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ORIGINAL ARTICLE

Favorable impact of long-term SGLT2 inhibitor for NAFLD complicated by diabetes mellitus: A 5-year follow-up study

Norio Akuta[1] | **Yusuke Kawamura**[1] | **Shunichiro Fujiyama**[1] | **Satoshi Saito**[1] | **Nozomu Muraishi**[1] | **Hitomi Sezaki**[1] | **Tetsuya Hosaka**[1] | **Masahiro Kobayashi**[1] | **Mariko Kobayashi**[2] | **Yasuji Arase**[1] | **Kenji Ikeda**[1] | **Fumitaka Suzuki**[1] | **Yoshiyuki Suzuki**[1] | **Hiromitsu Kumada**[1]

[1]Department of Hepatology, Toranomon Hospital and Okinaka Memorial Institute for Medical Research, Tokyo, Japan

[2]Liver Research Laboratory, Toranomon Hospital, Tokyo, Japan

Correspondence
Norio Akuta, Department of Hepatology, Toranomon Hospital, 2-2-2 Toranomon, Minato-ku, Tokyo, 105-8470, Japan.
Email: akutanorio@gmail.com

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Abstract

The aim of this study was to determine the impact at 5 years of sodium-glucose cotransporter 2 inhibitor (SGLT2i) in nonalcoholic fatty liver disease (NAFLD) with type 2 diabetes mellitus (T2DM) on liver histopathology and clinical features. In this retrospective study, the histological impacts at 5 years after the start of SGLT2i in NAFLD with T2DM were investigated. Six patients with NAFLD and T2DM were treated for the long term with canagliflozin of SGLT2i, and liver biopsies were obtained at the points of the pretreatment, 24 weeks, 3 years, and 5 years after the start of treatment. The primary outcome was liver histopathological changes at 5 years (defined as decrease in NAFLD activity score of one point or more without worsening in fibrosis stage, compared with the pretreatment). The additional treatment of glucagon-like peptide 1 receptor agonist (GLP-1RA) was performed in 2 patients after the point of 3 years, and evaluated as histological worsening. As the primary outcome, histological improvement, no change, and worsening were 50%, 17%, and 33% at 5 years, respectively. Overall, the scores of steatosis, lobular inflammation, ballooning, and fibrosis stage decreased at 5 years in 67%, 33%, 0%, and 33%, respectively. As the secondary outcomes, homeostasis model assessment of insulin resistance and serum ferritin decreased significantly at 5 years. None developed 3-point major adverse cardiovascular events. Two patients with the addition of GLP-1RA on SGLT2i did not show the worsening of steatosis, ballooning, and fibrosis stage at 5 years compared with 3 years. *Conclusion*: A 5-year follow-up study with SGLT2i indicated the favorable histological impact on NAFLD with T2DM.

INTRODUCTION

The most common worldwide liver disease is nonalcoholic fatty liver disease (NAFLD).[1–4] Its pathology ranges from the typically benign nonalcoholic fatty liver to nonalcoholic steatohepatitis (NASH), which may progress to liver cirrhosis, liver cancer, and finally, to liver failure.[2] Furthermore, The American Association for the Study of Liver Diseases reported that the most common cause of death in patients with NAFLD is related to cardiovascular diseases (CVDs), independent of other metabolic comorbidities. Liver-related mortality was reported to be the second or third cause of death, and cancer-related mortality was among the top three causes of death.[3]

Sodium-glucose cotransporter 2 inhibitor (SGLT2i) improves the outcome of patients with type 2 diabetes mellitus (T2DM) by enhancing urinary glucose excretion, and reducing the risk of renal complications and three-point major adverse cardiovascular events (3-point MACE), including death from cardiovascular causes, nonfatal myocardial infarction, or nonfatal stroke.[5,6] We have also reported that canagliflozin, SGLT2i, improved the histopathological findings in NAFLD complicated by T2DM by reducing the scores of steatosis, lobular inflammation, ballooning, and fibrosis stage at 24 weeks and 3 years, compared with the pretreatment scores (histopathological improvement represented ≥1-point reduction in NAFLD activity score [NAS], without worsening in fibrosis stage).[7–9] Similar results with the use of other SGLT2i were also reported by one study with empagliflozin (significant improvements at 24 weeks in steatosis, ballooning, and fibrosis)[10] and the other one with ipragliflozin (significant improvements at 72 weeks in ballooning and fibrosis),[11] highlighting the therapeutic potential of SGLT2i as effective agents against NAFLD complicated with T2DM.

The aim of the present retrospective study was to determine the long-term impact at 5 years of 100 mg/day canagliflozin, a SGLT2i, on the liver histopathology and clinical features including 3-point MACE of patients with NAFLD complicated with T2DM.

METHODS

Study design and participants

In this retrospective study, 6 Japanese patients with NAFLD and T2DM were treated for the long term with 100 mg canagliflozin/day at our hospital between November 2015 and March 2022, and the clinical and histopathological effects (e.g., steatosis, lobular inflammation, ballooning, fibrosis stage) of such long-term treatment were assessed at the four points of the pretreatment, and 24 weeks, 3 years, and 5 years after the start of treatment. The results of liver biopsies at pretreatment and 24 weeks were based on our previous prospective clinical trial UMIN000018166 (https://upload.umin.ac.jp/cgi-open-bin/ctr/index.cgi),[7,8] and the result of liver biopsy at 3 years was based on our previous retrospective study.[9] Patients with histopathological changes of steatosis in at least 5% of hepatocytes and those with history of alcohol intake of <20 g/day were included in the analysis. None of the selected patients had other underlying liver disease (e.g., autoimmune hepatitis, primary biliary cholangitis, drug-induced liver disease, viral hepatitis), systemic autoimmune diseases (e.g., rheumatoid arthritis, systemic lupus erythematosus), or metabolic diseases known to affect the liver (e.g., α-1-antitrypsin deficiency, Wilson disease, hemochromatosis).

The study protocol was approved by the Toranomon Hospital institutional review board (#953). The study was conducted in compliance with the International Conference on Harmonization Guideline for Good Clinical Practice (E6) and the 2013 Declaration of Helsinki. Written informed consent for liver biopsy was provided by all patients.

Liver histology

Liver specimens were obtained with a 16-gauge core tissue biopsy needle (Bard Peripheral Vascular Inc.), a 14-gauge modified Vim Silverman needle (Tohoku University style, Kakinuma Factory), or surgical resection. Specimen was fixed in 10% formalin, and the prepared sections were stained with hematoxylin and eosin, periodic acid–Schiff after diastase digestion, Masson trichrome, or silver impregnation. An adequate liver biopsy sample was defined as a specimen longer than containing more than 11 portal tracts and/or 1.5 cm.[4] Four pathologists (K.K., T.F., F.K., and T.F.), who were blinded to the clinical findings, evaluated each specimen, and the final assessment by consensus was reported.[4]

Steatosis scores of 0, 1, 2, and 3 corresponded to hepatocyte steatosis levels of <5%, ≥5 to <33%, ≥33 to <66%, and ≥66%, respectively. Lobular inflammation scores of 0, 1, 2, and 3 corresponded to no, <2, 2–4, and ≥4 foci per ×200 field, respectively. Hepatocyte ballooning scores of 0, 1, and 2 corresponded to none, few, and many cells, respectively. The sum of the steatosis, lobular inflammation, and hepatocyte ballooning scores (range 0–8 points) is termed the NAS.[12] Fibrosis stage was defined as 0, 1, 2, 3, or 4.[12,13]

Outcomes

The primary outcome measure included histopathological changes in the individual histological components of NASH from the pretreatment to 5 years after the start of treatment. Histopathological improvement was defined as a decrease in NAS of 1 or more points without worsening of the fibrosis stage relative to the pretreatment. No change was at the same point in NAS and fibrosis stage relative to the pretreatment. Worsening was an increase

TABLE 1 Histological findings at the time of liver biopsies

	Case 1				Pretreatment vs. 5 years[a]	Case 2	
Age at pretreatment (years)	64					44	
Sex	Male					Male	
Time of biopsy	Pretreatment	24 weeks	3 years	5 years		Pretreatment	24 weeks
Steatosis (%)	2 (50)	1 (30)	2 (40)	2 (40)		2 (40)	1 (20)
Lobular inflammation	2	2	1	2		2	2
Ballooning	1	1	1	1		1	1
Stage	1	1	1	1		2	2
NAS	5	4	4	5		5	4
Treatment efficacy[b]		Improvement	Improvement	No change			Improvement
	Case 4				**Pretreatment vs. 5 years[a]**	**Case 5**	
Age at pretreatment (years)	63					60	
Sex	Female					Male	
Time of biopsy	Pretreatment	24 weeks	3 years	5 years		Pretreatment	24 weeks
Steatosis (%)	3 (80)	1 (30)	1 (20)	1 (20)	↓	2 (60)	1 (30)
Lobular inflammation	2	2	2	2		2	1
Ballooning	2	1	1	2		1	1
Stage	4	3	3	3	↓	2	1
NAS	7	4	4	5	↓	5	3
Treatment efficacy[b]		Improvement	Improvement	Improvement			Improvement

Note: Results at pretreatment, 24 weeks, and 3 years are based on previous reports.[7–9]

[a]Factors that tended to decrease at 5 years, relative to the pretreatment, are indicated by black arrow.

[b]Improvement: a decrease in NAS of 1 point or more without worsening in fibrosis stage, relative to the pretreatment. No change: no change in NAS and fibrosis stage, relative to the pretreatment. Worsening: an increase in NAS and/or fibrosis stage of 1 point or more, relative to the pretreatment. The addition of drugs with high evidence level for NAFLD was also evaluated as worsening.

[c]Cases 3 and 6 were evaluated as worsening at 5 years, due to addition of GLP-1RA at 3 years after the start of SGLT2i.

in NAS and/or fibrosis stage of 1 point or more relative to the pretreatment. Furthermore, the addition of drugs with high evidence level for NAFLD (vitamin E, pioglitazone, and glucagon-like peptide 1 receptor agonist [GLP-1RA])[3] was also evaluated as histological worsening. The secondary outcomes included changes from the pretreatment to 5 years after the start of treatment in clinical parameters (including physical examination, laboratory data, glucose metabolism, imaging finding, and the incidence of 3-point MACE and malignancies).

Clinical parameters

The following normal range values of our hospital were used for the assessments: alanine aminotransferase (ALT), 8–42 U/L; aspartate aminotransferase (AST), 13–33 U/L (male); and 6–27 U/L (female). Fibrosis-4 index as a noninvasive test for the assessment of progression of liver fibrosis[3] was evaluated using the following formula: (age [year] × AST [U/L])/(platelet count [10^9/L] × √ALT [U/L]). T2DM was diagnosed in cases with a high fasting plasma glucose (FPG, ≥126 mg/dl), high glycated hemoglobin type A1c (HbA1C, ≥6.5%), use of glucose-lowering agents, or a self-reported history of a clinical diagnosis. Obesity was defined as a body mass index (BMI) of more than 25.0 kg/m^2. A normal waist circumference (WC) was defined as 85 cm for male and 90 cm for female.

Meal tolerance test

Each subject underwent the meal tolerance test (MTT), which was used to evaluate the amount of insulin secreted. The test involved the consumption of 500 kcal of a typical Japanese breakfast, followed by the measurement of the plasma glucose and C-peptide immunoreactivity (CPR) levels at baseline, 60 min, and 120 min, as described in detail previously.[14–16]

Insulin resistance and insulin secretion capacity

Insulin resistance was defined as homeostasis model assessment of insulin resistance (HOMA-IR) ≥2.5, and a low insulin secretion capacity was defined as HOMA-β < 30%.[17]

		Pretreatment vs. 5 years[a]	Case 3				Pretreatment vs. 5 years[a]
			60				
			Female				
3 years	5 years		Pretreatment	24 weeks	3 years	5 years	
1 (5)	1 (8)	↓	1 (30)	1 (5-10)	2 (50)	1 (5)	
2	1	↓	1	1	1	1	
1	1		1	0	1	1	
1	1	↓	1	1	1	1	
4	3	↓	3	2	4	3	
Improvement	Improvement			Improvement	Worsening	Worsening[c]	

		Pretreatment vs. 5 years[a]	Case 6				Pretreatment vs. 5 years[a]
			55				
			Male				
3 years	5 years		Pretreatment	24 weeks	3 years	5 years	
2 (40)	1 (30)	↓	3 (70)	2 (40)	2 (50)	2 (50)	↓
1	1	↓	1	1	1	2	
1	1		1	1	1	1	
2	2		3	3	3	3	
4	3	↓	5	4	4	5	
Improvement	Improvement			Improvement	Improvement	Worsening[c]	

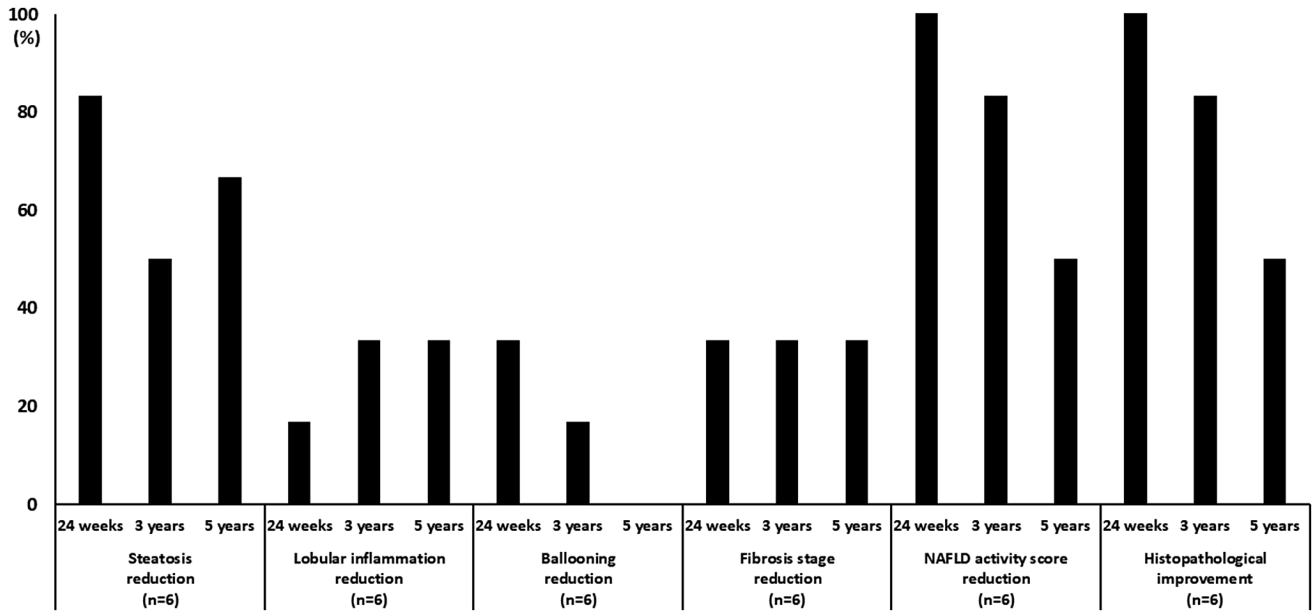

FIGURE 1 Changes from baseline to 24 weeks, 3 years, and 5 years after the start of treatment with sodium-glucose cotransporter 2 inhibitor (SGLT2i) in individual histopathological components of nonalcoholic fatty liver disease (NAFLD). Histopathological improvement was defined as a decrease in NALD activity score (NAS) of 1 point or more without worsening of the fibrosis stage, at 5 years after the start of treatment compared with baseline. The results of the first three liver biopsies have already been reported in our previous study.[7–9]

TABLE 2 Clinical parameters at the time of liver biopsies

	Case 1		Case 2		Case 3	
	Pretreatment	5 years	Pretreatment	5 years	Pretreatment	5 years
Physical examination						
Body mass index (kg/m^2)	23.5	23.0	25.3	29.3	27.9	27.2
Waist circumference (cm)	76.6	79.6	96.9	97.2	88.1	83.5
Skeletal muscle mass index (kg/m^2)	7.07	6.89	8.10	9.66	7.35	6.95
Combination drugs[b]	No	No	No	No	No	GLP-1RA
Laboratory data						
Serum aspartate aminotransferase (U/L)	22	27	10	9	19	14
Serum alanine aminotransferase (U/L)	24	29	17	8	23	11
Gamma-glutamyl transpeptidase (U/L)	42	49	42	22	23	17
Estimated glomerular filtration rate (ml/min/1.73m^3)	64.4	59.5	112.5	51.7	88.7	78.2
Fasting plasma glucose (mg/dl)	126	132	265	117	180	130
C-peptide (ng/ml)	2.30	2.34	2.10	1.36	3.47	2.03
HbA1C (%)	6.6	7.1	12.0	9.3	7.5	6.7
HOMA-IR	4.9	2.5	not done	1.7	3.2	1.4
HOMA-β (%)	65	44	not done	13	21	38
Total cholesterol (mg/dl)	168	175	217	242	188	148
Triglycerides (mg/dl)	232	231	153	228	191	171
High-density lipoprotein cholesterol (mg/dl)	40	44	37	39	45	54
Low-density lipoprotein cholesterol (mg/dl)	84	93	149	171	97	65
Uric acid (mg/dl)	6.7	5.9	4.4	6.2	5.9	4.9
Hyaluronic acid (μg/L)	16	21	17	17	42	34
Type IV collagen 7S (ng/ml)	4.4	4.8	4.4	4.7	3.8	4.4
Procollagen III peptide (U/ml)	0.54	0.60	<0.50	0.80	<0.50	0.60
Highly sensitive C-reactive protein (mg/dl)	0.081	0.063	0.026	0.239	0.062	0.041
Serum ferritin (μg/L)	233	194	259	82	202	50
Fibrosis-4 index	1.15	1.58	0.55	0.43	1.04	0.93
Genetic variations						
PNPLA3 rs738409	CG		CC		CC	
TM6SF2 rs58542926	CC		CC		CC	
HSD17B13 rs6834314	AA		AG		AG	
Imaging findings						
Liver stiffness measurement with transient elastography (kPa)	5.8	4.7	4.2	5.6	5.1	4.2
Liver fat with magnetic resonance spectroscopy (%)	12.1	10.4	13.6	7.4	8.9	5.5
Incidence of 3-point MACE	Absence	Absence	Absence	Absence	Absence	Absence
Incidence of malignancies	Absence	Absence	Absence	Absence	Absence	Colon cancer

Note: Results at pretreatment are based on previous reports.[7–9]

Abbreviations: HbA1C, hemoglobin type A1c; HOMA-IR, homeostasis model assessment of insulin resistance; *HSD17B13* (hydroxysteroid 17-beta dehydrogenase 13; MACE, major adverse cardiovascular event; *PNPLA3*, patatin-like phospholipase domain containing 3; *TM6SF2*, transmembrane 6 superfamily member 2.

[a]Wilcoxon test was used for comparison of paired samples of 6 patients.

[b]Combination drugs with high evidence level for NAFLD are shown, based on American Association for the Study of Liver Diseases (AASLD) practice guidance.[3]

Case 4		Case 5		Case 6		Pretreatment vs. 5 years
Pretreatment	5 years	Pretreatment	5 years	Pretreatment	5 years	p[a]
27.8	27.4	29.0	28.7	31.1	29.4	0.345
89.2	87.3	102.3	102.4	94.2	94.5	0.917
6.80	6.58	8.16	7.85	9.62	8.85	0.345
No	No	No	No	No	GLP-1RA	
39	39	32	30	53	48	0.336
63	51	50	44	83	78	0.058
36	29	46	29	42	43	0.207
64.5	66.2	102.9	67.3	62.8	51.4	0.046
114	115	134	126	133	117	0.116
4.30	3.81	3.79	3.66	2.18	3.11	0.416
7.9	6.8	7.3	6.7	6.5	6.8	0.066
9.4	3.9	5.0	2.8	6.8	4.1	0.043
186	82	90	97	84	88	0.686
210	153	144	132	157	131	0.173
105	88	256	173	219	160	0.249
37	38	57	46	35	39	0.344
145	102	52	61	72	67	0.752
5.5	5.4	6.4	6.5	6.9	5.7	0.666
102	106	18	101	13	12	0.500
4.9	5.3	21.2	25.1	4.1	3.3	0.197
0.70	0.70	1.10	1.40	1.00	0.80	0.345
0.075	0.030	0.020	0.012	0.050	0.040	0.225
602	286	1696	371	204	218	0.046
2.01	2.06	1.14	1.36	2.42	2.06	0.917
CG		CC		GG		
CC		CC		CC		
AG		AG		AA		
9.9	5.4	5.2	9.6	9.4	9.0	0.753
16.5	22.0	17.6	19.2	27.0	21.5	0.293
Absence	Absence	Absence	Absence	Absence	Absence	
Absence	Absence	Absence	Malignant lymphoma	Absence	Absence	

Determination of single-nucleotide polymorphism genotypes

The TaqMan single-nucleotide polymorphism genotyping assay (Applied Biosystems) was used for genotyping *PNPLA3* (patatin-like phospholipase domain containing 3) rs738409, *TM6SF2* (transmembrane 6 superfamily member 2) rs58542926, and *HSD17B13* (hydroxysteroid 17-beta dehydrogenase 13) rs6834314.

TABLE 3 Meal tolerance test at the liver biopsies of 4 patients without combination drugs[a]

	Case 1			Case 2		
	Pretreatment	5 years	Pretreatment vs. 5 years[b]	Pretreatment	5 years	Pretreatment vs. 5 years[b]
Plasma glucose (mg/dl)						
Baseline	126	132		265	117	↓
60 min	177	174	↓	370	130	↓
120 min	183	130	↓	349	112	↓
C-peptide (ng/ml)						
Baseline	2.30	2.34		2.10	1.36	↓
60 min	4.75	5.70		3.34	1.52	↓
120 min	7.26	4.69	↓	3.77	1.46	↓

Note: [a]Combination drugs with high evidence level for NAFLD are shown, based on AASLD practice guidance.[3]
[b]Factors that tended to decrease at 5 years, relative to pretreatment, are indicated by black arrow.

Body composition and the definition of sarcopenia

A bioelectrical impedance analysis performed with an Inbody770 multifrequency impedance body composition analyzer (Inbody Japan) to assess the body composition. The skeletal muscle mass index (SMI) represented the skeletal muscle mass of both arms and legs, expressed as (kg)/(height [m])^2. Based on the diagnostic criteria of sarcopenia by the Japan Society of Hepatology,[18] a low SMI was defined as <7.0 kg/m^2 for men and <5.7 kg/m^2 for women.

Image findings

Liver stiffness measurement with transient elastography was performed using the FibroScan-502 (Echosens) with the M-probe and XL-probe. Liver fat deposition was evaluated with magnetic resonance spectroscopy.

Statistical analysis

Wilcoxon test was used for comparison of paired samples. All *p*-values less than 0.05 by the two-tailed test

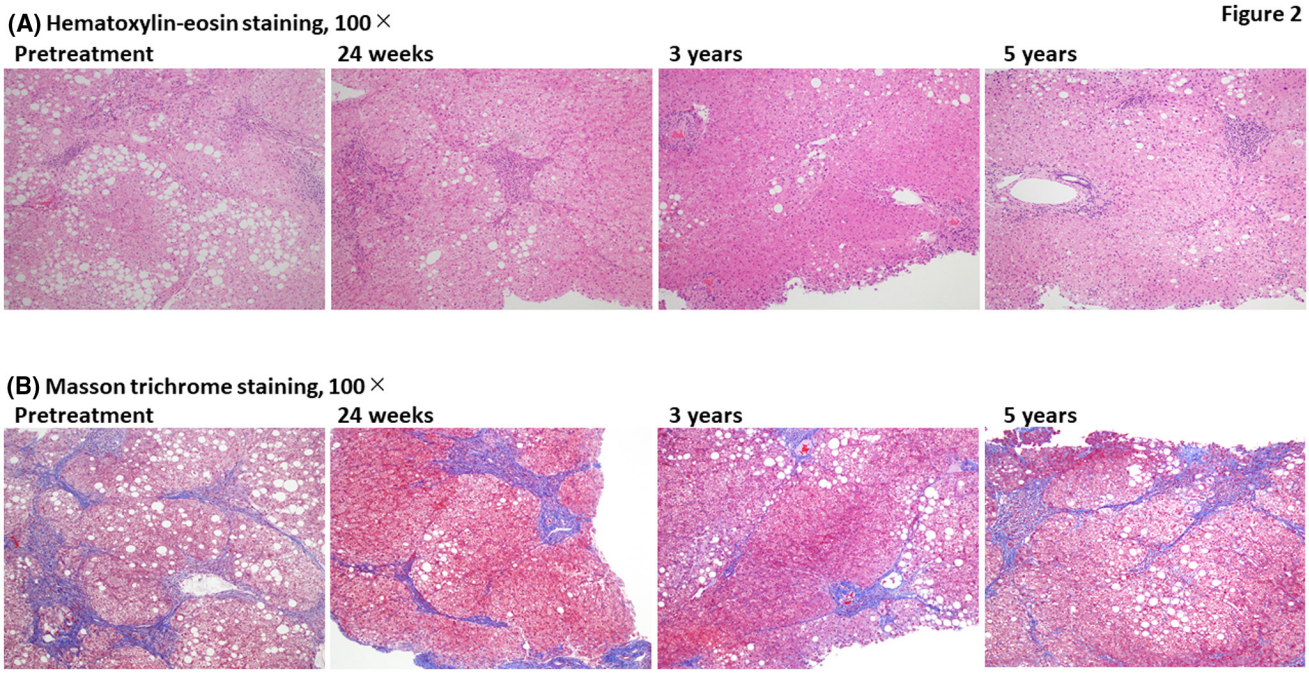

FIGURE 2 Representative pathological images of the improved case (Case 4) at 5 years. Histological findings at the four points of pretreatment, 24 weeks, 3 years, and 5 years after the start of SGLT2i. (A) Hematoxylin and eosin staining (×100). (B) Masson trichrome staining (×100).

Case 4			Case 5		
Pretreatment	5 years	Pretreatment vs. 5 years[b]	Pretreatment	5 years	Pretreatment vs. 5 years[b]
114	115		134	126	↓
210	211		209	214	
195	172	↓	203	224	
4.30	3.81	↓	3.79	3.66	↓
11.48	10.65	↓	8.31	8.45	
16.01	12.53	↓	11.27	12.44	

were considered significant. Statistical analyses were performed using the SPSS software (SPSS Inc.).

RESULTS

Histopathological changes

Table 1 and Figure 1 summarize the changes in histopathological scores at the time of the four liver biopsies. The results of the first three liver biopsies have already been reported in our previous study.[7–9] All 6 patients could continue SGLT2i until the time of fourth liver biopsy. Case 3 and Case 6 were performed with the addition of GLP-1RA on SGLT2i after the point of 3 years, and evaluated as histological worsening at 5 years.

As the primary outcome, histological improvement, no change, and worsening made up 50.0% (Cases 2, 4, and 5), 16.7% (Case 1), and 33.3% (Case 3, 6) of the 6 patients at 5 years, respectively (Figure 1). Overall, the scores of steatosis, lobular inflammation, ballooning, and fibrosis stage decreased at 5 years in 66.7% (Cases 2, 4, 5, and 6), 33.3% (Cases 2 and 5), 0%, and 33.3% (Cases 2 and 4) of the 6 patients (Figure 1). Representative pathological images of the improved case (Case 4) at 5 years is shown in Figure 2.

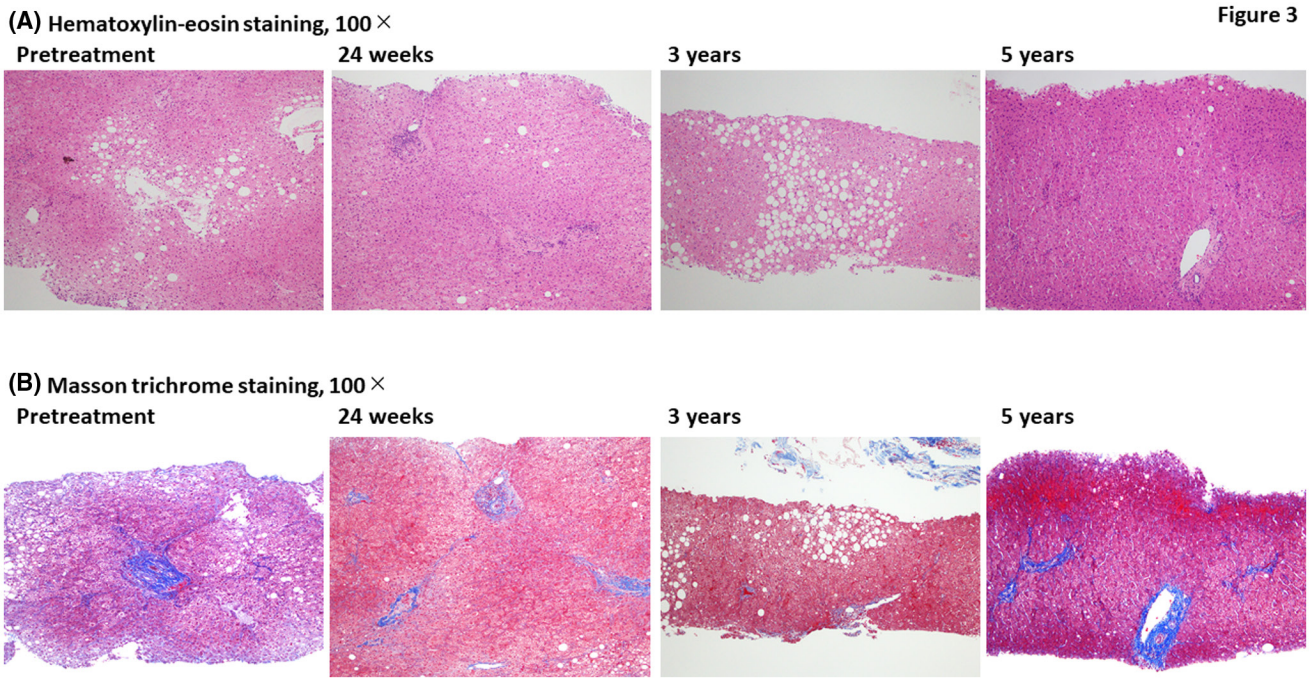

FIGURE 3 Pathological images of one decreased case of hepatocyte steatosis (Case 3), and the addition of glucagon-like peptide 1 receptor agonist (GLP-1RA) at 4 years after the start of SGLT2i. Histological findings at the four points of pretreatment, 24 weeks, 3 years, and 5 years after the start of SGLT2i. (A) Hematoxylin and eosin staining (×100×). (B) Masson trichrome staining (×100).

TABLE 4 Meal tolerance test at the liver biopsies of 2 patients who added GLP-1RA to SGLT2i after the point of 3 years

	Case 3			Case 6		
	3 years	5 years	3 years vs. 5 years[a]	3 years	5 years	3 years vs. 5 years[a]
Plasma glucose (mg/dl)						
Baseline	161	130	↓	125	117	↓
60 min	199	191	↓	262	220	↓
120 min	154	189		205	203	↓
C-peptide (ng/ml)						
Baseline	0.99	2.03		3.36	2.73	↓
60 min	3.33	3.25	↓	9.13	7.08	↓
120 min	3.50	3.98		12.03	8.57	↓

Note: Results at 3 years are based on previous reports.[9]

[a]Factors that tended to decrease at 5 years, relative to 3 years, are indicated by black arrow.

Changes in clinical parameters

Table 2 and Tables S1 and S2 summarize the changes in clinical parameters at the time of liver biopsies. The results of the first three liver biopsies have already been reported in our previous prospective study.[7–9] Five years of treatment with SGLT2i was associated with significant improvement in the laboratory data (HOMA-IR, $p = 0.043$; and serum ferritin, $p = 0.046$), compared with the pretreatment values.

Evaluation of glucose metabolism

Table 3 lists the results of MTT in 4 patients without combination drugs (Cases 1, 2, 4, and 5). At all three time points, 2 patients (Cases 2 and 4) indicated lower levels of CPR at 5 years compared with those at pretreatment, and this result suggests the improvement of insulin resistance. At two points, the other 2 patients indicated higher levels of CPR at 5 years compared with those at pretreatment (baseline and 60 min in Case 1, and 60 min and 120 min in Case 5), and this result partially suggests the improvement of insulin secretion function.

Impact of the addition of GLP-1RA on SGLT2I

Cases 3 and 6 were performed with the addition of GLP-1RA on SGLT2i after the point of 3 years.

In Case 3, levels of hepatocyte steatosis decreased from 50% (grade 2) at 3 years to 5% (grade 1) at 5 years, and accordingly, the addition of GLP-1RA at 4 years after the start of SGLT2i. The scores of lobular inflammation, ballooning, and fibrosis stage did not change at 5 years compared with 3 years (Table 1, Figure 3). Physical examination (BMI and WC) and glucose metabolism (FPG, HbA1C, and HOMA-β) showed improvement at 5 years compared with 3 years (Table S1). At two of three time points (baseline and 60 min) based on MTT, levels of plasma glucose at 5 years were lower than those at 3 years. Furthermore, at two of the three time points (baseline and 120 min), levels of CPR at 5 years were higher than those at 3 years. These results partially suggest the improvement of insulin secretion function (Table 4).

In Case 6, the scores of hepatocyte steatosis, ballooning, and fibrosis stage did not change at 5 years compared with 3 years, according the addition of GLP-1RA on SGLT2i since 2 months ago of the liver biopsy at 5 years (Table 1, Figure 4). Glucose metabolism (FPG, HbA1C, and HOMA-IR) showed improvement at 5 years compared with 3 years (Table S2). At all three time points (baseline, 60 min, and 120 min) based on MTT, levels of plasma glucose and CPR at 5 years were lower than those at 3 years, respectively, and these results suggested the improvement of insulin resistance (Table 4).

In conclusion, these patients did not show worsening of steatosis, ballooning, and fibrosis stage at 5 years compared with 3 years. Furthermore, glucose metabolism showed improvement.

DISCUSSION

This 5-year follow-up study with SGLT2i indicates the favorable histological and clinical impacts for NAFLD with T2DM. As the primary outcome, histological improvement was 50.0%. As the secondary outcomes, glucose metabolism improved significantly, and none developed 3-point MACE. This study reports the 5-year long-term impact of SGLT2i for NAFLD. Furthermore, the present results based on MTT indicate the long-term impact SGLT2i on glucose metabolism at 5 years in patients with NAFLD. However, we must pay attention

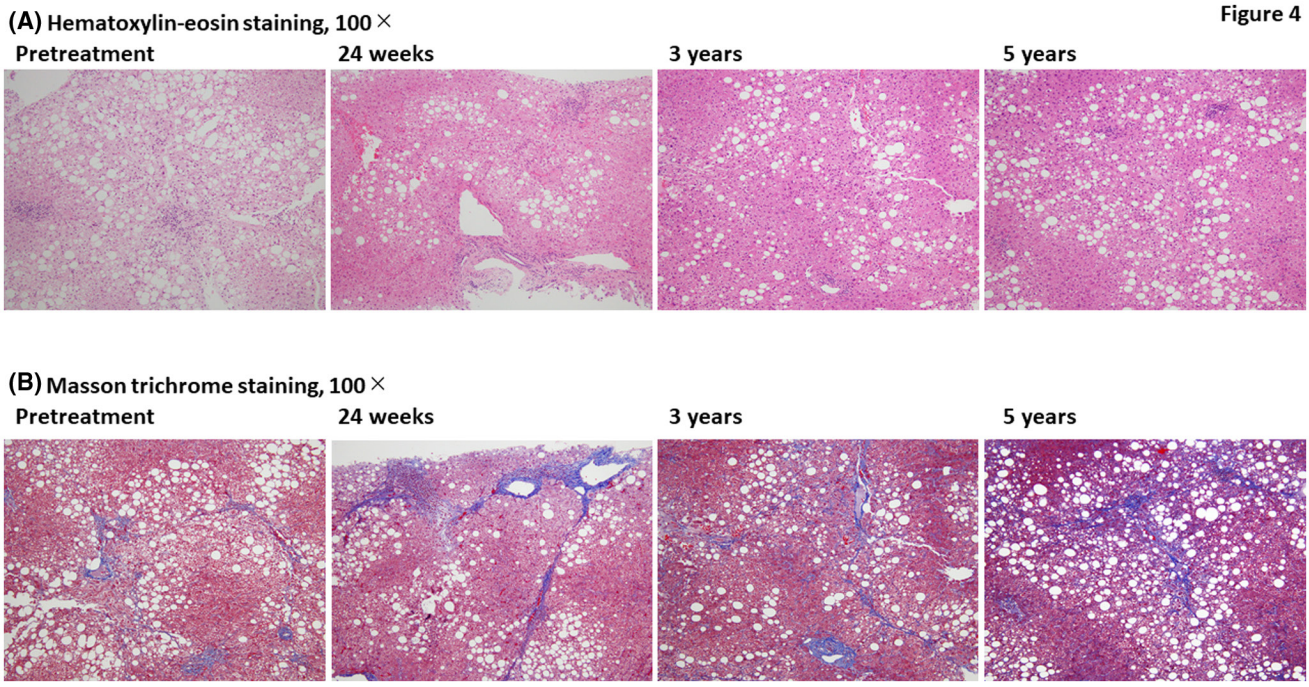

FIGURE 4 Pathological images of one case (Case 6) without worsening of hepatocyte steatosis, ballooning, and fibrosis stage, after the addition of GLP-1RA on SGLT2i since 2 months after the liver biopsy (at 5 years). Histological findings at the four points of pretreatment, 24 weeks, 3 years, and 5 years after the start of SGLT2i. (A) Hematoxylin and eosin staining (×100). (B) Masson trichrome staining (×100).

to the interpretation of these findings, because the study design was an uncontrolled, before–after study based on a small number of patients. Hence, further study based on a larger number of patients should be performed.

A previous report demonstrated that the stage of fibrosis in patients with NAFLD was one of the most important predictors for the incidence of the three complications (CVDs, malignancies except for liver cancer, and liver-related events).[3] However, realistically, improvement of fibrosis stage might not be necessarily suitable as the primary outcome of clinical trials, with the goal of development of novel drugs for NAFLD.[19] A recent report based on 1,398 Japanese patients with NAFLD showed that fibrosis stage was independently associated with liver-related events, but it was not associated with overall mortality after adjusting for confounders, such as histologic features of steatohepatitis. Especially, inflammation (steatosis, lobular inflammation, and ballooning) was more important than fibrosis stage in overall mortality.[20] Furthermore, a previous report based on 10,568 adults in Sweden with NAFLD confirmed by biopsy indicated that a significant excess mortality risk was found across all stages of fibrosis associated with NAFLD, and this increased risk was primarily due to deaths from malignancies except for liver cancer.[21] These results support that improvement of inflammation might be the more realistic primary outcome rather than that of fibrosis stage, in clinical trials aimed at improvement of overall survival in patients with NAFLD. Additionally, the previous reports with high evidence level support that SGLT2i can reduce the risk of 3-point MACE in patients with T2DM.[5,6] The present findings indicate that the reduction rates of inflammation (NAS) maintained at 50.0% at 5 years, even if those of fibrosis stage were not adequate (33.3%). Furthermore, none developed 3-point MACE. In the future, development of new treatment strategies centered on SGLT2i with the goal of suppressing 3-point MACE is expected in patients with NAFLD.

The present study suggests that the addition of GLP-1RA on SGLT2i might be one useful treatment option for histological improvement to refractory cases of SGLT2i. A recent report indicated that GLP-1RA resulted in a significantly higher percentage of patients with NASH resolution with no worsening of fibrosis than placebo, in NAFLD patients with or without T2DM.[22] Another previous report with high evidence level supports that GLP-1RA can reduce the risk of 3-point MACE in patients with T2DM.[23] This retrospective study has one limitation: Treatment durations of GLP-1RA were very short. Case 3 was evaluated for liver histology at 1 year after the start of GLP-1RA, and Case 6 was done at only 2 months. However, in Case 6, hepatocyte steatosis, ballooning, and fibrosis stage did not indicate any worsening, and physical examination and glucose metabolism showed improvement. Further large-scale, long-term, follow-up studies are needed to investigate the histological impact of the additional treatment regimens of GLP-1RA to refractory cases of SGLT2i.

In conclusion, this 5-year follow-up study with SGLT2i indicated a favorable histological impact for

NAFLD with T2DM. Further large-scale prospective studies are needed to determine the long-term impact for histological features and clinical outcome including 3-point MACE, and the usefulness adding GLP-1RA to refractory patients of SGLT2i.

AUTHOR CONTRIBUTIONS

All authors contributed to this work. *Data analysis*: N.A., Y.K., and S.F. *Manuscript draft*: N.A.

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CONFLICT OF INTEREST

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DISCLAIMERS

This paper has not been published or presented at national or international meetings, in part or in its entirety, and is not under consideration by another journal.

ETHICAL APPROVAL

The study protocol was approved by the Human Ethics Review Committee at Toranomon Hospital, and each patient provided a signed informed consent form at the time of liver histological diagnosis. The study was conducted in compliance with the International Conference on Harmonization Guidelines for Good Clinical Practice (E6) and the 2013 Declaration of Helsinki.

ORCID

Norio Akuta https://orcid.org/0000-0001-5386-9672
Tetsuya Hosaka https://orcid.org/0000-0003-0302-2275

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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