Keywords GERD · Insomnia · Nonalcoholic fatty liver disease · Proton-pump inhibitor

Abbreviations

BMI	Body mass index
GERD	Gastro-esophageal reflux disease
NAFLD	Nonalcoholic fatty liver disease
NASH	Nonalcoholic steatohepatitis

Introduction

Nonalcoholic fatty liver disease (NAFLD) [1] is the most common chronic liver disease in many developed countries and results in serious public health problems worldwide. NAFLD includes a wide spectrum of liver diseases, ranging from nonalcoholic fatty liver (NAFL), which is usually benign, to nonalcoholic steatohepatitis (NASH), which may progress to liver cirrhosis (LC), hepatic failure and hepatocellular carcinoma (HCC) in the absence of significant alcohol consumption [2]. A large proportion of NAFLD patients are asymptomatic, but some occasionally experience fatigue, anxiety, and/or insomnia, resulting in a significant decrement in quality of life (QOL) [3]. In middle-aged Koreans, short sleep duration and poor sleep quality were found to be significantly associated with an increased risk of NAFLD [4]. Similarly, short sleep duration was associated with NAFLD in the general Japanese population [5]. However, the mechanisms underlying the association between insomnia and NAFLD remain unknown. Sleep is important to maintain body homeostasis, with sleep problems associated with all-cause mortality [6].

In addition to being associated with sleep problems, NAFLD was found, in two recent studies from Japan and Italy, to be associated with a high prevalence of the symptoms of gastro-esophageal reflux disease (GERD) [7, 8]. Evidence has emerged suggesting a link between metabolic syndrome, specifically obesity and visceral fat accumulation, and the onset of GERD. Studies throughout the world have shown that GERD is associated with sleep problems [9–14]. For example, a population-based study from Sweden showed positive associations among the presence of insomnia, sleeplessness, problems falling asleep, and risk of GERD [10]. In addition, an analysis of 19864 healthy adults in Japan found that poor sleep quality and irregular dietary habits were strong risk factors for high scores on the frequency scale for the symptoms of GERD (FSSG) [15]. Thus, it can be hypothesized that GERD symptoms may be responsible for insomnia in patients with NAFLD. To our knowledge, no study to date has assessed the prevalence of insomnia or GERD, or their association, in patients with biopsy-proven NAFLD.

Rabeprazole (RPZ), a proton pump inhibitor (PPI), is a potent and irreversible inhibitor of the H(+)/K(+)-ATPase gastric pump and is indicated for the treatment of GERD, Zollinger–Ellison syndrome, and duodenal and gastric ulcers. Moreover, the combination of RPZ and antibiotics is indicated for the eradication of *Helicobacter pylori*. RPZ is therefore expected to be effective in the treatment of GERD patients with sleep disturbances [16, 17]. This study was designed to evaluate the prevalence of insomnia and GERD in patients with biopsy-proven NAFLD; to compare the rates of insomnia and GERD in patients with NASH and NAFL; to determine independent predictors of insomnia, including FSSG score, among these patients; and to evaluate the effect of RPZ on insomnia.

Methods

Study population

The study included a total of 123 patients with well-characterized, liver biopsy–confirmed NAFLD who completed the FSSG questionnaire assessing symptoms of GERD and the Athens Insomnia Scale (AIS) questionnaire. All patients underwent biopsies at one of the seven hepatology centers included in the Japan Study Group of NAFLD (JSG-NAFLD): Center for Digestive and Liver Diseases, Nara City Hospital; Division of Gastroenterology, Yokohama City University Graduate School of Medicine; Department of Medicine and Molecular Science, Graduate School of Biomedical Sciences, Hiroshima University; Department of Gastroenterology and Hepatology, Kochi Medical School; Department of Internal Medicine, Saga Medical School, Saga University; Department of Hepatology, Graduate School of Medicine, Osaka City University; and the Department of Gastroenterology and Hepatology, Kyoto Prefectural University of Medicine.

NAFLD was diagnosed based on liver biopsy findings of steatosis in $\geq$ 5 % of hepatocytes and the exclusion of other liver diseases, including viral hepatitis, autoimmune hepatitis, drug-induced liver disease, primary biliary cirrhosis, biliary obstruction, hemochromatosis, Wilson's disease, and α-1-antitrypsin-deficiency-associated liver disease. Patients consuming more than 20 g of alcohol per day, those with evidence of decompensated LC or HCC, those with psychiatric disorders or psychiatric drug users, and those taking PPIs and/or histamine H2-receptor antagonists were excluded. All patients provided written informed consent at the time of liver biopsy, and the study was conducted in conformance with the Declaration of Helsinki.

Laboratory and clinical parameters

Venous blood samples were taken in the morning after a 12-h overnight fast. Laboratory assays included blood cell counts and measurements of serum concentrations of aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyl transpeptidase (GGT), cholinesterase (ChE), total cholesterol, triglycerides, fasting plasma glucose (FPG), immunoreactive insulin (IRI), ferritin, hyaluronic acid, and type IV collagen 7S. These parameters were measured using the standard techniques of clinical chemistry laboratories.

Body mass index (BMI) was calculated as weight in kilograms/(height in meters)^2, with obesity defined as a BMI > 25 kg/m^2, according to the criteria of the Japan Society for the Study of Obesity [18]. Patients taking oral hypoglycemic medication, and those with a random glucose concentration > 200 mg/dl or a fasting glucose concentration > 126 mg/dl, were regarded as positive for hyperglycemia [19]. Patients with serum cholesterol concentrations > 220 mg/dl or triglyceride concentrations > 160 mg/dl were diagnosed with dyslipidemia. Patients taking antihypertensive agents and those having a resting recumbent blood pressure $\geq 140/90$ mmHg on at least two occasions were regarded as having hypertension [20].

GERD score

The FSSG is a questionnaire widely used to diagnose GERD [21–24] and to evaluate the effectiveness of any treatment [21, 25].The FSSG consisted of 12 questions assessing the frequency of symptoms (never, 0; occasionally, 1; sometimes, 2; often, 3; and always, 4). Patients with FSSG scores ≥ 8 were considered positive for GERD; at this cut-off point, the FSSG had a sensitivity of 62 %, a specificity of 59 %, and an accuracy of 60 % in assessing GERD [21].

Insomnia scale

The intensity of sleep difficulty was evaluated using the AIS, a self-administered psychometric tool with high consistency, reliability and external validity (Table 1) [26, 27]. The AIS consists of eight items, five of which are used to assess insomnia, and the three used to assess well-being, functional capacity, and sleepiness during the day. The full eight-item version (AIS-8) was developed for clinical settings, while the five-item version (AIS-5) can be used to assess sleep quantity and quality. These first five questions (AIS-5) are used to assess difficulty with sleep induction, awakenings during the night, early morning awakening, total sleep time and overall quality of sleep. The last three items in the AIS-8 refer to

Table 1 Athens Insomnia Scale (AIS) [26]

Sleep induction (time it takes you to fall asleep after turning-off the lights)			
0: No problem	1: Slightly delayed	2: Markedly delayed	3: Very delayed or did not sleep at all
Awakening during the night			
0: No problem	1: Minor problem	2: Considerable problem	3: Serious problem or did not sleep at all
Final awakening earlier than desired			
0: Not earlier	1: A little earlier	2: Markedly earlier	3: Much earlier or did not sleep at all
Total sleep duration			
0: Sufficient	1: Slightly insufficient	2: Markedly insufficient	3: Very insufficient or did not sleep at all
Overall quality of sleep (no matter how long you slept)			
0: Satisfactory	1: Slightly unsatisfactory	2: Markedly unsatisfactory	3: Very unsatisfactory or did not sleep at all
Sense of well-being during the day			
0: Normal	1: Slightly decreased	2: Markedly decreased	3: Very decreased
Functioning (physical and mental) during the day			
0: Normal	1: Slightly decreased	2: Markedly decreased	3: Very decreased
Sleepiness during the day			
0: None	1: Mild	2: Considerable	3: Intense

Instructions this scale is intended to record own assessment of any sleep difficulty you might have experienced. Please, check (by circling the appropriate number) the items above to indicate your estimate of any difficulty, provided that it occurred at least three times per week during the last month

The period of the self-assessment may vary, depending on the design of a given study. Whenever the self-assessment pertains to a period other than that of the last month, the second sentence of the instructions should be rephrased accordingly

daytime symptoms that often result from sleep disorders, such as narcolepsy and obstructive sleep apnea, in patients with insomnia. Each item on the AIS was rated from 0 (*no problem at all*) to 3 (*very serious problem*). Total scores can range from 0 to 24, with scores ≥ 6 and < 6 representing the presence and absence of insomnia, respectively. This cutoff point had a sensitivity of 93 %, a specificity of 85 % (90 % overall correct case identification), a positive predictive value (PPV) of 41 % and a negative predictive value (NPV) of 99 % [27].

Responders were asked to calculate their scores if they had experienced sleep difficulties at least three times a week during the previous month.

Liver histology

All enrolled patients underwent a percutaneous liver biopsy under ultrasonic guidance or peritoneoscopy. The liver specimens were embedded in paraffin and stained with hematoxylin and eosin, Masson-trichrome, reticulin silver stain, and Perls' Prussian blue. The specimens were evaluated by two hepatic pathologists (S.T. and Y.S.), who were blinded to the clinical findings. An adequate liver biopsy sample was defined as a specimen of length > 1.5 cm and/or having more than 6 portal tracts. NASH was defined as steatosis with lobular inflammation and ballooning degeneration, with or without Mallory–Denk body or fibrosis. Patients with liver biopsy specimens showing simple steatosis or steatosis with nonspecific inflammation were identified as the NAFL cohort [28]. Specimens with steatosis of < 5, 5–33, $>$ 33–66, and > 66 % were scored as having steatosis grades of 0, 1, 2, and 3, respectively [29]. Histological grade and stage were scored as described [30]. Necroinflammatory grades of 1, 2, and 3, were defined as mild, moderate and severe hepatocellular steatosis,

Table 2 Clinical characteristics of enrolled patients with NAFL and NASH

Clinical parameter	Total ($n = 123$ [100 %])	NAFL ($n = 40$ [33 %])	NASH ($n = 83$ [67 %])	P value
Age (years)	59 (14–82)	56 (20–78)	62 (14–82)	0.0025
Gender (female)	76 (62 %)	17 (43 %)	59 (71 %)	0.0030
BMI (kg/m^2)	26.6 (16.6–43.4)	26.6 (18.9–43.4)	27.3 (16.6–41.0)	0.1405
Obesity (BMI > 25)	87 (71 %)	27 (68 %)	60 (72 %)	0.6731
Dyslipidemia (yes [%])	46 (37 %)	12 (30 %)	34 (41 %)	0.3202
Hypertension (yes [%])	48 (39 %)	10 (25 %)	38 (46 %)	0.0310
Type 2 diabetes (yes [%])	55 (45 %)	13 (33 %)	42 (51 %)	0.0811
Hemoglobin (g/dl)	14.1 (10.5–18.3)	14.8 (10.6–18.3)	13.8 (10.5–16.7)	0.0728
Platelet count ($\times 10^4/\mu$l)	21.4 (4.6–78.5)	23.5 (13.0–78.5)	20.8 (4.6–45.4)	0.0125
AST (IU/l)	45 (17–186)	37 (17–151)	51 (18–186)	0.0001
ALT (IU/l)	69 (12–358)	61 (15–358)	71 (12–218)	0.2425
GGT (IU/l)	61 (20–391)	60 (20–319)	62 (21–391)	0.6382
Cholinesterase (IU/l)	371 (167–547)	378 (266–545)	370 (167–547)	0.2873
Total cholesterol (mg/dl)	209 (87–335)	218 (127–335)	203 (87–319)	0.0183
Triglyceride (mg/dl)	156 (61–659)	155 (61–416)	162 (66–659)	0.4849
HDL-C (mg/dl)	50 (23–290)	49 (31–77)	52 (23–290)	0.7727
Ferritin (ng/ml)	163 (5–1100)	113 (10–1100)	210 (5–923)	0.0160
FPG (mg/dl)	96 (60–452)	96 (60–161)	96 (60–452)	0.3571
IRI (μU/ml)	11.4 (1.59–49.5)	8.4 (1.6–46)	13.2 (2.8–49.5)	<0.0001
HOMA-IR	2.62 (0.38–33.04)	1.87 (0.38–13.63)	3.02 (0.65–33.04)	<0.0001
Hyaluronic acid (ng/ml)	37 (9–3480)	22 (9–149)	49 (9–3480)	0.0001
Type IV collagen 7S (ng/ml)	4.5 (2.7–13)	3.7 (2.8–7.1)	5.1 (2.7–13.0)	<0.0001
FSSG	4 (0–38)	4 (0–29)	3 (0–38)	0.5009
FSSG ≥ 8 (n [%])	31 [25 %]	10 [25 %]	21 [25 %]	1.0000
AIS	3 (0–15)	3 (0–12)	4 (0–15)	0.5591
AIS ≥ 6 (n [%])	34 [28 %]	10 [25 %]	24 [29 %]	0.8299

Results are presented as numbers with percentages in parenthesis for qualitative data or as mean ± SD for quantitative data

BMI body mass index, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *GGT* gamma glutamyl transpeptidase, *FPG* fasting plasma glucose, *IRI* immuno-reactive insulin

P values were calculated by t test or χ^2 analysis

ballooning and inflammation (acinar and portal), respectively. The severity of hepatic fibrosis (stage) was scored as: stage 1, zone 3 perisinusoidal fibrosis; stage 2, zone 3 perisinusoidal fibrosis with portal fibrosis; stage 3, zone 3 perisinusoidal fibrosis and portal fibrosis with bridging fibrosis; and stage 4, cirrhosis.

Treatment with RPZ

Thirteen NAFLD patients with GERD symptoms (11 females and 2 males) were administered 10 mg/day RPZ for 12 weeks. These patients completed both the FSSG and AIS before and after RPZ treatment.

Statistical analysis

Quantitative results are presented as medians and ranges, and qualitative results as numbers and percentages. Statistical differences in quantitative data were determined using the Mann–Whitney U test or Wilcoxon rank-sum test, and differences in qualitative data using Fisher's exact probability test or χ^2 analysis (Tables 2, 4, 5; Figs. 1, 2, 3, 4). Correlations were calculated by Spearman rank correlation analysis (Table 3). Multivariate logistic regression analysis was used to identify variables independently associated with the occurrence of insomnia (Table 6). Statistical significance was defined as a P value < 0.05.

Results

Characteristics of study subjects

Table 2 summarizes the clinical and laboratory data of the patient population. Of the 123 patients with NAFLD, 76 (62 %) were female, and 87 (71 %) were obese (BMI > 25 kg/m^2). Histologically, 83 patients (67 %) were diagnosed with NASH, and 40 (33 %) with NAFL. Patients with NASH were significantly older; were more predominantly female; were more likely to have hypertension and type 2 diabetes; had lower platelet counts and total cholesterol concentrations; and had higher levels of AST, ferritin, IRI, HOMA-IR, hyaluronic acid, and type IV collagen 7S. Of the 83 patients with NASH, 41 (49 %), 22 (27 %), 13 (16 %), and 7 (8 %) had stage 0–1, 2, 3, and 4 fibrosis, respectively.

Comparisons between NASH and NAFL

The distribution of AIS scores in patients with NAFL and NASH is shown in Fig. 1. Overall, 34 of the 123 patients (28 %) with NAFLD had AIS scores ≥ 6, diagnostic of insomnia, including 10 of 40 (25 %) patients with NAFL and 24 of 83 (29 %) with NASH ($P = 0.8299$). Males and females had similar median AIS scores [3 (range 0–13) vs. 3 (range 0–15), $P = 0.7954$] and a similar prevalence of insomnia [26 % (12/47] vs. 29 % (22/76), $P = 0.8359$].

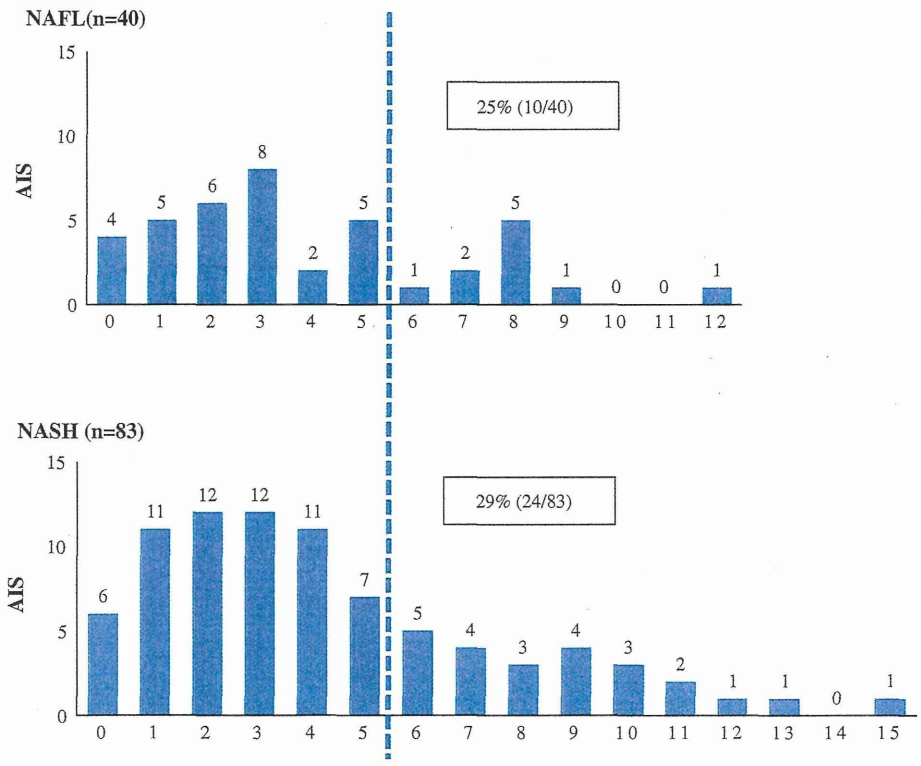

Fig. 1 The distribution of Athens Insomnia scale (AIS)

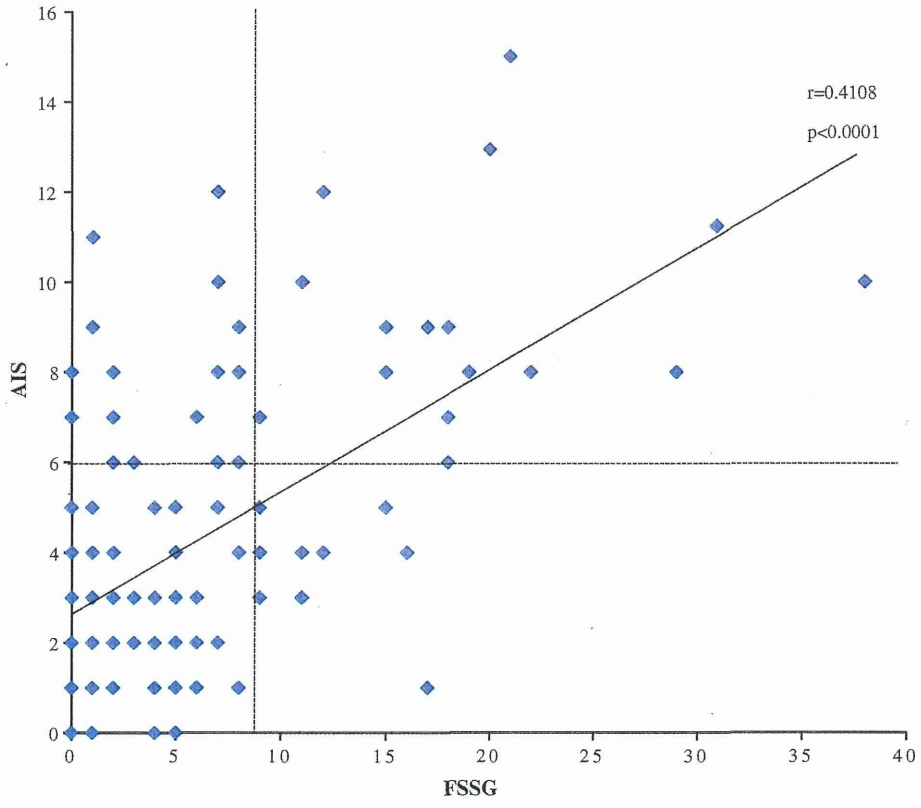

Fig. 2 Correlation between FSSG and AIS. A significant positive correlation was found between AIS and FSSG

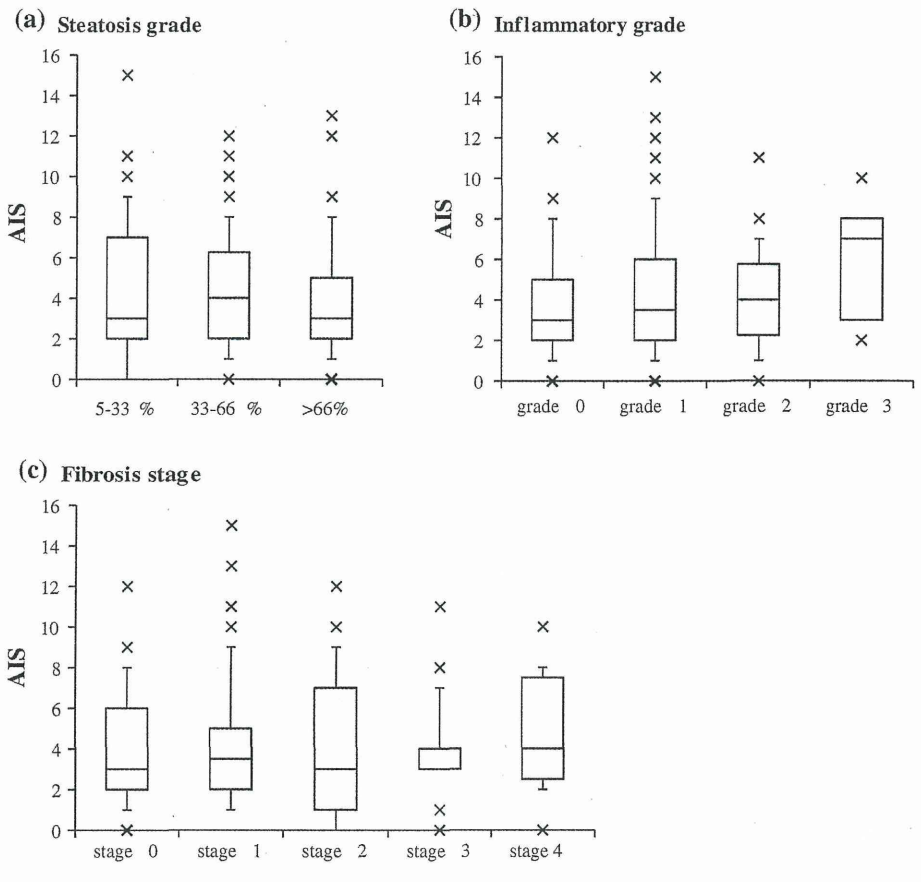

Fig. 3 Relationship between AIS and histological findings. The *box* represents the interquartile ranges (25 and 75 %) from the median (*horizontal line*). The *bars* indicate the 10 and 90 %

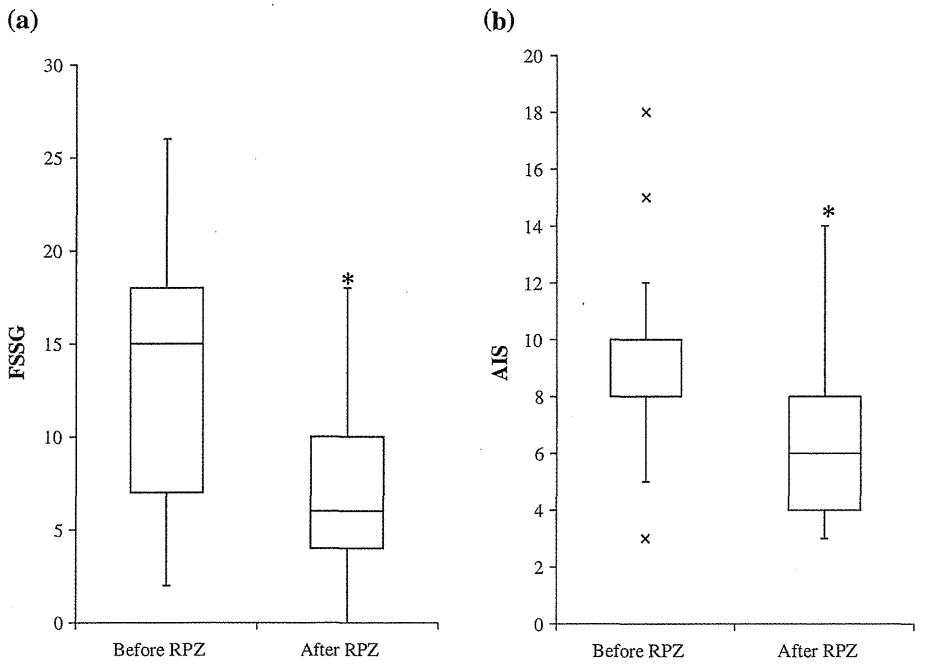

Fig. 4 Effects of rabeprazole (RPZ) on the improvement of GERD symptoms and insomnia. The *box* represents the interquartile ranges (25 and 75 %) from the median (*horizontal line*). The *bars* indicate the 10 and 90 %. **a** Change in the total FSSG score. RPZ significantly reduced total FSSG scores. $*P = 0.0071$ compared to baseline response before treatment. **b** Change in the total AIS. RPZ significantly reduced total AIS. $*P = 0.0144$ compared to baseline response before treatment

Table 3 Correlation between AIS/FSSG and clinical parameters in 123 patients with biopsy-proven NAFLD

Variables	AIS		FSSG	
	Correlation coefficient	*P* value	Correlation coefficient	*P* value
Age	−0.0431	0.6363	−0.2363	0.0085
BMI	−0.0075	0.9345	0.091	0.3128
Hemoglobin	0.0104	0.9100	0.0328	0.7220
Platelet	0.1079	0.2407	0.2197	0.0159
AST	0.0588	0.5197	0.0015	0.9868
ALT	0.0284	0.7558	0.1148	0.2080
AST/ALT ratio	−0.0046	0.9597	−0.1254	0.1689
γGT	0.1545	0.0935	−0.1272	0.1681
Cholinesterase	0.1366	0.1646	0.1683	0.0861
Prothrombin time	0.0359	0.6998	0.1403	0.1296
Cholesterol	0.1366	0.7233	0.0624	0.5039
Triglyceride	−0.0343	0.7132	0.0506	0.5879
HDL-C	−0.0128	0.8943	−0.1166	0.2230
FPG	0.0442	0.6316	−0.2010	0.0277
IRI	−0.1073	0.2412	−0.1570	0.0855
HOMA-IR	−0.0993	0.2806	−0.1948	0.0330
Ferritin	0.0934	0.3081	−0.0490	0.5932
Hyaluronic acid	−0.0340	0.7088	−0.2246	0.0125
Type IV collagen 7S	−0.0193	0.8323	−0.1277	0.1592
FSSG	0.4108	<0.0001	–	–

P values are based on Spearman's non-parametric correlation analysis

FSSG scored did not differ in NAFL and NASH patients, with the prevalence of GERD being 25 % in each group.

Factors positively correlating with AIS and FSSG scores

AIS score was positively correlated with FSSG score ($r = 0.4108$, $P < 0.001$) (Fig. 2), but not with any other parameter (Table 3). FSSG score was positively correlated with platelet count ($r = 0.2197$, $P = 0.0159$), and negatively correlated with age ($r = -0.2363$, $P = 0.0085$), FPG concentration ($r = -0.2010$, $P = 0.0277$), HOMA-IR score ($r = -0.1948$, $P = 0.0330$) and hyaluronic acid concentration ($r = -0.2246$, $P = 0.0125$). BMI, transaminase activities, lipid profiles, and iron parameters were not correlated with AIS or FSSG score.

Correlation between histological findings and AIS scores

Assessment of histological findings in the 123 patients with NAFLD showed that 53 (43 %), 40 (33 %), and 30 (24 %) had steatosis grades 1, 2, and 3, respectively; 30 (24 %), 70 (57 %), 18 (15 %), and 5 (4 %) had inflammation grades 0, 1, 2, and 3, respectively; and 44 (36 %), 38 (31 %), 21 (17 %), 13 (11 %), and 7 (6 %) had fibrosis grades 0, 1, 2, 3, and 4, respectively. Evaluation of correlations between AIS scores and histological findings showed that AIS score was not correlated with steatosis, inflammation, or fibrosis grade (Fig. 3).

Clinical findings in patients with and without insomnia

Comparisons of clinical and laboratory findings in patients with and without insomnia showed that γGT concentrations and FSSG scores were higher, and the prevalence of hypertension and IRI and HOMA-IR scores were lower, in patients with insomnia (Table 4). Moreover, GERD symptoms were significantly more prevalent in patients with than without insomnia (56 vs. 13 %, $P < 0.0001$).

Drug usage

Drug usage in patients involved in this study was shown in Table 5. Beta-blockers users were more prevalent in patients with GERD compared to those without. The prevalence of other drug users was not different between patients with GERD/insomnia and those without.

Table 4 The comparison between insomniacs and non-insomniacs

Clinical parameter	Insomniacs ($n = 34$ [28 %])	Non-insomniacs ($n = 89$ [72 %])	P value
Age (years)	56 (35–74)	60 (14–82)	0.8100
Gender (female)	22 (65 %)	54 (61 %)	0.8359
BMI (kg/m^2)	26.4 (21.9–38.6)	26.6 (16.6–43.4)	0.5028
Obesity (BMI > 25)	25 (74 %)	62 (70 %)	0.8252
Dyslipidemia	21 (62 %)	56 (63 %)	1.0000
Hypertension (yes)	8 (24 %)	40 (45 %)	0.0385
Type 2 diabetes (yes)	16 (47 %)	39 (44 %)	0.8400
Hemoglobin (g/dl)	14.4 (11.0–18.3)	14.0 (10.5–17.1)	0.9072
Platelet count (× 10^4/μl)	22.3 (8.7–33.5)	21.1 (4.6–78.5)	0.7137
AST (IU/l)	47 (20–182)	44 (17–186)	0.4682
ALT (IU/l)	71 (12–358)	69 (15–218)	0.6233
GGT (IU/l)	77 (24–391)	59 (20–268)	0.0063
Cholinesterase (IU/l)	373 (208–547)	371 (167–545)	0.7992
Total cholesterol (mg/dl)	206 (125–314)	214 (87–335)	0.3670
Triglyceride (mg/dl)	146 (68–424)	164 (61–659)	0.3864
Ferritin (ng/ml)	128 (11–1100)	170 (5–923)	0.7315
FPG (mg/dl)	94 (70–452)	97 (60–171)	0.7708
IRI (μU/ml)	9.8 (2.8–11.2)	12.0 (1.6–49.5)	0.0326
HOMA-IR	2.10 (0.65–33.04)	2.96 (0.38–16.34)	0.0335
Hyaluronic acid (ng/ml)	32 (9–392)	37 (9–3480)	0.9774
Type IV collagen 7S (ng/ml)	4.7 (2.7–13.0)	4.4 (2.8–10.0)	0.9054
FSSG	8 (0–38)	3 (0–17)	<0.0001
FSSG ≥ 8 (n [%])	19 [56 %]	12 [13 %]	<0.0001
NASH (n [%])	24 [71 %]	59 [66 %]	0.8299

Results are presented as numbers with percentages in parenthesis for qualitative data or as mean ± SD for quantitative data

BMI body mass index, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *GGT* gamma glutamyl transpeptidase, *FPG* fasting plasma glucose, *IRI* immuno-reactive insulin

P values were calculated by t test or χ^2 analysis

Table 5 Drug usage

Drug usage	FSSG < 8 ($n = 92$)	FSSG ≥ 8 ($n = 31$)	*P* value	Insomniacs ($n = 34$)	Non-insomniacs ($n = 89$)	*P* value
Use of antihypertensive drugs						
Calcium antagonists	26 [28 %]	10 [32 %]	0.6560	7 [21 %]	29 [33 %]	0.2680
ARBs	4 [4 %]	2 [6 %]	0.6412	2 [6 %]	4 [4 %]	0.6679
Beta-blockers	2 [2 %]	4 [13 %]	0.0348	2 [6 %]	4 [4 %]	0.6679
Use of NSAIDs	10 [11 %]	2 [6 %]	0.7285	1 [3 %]	11 [12 %]	0.1762
Use of anticoagulants	2 [2 %]	0 [0 %]	1.0000	0 [0 %]	2 [2 %]	1.0000
Use of digestive drugs	2 [2 %]	0 [0 %]	1.0000	0 [0 %]	2 [2 %]	1.0000

ARBs angiotensin receptor blockers, *NSAIDs* non-steroidal anti-inflammatory drugs

Table 6 Results of multivariate analysis: independent predictors of insomniacs

Variables	Adjusted (multivariate)		
	OR	95 % CI	*P* value
FSSG	1.2315	1.1221–1.3516	<0.0001
GGT	1.0109	1.0031–1.0189	0.0063
IRI	0.9515	0.8908–1.0164	0.1396
Hypertension	0.5503	0.1823–1.6610	0.2893

OR odds ratio, *CI* confidence interval, *FSSG* frequency scale for the symptoms of GERD, *GGT* gamma glutamyl transpeptidase, *IRI* immuno-reactive insulin

Factors independently predictive of insomnia in patients with NAFLD

Multivariate logistic regression analysis showed that plasma GGT concentration [odds ratio (OR) 1.2315, 95 % confidence interval (CI) 1.1221–1.3516, $P < 0.0001$] and FSSG score (OR 1.0109, 95 % CI 1.0031–1.0189, $P = 0.0063$) were significant independent predictors of insomnia (Table 6). In contrast, IRI and HOMA-IR scores and hypertension were not predictive.

Effects of RPZ on insomnia

RPZ treatment of patients with GERD significantly reduced FSSG and AIS scores (Fig. 4). Of the 9 patients with insomnia treated with RPZ, 4 (44 %) showed resolution of insomnia after treatment.

Discussion

This study of Japanese NAFLD patients demonstrated that (1) 28 % had AIS scores ≥ 6, indicative of insomnia; (2) 25 % had FSSG scores ≥ 8, indicative of GERD; (3) AIS and FSSG scores did not differ significantly in patients with NASH and NAFL; (4) FSSG score was independently associated with AIS score; and (5) insomnia could be relieved after treatment with RPZ.

The precise prevalence of insomnia in NAFLD patients has been unclear. Using the AIS, we found that 28 % of patients with biopsy-proven NAFLD had insomnia. Although sleep dysfunction has been defined as a Pittsburgh Sleep Quality Index (PSQI) score > 5.5 in other studies [17, 31], the AIS is satisfactorily validated, simple to perform, and well accepted based on ICD-10 criteria. The AIS has been used to assess insomnia in the general population in Japan. For example, a study of approximately 3000 individuals found that 21.4 % had experienced insomnia during the previous month [32]. The prevalence of insomnia in our NAFLD patients does not seem to be markedly different from that in the general population. Though this precise reason is unknown, one plausible explanation is that about 20–30 % of the general population is estimated to have NAFLD. We should obtain data from sex- and age-matched non-NAFLD population to clarify whether the prevalence of insomnia in NAFLD is really higher compared to that in the general population. Assessments of employees of two local governments in Japan found that 1382 of 5951 males (23.2 %) and 465 of 1500 females (31.0 %), aged 34–59 years, had insomnia [33, 34]. Assessments of middle-aged women found that 27.5–43.6 % had AIS scores ≥ 6 [35, 36]. Taken together, these findings indicate that the prevalence of insomnia is higher in women than in men, across countries and cultures. In contrast, we observed no differences in AIS scores between men and women with NAFLD. Since the discrepancy between our results and previous studies can be explained by a small number of patients involved in this study, a larger number of patients should be examined in the future. Since Yoshioka et al. [34] showed that the gender difference disappeared after adjustment for paid work and family responsibilities, detailed characteristics of patients should be considered to clarify the gender differences. In Japanese studies in which insomnia was

diagnosed using AIS, factors associated with insomnia included work at visual display terminals for ≥ 6 h per day [37], job stress [38, 39], and reduced illumination in the workplace [40]. In this study, however, we did not evaluate these work environmental factors.

The mechanisms by which insomnia arises in patients with NAFLD have never been clarified. We found that AIS scores and the incidence of insomnia were similar in patients with NAFL and NASH. AIS scores did not correlate with any histological findings, such as steatosis, inflammation, and fibrosis scores, indicating that histological severity is not important in the pathogenesis of insomnia in patients with NAFLD. In contrast, many studies have explored the associations between life-style related disorders/obesity and sleep disturbance. Changes in secretion of the hormones cortisol, leptin, and ghrelin, and increased insulin resistance due to short sleep duration were found to increase the risks of obesity and diabetes [41–43]. In contrast, insomniacs were more likely to have insulin resistance than non-insomniacs. This study demonstrated that FSSG score was significantly correlated only with AIS score. Multivariate analysis showed that FSSG was an independent risk factor associated with insomnia, suggesting that GERD symptoms are responsible for insomnia in NAFLD patients, findings consistent with previously reported results [9, 17]. For example, sleep disorders, such as inability to sleep, difficult falling asleep, and awakening during the night, were observed in 56.3 % of patients with heartburn [9]. Similarly, we found that 61.3 % (19/31) of NAFLD patients with GERD had insomnia. A study in 134 Japanese patients with GERD found that FSSG score was significantly positively correlated with PSQI score [11]. GERD can affect sleep through two primary mechanisms. First, nighttime reflux, which occurs in 47–79 % of patients with GERD, can cause awakening during the night. Second, GERD can cause short, amnestic arousals (approximately 30 s), resulting in sleep fragmentation. However, recent studies also suggested that the link between GERD and sleep problems may be bidirectional. Sleep stage may influence the esophago-upper esophageal sphincter contractile reflex [44]. Sleep disturbance may reinforce the perception of intra-esophageal acid [45]. The association of NAFLD with GERD has been assessed in only two studies, which reported that 37 and 51 % of patients with NAFLD had GERD symptoms [7, 8], percentages higher than observed in the present study. Plausible explanations of a lower prevalence (25 %) of GERD in our NAFLD patients were the difference of ethics, sex/age distribution, and the diagnostic method of GERD or NAFLD between previous studies [7, 8] and ours. Another explanation is the possibility that our NAFLD patients receiving dietary educations might avoid irregular diet habits, which are known to be the most significant risk factors for GERD symptoms [15]. In the future, sex- and age-matched controlled studies using a larger population is essential to draw conclusions. A recent study of Japanese patients with NAFLD found that GERD symptoms were significantly more severe in the group with higher than lower total cholesterol (T-CHO) and triglyceride (TG) levels [7]. In contrast, we observed no correlation between GERD symptoms and either T-CHO or TG. These conflicting results may be due to our inclusion of only patients with biopsy-proven NAFLD, who are not representative of the general population of patients diagnosed with NAFLD. In contrast, the patients included in the previous study were diagnosed with NAFLD by ultrasound [7]. Moreover, that study did not assess the association of insomnia with GERD symptoms. Thus, to our knowledge, our study is the first to clarify the relationship between insomnia and GERD symptoms in patients with NAFLD.

Previous studies suggested that acid suppression can improve sleep problems in GERD patients. For example, a prospective randomized clinical trial found that a significantly higher percentage of patients treated with esomeprazole than placebo showed resolution of GERD-related sleep disturbances [46]. RPZ treatment also significantly improved subjective indices of sleep quality over placebo [16]. Moreover, an 8-week course of RPZ treatment significantly decreased both FSSG and PSQI scores in Japanese patients [11]. Consistent with these findings, we found that treatment with RPZ significantly decreased both AIS and FSSG scores. These results also indicate that GERD symptoms are at least partly responsible for the occurrence of insomnia in NAFLD patients.

In addition to AIS score, GGT concentration was found to be an independent predictor of insomnia in patients with NAFLD. Serum GGT activity is a marker of oxidative stress [47]. The primary function of GGT is to maintain intracellular concentrations of glutathione, a critical anti-oxidant molecule. Thus, increased GGT activity can be regarded as a response to oxidative stress, aimed at increasing the intracellular concentration of glutathione. Although little is known about the relationship between insomnia and oxidative stress, a preliminary study showed that anti-oxidant activity was significantly lower and lipid peroxidation levels significantly higher in patients with primary insomnia than in controls [48]. Serum concentrations of GGT and 8-hydroxydeoxyguanosine are correlated in patients with NAFLD, indicating the occurrence of oxidative stress [49]. GGT has also been associated with the occurrence of metabolic syndrome, early atherosclerosis, and cardiovascular events [50]. Moreover, GGT concentrations are higher in patients with obstructive sleep

apnea syndrome (OSAS) than in controls [51] and have been associated with nocturnal arterial oxygen desaturation. Furthermore, continuous positive airway pressure treatment has been shown to decrease GGT, further suggesting that the increase in GGT is directly associated with OSAS. OSAS has also been associated with the presence and severity of NAFLD [52], suggesting that insomnia in patients with NAFLD is at least partly associated with the occurrence of OSAS. Our finding, that elevated GGT is an independent predictor of insomnia, suggests that these patients also have OSAS, although this was not directly evaluated in our patient population.

This study had several limitations. First, its design was cross-sectional, making it difficult to establish a cause–effect relationship, and suggesting the need for prospective studies. Second, the AIS is a self-administered questionnaire and subjective measure of insomnia, suggesting the need for assessment of objective sleep variables such as those obtained during polysomnography. Third, GERD was diagnosed based on FSSG scores alone, without endoscopic examination or 24 h pH monitoring. Because all participants were Japanese, there is a possibility that our results may not be applicable for NAFLD patients of other races or ethnic groups. The current study also did not assess the effects of smoking habits, mental status, dietary habit, and work environments. Additional studies, in larger populations, are needed to assess these variables.

In conclusion, we found that about 30 % of Japanese patients with biopsy-proven NAFLD have insomnia. GERD symptoms may be important in the development of insomnia. PPIs may be clinically useful for treating insomnia in NAFLD patients.

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