Table 3 Demographic and baseline characteristics of patients in the groups 1 and 2

	Group 1 ($n = 6$)	Group 2 ($n = 6$)	P
Age (years)	72.5 (66–76)	67.5 (56–77)	0.26†
Sex			0.50*
Male	4 (66.7%)	5 (83.3%)	
Female	2 (33.3%)	1 (16.7%)	
Bodyweight (kg)	65.4 (49.9–73.3)	70.5 (56.4–78.5)	0.26†
Liver disease			0.56*
Hepatitis C	4 (66.7%)	3 (50%)	
Alcohol	2 (33.3%)	3 (50%)	
Child–Pugh			0.56*
B	3 (50%)	2 (33.3%)	
C	3 (50%)	4 (66.7%)	
Serum albumin (g/dL)	2.8 (2.3–3.6)	2.65 (2.2–3.7)	0.81†
Total bilirubin (mg/dL)	2.2 (1.0–4.5)	1.1 (0.8–2.2)	0.06†
PT (%)	59.8 (43.9–83.4)	49.1(13.8–71.6)	0.26†
Platelet (10^4 counts/μL)	5.45 (2.30–12.4)	11.5 (6.6–19.8)	0.08†
Serum creatinine (mg/dL)	1.31 (1.00–1.55)	1.15 (0.80–1.80)	0.63†
Hepatocellular carcinoma**	4/6 (66.7%)	3/6 (50%)	0.56*
Esophageal varix**	5/6 (83.3%)	5/6 (83.3%)	1.00*
Splenomegaly	5/6 (83.3%)	5/6 (83.3%)	1.00*

Data presented as median (range) or n (%) patients.
†Mann–Whitney U-test.
*χ^2-Test.
**Present or past complications.
PT, prothrombin time.

portal hypertensive state. Hence, in case 2, high portal hypertension might have been the cause of tolvaptan non-efficacy. Case 3 developed SBP, which induces endotoxemia and worsens splanchnic vasodilatation. Patients with SBP frequently develop a rapidly progressive impairment of systemic hemodynamics, leading to severe renal and hepatic failure and aggravation of portal hypertension.[12] SBP-related portal hypertension could have been the reason for tolvaptan non-efficacy in this patient. Accordingly, we suggest that ascites or pleural effusion should be evaluated when tolvaptan is not effective to reduce fluid accumulation, because chylous or inflammatory findings may help predict tolvaptan efficacy.

During the intermediate-term administration of tolvaptan, the differences in median weight between days 0 and 7 and days 0 and 42 were statistically significant, but not between days 7 and 42 ($P = 0.345$). This result suggests that the change in weight during the introduction phase is maintained by the intermediate-term administration of tolvaptan, without sodium imbalance and renal dysfunction. Another advantage of intermediate-term tolvaptan administration seemed to be the possibility of its use as an alternative therapy for CART. In one of our cases, the frequency of CART was decreased from monthly to once in 4 months after initiating tolvaptan therapy.

One of the disadvantages of intermediate-term administration was thought to be development of hepatic coma. In this study, two cases experienced hepatic coma and needed hospitalization because of loss of consciousness. Both these cases were Child–Pugh classification C. In comparison with the median total bilirubin (1.5 mg/dL) of the 15 enrolled patients (Table 1), the total bilirubin values of these two cases were high (4.5 and 2.8 mg/dL, respectively). It has been thought that use of tolvaptan in the setting of serious liver damage would induce hepatic coma. *In vitro* studies indicated that CYP3A4 alone was responsible for tolvaptan metabolism, and Shoaf *et al.* described the change in tolvaptan pharmacokinetics and pharmacodynamics following inhibition or induction of CYP3A4.[13] Therefore, presence of serious liver damage in these cases might have hindered tolvaptan metabolism, prolonging its effects, including the aquaretic effect.

We used tolvaptan according to the manufacturer's medical information and the results of the dose-finding trial.[10] A daily dose of 7.5 mg was approved in the treatment of patients with cirrhosis, and a higher dose of 15 mg was recommended in patients with congestive heart failure. Among the patients with increments of tolvaptan dose, only one patient without congestive heart failure received 11.25 mg/day of tolvaptan, after a medical consultation regarding resistant massive ascites.

According to our study, 40% of patients initiating tolvaptan did not require additional treatments for ascites for a median follow-up period of 6 weeks. No significant demographic or clinical characteristics of patients were found to account for the difference between groups 1 and 2. Further studies are essential to elucidate such factors. To distinguish responders and non-responders, urine osmolality may be an important factor. Imamura *et al.* showed a more than a 26% decrease in urine osmolality from a baseline of more than 352 mOsm/L for the first 4–6 h predicts responders of congestive heart failure to tolvaptan.[14] Moreover, vasopressin-sensitive water channel, aquaporin 2 (AQP-2), whose urinary excretion corresponded to osmotic alteration and severity of cirrhosis, was supposed to be another predictor for the responsiveness of tolvaptan for liver cirrhosis.[15,16] However, we did not measure the urine osmolality or urinary AQP-2 in this study. Further evaluations are necessary to understand the relevance of such factors with respect to the responsiveness of tolvaptan.

This study could not be used to evaluate the effects of long-term administration of tolvaptan regarding requirements for additional treatments, because in group 1, one patient quit taking tolvaptan, three needed additional therapy and two developed hepatic coma during 2 months of follow up. The long-term administration of tolvaptan without use of additional treatments is thought to be less feasible. It remains to be demonstrated that it is possible to take tolvaptan long term and undergo concurrent additional treatments, including CART or i.v. albumin infusion.

In conclusion, the addition of tolvaptan to conventional diuretics contributed to the bodyweight reduction of cirrhosis patients with fluid retention during the introduction phase (the first week of therapy). The findings of paracentesis or thoracentesis may predict the efficacy of tolvaptan. A total of 40% of patients initiating tolvaptan did not require additional treatments for ascites during a median follow-up period of 6 weeks. The intermediate-term administration of tolvaptan can play an important role to maintain reduced bodyweight without renal dysfunction or sodium imbalance. However, especially for patients with severe liver damage, the possibility of serious adverse events, including hepatic coma, should be kept in mind.

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Randomized Controlled Trial Comparing the Efficacy of Impedance Control and Temperature Control of Radiofrequency Interstitial Thermal Ablation for Treating Small Hepatocellular Carcinoma

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Key Words
Radiofrequency ablation · Hepatocellular carcinoma · Impedance control · Temperature control · Randomized controlled trial

Abstract
Objectives: A randomized controlled trial was conducted to evaluate the efficacy of impedance control of a radiofrequency interstitial thermal ablation system (RITA) used to treat hepatocellular carcinoma (HCC). ***Methods:*** Fifteen patients with hypervascular HCCs <20 mm in diameter were randomly treated with radiofrequency ablation (RFA) using conventional temperature control (group A) or impedance control methods (group B). RITA needle electrodes were used in all cases. We compared ablation time, extent of lesion ablation, and energy use between the two groups. ***Results:*** The median long and short diameters of the axial cross sections of radiofrequency-induced necrotic areas visualized by CT were 32 mm (range, 26–36) and 25 mm (20–31) in group A and 32 mm (28–40) and 31 mm (24–37) in group B, respectively. The short diameter of group B patients was significantly greater than that of group A patients ($p = 0.029$). The median ablation time was 18.8 min in group A and 13.4 min in group B, thus significantly shorter in group B ($p = 0.001$). The energy requirement did not differ significantly between the groups. ***Conclusions:*** Impedance control of the RITA system resulted in an increased size of the ablation zone and a decreased ablation time.

Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignant diseases worldwide [1]. In Japan, more than 30,000 patients die of HCC each year, and HCC ranks third and fifth among men and women, respectively, as the cause of death from malignant neoplasms [2]. Therefore, improved curative strategies are needed. Surgical resection is one effective treatment option, but some patients are not candidates for hepatectomy because of underlying liver disease, large tumor size, inaccessible tumor location, or cancer multifocality. Therefore, other less invasive (but potentially equally effective) treatments are under investigation. Percutaneous

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ethanol injection (PEI) is effective in this regard and is preferred to surgery in many patients [3, 4]. However, several treatment sessions may be required to achieve complete tumor necrosis [5, 6].

Radiofrequency ablation (RFA) is another therapeutic option that requires fewer treatment sessions than PEI because the extent of necrosis created in a single RFA session is greater than that afforded by PEI [5, 6]. The most appropriate indication for RFA is an HCC <30 mm in diameter [5–7], and RFA systems are designed to ablate such HCCs. However, if radical therapeutic effects are required, it is often difficult to define a 'safety margin' (an area of coagulation outside the tumor, analogous to a surgical margin) when the tumor diameter exceeds 30 mm. Thus, attempts have been made to expand the use of RFA to the treatment of larger HCCs by increasing the diameter of the expandable probe [8], by using internally cooled electrodes [9], by injecting saline to reduce tissue impedance [10], by the concomitant use of transcatheter arterial embolization [11], and by combining RFA with PEI [12, 13].

If RFA is to be effective, the radiofrequency (RF) electrode and generator must be very carefully designed. Four RFA devices have been used to date in Japan: a RF tumor coagulation system (RTC system; Boston Scientific, Natick, Mass., USA), a cool-tip RF system (Valleylab, a division of Tyco Healthcare Group, Boulder, Colo., USA), a multipolar RF system (CelonLabPOWER system; Celon AG Olympus, Berlin, Germany), and an RF interstitial thermal ablation system (RITA; AngioDynamics, Latham, N.Y., USA). The maximum RF power available, the methods of monitoring impedance and/or temperature during RFA, and the procedural end points differ among the four devices. Thus, device effectiveness in terms of complete tumor necrosis, inhibition of local tumor progression, and duration of required ablation may also differ. Of the above RFA instruments, the first three devices employ impedance control methods, and only RITA features temperature control. An impedance control method can be used with RITA. We compared the effectiveness of RITA therapy delivered using impedance and temperature control methods.

Patients and Methods

Patients and HCC Diagnosis

From October 2011 to February 2013, 15 consecutive patients diagnosed with HCC at our hospital were enrolled in the present study. Patients had to meet the following criteria.

(1) The nodular HCC diameter did not exceed 20 mm; (2) the HCC was evident only in the liver (i.e., no metastatic lesion was noted); (3) the HCC had not regressed when other treatments had been given; (4) the patient was able to care for himself or herself; (5) the liver was able to tolerate the planned treatment; (6) general organ function was good, fulfilling particular criteria (WBC $\geq$1,500/mm^3, platelets $\geq 5 \times 10^4$/mm^3, Hg $\geq$9.0 g/dl, Cr $\leq$1.5 mg/dl, BUN $\leq$30 mg/dl, and PT $\geq$50% of the normal values); (7) age >20 years, and (8) an estimated survival duration of over 3 months and an ability of the patient to remain in hospital if necessary. Gender was irrelevant. The exclusion criteria were: (1) another active cancer; (2) a prior history of severe allergy; (3) pregnancy; (4) a prior history of heart disease; (5) any severe renal problem; (6) any severe complication; (7) a fitted pacemaker; (8) a metal implant, and (9) any other cogent reason.

Patients were required to have been definitively diagnosed with HCC using CT or MRI. No included patient was excluded during the study period. The 15 patients treated with RFA included 8 men and 7 women, with a median age of 65.5 years (range, 60–85). Thirteen patients were of Child-Pugh class A and the other 2 of Child-Pugh class B. The median tumor diameter was 16 mm (range, 11–20). Table 1 summarizes the clinical data on all patients.

Written informed consent was obtained from all patients, and our randomized controlled trial was approved by our institutional ethics committee, which oversaw our work throughout the entire study.

Study Design and RFA Protocol

We used an RF model 1500a RF interstitial thermal ablation system (RITA; AngioDynamics). RITA features an expandable needle with an insulated outer cannula housing 7–9 curved electrodes of different lengths which can be deployed from the tip of the trocar.

Patients were randomly divided into two groups. Patients in group A were treated using RITA featuring conventional temperature control. The needle shaft was inserted into the tumor with the electrode array retracted using real-time ultrasonic guidance, and the electrode array was then deployed from the tip to a depth of 2 cm into the tumor. The maximum power delivered was 90 W until the temperature attained 105°C, which was maintained for 7 min. Subsequently, the array was further advanced 1 cm, and the above procedure was repeated. Patients in group B were treated with RFA using an impedance control method. After satisfactory deployment of the array to 2 cm into the tumor, 10 W of power was initially applied, and this power level was increased twice, at 1-min intervals, in 10-watt increments. This pulsed series of treatments was repeated until power roll-off was evident. This was a precipitous drop in output caused by a marked increase in tissue impedance in turn triggered by the development of coagulative necrosis. If power roll-off did not occur within 15 min, treatment was terminated. The power delivered and tissue impedance were continuously monitored. Next, the electrode array was advanced by 1 cm and the above procedure was repeated.

Image Analysis

Contrast-enhanced CT or MRI was performed before RITA and 1–6 days thereafter. Dynamic CT scans were obtained using nonionic contrast material unless a patient was allergic to iodine; such patients were examined by MRI. Dynamic CT was performed in the arterial phase (30-second delay after starting the injection), the hepatic portal phase (60 s), and the hepatic venous phase (120 s). The slice thickness was 5 mm. Contrast-enhanced MRI (EOB-

Table 1. Data on group A and B patients

	Group A	Group B	p
Demographic and background information			
Men:women	5:3	3:4	n.s.
Age, years	71.5 (62–82)	64 (60–85)	n.s.
HBV:HCV:NBNC status	1:7:0	1:5:1	n.s.
Child-Pugh score (A:B)	6:2	7:0	n.s.
Laboratory data			
Albumin, g/dl	3.7 (2.3–4.3)	3.7 (3.3–4.1)	n.s.
Bilirubin, mg/dl	0.7 (0.6–2.2)	0.9 (0.5–1.2)	n.s.
AST, IU/l	33 (23–127)	42 (19–91)	n.s.
ALT, IU/l	30.5 (16–61)	38 (14–107)	n.s.
Platelets (×1,000/mm^3)	13.05 (61–179)	12.1 (42–151)	n.s.
PT, % of normal	79.5 (58.7–101.7)	78 (71.9–98.9)	n.s.
AFP, ng/ml	18.55 (7.7–79.6)	11.9 (3.4–830)	n.s.
DCP, AU/l	12.5 (8–25)	25 (14–54)	0.013
Tumor			
Tumor diameter, mm	13.5 (11–19)	16 (11–21)	n.s.
Location (superficial:deep)	4:4	3:4	n.s.

Values are presented as medians (ranges) unless otherwise indicated. HBV = Hepatitis B virus; HCV = hepatitis C virus; NBNC = nonviral hepatitis; ALT = alanine transaminase; AFP = alpha-fetoprotein; DCP = des-gamma carboxyprothrombin; n.s. = not significant.

MRI) was performed after intravenous injection of the contrast material Gd-EOB-DTPA. Dynamic MRI was performed in the arterial phase (30-second delay), the hepatic portal phase (60-second delay), the hepatic venous phase (120- and 180-second delays), and the hepatocyte-specific phase (>20-min delay). The slice thickness was 5 mm in the first three phases and 3 mm in the last phase.

Statistical Analysis

The ablation durations, the energy used, and the diameters of ablated lesions were compared using the Mann-Whitney U test. All values were expressed as medians. A p value <0.05 upon two-tailed testing was considered to be significant.

Table 2. Ablation times, lesion dimensions, and energy delivered

	Group A	Group B	p
Ablation time, min	18.8 (17.5–20.5)	13.4 (9.7–14.9)	0.001
Energy delivered, J	36 (26–52)	30.6 (15–39)	n.s.
Diameter, mm			
Long	32 (26–36)	32 (28–40)	n.s.
Short	25 (20–31)	31 (24–37)	0.038

Values are medians (ranges). n.s. = Not significant.

Results

Sizes and Shapes of Ablated Areas

The HCCs of all patients were completely ablated as revealed by dynamic CT or MRI performed 1–6 days after treatment. Table 3 shows the sizes and shapes of ablated areas. The median long diameters of axial cross-sections of RF-ablated areas measured by CT were 32 mm (range, 26–36) in group A and 32 mm (28–40) in group B. These values did not differ significantly between the two groups. The median short diameters were 25 mm (20–31) in group A and 31 mm (24–37) in group B. The short diameter of Group B patients was significantly greater than that of Group A patients (p = 0.029). Regarding post-RF lesion shape, 5 of the 7 lesions in group B were spherical, as were 3 of 8 in group A. This between-group difference was not statistically significant. Almost all patients were adequately ablated. Figure 1 shows representative data from both groups. The lesion dimensions were 36 × 28 mm (fig. 1a; temperature control) and 34 × 37 mm (fig. 1b; impedance control). The ablated areas of patients in group B were thus larger and more spherical in shape than those of patients in group A patients.

Times and Energies Required for Coagulation

Table 2 shows the treatment times and energies required for coagulation. The total ablation time was 18.8 min (17.5–20.5) in group A and 13.4 min (9.7–14.9) in

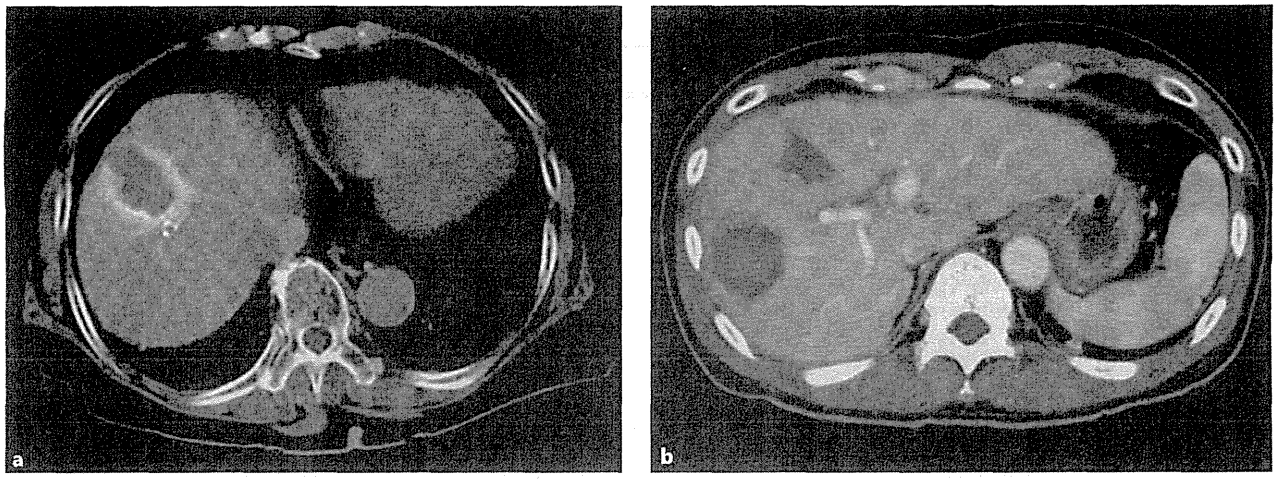

Fig. 1. Enhanced CT images of lesions created by RFA using a temperature control (**a**) and an impedance control method (**b**).

Table 3. Adverse effects

	Group A (n = 8)		Group B (n = 7)	
	all grades	grades 3 and 4	all grades	grades 3 and 4
Fever	4 (50)	0 (0)	4 (57.1)	0 (0)
Abdominal pain	8 (100)	0 (0)	8 (100)	0 (0)
Loss of appetite	2 (25)	0 (0)	2 (28.5)	0 (0)
Increased bilirubin	6 (75)	0 (0)	6 (85.7)	0 (0)
Increased AST	8 (100)	3 (37.5)	8 (100)	5(71.4)
Increased ALT	8 (100)	0 (0)	8 (100)	0 (0)

Values denote numbers of subjects with percentages in parentheses. Treatment-related toxicity was assessed using the National Cancer Institute Common Terminology Criteria 4.0. n.s. = Not significant.

group B; thus, the ablation time of group B was significantly shorter than that of group A ($p = 0.001$). The total energy requirement during each session of ablation was 36,075 J (26,700–52,425) in group A and 30,600 J (15,000–39,000) in group B. This difference did not attain statistical significance. In addition, no association was evident between energy requirement and the extent of RF-induced coagulation in either group, but the ablated volume per unit of energy was significantly higher in group B than in group A.

Complications

No severe complications were noted. Fever, abdominal pain, anorexia, and elevated serum transaminase levels were observed in most patients after RFA (table 3). An increased aspartate aminotransferase (AST) level (grades 3–4) was observed in 3 patients in group A (37.5%) and in 5 patients in group B (71.4%); the conditions resolved within 1 week. No significant difference in the prevalence of adverse effects was observed between the two groups. We did not encounter skin burn, intrahepatic abscess, intraperitoneal bleeding, or renal failure.

Overall Utility of Our Procedure

We planned to enroll 30 patients, but upon interim analysis of data from 15 patients, it was clear that the extent of the ablated area, the ablation time, and the ablated volume per unit of input energy were all significantly bet-

ter in group B than group A patients. Therefore, the trial was terminated at that point and all subsequent patients have been treated using the group B protocol.

Discussion

RFA has been widely used to percutaneously treat both primary and secondary hepatic malignancies, but RFA is known to be most effective for lesions <3 cm in diameter. Much work has been devoted to the enlargement of the necrotic area. Blood flow around the tumor must be considered as any curative effect diminishes when an HCC is adjacent to a large vessel. This phenomenon is termed 'heat sink effect' [14, 15]. As might be expected, the effects of RFA are enhanced by vascular occlusion [16–19]. Kobayashi et al. [20] found that balloon-induced hepatic artery occlusion during RFA increased lesion ablation, and tumor recurrence tended to decrease. De Baere et al. [21] showed that temporary percutaneous hepatic vein or portal branch occlusion enhanced the efficacy of RFA. It is known that effective ablation procedures must be planned in minute detail. The RTC system uses expandable needles and takes a creative approach toward improving RFA efficacy. Hirakawa et al. [22] developed a new ablation procedure featuring stepwise hook extension from a Super-Slim needle. RFA outcomes may be enhanced by increased energy deposition and pulsed energy delivery. Goldberg et al. [23] showed that pulsing of RF current increased the extent of coagulative necrosis. Solazzo et al. [24] showed that large RFA volumes may be attained using optimized pulsing algorithms. Current and power delivery rose and the surface area of the electrode could be increased.

In the present study, we compared the effectiveness of RITA therapy delivered using an impedance control and a temperature control method. RFA creates heat in the vicinity of an electrode via ion oscillation triggered by the electric field produced by high-frequency alternating current, in turn causing coagulative necrosis of the adjacent tissue. When an impedance control method is used, power settings are gradually increased to minimize tissue desiccation and charring and to prevent a rapid rise in impedance. When the tissue temperature rises, blood vessels delivering heat energy to nearby regions become carbonized and impedance rapidly increases, inhibiting further ablation. When temperature control is used, the program monitors the temperature at the tips of electrodes and delivers peak power until a preselected target temperature is exceeded. Liver tissue carbonizes and becomes desiccated when the temperature climbs over 100°C. Thus, temperature control methods seek to hold liver tissue at a temperature at or over 105°C (the standard setting is 105°C).

We found that use of an impedance control method was associated with a shorter ablation time and a lower energy requirement, yet afforded a larger coagulation area, than when temperature control was employed. Several possible explanations may be advanced. First, an average electrode tip temperature of 105°C may not be adequate to ablate liver tissue. The use of an impedance control method is associated with attaining temperatures of over 105°C in the last phase of ablation. Thus, ablation in Group A patients required more time and energy than that in Group B patients. The use of only four electrodes is not adequate to ablate tissue if it is difficult to raise the temperature of that tissue. If the tissues to be ablated were located in proximity to large vessels, fat deposits, and tumor capsules, all of which are known to adversely affect ablation efficiency, not all electrode tips may have attained 105°C when a temperature control method was used, although the average tip temperature was indeed 105°C. Thus, ablation may have been ineffective in part. On the other hand, the impedance control method monitors impedance in all regions of ablation. All electrodes ablate tissue uniformly, and the shape of a coagulated region is thus spherical, in contrast to what was noted in Group A patients.

No severe complication was noted in patients treated using either control method. Thus, our new procedure is safe. The transient elevation in AST levels in some Group B patients may be explained by the fact that the extent of coagulation was greater in Group B than in Group A patients.

Although we studied only a relatively small number of patients, we found that the extent of coagulation was enhanced upon use of an impedance control method, particularly in terms of extension of the short axis of the lesion. In other words, coagulated regions were more spherical in Group B than Group A patients. As most hepatic tumors, including HCC, are spherical in shape, it is important that ablated areas should also be spherical to obtain adequate safety margins and to ensure radical therapeutic effects.

In conclusion, we found that the use of an impedance control method reduced ablation time and energy and achieved more coagulation compared to the use of a temperature control method. However, both methods afforded complete tumor necrosis. Long-term follow-up studies are necessary to confirm the efficacy of our method and the effect on prognosis.

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INTERNAL MEDICINE

□ ORIGINAL ARTICLE □

Significance of Oral Glucose Tolerance Tests in Non-alcoholic Fatty Liver Disease Patients with a Fasting Plasma Glucose Level of <126 mg/dL and HbA1c Level of ≤6.4% in Japan

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Abstract

Objective The aim of this study was to clarify the indications for oral glucose tolerance tests (OGTT) in non-alcoholic fatty liver disease (NAFLD) subjects with a HbA1c level of ≤6.4%, fasting plasma glucose (FPG) level of <126 mg/dL and no history of diabetes.

Patients A total of 569 NAFLD subjects underwent 75-g OGTT. The plasma glucose and insulin levels were analyzed periodically for three hours during the OGTT examinations. Impaired fasting glucose (IFG) was defined as a plasma glucose level of ≥100 mg/dL to <126 mg/dL. Diabetes was defined as a two-hour post-load plasma glucose level of ≥200 mg/dL. Elevated insulin resistance was defined as a homeostasis model assessment-insulin resistance (HOMA-IR) of ≥2.5. Insulin secretory insufficiency was defined as an insulinogenic index of <0.4.

Results The prevalence of diabetes on the OGTT was 7.7% (44/569) among the NAFLD patients with an HbA1c level of ≤6.4%, FPG level of <126 mg/dL and no history of diabetes. A multivariate analysis showed that diabetes occurred more frequently when the subjects had IFG [odds ratio (OR) 5.13; 95% confidential interval (CI) 3.01-8.76; p<0.001] and an HbA1c level of 5.7-6.4% (OR 5.45; 95% CI 3.33-8.93; p<0.001). Of the NAFLD subjects with both IFG and an HbA1c level of 5.7-6.4%, 22.8% (28/123) exhibited a pattern of diabetes on OGTT. Regarding insulin dynamics, among the NAFLD subjects with both IFG and an HbA1c level of 5.7-6.4%, 25.2% (31/123) had elevated IR alone, 25.2% (31/123) had insulin secretory deficiency alone and 27.6% (34/123) had both elevated insulin resistance and insulin secretory deficiency.

Conclusion NAFLD subjects with IFG and an HbA1c level of 5.7-6.4% should undergo OGTT in order to determine whether they have diabetes and/or abnormal insulin dynamics.

Key words: non-alcoholic fatty liver disease, oral glucose tolerance test, type 2 diabetes mellitus

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Introduction

Non-alcoholic fatty liver disease (NAFLD) is one of the more common causes of chronic liver disease in the Western world and many Asian nations (1-6). NAFLD is considered to be the liver component of metabolic syndrome (7-9) and is associated with obesity, dyslipidemia, pituitary dysfunction, hypertension, sleep apnea, chronic kidney disease and type 2 diabetes (T2DM) (10-16). In addition, the presence of NAFLD is associated with a high risk of developing cardiovascular disease and stroke (17, 18). Hence, NAFLD is

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emerging as a new significant health problem in many countries. A substantial problem is that NAFLD is strongly associated with T2DM. The coexistence of NAFLD and T2DM is clinically important for the following reasons: 1) T2DM is an independent predictor of hepatic fibrosis progression in patients with NAFLD (19) and 2) the presence of T2DM is pivotal with respect to increased risks of cardiovascular disorders and the development of hepatocellular carcinoma in the setting of NAFLD (20, 21). Therefore, early intervention to prevent or improve T2DM is required in order to obtain a good prognosis in NAFLD patients. The physicians in charge of NAFLD patients should thus detect T2DM in the early stage. According to population-based studies conducted in Asia, only 37% of diabetic patients fulfill both the fasting and two-hour plasma glucose criteria (22). Although oral glucose tolerance tests (OGTT) are adequate for making a strict diagnosis of T2DM, they are not routinely applied in NAFLD subjects due to the high cost and inconvenience.

Hence, it is an urgent issue to determine the indications for OGTT in NAFLD subjects. This topic must be addressed in studies with large numbers of NAFLD patients in whom the glucose and insulin levels are examined after oral glucose loading.

Against this background, we evaluated the prevalence of impaired glucose tolerance (IGT) and diabetes based on the findings of OGTT in Japanese subjects with NAFLD. In addition, we assessed the level of insulin secretion and degree of insulin resistance in the study participants. The strengths of the current study are the large sample size and comparison with control subjects exhibiting normal aminotransferase levels without fatty liver.

Materials and Methods

Subjects

A total of 823 Japanese subjects diagnosed with fatty liver on ultrasonography (23) and examined for oral glucose tolerance using 75-g glucose loading between January 1997 and December 2007 at the Department of Hepatology and Toranomon Hospital Health Management Center were enrolled in this study. Of the 823 subjects, 569 satisfied the following inclusion criteria: 1) a HbA1c national Glycohemoglobin Standardization Program (NGSP) equivalent value (%) level of ≤6.4% and fasting plasma glucose (FPG) level of <126 mg/dL; 2) no history of diabetes; 3) a current and past daily alcohol intake of <20 g/day; 4) negativity for hepatitis B surface antigens (HBsAg), anti-hepatitis C virus (HCV), antinuclear antibodies or antimitochondrial antibodies in the serum, as determined on a radioimmunoassay, enzyme-linked immunosorbent assay or indirect immunofluorescence assay; 4) no underlying neoplasms or systemic disease, such as systemic lupus erythematosus or rheumatic arthritis; 5) the absence of malignancy on gastrofiberscopy, abdominal ultrasonography, chest X-ray and/or chest computed tomography; 6) levels of the tumor markers carcinoembryonic antigen, alpha-fetoprotein and prostate-specific antigen with the normal range. Subjects meeting the above criteria were enrolled regardless of whether their serum level of aminotransferase was within the normal range. Subjects meeting any of the following criteria were excluded from the study: secondary causes of steatohepatitis or drug-induced liver disease, alcoholic liver disease, viral hepatitis, autoimmune hepatitis, primary biliary cirrhosis, hemochromatosis, Wilson's disease and biliary obstruction.

All subjects underwent 75-g OGTT examinations after a 12-hour fast. The plasma glucose and insulin levels were analyzed before and 30, 60, 90, 120 and 180 minutes after oral glucose loading. Blood samples were obtained from all subjects six times during the OGTT after oral glucose loading. IGT was defined as a two-hour post-load plasma glucose level of 140-199 mg/dL. Diabetes was defined as a two-hour post-load plasma glucose level of 200 mg/dL (24). Impaired fasting glucose (IFG) was defined as a plasma glucose level of ≥100 mg/dL to <126 mg/dL. The index of insulin resistance was calculated for fasting glucose and insulin according to the homeostasis model for insulin resistance (HOMA-IR). Elevated insulin resistance (IR) was defined as a HOMA-IR of ≥2.5 (25). Insulin secretion was calculated according to the insulinogenic index (IGI), as follows: IGI = (Ins30-Ins0) / (Glc30-Glc0), Ins0: fasting plasma insulin (mU/L); Ins30: insulin 30 minutes after glucose intake (IU/mL); Glc0: FPG (mg/dL); and Glc30: plasma glucose 30 minutes after glucose intake (mg/dL). Insulin secretory insufficiency was defined as an IGI of <0.4 (26). The pattern of insulin dynamics was divided into the following four types based on the differences in HOMA-IR and IGI: 1) normal insulin dynamics, IGI≥0.4 and HOMA-IR<2.5; 2) insulin secretory insufficiency, IGI<0.4 and HOMA-IR<2.5; 3) elevated insulin resistance, IGI<0.4 and HOMA-IR≥2.5; 4) combination of insulin secretory insufficiency and insulin resistance, IGI<0.4 and HOMA-IR≥2.5. Fatty liver was diagnosed based on the presence of an ultrasonographic pattern consistent with a bright liver with stronger echoes in the hepatic parenchyma than in the renal or splenic parenchyma (23).

Clinical and laboratory analysis

The laboratory analysis was performed according to standard laboratory methods. Anti-HCV was detected using a second-generation enzyme-linked immunosorbent assay (ELISA II) (Abbott Laboratories, North Chicago, USA). HBsAg was assessed using a radioimmunoassay (Abbott Laboratories, Detroit, USA). The serum biochemical parameters included aspartate aminotransferase (AST), alanine aminotransferase (ALT), ganma-glutamyltransferase (GGT), total cholesterol, high-density lipoprotein cholesterol (HDL-C), triglycerides, FPG, insulin and HbA1c. The serum insulin levels were measured with a solid-phase radioimmunoassay (Diagnostic Products Corporation, Los Angeles, USA). The HbA1c level (%) was estimated as the NGSP equivalent

Table 1. **Clinical Characteristics***

	NAFLD subjects
n	569
Age (y.o)	59.8±10.1
Gender (male/female)	428/141
Height(cm)	166.1±8.7
Weight(kg)	70.0±11.1
Body mass index	26.3±2.8
HbA1c (NGSP, %)	4.99±0.34
FPG (mg/dL)	95.5±7.5
Triglyceride(mg/dL)	153±121
Total cholesterol (mg/dL)	207±39
HDL cholesterol (mg/dL)	52±14
Albumin (g/dL)	4.0±0.2
AST(IU/L)	28±15
ALT(IU/L)	31±20
GGT(IU/L)	63±109

*Data are number of subjects or mean ± standard deviation. ALT: alanine aminotransferase, AST: aspartate aminotransferase, FPG: fasting plasma glucose, FPI: fasting plasma insulin, GGT: gamma-glutamyltransferase, HDL: high density lipoprotein

value (%) calculated according to the formula HbA1c (%) = HbA1c (Japan Diabetes Society, JDS) +0.4%, considering the relational expression of HbA1c (JDS) (%) measured based on previous Japanese 165 standard materials and measurement methods and the HbA1c (NGSP) value (26). The anthropometric parameters included height, weight and body mass index (BMI), the latter of which was calculated as weight (kg) divided by the square of the height (m^2). All of analyses of the control group were performed retrospectively by collecting and analyzing data from the subjects' records. The study protocol was approved by the Institutional Review Board of our hospital.

Statistical analysis

The results are presented as the mean ± standard deviation (SD) or as numbers with percentages. Statistical differences in quantitative data were determined using the Mann-Whitney *U*-test, Fisher's exact probability test and Kruskal-Wallis test. Changes in the serum glucose and insulin levels between the NAFLD patients and control subjects during OGTT were analyzed using a one-way repeated measurement ANOVA. Significant predictors according to a univariate analysis were subsequently included in a forward, stepwise multiple logistic regression model in order to identify important predictive factors for the diabetes pattern on the OGTT. The Statistical Program for Social Sciences software package (SPSS 11.5 for Windows, SPSS, Chicago, USA) was used to perform all statistical analyses. A p value of <0.05 was considered to be statistically significant.

Results

Patient characteristics

Table 1 shows the characteristics on the day of the OGTT examinations in the NAFLD group. The mean age was 59.8 years, the mean BMI was 26.3, the mean FPG level was 95.5±7.5 mg/dL and the mean HbA1c level was 5.0±0.3%.

Prevalence and predictive factors of diabetes

According to the OGTT results, of the 569 NAFLD subjects with an HbA1c level of ≤6.4% and FPG level of <126 mg/dL, 198 (34.8%) were diagnosed as having IGT and 44 (7.7%) were diagnosed as having diabetes.

Table 2 shows the predictive factors for diabetes in the NAFLD subjects. The multivariate analysis showed that diabetes occurred more frequently among the NAFLD patients with a FPG level of 5.6-6.9 mg/dL [odds ratio (OR) 5.13; 95% confidential interval (CI) 3.01-8.76; p<0.001] and HbA1c level of 5.7-6.4% (OR: 5.45; 95% CI 3.33-8.93; p< 0.001).

Fig. 1 shows the distribution of the normal, IGT and diabetes pattern on OGTT in each group classified based on the differences in FPG and HbA1c. Among the 123 subjects with both IFG and an HbA1c level of 5.7-6.4%, 28 (22.8%) were diagnosed as having diabetes on OGTT. In addition, 67 patients (54.5%) were diagnosed as having IGT.

Insulinogenic index and HOMA-IR based on the differences in FPG and HbA1c

Fig. 2 shows the prevalence of insulin secretory insufficiency and elevated insulin resistance in each group classified based on the differences in FPG and HbA1c. Among the 123 NAFLD subjects with both IFG and an HbA1c level of 5.7-6.4%, 31 (25.2%) exhibited elevated insulin resistance, 31 (25.2%) had insulin secretory deficiency and 34 (27.6%) displayed both elevated insulin resistance and insulin secretory deficiency.

Discussion

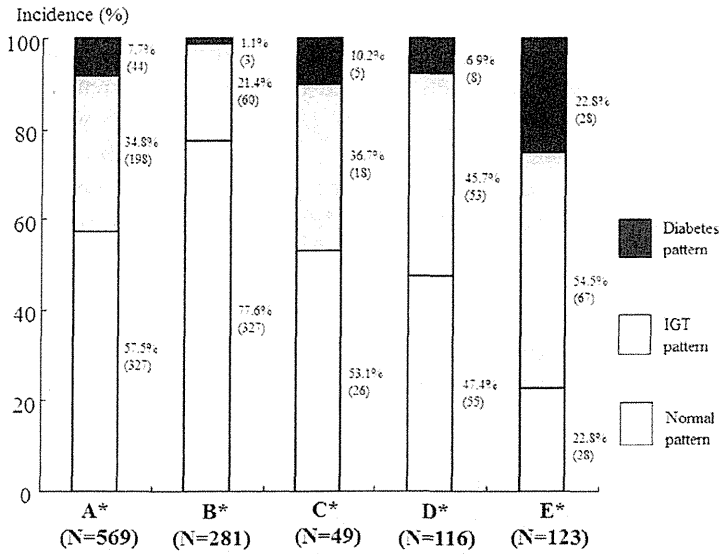
We herein described the state of glucose and insulin after OGTT in NAFLD Japanese subjects with an HbA1c level of ≤6.4% and FPG level of <126 mg/dL. The strengths of the present study include the following points: 1) the large number of subjects with NAFLD, 2) the evaluations of insulin resistance and insulin secretion in all enrolled patients.

The present study showed several important findings with regard to the prevalence of abnormal oral glucose tolerance in Japanese NAFLD subjects with an HbA1c level of ≤6.4% and FPG level of <126 mg/dL. First, approximately 40% of the NAFLD subjects with an HbA1c level of ≤6.4% and FPG level of <126 mg/dL showed abnormal glucose tolerance on OGTT. In particular, among the 123 subjects with both IFG and an HbA1c level of 5.7-6.4%, approximately one-fifth of the NAFLD patients were diagnosed as having diabetes on OGTT. Indeed, the HOMA-IR is also useful for screening diabetic patients, and if OGTT were not performed, diabetes cases may be missed. Wong et al. reported that T2DM and IGT cannot be accurately predicted by any fasting glucose cut-off values among Chinese NAFLD patients without a history of diabetes (27). The present investi-

Table 2. Predictive Factors for Diabetes Pattern in Oral Glucose Tolerance Test in NAFLD Patients*

Variables	Univariate analysis		Multivariate analysis	
	OR (95%CI)	p	OR (95%CI)	p
Age (per 10 years)	1.47(1.17-1.84)	0.001		
Gender (male / female)	1.72(1.07-2.76)	0.026		
Body mass index (per 5)	1.64(1.20-2.27)	0.002		
AST(IU/L, ≥34/<34)	0.97(0.44-2.13)	0.932		
ALT(IU/L, ≥42/<42)	1.57(0.82-3.03)	0.177		
GGT (IU/L, ≥109/<109)	1.17(0.47-2.93)	0.746		
Platelet ($\times 10^4/mm^3$, ≥20/<20)	0.63(0.23-1.74)	0.372		
APRI (≥0.5/<0.5)	0.67(0.290-1.536)	0.342		
Albumin (g/dL, ≥3.9/<3.9)	1.07(0.68-1.69)	0.762		
Triglyceride (mg/dL, ≥150/<150)	1.95(1.24-3.08)	0.004		
Total Cholesterol (mg/dL, ≥220/<220)	1.15(0.74-1.80)	0.533		
HDL Cholesterol (mg/dL, ≥40/<40)	0.59(0.34-1.03)	0.065		
FPG (mg/dL, ≥100-<126/<100)	8.38(5.03-13.96)	< 0.001	5.13(3.01-8.76)	< 0.001
HbA1c (NGSP %, 5.7-6.4/<5.6)	8.75(5.47-13.98)	<0 .001	5.45(3.33-8.93)	< 0.001

*ALT: alanine aminotransferase, AST: aspartate aminotransferase, CI: confidential Interval, DM: diabetes mellitus, FPG: fasting plasma glucose, GGT: gamma-glutamyltransferase, HbA1c: hemoglobin A1c, HDL: high density lipoprotein, OR: odds ratio

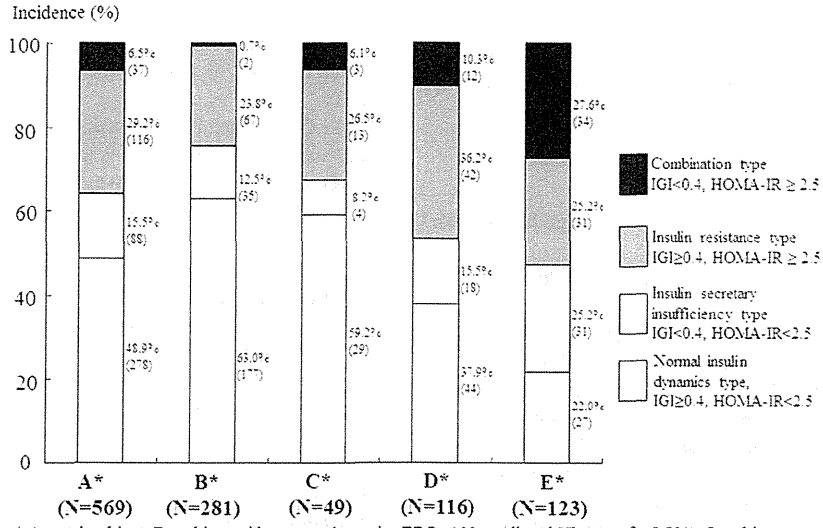


* A, total subject; B, subject with normoglycemia, FPG<100mg/dl and HbA1c of <5.7%; C, subject with HbA1c 5.7-6.4% alone (FPG<100mg/dl and HbA1c of 5.7-6.4%); D, subject with impaired fasting glucose (FPG of ≥100 to <126mg/dl) and HbA1c of <5.7%; E, subject with both impaired fasting glucose and HbA1c of 5.7-6.4%

Figure 1. Oral glucose tolerance pattern based on the differences in the fasting plasma glucose and HbA1c levels among the NAFLD patients with an HbA1c level of ≤6.4%, FPG level of <126mg/dL and no history of diabetes.

gations are in agreement with these results.

Second, the multiple regression analysis showed FPG and HbA1c to be independent predictors of a diabetes pattern on OGTT in the NAFLD subjects. These results suggest that IFG and an HbA1c level of 5.7-6.4% are associated with an increased prevalence of a diabetes pattern on OGTT in NAFLD subjects. In fact, of the NAFLD subjects with an HbA1c level of 5.7-6.4% and IFG, approximately 23% showed a diabetes pattern on the OGTT examinations. Although OGTT has the disadvantages of greater cost and inconvenience, physicians in charge of NAFLD patients with IFG and an HbA1c level of 5.7-6.4% should perform OGTT in order to evaluate the presence of diabetes. Yun et al. reported that OGTT should be conducted in young men with



* A, total subject; B, subject with normoglycemia, FPG<100mg/dl and HbA1c of <5.7%; C, subject with HbA1c 5.7-6.4% alone (FPG<100mg/dl and HbA1c of 5.7-6.4%); D, subject with impaired fasting glucose (FPG of ≥100 to <126mg/dl) and HbA1c of <5.7%; E, subject with both impaired fasting glucose and HbA1c of 5.7-6.4%

Figure 2. Insulin dynamics based on the differences in the fasting plasma glucose and HbA1c levels among the NAFLD patients with an HbA1c level of ≤6.4%, FPG level of <126mg/dL and no history of diabetes.

NAFLD who exhibit elevated liver enzymes and IFG in order to predict the risk of T2DM (28). In addition, Heianza et al. reported that the general population with IFG and an HbA1c level of 5.7-6.4% tends to have a greater risk of the onset of DM based on the findings of a large-scale follow-up study (29). Furthermore, the present results indicate that HbA1c and FPG are strong predictors for T2DM compared with liver enzymes. Although a recent study found a higher BMI and older age to be associated with impaired glucose metabolism in NAFLD patients (30), these values were not identified to be significant in the multivariate analysis in that study. We do not consider these factors to have had a sufficient impact on the rate of DM in this study.

Third, among the NAFLD patients with IFG and an HbA1c level of 5.7-6.4%, most demonstrated elevated insulin resistance and/or insulin secretory insufficiency; one-fourth had elevated insulin resistance, one-fourth had insulin secretory insufficiency and one-fourth had both elevated insulin resistance and insulin secretory insufficiency. Several other authors have reported that postprandial hyperinsulinemia is universal in non-diabetic patients with NAFLD (31-34). Therefore, it is recommended that NAFLD patients with IFG and an HbA1c level of 5.7-6.4% be examined using OGTT in order to evaluate whether they have elevated insulin resistance and/or insulin secretory insufficiency.

Recent studies have demonstrated that non-invasive serum markers can be used to predict the onset of DM (35-37). We previously estimated the aspartate aminotransferase platelet ratio index (APRI), calculated as the AST [ULN]/platelet count [10^3/mL] ×100, from a database (37). Unfortunately, our results did not show the APRI to be a predictor of DM, as most of our patients had either no or mild liver fibrosis.

The prevalence of NAFLD has increased dramatically in many nations, including Japan, over the past decades. At presence, according to Japanese annual health check reports, 9-30% of Japanese adults demonstrate evidence of NAFLD on ultrasonography. Since approximately 10% of individuals with NAFLD have non-alcoholic steatohepatitis (NASH), the prevalence of NASH is estimated to be 1-3% of the adult Japanese population (21). It was also recently reported that T2DM may occur in NAFLD patients (15, 16) and that NAFLD subjects with T2DM have an increased risk of hepatocellular carcinoma (20, 21). Therefore, in subjects with NAFLD and T2DM, the management of DM is very important for improving the prognosis. The present findings indicate that OGTT is a useful test for detecting diabetes, insulin resistance and insulin secretory insufficiency in NAFLD subjects with IFG and an HbA1c level of 5.7-6.4%.

The present study is associated with several limitations. First, the NAFLD subjects did not undergo liver biopsies. In addition, although NAFLD can be categorized into simple steatosis or steatohepatitis, the present study was undertaken without histologically differentiating between simple steatosis and steatohepatitis. Second, control subjects without NAFLD were not evaluated. Third, our cohort comprised Japanese subjects only; this heterogeneity makes it difficult to interpret the results of our study.

In conclusion, NAFLD subjects with IFG and an HbA1c level of 5.7-6.4% should undergo OGTT in order to determine whether they have diabetes, elevated insulin resistance and/or insulin secretory insufficiency.

The authors state that they have no Conflict of Interest (COI).

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Original Article

Potential impact of joint association of alanine aminotransferase and gamma-glutamyltransferase on insulin resistance in Japan: The Toranomon Hospital Health Management Center Study 19 (TOPICS 19)

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Aim: To investigate the potential impact of joint association of alanine aminotransferase (ALT) and γ-glutamyltransferase (GGT) on insulin resistance and β-cell dysfunction in healthy Japanese individuals with a normal range of liver enzymes.

Methods: This study included 1010 individuals (545 men and 465 women) aged 20–89 years who underwent an oral glucose tolerance test for health screening. Participants were divided into four groups on the basis of median values for ALT and GGT: (i) both ALT and GGT low (both-low); (ii) ALT high and GGT low (ALT-high); (iii) ALT low and GGT high (GGT-high); and (iv) both ALT and GGT high (both-high). Logistic regression analysis was used to investigate the relationship between liver enzyme and insulin dynamics, such as Homeostasis Model of Assessment – Insulin Resistance (HOMA-IR) and insulinogenic index (IGI). The insulin resistance was defined when HOMA-IR was 2.5 or more. IGI of less than 0.4 was considered to be decreased early-phase insulin secretion.

Results: Mean values of HOMA-IR in men was 1.5 in the both-low group, 1.8 in ALT-high, 1.8 in GGT-high and 2.8 in both-high. The mean HOMA-IR in women was 1.3 in the both-low group, 1.3 in ALT-high, 1.6 in GGT-high and 2.0 in both-high. HOMA-IR in the both-high group was significantly higher than that in the both-low group regardless of the difference of sex. Multivariate analysis showed that insulin resistance occurred when the patient had high liver enzymes.

Conclusion: Combining the two liver function markers would be effective for identifying individuals with insulin resistance.

Key words: fatty liver, insulin resistance, liver enzyme, OGTT

INTRODUCTION

THE PREVALENCE OF type 2 diabetes mellitus (T2DM) is increasing dramatically in newly developed and developing countries in many nations.[1] T2DM is a serious, costly disease. Early diagnosis for T2DM may prevent some of its devastating complications. In addition, various factors have been reported as risk factors for T2DM.[2–4] Several authors have reported that both alanine aminotransferase (ALT) and γ-glutamyltransferase (GGT) are associated with T2DM.[5–11]

Serum activities of aspartate aminotransferase (AST), ALT and GGT are the most common indicators of liver disease. Elevated levels of aminotransferase often suggest the existence of medical problems such as viral hepatitis, alcohol use, medication use, steatosis and steatohepatitis. Other various conditions, such as heart disease, thyroid disease, bile duct problems, infectious

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mononucleosis or myopathy can cause an augmentation.[12] Several studies have reported that elevated ALT levels are associated with T2DM.[5–9] However, other authors refute that association.[13,14] Next, serum GGT has been commonly used as a marker of the existence of medical problems such as diseases of the biliary system, large quantities of alcohol ingestion, medication use or congestive heart failure.[15] Lims *et al.* have also proposed that GGT is as a marker of oxidative stress.[16] In addition, serum GGT level could predict the development of T2DM in longitudinal studies.[10,11] Moreover, GGT is closely related to insulin resistance (IR), reduced β-cell function and deterioration of glucose tolerance.[17]

As described above, elevation of liver enzymes originates from various etiologies. However, it is often difficult to determine the normal range of liver enzymes. In particular, the mean values of aminotransferase and GGT in non-alcoholic fatty liver, which has been recently increasing, were within normal limits as reported by Kawamura *et al.*[18] Tanaka K *et al.* have reported that the upper limits of normal for ALT when considering lifestyle factors in Japanese subjects were 29 IU/L in men and 23 IU/L in women.[19] Thus, it is an emerging issue to evaluate the upper limits of liver enzymes for predicting IR fatty liver in Japanese people. In addition, whether joint association of ALT and GGT could be valuable for predicting IR and insulin secretion compared with ALT or GGT only is unclear. With this background in mind, we investigated the impact of combination of the two liver function markers on IR and β-cell dysfunction in Japanese healthy individuals by oral glucose tolerance test (OGTT). The strengths of the current study are the large numbers of patients included and the joint association of ALT and GGT for predicting IR, DM and fatty liver.

METHODS

Study population

THIS CROSS-SECTIONAL STUDY included 1526 individuals aged 20–89 years who consented to undergo an OGTT for health screening from 1997–2010 at the Health Management Center in Toranomon Hospital. Of these, 1010 Japanese patients (545 men and 465 women) satisfied the following enrolled criteria: (i) normal range in marker of AST, ALT and GGT; (ii) daily alcohol intake of less than 20 g/day; and (iii) negativity for hepatitis B surface antigens or hepatitis C virus antibodies in serum, as determined by radioimmunoassay or enzyme-linked immunosorbent assay. Patients with either of the following criteria were excluded from the study: (i) presence of underlying systemic disease, such as diabetes, cardiovascular disease, chronic lung disease, chronic liver disease or collagen disease; and (ii) the presence of neoplasm by gastrofiberscope (or gastrography), abdominal ultrasonography, chest X ray and/or computed tomography. Subsequently, 545 men and 465 women (mean [SD] age, 58.4 years [11.4]) were eligible for the current analysis. As for liver enzyme, the normal values of AST, ALT and GGT were the following in our hospital: (i) AST, 13–33 IU/L in both sexes; (ii) ALT, 8–42 IU/L in men and 6–27 IU/L in women; and (iii) GGT, 9–109 IU/L in both sexes.

Participants were divided into four groups on the basis of median values for ALT and GGT: (i) both ALT and GGT low (ALT and GGT of less than median value, both-low group); (ii) ALT high and GGT low (ALT of median value or higher and GGT of less than median value, ALT-high group); (iii) ALT low and GGT high (ALT of less than median value and GGT of median value or more, GGT-high group); and (iv) both ALT and GGT high (ALT and GGT of median value or more, both-high group). IR and insulin secretion were evaluated by OGTT in these four groups. All of the studies were performed retrospectively by collecting and analyzing data from the patient records. The study protocol was consistent with the Japanese Government's Ethical Guidelines Regarding Epidemiological Studies in accordance with the Declaration of Helsinki and was reviewed by the Institutional Review Board at Toranomon Hospital.

Medical evaluation

A 75-g OGTT was undergone after a 12-h fast. Plasma glucose and insulin levels were analyzed before and 30, 60, 90, 120 and 180 min after oral glucose loading. Blood samples were obtained from all subjects six times during the OGTT. The following indices of insulin secretion and insulin sensitivity or resistance were calculated in this study: (i) insulinogenic index (IGI) = (Ins30 – Ins0) / (Gluc30 – Gluc0), where Insγ and Gluγ represent values at time γ min during the OGTT; and (ii) Homeostasis Model of Assessment – Insulin Resistance (HOMA-IR) = Glu0 × Ins0 / 405.[20–22] In calculation, the used unit was μU/mL in serum insulin level and mg/dL in glucose level.

Serum insulin was measured by chemiluminescent enzyme immunoassay (Fujirebio, Tokyo, Japan) and plasma glucose was measured by enzymatic methods (Roche Diagnostics, Tokyo, Japan). Hemoglobin A1c (HbA1c) was examined by high-performance liquid chromatography (Tosoh, Tokyo, Japan). The IR was

defined when HOMA-IR was 2.5 or more.[20] IGI is less than 0.4 is considered to be decreased early-phase insulin secretion according to the "Report of The Committee on the Classification and Diagnostic Criteria of Diabetes Mellitus" by the Committee of the Japan Diabetes Society on the Diagnostic Criteria of Diabetes Mellitus.[23] Serum low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald equation, except where triglycerides exceeded 400 mg/dL, in which case LDL-C data were treated as "missing". This was applicable to 15 subjects.

The diagnosis of fatty liver was based on the ultrasonographic images, which were stored as photocopies by trained technicians. A gastroenterologist reviewed the photocopies and made the diagnosis of fatty liver without reference to any of the participant's other individual data. When the individuals had a pattern consistent with bright liver (brightness and posterior attenuation) with stronger echoes in the hepatic parenchyma than in the renal or spleen parenchyma, they were given a diagnosis of fatty liver.[24]

A standardized questionnaire was administered to all participants by trained nurses of interviewers. The information on demographic characteristics, medical history, and health-related habits including questions on alcohol intake and physical exercise were evaluated. The physical activity other than daily work was evaluated by the Metabolic Equivalent of Task (MET).[25,26] The patients were asked the average frequency (times/week) and duration (min/time) of normal walking, brisk walking, jogging and other miscellaneous exercise. The duration engaged in each activity in min/time was multiplied by that activity's typical energy expenditure, expressed in MET. Thus, overall activity was summed to yield a MET h score per day. Height and weight were recorded at baseline and the body mass index (BMI) was calculated as weight (in kg) / height (in m^2). Patients were defined as hypertensive when blood pressure was 140/90 mmHg or more or pharmacological treatment for high blood pressure was given.

Statistical analysis

All analyses were performed in men and women separately. Non-parametric procedures were employed for the analysis of background features of the patients in the ALT-high, GGT-high and both-high groups compared with the both-low group, including the Mann–Whitney *U*-test or Fisher' exact test. Logistic regression analysis was used to investigate factors associated with the presence of HOMA-IR of 2.5 or more. A multivariate regression model was also performed using the factors of $P < 0.1$ by univariate regression analysis. The model was calculated with a backward elimination method. In addition, Spearman's rank correlation coefficients were used for the relationship between liver enzymes and glycemic, IR and insulin secretion parameters.

Statistical analyses were performed using IBM SPSS Statistics version 19 (IBM, Armonk, NY, USA), and statistical significance was considered at $P < 0.05$.

RESULTS

Demographic characteristics

MEN HAD A median ALT level of 23 IU/L and median GGT level of 34 IU/L. Women had a median ALT level of 16 IU/L and median GGT level of 20 IU/L. The participants were divided into four groups on the basis of the serum median level of ALT and GGT in men and women, respectively. Table 1 shows the baseline characteristics of the 1010 individuals based on the difference of sex and serum median level of liver enzyme (ALT and GGT). BMI and serum triglyceride level were higher in the both-high group than in the both-low group in both men and women. The incidence of fatty liver in men was 16.1% (12/199) in the both-low group and 64.8% (125/193) in the both-high group. The incidence of fatty liver in women was 4.3% (7/164) in the both-low group and 26.4% (42/159) in the both-high group. The prevalence of fatty liver in the both-high group was higher than that in the both-low group regardless of the difference of sex.

Change of plasma glucose and insulin level in OGTT

Figure 1 shows the change of plasma level of glucose and insulin by OGTT based on difference of liver enzymes and sex. The plasma levels of glucose and insulin at 0, 30, 60, 90, 120 and 180 min after oral glucose loading in the both-high group were elevated compared to the both-low group with statistical significance regardless of difference of sex.

Relationship between liver enzymes and glycemic parameters

Table 2 shows glycemic parameters based on the difference of ALT and GGT. Fasting plasma glucose (FPG) and HbA1c were higher in the both-high group than in the both-low group. However, FPG in the ALT-high or GGT-high groups was not statistically higher than that in the both-low group.

The mean (SD) HOMA-IR of men was 1.5 (1.2) in the both-low group and 2.8 (1.6) in the both-high group.

Table 1 Characteristics of the study participants by a combination of GGT and ALT

	Men ($n = 545$)				Women ($n = 465$)			
	Both-low GGT <34 U/L	ALT-high	GGT-high GGT ≥34 U/L	Both-high	Both-low GGT <20 U/L	ALT-high	GGT-high GGT ≥20 U/L	Both-high
	ALT <23 U/L	ALT ≥23 U/L	ALT <23 U/L	ALT ≥23 U/L	ALT <16 U/L	ALT ≥16 U/L	ALT <16 U/L	ALT ≥16 U/L
n	199	75	79	193	164	72	70	159
Age, years†	62.6 (11.3)	57.0 (12.6)**	62.9 (10.7)	57.7 (11.2)**	57.9 (12.7)	58.6 (9.4)	61.8 (9.8)*	60.7 (9.8)*
BMI†	22.5 (2.9)	23.4 (3.3)**	23.1 (2.7)*	25.6 (2.9)**	21.3 (2.7)	21.2 (2.9)	22.3 (2.3)**	23.0 (3.9)**
SBP, mmHg†	118 (15)	118 (13)	122 (15)	122 (13)	110 (14)	109 (11)	117 (17)	117 (14)
DBP, mmHg†	73 (10)	74 (10)	77 (10)	77 (9)	68 (9)	68 (8)	70 (9)	71 (9)
Triglycerides, mg/dL†	103 (87)	114 (64)	132 (73)**	179 (1.94)**	78 (32)	86 (37)	99 (56)**	104 (51)**
Total-C, mg/dL†	201 (33)	195 (32)	200 (34)	206 (32)	206 (32)	210 (32)	215 (33)	217 (39)
HDL-C, mg/dL†	52 (14)	48 (11)*	51 (14)	47 (13)**	63 (13)	64 (15)	62 (14)	61 (15)
LDL-C, mg/dL†	126 (27)	124 (27)	126 (30)	129 (27)	127 (28)	128 (28)	134 (29)	136 (33)*
Albumin, g/dL†	4.0 (0.2)	4.0 (0.2)	4.0 (0.2)	4.1 (0.2)	3.9 (0.2)	3.9 (0.2)	3.9 (0.2)	4.0 (0.2)
Uric acid, mg/dL†	5.7 (1.1)	5.6 (1.2)	6.2 (1.2)	6.5 (1.3)	4.4 (0.9)	4.7 (0.9)	4.8 (1.0)	5.0 (1.0)
AST, IU/L†	20 (4)	26 (10)	21 (4)	33 (15)**	19 (3)	23 (4)	19 (3)	25 (6)*
ALT, IU/L†	16 (4)	32 (13)**	18 (4)*	35 (24)**	13 (2)	20 (5)**	14 (2)	25 (8)**
GGT, IU/L†	23 (6)	25 (6)**	52 (19)**	73 (36)**	14 (3)	15 (2)	26 (9)**	38 (21)**
Platelets ($\times 10^4/mm^3$)	21.2 (4.2)	20.9 (5.4)	22.5 (5.3)	21.5 (5.5)	23.6 (5.3)	23.1 (4.9)	25.5 (6.8)	23.6 (5.1)
Hypertension (%)	21 (10.6%)	11 (14.7%)	16 (20.3%)*	36 (18.7%)*	11 (6.7%)	6 (8.3%)	10 (14.3%)	24 (15.1%)*
Fatty liver (%)‡	32 (16.1%)	34 (45.3%)**	27 (34.2%)**	125 (64.8%)**	7 (4.3%)	8 (11.1%)*	9 (12.9%)*	42 (26.4%)**
Ethanol (≥20 g/day)	45 (22.6%)	22 (29.3%)	25 (31.6%)	61 (31.6%)	20 (12.2%)	9 (12.5%)	11 (15.7%)	19 (11.9%)
Exercise (≥2 MET/day)	105 (52.8%)	29 (40.0%)	34 (43.0%)	70 (36.3%)**	74 (45.1%)	37 (51.4%)	36 (51.4%)	68 (42.8%)

Non-parametric procedures were employed for the analysis of background features of the patients in the ALT-high, GGT-high, and both-high groups compared with the both-low group, including the Mann–Whitney *U*-test or Fisher' exact test. *$P < 0.01$ vs both-low. **$P < 0.001$ vs both-low.

†Data are mean (standard deviation). ‡Data are number of patients (%).

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CI, confidence interval; DBP, diastolic blood pressure; FPG, fasting plasma glucose; GGT, γ-glutamyltransferase; HDL-C, high-density lipoprotein cholesterol; MET, Metabolic Equivalent of Task; SBP, systolic blood pressure; Total-C, total cholesterol.

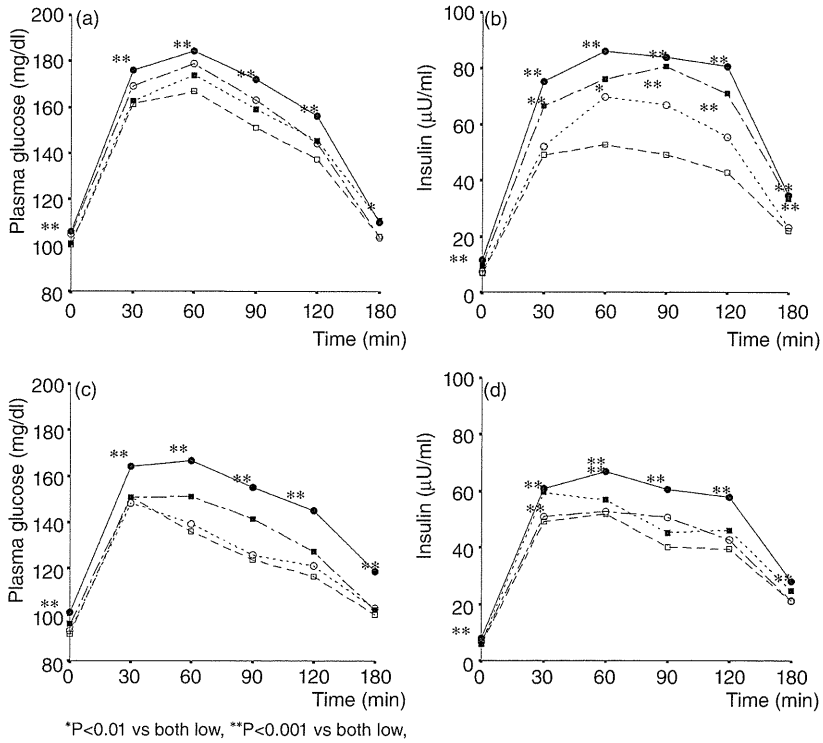

Figure 1 Comparative values of levels of plasma glucose and serum insulin during oral glucose tolerance test in males and females based on the difference of liver enzymes and presence or absence of fatty liver. (a) Plasma glucose level in males. (b) Serum insulin level in males. (c) Plasma glucose level in females. (d) Serum insulin level in females. ——●, both high; - - - -■, alanine aminotransferase high; ——- ○, γ-glutamyltransferase high; — — □, both low.

The mean HOMA-IR of women was 1.3 (0.6) in the both-low group and 2.0 (1.4) in the both-high group. HOMA-IR significantly increased in individuals of the both-high group. On the other hand, IGI did not change based on ALT and GGT values. Table 3 shows Spearman's rank correlation coefficients for the relationship between liver enzymes and glycemic, IR and insulin secretion parameters. Liver enzymes were strongly correlated with HOMA-IR.

Predictive factors for IR

The predictive factors for IR of HOMA-IR of 2.5 or more by logistic regression model in men and women are shown in Table 4. Multivariate analysis shows that IR in men occurred when patient had high FPG of 100 mg/dL or more (odds ratio, 5.23; 95% confidence interval [CI], 3.08–8.86; $P < 0.001$], high liver enzymes (odds ratio of both-high/ALT-high or GGT-high/both-low, 2.51; 95% CI, 1.68–3.75; $P < 0.001$), elevated BMI (odds ratio per increment of 5 kg/m^2, 1.57; 95% CI, 1.07–2.41; $P = 0.042$), and presence of fatty liver (odds ratio, 2.32; 95% CI 1.33–4.03; $P = 0.003$). The IR in women occurred when a patient had high FPG of 100 mg/dL (odds ratio, 7.93; 95% CI, 4.11–15.29; $P < 0.001$), high liver enzymes (odds ratio of both-high/ALT-high or GGT-high/both-low, 2.21; 95% CI, 1.41–3.44; $P < 0.001$), elevated BMI (odds ratio per increment of 5 kg/m^2: 1.86; 95% CI 1.11–3.10; $P = 0.018$) and presence of fatty liver (odds ratio, 3.80; 95% CI, 1.73–8.32; $P = 0.001$).

Figure 2 shows the HOMA-IR based on the differences of liver enzymes and presence or absence of fatty liver. In men without fatty liver, mean (SD) HOMA-IR was 1.29 (0.73) in the both-low group and 2.35 (1.65) in the both-high group. In men with fatty liver, HOMA-IR was 1.93 (1.05) in the both-low group and 2.99 (1.45) in the both-high group. In women without fatty liver, HOMA-IR was 1.29 (0.62) in the both-low group and 1.74 (1.06) in the both-high group. In women with fatty liver, mean HOMA-IR was 1.52 (1.05) in the both-low group and 2.84 (1.74) in the both-high group. HOMA-IR in the both-high group was statistically higher than that in the both-low group regardless of sex and presence or absence of fatty liver.

Predictive factors for insulin secretion

The predictive factors for IGI of less than 0.4 by logistic regression model in men and women are shown in