and lower panel revealed the second biopsy of the same patient (Case No-01, ID NO.41/40). NAS score improvement from 7 to 4 and fibrosis score improvement from 2 to 1. (**A**) Histological section showing marked steatosis and NAS of 7 with H&E ×440. (**B**) Histological section showing marked reduction of steatosis and reduction of NAS to 4 H&E ×220. (**C**) Histological section showing fibrous bands in the portal area Masson's trichrome ×220. (**D**) Histological section showing fibrosis markedly improved Masson's trichrome ×220.
Abbreviation: NAS, nonalcoholic fatty liver disease activity score.

improvement of NAS. Binary logistic regression analysis explored that sitagliptin had odds of 6.38 and weight reduction had odds of 4.51 for NAS improvement (Table 4).

Adverse events

Treatment was generally well tolerated. Only three patients in the SL group and one patient in the L group noted dyspepsia. Two patients in the SL group and one patient in the L group developed constipation. There was no statistically significant difference between the two groups. There was no sufficient side effect in the SL group which necessitated a dose reduction of sitagliptin or cessation of the drug. None of the patients experienced pancreatitis, diarrhea, or respiratory tract infection.

Discussion

This is the first open-label RCT reported with dual liver biopsy for NASH patients intervened with sitagliptin 100

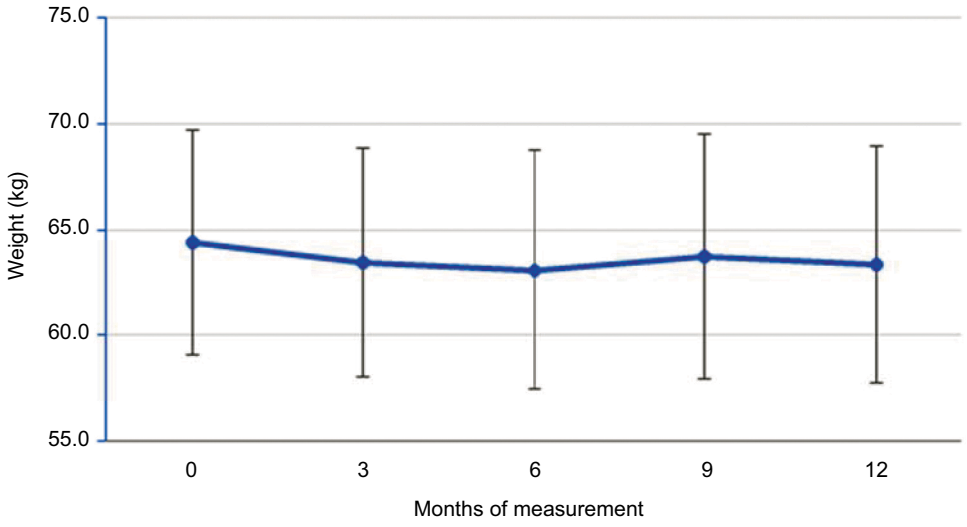

Figure 3 Trend of weight during the study period.

Table 3 Comparison of anthropometric, biochemical, and histological changes between the sitagliptin and control groups

Variable improvement (mean±SD)	Sitagliptin (SL) (n=20)	Control (L) (n=20)	*P*
NAS	1.9±1.4	0.7±1.1	0.006
Fibrosis	0.1±1.2	0.3±1.1	0.580
Steatosis	1.1±0.9	0.6±0.8	0.053
Ballooning	0.5±0.6	0.1±0.7	0.034
Lobular inflammation	0.3±0.6	0.1±0.6	0.432
ALT, U/L	38.1±44.8	21.2±26.1	0.152
AST, U/L	17.9±22.9	6.9±14.1	0.076
GGT, U/L	25.0±32.4	−1.2±33.4	0.018
Weight, kg	1.1±3.3	1.0±2.3	0.978
BMI, kg/m^2	0.4±1.1	0.4±1.0	0.890
Waist circumference, cm	1.2±5.6	−1.4±16.1	0.503
Serum cholesterol, mg/dL	59.7±59.3	11.8±77.4	0.040
LDL, mg/dL	52.1±43.4	24.6±31.8	0.061
HDL, mg/dL	−2.7±6.4	−0.9±13.7	0.610
Triglyceride, mg/dL	36.3±92.6	−36.6±202.3	0.157
FBS, mmol/L	1.8±3.3	−0.14±1.5	0.030

Note: SL, sitagliptin plus lifestyle modification; L, only lifestyle modification.
Abbreviations: NAS, nonalcoholic fatty liver disease activity score; ALT, alanine aminotransferase; AST, aspartate-aminotransferase; GGT, gamma-glutamyl transpeptidase; BMI, body mass index; LDL, low density lipoprotein; HDL, high density lipoprotein; FBS, fasting blood sugar.

mg daily for 1 year. Sitagliptin, a DPP- 4 inhibitor, was prescribed for the NASH patients, irrespective of the presence of diabetes. Although NAFLD and diabetes frequently co-exist, expression of DPP-4 in hepatocytes was reported to be greatly increased in NAFLD[14–16] and increased in chronic HCV infection,[11,17] cirrhosis,[18] hepatocellular carcinoma,[19] and stem cell.[20] This excessive expression of DPP-4 is not always associated with the presence of diabetes or prediabetic conditions. This baseline knowledge made us confident to include the NASH patients with diabetes and prediabetic conditions as in previous studies. Therefore, of our inclusion criteria of NASH patients could be justified, irrespective of the presence of diabetes.

This randomized control trial has demonstrated that sitagliptin 100 mg daily, a DPP-4 inhibitor for 1 year, ameliorates steatosis and hepatocyte ballooning in NASH patients. These two changes lead to a significant reduction in the NAS in paired biopsy samples. Fibrosis was unchanged with this intervention. NAS was also decreased in the control group by reduction of steatosis, but the hepatocyte ballooning was unchanged in this group. Reduction in steatosis and NAS was significantly higher in the SL group than in the L group.

In an animal study, sitagliptin decreased the liver steatosis, β-cell apoptosis, and insulin resistance in fructose-fed rats that developed metabolic syndrome.[21] Another recent animal study from Japan demonstrated that sitagliptin can ameliorate hepatic steatosis in high-fructose diet-fed ob/ob mice and can prevent development of NAFLD by inhibiting inflammatory cytokines and expression of genes related to lipid synthesis in the liver.[22] The most significant finding in this study was that sitagliptin induced a decrease in the grade of hepatocyte ballooning hepatic histology. Ballooning degeneration, which

Table 4 Factors associated with NAS improvement

Variable	Univariate analysis		*P*	Multivariate analysis Odds ratio	*P*
	NAS≥2 improvement	NAS<2 improvement			
Sitagliptin (SL)/control (L)	13/5	7/15	0.01	6.38	0.012
Age, years	39.3±10.4	38.0±7.0	0.63		
Sex (male/female)	6/12	6/16	0.67		
Diabetes, present/absent	7/11	10/12	0.67		
Hypertension, present/absent	8/10	8/14	0.60		
BMI, kg/m^2	25.7±4.2	27.1±4.3	0.28		
Weight, kg	63.1±12.1	65.4±9.5	0.50		
Metabolic syndrome present/absent	13/5	14/8	0.56		
HOMA IR	2.8±2.3	2.5±1.5	0.61		
Baseline NAS	5.7±.8	5.4±.7	0.20		
Baseline ALT, U/L	69.6±44.0	56.3±25.8	0.24		
Baseline AST, U/L	46.8±27.4	38.3±16.2	0.26		
Baseline GGT, U/L	61.5±38.9	47.1±27.0	0.17		
Weight reduction, kg	2.1±2.6	0.1±2.7	0.02	4.51	0.034
BMI, kg/m^2 reduction	0.7±1.1	0.1±1.0	0.09		
ALT change, U/L	38.0±46.9	22.8±26.2	0.20		
AST change, U/L	17.9±24.4	7.9±13.7	0.11		
GGT change, U/L	26.2±33.7	0.3±32.5	0.02		
Fibrosis improvement	0.3±1.2	0.1±1.1	0.52		

Note: SL, sitagliptin plus lifestyle modification; L, only lifestyle modification.
Abbreviations: NAS, nonalcoholic fatty liver disease activity score; BMI, body mass index; HOMA IR, homeostasis model assessment insulin resistance; ALT, alanine aminotransferase; AST, aspartate-aminotransferase; GGT, gamma-glutamyl transpeptidase.

has been pointed as a hallmark of steatohepatitis, associates with cell swelling and has been linked to cytoskeletal injury in NASH.[23,24] Therefore, it is tempting to speculate that DPP-4 inhibitors may exert a beneficial effect on histological activity by reducing steatosis and ballooning. Similar histologically proven benefit was observed in another uncontrolled pilot study from Turkey.[25] A recent randomized, double-blind, allocation-concealed, placebo-controlled trial explored that sitagliptin was not significantly better than placebo in reducing liver fat measured by MRI-derived proton density-fat fraction (mean difference between sitagliptin and placebo arms=−0.3%, P=0.4).[26] This dissimilarity may be due to the lack of microscopic assessment in this study, which is considered a gold standard for evaluation. Reduction of fibrosis was not significant in our study, for which longer treatment periods of >1 year would probably be needed.

These findings show that the use of sitagliptin DPP-4 inhibitors in NASH is promising and may have several advantages in terms of efficacy and tolerability compared with thiazolidinediones, which have been shown to induce weight gain.[27]

ALT, AST, GGT, and HOMA IR were improved in the sitagliptin group, whereas ALT and AST, but not GGT or HOMA IR, improved in the control group. ALT and AST were not correlated with histological improvement, but GGT had a significant correlation with improvement of NAS. ALT and AST were decreased in a previous study with sitagliptin.[27] However, GGT was previously described as a better baseline and dynamic marker of histological changes than ALT and AST in NASH.[28,29] It is plausible that sitagliptin improved HOMA IR and fasting blood sugar in this study, in accordance with previous studies.[12]

In this randomized control trial, overall weight reduction was significant, and weight reduction was significantly higher in NAS≥2 improvement than that of NAS<2 improvement. Weight reduction had odds of 4.51 for NAS improvement. Beneficial effect weight reduction in NASH has been proven and established in meta-analysis and the recommended intervention for NASH.[30] We had not quantified the weight reduction that had influenced the improvement of NAS. However, novel findings of our study are that sitagliptin had stronger efficacy than that of weight reduction in ameliorating NAS, irrespective of diabetes.

The study limitations of small sample size, lack of concealment, and all patients recruited in this study were from a single tertiary-level hospital. So, the present study suffered from lack of multicenter, different ethnic categories of patients.

Conclusion

Sitagliptin 100 mg once daily for 1 year ameliorates NAS by improving steatosis and hepatocyte ballooning, irrespective of diabetic status. Sitagliptin has a stronger effect than that of weight reduction. Safety and tolerability of sitagliptin is similar to the control. Future large, double blind, randomized control clinical trials are recommended to confirm and establish these findings.

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Disclosure

The authors report no conflicts of interest in this work.

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