表1 厚生労働省指定難病における副甲状腺機能低下症の診断基準

A. 症状
1. 口周囲や手足などのしびれ，錯感覚
2. テタニー
3. 全身痙攣
B. 検査所見
1. 低 Ca 血症，正または高リン血症
2. eGFR 30 mL/min/1.73 m^2 以上
3. intact PTH 30 pg/mL 未満
〈除外項目〉
1. 二次性副甲状腺機能低下症
・頸部手術後
・放射線照射後
・悪性腫瘍の浸潤
・肉芽腫性疾患
・ヘモクロマトーシス
・ウィルソン病
・母体の原発性副甲状腺機能亢進症(新生児・一過性)
2. マグネシウム補充により治癒する場合
〈重症度分類〉
主要徴候により分類される.
軽　症：生化学異常を認めるものの，感覚異常やテタニーなどの症候を認めず，日常生活に支障がない.
中等症：低 Ca 血症を認め，しびれなどの感覚異常を認め，日常生活に支障がある.
重　症：低 Ca 血症を認め，テタニーや痙攣などにより，日常生活に著しい支障がある.

(http://www.nanbyou.or.jp/entry/4427 より作成)

臨床試験において，rhPTH(1-84)1日1回皮下投与の有効性が報告されており[5]，米国で2015年に承認された．rhPTH(1-34)についても有用性が報告されている．PTH補充療法は，より生理的状況に近づいた治療法であり，臨床応用が期待される．

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ORIGINAL ARTICLE

Treatment and management of thyroid storm: analysis of the nationwide surveys

The taskforce committee of the Japan Thyroid Association and Japan Endocrine Society for the establishment of diagnostic criteria and nationwide surveys for thyroid storm

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Summary

Objective Thyroid storm (TS) is a life-threatening endocrine emergency. This study aimed to achieve a better understanding of the management of TS by analyzing therapeutic modalities and prognoses reported by nationwide surveys performed in Japan.

Design, patients and measurements Retrospective analyses were performed on clinical parameters, outcomes, and treatments in 356 TS patients.

Results Patient disease severities assessed via Acute Physiology and Chronic Health Evaluation (APACHE) II and Sequential Organ Failure Assessment (SOFA) scores significantly correlated with mortality. Free triiodothyronine (FT3) and the FT3/free thyroxine (FT4) ratio inversely correlated with disease severity. Methimazole (MMI) was used in the majority of patients (78·1%), and there were no significant differences in mortality or disease severity between those treated with MMI and those receiving propylthiouracil (PTU). Patients who received inorganic iodide (KI) demonstrated higher disease severity but no change in mortality compared to those who did not. Patients treated with corticosteroids (CSs) demonstrated significantly higher disease severity and mortality than those who were not. Disease severity in patients treated with intravenous administration of beta-adrenergic antagonists (AAs) was significantly higher than those treated with oral preparations, although no significant difference in mortality was observed between these groups. In addition, mortality was significantly higher in patients treated with non-selective beta-AAs as compared with other types of beta-AAs.

Conclusion In Japan, MMI was preferentially used in TS and showed no disadvantages compared to PTU. In severe TS, multimodal treatment, including administration of antithyroid drugs, KI, CSs and selective $beta_1$-AAs may be preferable to improve outcomes.

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Introduction

Thyroid storm (TS) is a life-threatening endocrine emergency originating from thyrotoxic conditions such as uncontrolled Graves' disease, or destructive thyroiditis in the presence of various triggers.[1] TS is characterized by decompensation of multiple organs, resulting in severe clinical manifestations such as disturbed consciousness, high fever, marked tachycardia, congestive heart failure (CHF), and gastrointestinal and hepatic disturbances.[1] The diagnosis of TS has been made using the diagnostic criteria and a clinical scoring system first introduced by Burch and Wartofsky.[1] Based on newly formulated diagnostic criteria of TS, we recently conducted nationwide surveys to clarify the incidence and clinical features of TS in Japan.[2] These surveys revealed that the incidence of TS in hospitalized patients was an estimated 0·20 per 100 000 per year, and the mortality of TS was approximately 10%. Shock, disseminated intravascular coagulation (DIC), and multi-organ failure significantly contributed to mortality. The levels of inpatient care and the scores of the Acute Physiology and Chronic Health Evaluation (APACHE) II and Sequential Organ Failure Assessment (SOFA)

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instruments were significantly higher in non-survivors. Because of the rarity of TS, large prospective clinical trials to validate the management of TS are difficult to conduct, and therefore treatment of TS has been empirically performed. Severe thyrotoxicosis in TS originating from Graves' disease has been managed using a combination of large doses of antithyroid drugs (ATDs), inorganic iodide (KI), antipyretics, corticosteroids (CSs) and beta-adrenergic antagonists (AAs).[1] In some TS cases that are refractory to conventional drug therapy, plasmapheresis has been utilized to remove circulating excess thyroid hormones.[2] However, the relationship between prognosis and the choice of treatment for TS has not yet been evaluated in even a retrospective manner. In the present study, as an initial step to establishing the evidence-based management of TS, we comprehensively analysed the clinical manifestations, therapeutic management and prognosis of Japanese TS patients as reported in nationwide surveys.

Materials and methods

Subjects

We analysed the clinical features of 356 TS patients whose data were reported between 2004 and 2008 by the nationwide surveys performed in Japan.[2] Based on our diagnostic criteria for TS, 282 and 74 patients, respectively, were defined as definite cases (TS1) and suspected cases (TS2). Since no statistical difference in mortality was observed between the TS1 and TS2 patients, we combined the data of both groups for further analyses, although both APACHE II[3] and SOFA scores[4,5] indicated significantly greater disease severity in TS1 patients, as previously reported.[2] The surveys examined clinical findings and laboratory data, including free thyroxine (FT4), free triiodothyronine (FT3) and thyrotropin (TSH) levels, measured at each facility where treatment was conducted.[2] The surveys and analyses were performed by the Research Program of Intractable Diseases of the Ministry of Health, Labour and Welfare of Japan, and the present study was approved by the ethics committee of the Jichi Medical University, as reported previously.[2]

Statistical analyses

Clinical parameters, including patients' mortality and disease severity, were compared among different treatments using Fisher's exact test and the Wilcoxon and Kruskal–Wallis tests. We also used multivariate analysis to assess the relationships between these parameters using Spearman's rank correlation coefficient. To identify factors independently associated with treatment choice or mortality, logistic regression analysis or multiple regression analysis with the stepwise method was employed after the possible clinical parameters had been transformed to a normal distribution by the Box-Cox method. $P < 0{\cdot}05$ was regarded as being statistically significant. All statistical analyses were carried out using the software package JMP 11 PRO (SAS Institute, Cary, NC, USA).

Results

Serum FT4 and FT3 levels, FT3/FT4 ratio, APACHE II and SOFA scores, and mortality

In order to establish effective therapies for TS, we evaluated serum FT4 and FT3 levels and FT3/FT4 ratios in TS patients in relation to factors contributing to mortality and disease severity. Higher APACHE II and SOFA scores, indicating greater disease severity, were significantly correlated with patient mortality (Table 1), whereas serum FT4 and FT3 levels and FT3/FT4 ratio were not. Serum FT3 level and FT3/FT4 ratio were negatively correlated with APACHE II and SOFA scores (Table 1 and Figure S1), whereas FT4 level had no correlation with these scores. These findings suggested that T4 to T3 conversion was inhibited in patients with severe disease.

In addition to thyrotoxicosis, 248 patients had precipitating factors as we previously reported.[2] As shown in Supplementary Table 1, the mortality of patients with these factors was similar to that of patients without them. Only APACHE II score was significantly higher in these patients, but SOFA score, levels of FT4 and FT3 or FT3/FT4 ratio showed no difference (Tables S1 and S2). The mortality of TS patients with infection (89 patients) was not significantly different from that of patients without infection (Table S1), either. Although the levels of FT3 and FT3/FT4 ratio were significantly lower in TS patients after non-thyroidal surgery (eight patients), other parameters showed no significant difference. FT4 levels were significantly higher in patients after radioactive iodine therapy (six patients), but other parameters showed no significant difference (Table S2).

Therapeutic strategies, mortality and disease severity

More than half of the patients were treated with a combination of ATDs, KI and CSs (Table 2). Only eight patients were not treated with any of these drugs. The diagnostic clinical score by Burch and Wartofsky[1] ranged from 30 to 90 points in these patients, and six and two of them were judged as TS1 and TS2 by JTA criteria, respectively. The majority of these patients were not treated with ATD, KI or CSs, since thyrotoxicosis was not recognized at the time of admission or on the first visit to hospital and the critical conditions were managed without these drugs. These patients were diagnosed as TS based their thyroid hormone data reported later. However, the mortality of "None" group was very similar to that of "ATDs + KI +CSs" group (13·3% *vs* 12·5%).

We then investigated the pairwise relationships between therapeutic strategies, mortality and disease severity in patients with TS. As shown in Table 3, ATD therapy had no relationship with mortality, APACHE II score or SOFA score. The use of KI, however, was positively associated with higher APACHE II and SOFA scores, but not with mortality. Treatment with CSs was associated with significantly higher mortality and APACHE II and SOFA scores (Table 3), as well as with treatment with KI, but not with ATD therapy.

Table 1. Relationships between mortality, disease severity as assessed by APACHE II and SOFA scores, and thyroid hormone levels

	APACHE II	SOFA	FT4	FT3	FT3/FT4
Mortality	**0·1914** $P = 0·0003$	**0·2748** $P < 0·0001$	0·0847 $P = 0·1132$	0·0615 $P = 0·0627$	−0·0210 $P = 0·7028$
APACHE II	–	**0·5500** $P < 0·0001$	−0·0520 $P = 0·3364$	**−0·1984** $P = 0·0003$	**−0·2237** $P < 0·0001$
SOFA		–	−0·0418 $P = 0·4393$	**−0·1418** $P = 0·0101$	**−0·1631** $P = 0·0031$
FT4			–	**0·6191** $P < 0·0001$	−0·0352 $P = 0·5217$
FT3				–	**0·7088** $P < 0·0001$

Data are the result of Spearman rank correlation analysis; correlation coefficient values (ρ) and P values are indicated.

Table 2. Treatment with ATDs, KI and CSs: patient numbers and mortality

	ATDs + KI + CSs	ATDs + KI	ATDs + CSs	ATDs	KI + CSs	KI	CSs	None	Sum
Total	181	94	15	33	17	5	3	8	356
Survivor	157	89	13	31	13	5	3	7	318
Non-survivor	24	5	2	2	4	0	0	1	38
Mortality (%)	13·3	5·3	13·3	6·0	23·5	0·0	0·0	12·5	10·7

ATDs, antithyroid drugs; KI, inorganic iodide; CSs, corticosteroids.

Table 3. Relationships of therapeutic strategies with mortality, APACHE II score and SOFA score

	ATDs	KI	CSs
Mortality	0·0463 $P = 0·3883$	0·0317 $P = 0·5505$	**0·1293** $P = 0·0146$
APACHE II	0·0413 $P = 0·4425$	**0·1591** $P = 0·0029$	**0·1868** $P = 0·0005$
SOFA	0·0726 $P = 0·1769$	**0·1901** $P = 0·0004$	**0·1646** $P = 0·0021$
ATDs	–	**0·1441** $P = 0·0065$	0·0004 $P = 0·9933$
KI		–	**0·2752** $P < 0·0001$
CSs			–

ATDs, antithyroid drugs; KI, inorganic iodide; CSs, corticosteroids.

ATD treatment

Of the 356 TS patients, 323 were treated with ATDs and 33 were not (Table 2), with no statistically significant difference ($P = 0·2674$, Fisher's exact test) in mortality between the two groups (33 of 323 *vs* 5 of 33) (Tables 3 and 4). Similarly, APACHE II and SOFA scores did not differ significantly between patients treated with and without ATDs ($P = 0·4417$ and $P = 0·1765$, respectively, Wilcoxon/Kruskal–Wallis test). FT4 and FT3 levels and FT3/FT4 ratio were also not affected by ATD therapy, as shown in Table S3.

Regarding types of ATDs, 276 patients were treated with methimazole (MMI) alone (85·4%), 45 were treated with propylthiouracil (PTU) alone (13·9%) and two were treated with both MMI and PTU (0·6%). APACHE II and SOFA scores in patients treated with MMI alone were not significantly different from those treated with PTU alone ($P = 0·2187$ and $P = 0·7170$, respectively, Wilcoxon/Kruskal–Wallis test). No significant difference in mortality was observed between these patients ($P = 0·5058$, Fisher's exact test) (Table 4). Serum FT4 and FT3 levels and FT3/FT4 ratio showed no differences either (Figure S2). The initial doses of MMI were significantly correlated with serum FT4 and FT3 ($r^2 = 0·067$, $P = 0·0001$ and $r^2 = 0·045$, $P = 0·0005$, respectively; Box-Cox methods), while those of PTU were not (Figure S2).

Parenteral administration is generally preferable to oral administration for the treatment of critically ill patients, and an intravenous (IV) preparation of MMI is commercially available in Japan. We therefore analyzed the clinical features of the 47 TS patients (of 278 total; 16·9%) treated with IV MMI. Both APACHE II and SOFA scores were significantly higher in patients treated with MMI injection ($P = 0·0053$ and $P = 0·0086$, respectively, Wilcoxon/Kruskal–Wallis test) (Table 4). The IV preparation was associated with a significantly higher mortality than oral administration ($P = 0·0090$), although serum FT4 and FT3 levels and FT3/FT4 ratio did not differ between these patients. Multivariate logistic regression analysis with the stepwise method revealed that APACHE II scores correlated with the choice of IV MMI administration. As for the dose of MMI, no significant difference was observed between oral and IV preparations. Stepwise multivariate regression analysis revealed that APACHE II score and FT4 level significantly contributed to the MMI dose in both preparations.

KI treatment

Of the 356 TS patients, 297 and 59 were treated with and without KI, respectively (Table 2). The doses of KI ranged between 10 and 2000 mg (median, 100 mg). Compatible with the analysis in Table 3, APACHE II and SOFA scores were significantly higher in the KI group than the non-KI group ($P = 0·0030$ and $P = 0·0004$, respectively, Wilcoxon/Kruskal–Wallis test) (Table 4), while no significant difference

Table 4. Treatment comparisons in terms of disease severity and mortality

Treatment comparisons (# of patients)	APACHE II score median (range)†	SOFA score median (range)†	Mortality (%)‡
ATD: with (323) *vs* without (33)	10 (0–33) *vs* 10 (2–37)	2 (1–20) *vs* 2 (0–11)	10·2% *vs* 15·1%
ATD: MMI only (276) *vs* PTU only (45)	10 (0–33) *vs* 10 (2–32)	2 (0–20) *vs* 2 (0–10)	10·1% *vs* 11·1%
MMI: Oral (231) *vs* IV (47)	**9 (0–33) *vs* 14 (2–33)***	**2 (0–20) *vs* 3 (0–12)***	**7·8% *vs* 21·2% ***
KI: with (297) *vs* without (59)	**10 (0–37) *vs* 7 (1–25) ***	**2 (0–6) *vs* 1 (0–7) ***	11·8% *vs* 8·5%
CS: with (216) *vs* without (140)	**10 (0–37) *vs* 8 (1–33)***	**2 (0–12) *vs* 2 (0–20) ***	**13·9% *vs* 5·7%***
Beta-AA: with (286) *vs* without (51)	10 (0–37) *vs* 10 (1–33)	2 (0–20) *vs* 2 (0–9)	11·2% *vs* 7·8%
Beta-AA: Oral (228) *vs* IV (58)	**9 (0–37) *vs* 15 (2–33)***	**2 (0–20) *vs* 4 (0–12)***	10·1% *vs* 15·5%
Non-selective beta-AA: with (193) *vs* without (93)	10 (0–33) *vs* 10 (2–37)	2 (0–12) *vs* 2 (0–20)	**14·5% *vs* 4·3% ***

ATD, antithyroid drug; MMI, methimazole; PTU, propylthiouracil; IV, intravenous; KI, inorganic iodide; CS, corticosteroid; beta-AA, beta-adrenergic antagonist.
*$P < 0.05$.
†Compared by Wilcoxon/Kruskal–Wallis test.
‡Compared by Fisher's exact test.

was observed in the mortality of these patients ($P = 0.3704$, Fisher's exact test). These findings suggest that KI treatment may reduce the mortality of patients with severe TS. The analysis of the relationships revealed that FT4 and FT3 levels were associated with KI therapy (Table S3). Stepwise multivariate logistic regression analysis showed that SOFA score and FT3 level contributed to the choice of KI therapy, while none of the clinical parameters, namely mortality, APACHE II score, SOFA score, and thyroid hormone levels, contributed to KI dose.

CS treatment

Two hundred sixteen and 140 patients were treated with and without CSs, respectively (Table 2). The CS doses initially administered ranged from 30 to 1200 mg for hydrocortisone, from 5 to 60 mg for prednisolone, from 1·5 to 16 mg for dexamethasone, and from 80 to 1000 mg for methylprednisolone. The doses of prednisolone, dexamethasone and methylprednisolone were converted to hydrocortisone-equivalent doses (the ratios of hydrocortisone to prednisolone, dexamethasone and methylprednisolone were 4, 25 and 5, respectively). The CS doses converted to hydrocortisone ranged from 20 to 5000 mg (median, 200 mg) (Figure S3). Treatment with CSs showed no relation to ATD therapy ($P = 0.5665$, Fisher's exact test) but was positively associated with KI therapy ($P < 0.0001$; Spearman rank correlation analysis) (Table 3). APACHE II and SOFA scores were higher in patients treated with CSs than in those who were not ($P = 0.0005$ and $P = 0.0022$, respectively, Wilcoxon/Kruskal–Wallis test) (Table 4). Mortality was also higher in those receiving CSs ($P = 0.0099$, Fisher's exact test). FT3 level correlated with choice of CS therapy, while FT4 level and FT3/FT4 ratio showed no correlation. Stepwise multivariate analysis of factors contributing to the use of CSs revealed that APACHE II score significantly contributed to the use of CS. However, other factors including outcome as death and serum thyroid hormone levels did not contribute to the choice of CS therapy. Multivariate analysis showed that doses of CSs correlated with SOFA score but not with APACHE II score, and were inversely correlated with FT3 level. Stepwise multivariate analysis indicated that both FT3 level and SOFA score significantly contributed to CS dose.

Beta-AA treatment

Two hundred eighty-six and 51 TS patients were treated with and without beta-AAs, respectively (Tables 4 and S4). In the remaining 19 patients, it was unknown whether beta-AAs were used or not. Treatment with beta-AAs was not associated with any significant differences in APACHE II score, SOFA score or mortality.

We first investigated the selectivity of beta-AAs administered to TS patients. Sixty-six patients were treated only with selective $beta_1$-AAs (atenolol, bisoprolol, betaxolol, metoprolol and landiolol), 3 were treated with both a selective and a non-selective $beta_1$-AA (landiolol with propranolol) and 190 were treated only with non-selective beta-AAs (propranolol and carteolol) (Tables 4 and S4). Eighteen patients were treated with alpha/beta-AAs (carvedilol and arotinolol), while in nine patients the type of beta-AA used was unknown. Stepwise multivariate logistic regression analysis using Box-Cox–transformed APACHE II and SOFA scores, in addition to the types of beta-AAs, revealed that SOFA score, treatment with non-selective beta-AAs alone, and combined treatment with non-selective beta-AAs and selective $beta_1$-AAs contributed to mortality. Consistent with this analysis, the mortality of patients treated with non-selective $beta_1$-AAs with or without selective $beta_1$-AAs was significantly higher than those treated with other types of beta-AAs (28 of 193 *vs* 4 of 93; $P = 0.0063$, Fisher's one-tailed test). However, no significant differences in APACHE II or SOFA score were observed among patients treated with non-selective $beta_1$-AAs and those treated with other types of beta-AAs ($P > 0.05$, Wilcoxon/Kruskal–Wallis test) (Table 4). Similarly, these two groups showed no significant differences in serum FT4 or FT3 level or FT3/FT4 ratio.

Beta-AAs were administered parenterally in 58 of the 286 patients treated with these drugs (Table 5). Forty-six patients

Table 5. Mortality of TS patients treated with oral or intravenous beta-AA preparations

Selectivity of beta-AA	Oral administration	Intravenous administration	Total
Non-selective beta-AA	20/144 (13·89%)	7/46 (15·22%)	27/190 (14·21%)
Non-selective + Selective beta1-AA	0/0 (0·00%)	1/3 (33·33%)	1/3 (33·33%)
Selective beta1-AA	3/60 (5·00%)	1/6 (16·67%)	4/66 (6·06%)
Alpha & beta-AA	0/18 (0·00%)	0/0 (0·00%)	0/18 (0·00%)
Unknown	0/6 (0·00%)	0/3 (0·00%)	0/9 (0·00%)
Total	23/228 (10·09%)	9/58 (15·51%)	32/286 (12·50%)

beta-AA, beta-adrenergic antagonist.

were treated with non-specific beta-AAs and five were treated with selective $beta_1$-AAs. Three patients were treated with IV selective $beta_1$-AAs together with oral non-selective beta-AAs. The kind of beta-AA was unknown in three patients. APACHE II and SOFA scores were significantly higher in patients treated with IV beta-AAs than in those who received oral preparations ($P < 0·0002$ and $P < 0·0001$, respectively, Wilcoxon/Kruskal-Wallis test) (Table 4). However, these two groups did not differ significantly in mortality (9 of 58 *vs* 23 of 228). As for the degree of CHF, 21 of 30 patients were graded as Killip class III or IV in patients treated with IV beta-AAs, compared to 39 of 113 patients treated with oral preparations only ($P = 0·0008$, Fisher's exact test) (Table S5). However, there were no significant differences between these patients in FT4 or FT3 level, FT3/FT4 ratio, or mortality. Stepwise multivariate logistic analysis using Killip classification, transformed APACHE II score, and transformed SOFA score revealed that Killip classification (III and IV) and SOFA score contributed to the choice of IV beta-AA therapy.

As CHF influences the outcome of TS patients as well as both the effectiveness and choice of beta-AAs, we analysed the relationship between mortality and type of beta-AA in patients with severe CHF. Of 12 patients in Killip Class IV, 4 who were treated with a non-selective beta-AA (propranolol) died, a 33·3% mortality rate (Table 6). Although there was no significant difference in mortality between patients treated with non-selective beta-AAs and those receiving other types of beta-AAs ($P = 0·1177$, Fisher's one tailed exact test), stepwise multivariate logistic regression analysis using type of beta-AA, transformed APACHE II score, and transformed SOFA score revealed that use of propranolol without landiolol significantly contributed to increased mortality (odds ratio, 7·6381; $r^2 = 0·2189$, $P = 0·0386$).

The doses of beta-AAs used for the treatment of TS, including oral propranolol, IV propranolol, metoprolol, atenolol and landiolol, did not differ significantly between survivors and non-survivors (Table S6).

Table 6. Mortality of beta-AA-treated patients with congestive heart failure graded as Killip's class IV

Selectivity of beta-AA	Oral administration	Intravenous administration	Total
Non-selective beta-AA	3/8 (37·50%)	1/4 (25·00%)	4/12 (33·33%)
Non-selective + Selective beta1-AA	0/0 (0·00%)	0/1 (0·00%)	0/1 (0·00%)
Selective beta1-AA	0/1 (0·00%)	0/1 (0·00%)	0/2 (0·00%)
Alpha & beta-AA	0/3 (0·00%)	0/0 (0·00%)	0/3 (0·00%)
Unknown	0/0 (0·00%)	0/1 (0·00%)	0/1 (0·00%)
Total	3/12 (25·00%)	1/7 (14·29%)	4/19 (21·05%)

beta-AA, beta-adrenergic antagonist.

Antipyretic agents

Ninety-six patients received antipyretic agents while 225 did not. In the remaining 35 patients, it was unknown whether antipyretic agents were used. Body temperature was significantly higher in patients who received antipyretic agents than in those who did not (38·56 ± 1·16 °C *vs* 37·86 ± 1·24 °C, $P < 0·05$, unpaired Student's *t*-test). However, there were no significant differences between these patients in terms of mortality, APACHE II or SOFA score, FT4 or FT3 level or FT3/FT4 ratio.

We also compared the influence of acetaminophen with that of other antipyretic agents because some of these agents have been shown to increase free thyroid hormone levels in TS patients.[6] Forty-nine patients were treated with acetaminophen, while 40 were not. In seven patients, the type of antipyretic agent used was unknown. No significant difference in body temperature was observed between patients treated with acetaminophen and those treated with other antipyretic agents (38·72 ± 1·06 °C *vs* 38·43 ± 1·08 °C), and there were also no significant differences in mortality, APACHE II score or SOFA score.

Extraordinary interventions and treatments for cardiovascular collapse

Because organ-specific extraordinary intervention is essential in critically ill patients such as those with TS, we analysed the nationwide surveys' data on these interventions. Sixteen patients were treated with plasmapheresis, which may decrease circulating thyroid hormone levels as well as levels of anti-TSH receptor antibody and proinflammatory cytokines.[7] These patients demonstrated significantly worse mortality (6/16) and disease severity (as assessed by APACHE II and SOFA scores) compared to the rest of the patients (Table S7). Both mortality and disease severity were also significantly greater in 90 patients with respiratory failure who underwent endotracheal intubation or administration of biphasic positive airway pressure (BIPAP), and in 20 patients who underwent haemodialysis. Cardiopulmonary bypass was used in nine patients. While their mortality was significantly higher than in the remaining patients, they did not differ in terms of disease severity (Table S7). Neither FT4 and FT3 levels

nor FT4/FT3 ratio were significantly different in the patients treated with these interventions.

Cardiovascular collapse was identified as a major contributor to TS-related mortality in our nationwide surveys.[2] The use of catecholamines as vasopressor agents was associated with significantly increased mortality and disease severity (Table S8), as well as with lower FT3 level and FT3/FT4 ratio. The use of diuretics or human alpha-atrial natriuretic peptide (hANP) was significantly related to mortality, but not to disease severity. The use of digoxin and verapamil was not related to either mortality or disease severity (Table S8). No significant relationship was observed between thyroid hormone levels and the use of diuretics, hANP, digoxin or verapamil (data not shown).

Discussion

The present retrospective analysis of the management of TS as reported in nationwide surveys revealed that combined modality therapy, including ATDs, was employed in 81·4% of patients. In addition, MMI rather than PTU was used in 86·1% of TS patients in Japan. A clinical trial recently conducted in Japan revealed that the treatment of non-storm Graves' hyperthyroidism with MMI could improve thyrotoxicosis more rapidly than PTU.[8] The preferential use of MMI in the treatment of TS in Japan may be reasonable, because according to the recommendation by the Japan Thyroid Association MMI is used in the treatment of non-storm Graves' disease more frequently than PTU.[8]

As for the choice of ATD, the guidelines of the American Thyroid Association and American Association of Clinical Endocrinologists recommend the use of PTU, because it blocks the conversion of T4 to T3 in addition to inhibiting new hormone synthesis.[9] However, the present analyses demonstrated that both APACHE II and SOFA scores, which significantly correlated with the mortality of TS patients, inversely correlated with FT3 level and FT3/FT4 ratio, suggesting that the conversion of T4 to T3 had been already inhibited in patients with severe TS.

This study also showed that disease severity was the primary factor influencing the choice of IV MMI administration. Although IV preparations of MMI are commercially available in Japan, in other countries, including the United States and United Kingdom, they are not. Hodak *et al.*, however, reported a method for preparing MMI injections and the effectiveness of IV administration of MMI in refractory TS patients.[10] MMI dose correlated with FT4 level as well as SOFA score, and multivariate analysis revealed that FT4 level was an independent factor influencing both MMI dosing and SOFA score. Therefore, serum FT4 levels might influence physicians' choices of MMI doses.

Like MMI, KI was also used in severe patients; however, the mortality of patients who were treated with KI showed no difference compared to those who were not. CSs were also used in patients with severe disease and severity of patients was the choice of CS therapy. The doses of CSs inversely correlated with FT3 level and FT3/FT4 ratio. However, only severity of patients contributed to the mortality of patients according to the stepwise multivariate analysis and the contribution of CS therapy to the mortality was not significant. Although sufficient evidence for that the use of CS and KI was effective for reducing the mortality of TS patients was not obtained from this study, enough doses of CS with KI might be used in patients with severe conditions, along with other modalities such as high-dose ATD.

Although the mortality of patients treated with non-selective beta-AAs did not differ significantly from those receiving other types of beta-AAs, 4 of 12 patients with CHF graded as Killip's class IV treated with non-selective beta-AA died. In addition, stepwise multivariate logistic regression analysis revealed that the use of propranolol without landiolol significantly contributed to the increased mortality of these patients (odds ratio, 7·6381; $r^2 = 0{\cdot}2189$, $P = 0{\cdot}0386$). The use of non-selective beta-AAs, particularly propanol, will require caution.

With regard to antipyretic agents, we could not demonstrate the superiority of acetaminophen over other antipyretics. However, since acetaminophen does not affect free thyroid hormone levels, it may be used to cool patients with TS and thus improve their general condition.[6]

Extraordinary interventions are essential for the management of decompensated organ function. The mortality of patients treated with therapeutic plasmapheresis, endotracheal intubation, hemodialysis, or cardiopulmonary bypass was higher than that of TS patients who did not undergo these procedures, probably because their conditions were more severe at the outset. The administration of catecholamines was also significantly associated with higher mortality probably due to greater disease severity. The limitation of the present study is retrospectively analysed. Retrospective analysis may have more potential sources of bias and confounding factors than prospective one. Further prospective interventional studies using more patients are obviously needed to determine whether these modalities are effective in the management of severe TS.

Although above findings and following conclusions were based on the analysis from observational study, the nationwide surveys in Japan demonstrated that the MMI was the chosen ATD in 86·1% of patients. PTU showed no superiority over MMI, perhaps because conversion of T4 to T3 in patients with severe TS might already be decreased. Larger doses or IV administration of MMI by itself did not improve outcomes, but simultaneous administration of KI may help by decreasing thyroxine levels. It is also suggested that administration of $beta_1$-selective AAs may be effective in patients with severe CHF. Therefore, multimodal treatment including KI, CSs, selective $beta_1$-AAs and antipyretic agents, in addition to ATD, may be preferable to improve the outcomes of patients with severe TS.

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Conflict of interest

Authors have no conflict of interest.

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Clinical Study

Liver Dysfunction Associated with Intravenous Methylprednisolone Pulse Therapy in Patients with Graves' Orbitopathy

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Intravenous methylprednisolone (IVMP) pulse therapy is the first-line treatment for the active phase of moderate to severe Graves' orbitopathy (GO). However, acute and severe liver damage has been reported during and after IVMP therapy. In this retrospective study, we investigated risk factors for liver dysfunction during and after IVMP therapy based on 175 Japanese patients with moderate to severe GO and treated at our center between 2003 and 2011. The results showed that seven patients developed severe liver dysfunction with elevated serum alanine aminotransferase (ALT > 300 U/L). Mild (40–100 U/L) and moderate (100–300 U/L) increases of ALT occurred in 62 patients (35%) and 10 patients (6%), respectively. Liver dysfunction was more frequently observed in males, in patients receiving high-dose methylprednisolone, and patients aged over 50 years. Preexistent viral hepatitis was significantly associated with liver dysfunction (65% in patients positive for hepatitis B core antibody and patients positive for hepatitis C virus antibodies). Our study confirmed the association of liver dysfunction with IVMP during and after treatment. It suggests that, in patients with GO, evaluation of preexisting risk factors—including viral hepatitis—and careful weekly monitoring of liver function during IVMP therapy and monthly thereafter for 12 months are warranted.

1. Introduction

Intravenous methylprednisolone (IVMP) pulse therapy is the first-line treatment for patients with active-phase moderate to severe Graves' orbitopathy (GO) [1]. IVMP is widely used because it is more effective and better tolerated than oral steroids [2, 3]. However, acute and severe liver damage has been reported after pulse therapy, with a roughly estimated morbidity and mortality of 0.8% and 0.3%, respectively [4]. The cumulative dose of IVMP in four patients with fatal liver failure was 8.3–15 g [4, 5] but slightly higher in three patients who died (10.8 ± 3.6 g) than in four patients who recovered (7.9 ± 2.9 g) [4]. Therefore, the European Group of Graves' Orbitopathy (EUGOGO) now recommends that the cumulative dose of MP should be less than 8 g [1, 6].

The causes of IVMP-associated liver damage are incompletely understood. Thus, the aim of the present study was to investigate the risk factors for liver dysfunction during and after IVMP pulse therapy for GO.

2. Materials and Methods

2.1. Study Population. This was a retrospective study of 175 Japanese patients with moderate to severe GO who were treated in one center from 2003 to 2013. The mean age of the 118 females and 57 males was 51.7 ± 15.5 years. They

had been admitted to our university hospital for GO and were treated with an intravenous injection of 1 g of MP daily for 3 consecutive days per week, repeated for three to six cycles, and followed by a tapering dose of oral prednisolone (20 mg/day for 4 weeks, 15 mg/day for 2 weeks, 10 mg/day for 2 weeks, 5 mg/day for 2 weeks, and 5 mg/2 days for 2 weeks). The daily dose of MP was reduced to 0.5 g except in cases with optic neuropathy after the recommendation by EUGOGO in 2008 [1]. Heart rate and ECG were monitored during the intravenous infusion of MP, administered every 2-3 h. In addition, 100 of the 175 patients were treated with orbital irradiation therapy (2 Gy/day, 10 times; total dose = 20 Gy) either during or after IVMP pulse therapy. All patients were given artificial tear drops to protect the cornea. Histamine receptor 2 antagonists or proton pump inhibitors were administered for all the cases. Bisphosphonates were administered in 82 patients to protect steroid-induced osteoporosis.

2.2. Biochemical Examination and Diagnosis of Thyroid Diseases. Thyroid diseases were diagnosed by measuring serum-free triiodothyronine (FT3), free thyroxine (FT4), thyroid-stimulating hormone (TSH), thyroglobulin, anti-thyroglobulin antibody, anti-thyroid peroxidase antibody, and anti-thyrotropin receptor antibodies (TRAbs). TRAbs were measured using three commercial kits: TRAb 1st generation (TRAb Cosmic III, Cosmic, Tokyo, Japan), TRAb 2nd generation, human TRAb (Yamasa, Tokyo, Japan) and TSAb (Yamasa TSAb kit), and thyroid ^{123}I uptake on ^{123}I scintigraphy. Orbitopathy was estimated by ophthalmologists using a modified NOSPECS classification [7] and the clinical activity score (CAS) [1]. Magnetic resonance imaging was also performed before and after pulse therapy, as previously reported [8]. Graves' disease was detected in 139 patients, 29 patients were euthyroid without a history of Graves' disease, and 7 patients had hypothyroidism without a history of Graves' disease. Orbitopathy with NOSPECS class VI was determined in 8 patients, class V in 3 patients, class IV in 139 patients, class III in 23 patients, and class II in 2 patients.

Liver function tests were performed once a week during pulse therapy and repeated at every visit thereafter for 1 year. Hepatitis B surface antigen (HBsAg), hepatitis B surface antibody (HBsAb), hepatitis B core antibody (HBcAb), and hepatitis C virus antibody (HCVAb) were measured before pulse therapy. The one patient who was HBsAg-positive consulted with a hepatologist, who prescribed 0.5 mg of entecavir, during and after pulse therapy. In addition, 43 patients were HBcAb-positive and 17 were HCVAb-positive. They likewise consulted with hepatologists before pulse therapy. Serum HBV-DNA was not detected in any patient. HBV-DNA and HCV-RNA were also monitored. Liver dysfunction was classified based on serum alanine aminotransferase (ALT) and total bilirubin levels as mild (ALT: 40–100 U/L), moderate (ALT: 100–300 U/L), or severe (ALT > 300 U/L or total bilirubin: >3 mg/dL).

2.3. Clinical Characteristics of Patients with GO. Female and male GO patients significantly differed with respect to age, body mass index (BMI), smoking habits, alcohol habits, and HBcAb positivity before pulse therapy (Table 1). Human TRAb and TSAb levels were significantly higher in female than in male patients before IVMP pulse therapy.

Table 1: Clinical characteristic of patients with Graves' orbitopathy.

	Total	Male	Female	Male versus female
	$N = 175$	$N = 57$	$N = 118$	P value
Age (yr)	51.7 ± 15.5	55.9 ± 16.3	49.6 ± 11.9	0.012
BMI (kg/m^2)	22.5 ± 3.7	23.4 ± 3.8	22.1 ± 2.7	0.021
HBcAb (+)	43 (25%)	21 (37%)	22 (19%)	0.0102
HCVAb (+)	17 (10%)	9 (16%)	8 (7%)	0.0677
HBcAb (−) HCVAb (−)	122 (70%)	31 (54%)	91 (77%)	—
IVMP > 8 g	118 (67%)	40 (70%)	78 (66%)	0.5884
Smoking (+)	57 (33%)	27 (47%)	30 (25%)	0.0041
Alcohol (+)	48 (27%)	28 (49%)	20 (17%)	0.0001
CAS	3.1 ± 1.7	3.2 ± 1.8	3.0 ± 1.7	0.8263
TRAb (%)	29.3 ± 27.3	25.8 ± 26.1	30.8 ± 27.8	0.2987
hTRAb (IU/L)	17.7 ± 44.4	8.10 ± 11.4	21.9 ± 52.2	0.0407
TSAb (%)	1262 ± 1480	931 ± 1229	1404 ± 1559	0.0346
				Mean ± SD

BMI, body mass index; HBcAb, anti-hepatitis B core antibody; HCVAb, anti-hepatitis C virus antibody; IVMP, intravenous injection of methylprednisolone; CAS, clinical activity score; TRAb, anti-thyrotrophin antibody; hTRAb, human TRAb; TSAb, thyroid stimulating antibody.

2.4. Statistical Analysis. Statistical analysis was performed using JMP Pro software (version 11.0.0, SAS Institute, USA). Data are expressed as the mean ± standard deviation. Statistical comparisons were performed using Student's t-test, one-way ANOVA, or Mann-Whitney U test for the analysis of continuous variables. The χ^2 test or Fisher's exact probability test was used to analyze 2 × 2 or 2 × 4 tables. Multivariate logistic regression analyses were carried out to evaluate the risk factors for liver dysfunction, using exact method (LogXact, Cytel Inc., USA). In all tests, a P value < 0.05 was considered to indicate significance.

3. Results

3.1. Liver Dysfunction. Increases of ALT during and/or after pulse therapy were detected in 79 patients (45%) (Table 2). Mild (ALT 40–100 U/L), moderate (ALT 100–300 U/L), and severe (ALT > 300 U/L) increases of serum ALT were measured in 62 patients (35%), 10 patients (6%), and 7 patients (4%), respectively. All patients with severe liver dysfunction were female and most of them were female. Two were HBcAb positive and one of them developed jaundice with ALT 945 U/L, one day after the cessation of IVMP. Her total bilirubin 8 weeks after the cessation of IVMP was 18.45 mg/dL. Single and cumulative doses of IVMP were 0.5 g and 3.5 g, respectively. HBV-DNA was not detected in patients with severe liver dysfunction. Anti-nuclear and

Table 2: Risk factors associated with liver dysfunction during and/or after intravenous methylprednisolone pulse therapy for Graves' orbitopathy.

	Number of patients	Liver dysfunction ALT (IU/L)				Univariate	Multivariate analysis		
						Fisher's exact probability test	Multinomial logit model for an unordered response		
							95% CI		2 sided
		<40	40–100	100–300	>300	2 × 4	Lower	Upper	*P* value
Total	175	96 (55%)	62 (35%)	10 (6%)	7 (4%)				
Male	57	21 (37%)	31 (54%)	5 (9%)	0 (0%)	$\chi^2 = 18.34$			
Female	118	75 (64%)	31 (26%)	5 (4%)	7 (6%)	$P = 0.000278$	0.1348	1.646	0.01895
HBcA b (+)	43	15 (35%)	24 (56%)	2 (5%)	2 (5%)	$\chi^2 = 11.01$ $P = 0.01125$			
HCVAb (+)	17	6 (35%)	6 (35%)	4 (24%)	1 (6%)	$\chi^2 = 11.94$ $P = 0.01132$			
HBcAb (+) and/or HCVAb (+)	53	19 (36%)	26 (49%)	5 (9%)	3 (6%)	$\chi^2 = 11.36$ $P = 0.008867^*$	−0.245	1.417	0.1846
HBcAb (−) HCVAb (−)	122	77 (63%)	36 (30%)	5 (4%)	4 (3%)	—			
Age									
>50 yr	95	41 (43%)	45 (47%)	8 (8%)	1 (1%)	$\chi^2 = 20.72$	0.03396	1.553	0.03972
≦50 yr	80	55 (69%)	17 (21%)	2 (3%)	6 (8%)	$P = 0.00004$			
IVMP									
>8 g	118	57 (48%)	47 (40%)	9 (8%)	5 (4%)	$\chi^2 = 7.187$	0.1012	1.640	0.02426
<8 g	57	39 (89%)	15 (26%)	1 (2%)	2 (4%)	$P = 0.06052$			
Smoking									
(+)	57	26 (46%)	24 (42%)	4 (7%)	3 (5%)	$\chi^2 = 2.969$			
(−)	118	70 (59%)	38 (32%)	6 (5%)	4 (3%)	$P = 0.3887$			
Alcohol									
(+)	48	24 (50%)	19 (40%)	3 (6%)	2 (4%)	$\chi^2 = 0.6445$			
(−)	127	72 (57%)	43 (34%)	7 (6%)	5 (4%)	$P = 0.9176$			

*Compared to patients without HBcAb or HCVAb.
ALT, alanine aminotransferase; HBcAb, anti-hepatitis B core antibody; HCVAb, anti-hepatitis C virus antibody; IVMP, intravenous injection of methylprednisolone.

anti-single-stranded DNA antibodies were also negative, as were anti-smooth muscle antibody, and anti-double-stranded DNA antibody in those patients. The HBV carrier taking entecavir prescribed by the hepatologist did not show the elevation of ALT.

3.2. Factors Associated with Liver Dysfunction during or after Pulse Therapy for GO. Liver dysfunction occurred more frequently in male patients ($P < 0.0012$) and in patients over the age of 50 years ($P < 0.0009$). BMI was significantly higher in patients with mild liver dysfunction than in those without liver dysfunction ($23.3 \pm 4.33\,kg/m^2$ versus $22.0 \pm 3.39\,kg/m^2$, Student's t-test, $P = 0.043$, data not shown). Liver dysfunction was not associated with smoking or alcohol habit but it was associated with a high dose of MP (cumulative dose >8 g versus <8 g, 2 × 2 table, $\chi^2 = 6.280$, $P = 0.0122$). Preexistent viral hepatitis was significantly associated with liver dysfunction during and after pulse therapy ($P = 0.0035$). HBcAb was positive in 43 GO patients (25%) before pulse therapy. In 28 of them (65%), ALT was significantly increased ($P = 0.01125$), although in most the increase was mild. HCVAb was positive in 17 GO patients (10%) before pulse therapy. In this group, 11 patients (65%) had increased ALT levels ($P = 0.01132$).

3.3. Multivariate Logistic Regression Analysis. Multivariate logistic regression analysis showed that age, gender, and cumulative MP dose (>8 g) were associated with liver dysfunction (Table 2).

4. Discussion

Although IVMP pulse therapy is widely used as the first-line treatment for active moderate-to-severe orbitopathy, severe related side effects have been reported, the most common of which is hepatotoxicity. In the recent review by Zang et al. [9], the morbidity and mortality of GO patients treated with IVMP pulse therapy were 6.5% and 0.6%, respectively. Fatal hepatotoxicity was reported to be associated with a cumulative dose of IVMP > 8 g. In two studies, the cumulative

doses were 8.3–15 g [4, 5]. EUGOGO now recommends that the cumulative dose of IVMP does not exceed 8 g [1].

In our series of 175 patients, seven patients (4.0%) developed severe liver dysfunction. The rate of morbidity was similar to the previous report [9]. The cumulative doses of MP were more than 8 g in 5 out of seven patients. However, single and cumulative doses of IVMP in a patient with jaundice were 0.5 g and 3.5 g, respectively.

Koga et al. [10] reported two fatal cases of HBV carriers after corticosteroid therapy, and the frequent reactivation of HBV after immune suppressive therapy, such as with rituximab, was noted [11]. Therefore, in GO patients during IVMP therapy, the reactivation of HBV leading to acute liver failure remains a concern, although its occurrence is rare [4, 9, 12, 13]. Indeed, in the series of Le Moli et al. [12], none of the 27 patients with GO suffered serious liver damage. Wichary and Gasińska [13] concluded that the risk of HBV reactivation is low, based on their experience with 30 patients treated with IVMP. Those studies suggest that it is difficult to predict who will develop severe liver failure, such that it is important to carefully monitor patients during and after IVMP therapy.

Our study identified risk factors for mild to moderate liver dysfunction during and after IVMP therapy for GO. Among male patients, a mild elevation of ALT was associated with a cumulative dose of IVMP > 8 g; in female patients, a moderate elevation of ALT was associated with age over 50 years. A history of HBV and HCV infection also contributed to a high prevalence of hepatotoxicity, as approximately 25% of our GO patients were HBcAb-positive and 10% were HCVAb-positive before pulse therapy. Within this group, 65% had increased ALT levels during and/or after pulse therapy. Multivariate logistic regression analysis showed that gender, age, and cumulative dose of MP were associated with liver dysfunction. Our study in Japanese patients suggests that viral hepatitis, gender, age, and cumulative dose are predisposing risk factors for hepatotoxicity during and after IVMP therapy. The current study also supports recommendations of a cumulative dose of MP < 8 g. However, as even this dose may not be completely safe, careful monitoring of GO patients receiving IVMP is recommended both during and 12 months after therapy.

Although the mechanisms of mild to severe hepatotoxicity remain unclear, reactivation of viral hepatitis [4, 10, 11], a direct toxicity of MP [12–14], and exacerbation of autoimmune hepatitis have been suggested [15, 16]. Le Moli et al. [12] reported that mild elevations in liver enzymes following IVMP were dose dependent. The toxic effect of glucocorticoids on hepatocytes, leading to drug-induced steatohepatitis, is thought to involve mitochondrial injury because of the impaired β-oxidation of fatty acids, with subsequent generation of reactive oxygen species and ATP depletion [17].

Salvi et al. [15] and Marinò et al. [16] reported the exacerbation of autoimmune hepatitis with severe liver dysfunction during IVMP therapy. In our series, two patients were positive for antinuclear and anti-smooth muscle antibodies but in both cases liver dysfunction was mild.

The drug-drug interaction may be another possible mechanism of liver dysfunction [18]. None of patients received aspirin in combination of Ramipril or clopidogrel.

There were several limitations to this study. First, it was retrospective in design. However, it allowed us to assess the effect of single and cumulative doses of IVMP, because in line with the EUGOGO's recommendation we reduced the single dose of IVMP from 1 g to 0.5 g. Another limitation of the study was the small number of patients, which prevented definite conclusions because of the low incidence of severe liver dysfunction. Additionally, no histopathological examinations were done and the effectiveness of IVMP for GO was not evaluated. Therefore, further prospective studies are indicated to assess the hepatotoxicity of IVMP during and after pulse therapy for GO.

In conclusion, liver dysfunction is frequently associated with pulse therapy for GO, both during and after treatment. Our study supports the careful evaluation of preexisting risk factors (especially viral hepatitis, age, gender, body mass index, and smoking history) before initiating IVMP therapy in GO patients. In these patients, strict monitoring of liver function once a week during pulse therapy and every month thereafter for the next 12 months is warranted.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Editorial

Graves' Orbitopathy

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Graves' orbitopathy (GO) is an autoimmune disorder of the orbit that is closely associated with autoimmune thyroid diseases (AITD). Although the primary autoantigen(s) and precise mechanisms underlying the association between GO and AITD remain unclear, TSH receptors are thought to be the primary target of autoimmune reactions in GO patients. However, other antigens such as insulin-like growth factor 1 receptor, thyroglobulin, thyroid peroxidase, calcium binding protein calsequestrin, and collagen XIII are also involved in the orbital reactions in GO. This special issue is a great opportunity for the reader to learn the latest and emerging findings on the management of GO.

Recently the analytical performance and clinical utility of the functional thyroid stimulating autoantibodies (TSAb) and thyroid blocking autoantibodies (TBAb) have been extensively evaluated. In this special issue, E. Kampmann et al. performed a prospective study on the clinical relevance of the functional TSH receptor autoantibodies in a large collective of patients with GO. They noted that TSAb, not TBAb, were highly prevalent in severe and active GO. Serum TSAb levels correlated with all specific ophthalmic signs of the thyroid eye disease and mirrored the severity of GO. Thus, TSAb may be regarded as useful and reliable biomarkers for Graves' disease and associated GO.

The current first-line treatment for patients with severe and active GO is the administration of intravenous infusions of methylprednisolone pulses (IVMP). The European Group on Graves' Orbitopathy (EUGOGO) recommends single doses of 0.5 g of IVMP per day and a maximal cumulative dose of 8 g. In this issue, H. Eguchi et al. performed a retrospective study to look for risk factors of liver dysfunction during and after the IVMP therapy in a single center. Liver dysfunction was more frequently observed in males, in patients receiving high-dose methylprednisolone, and in patients aged over 50 years. Preexistent viral hepatitis was significantly associated with liver dysfunction (65% in patients positive for hepatitis B core antibody and patients positive for hepatitis C virus antibodies). Therefore, evaluation of preexisting risk factors and careful weekly monitoring of liver function during IVMP therapy and monthly thereafter for one year are warranted.

Also in this issue, M. Lin et al. introduced in a pilot and open study the subantimicrobial dose of doxycycline (50 mg daily for 12 wks.) for patients with moderate to severe and active GO. Eight of 13 patients showed improvement at 24 wks.; unfortunately this study lacks a control group. Since the subantimicrobial dose of doxycycline displays an anti-inflammatory and immunomodulatory function, it might serve as a new promising therapeutic strategy for GO. Future multicenter, double blind, randomized, controlled trials are therefore needed.

Orbital decompression surgery is indicated for rehabilitative reduction of the GO-induced exophthalmos and for restoration of the visual function in dysthyroid optic neuropathy. In this issue, N. Fichter and R. F. Guthoff performed a retrospective study and proposed that lateral

wall decompression with orbital fat resection is the first choice in patients without disturbance of binocular functions and where moderate exophthalmos reduction is required.

Finally, the involvement of inferior rectus muscles (IRM) is a common but severe sequel in patients with GO. Recession of inextensible IRM is the first step in the correction of a restrictive hypotropia in GO. Y. Takahashi and H. Kakizaki performed a retrospective study to evaluate the predictive factors for the dose-effect relationship regarding unilateral IRM recession in GO. They found that the IRM thickness, the degree of intramuscular adipose changes, and the smoking status were relevant to it. Magnetic resonance imaging can detect both a thickened IRM and adipose change, enabling an accurate preoperative estimation.

In conclusion, the original articles in this special issue on Graves' orbitopathy provide new insights on diagnosis and management of GO. Future efforts to understand both the molecular pathology and mechanisms for the development of GO as well as to search for biomarkers of this complex disorder are indicated, and the performances of randomized clinical trials in patients with moderate to severe GO are keenly warranted.

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ORIGINAL

Serum free thyroxine levels are associated with the efficacy of weight reduction therapy in obese female patients

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Abstract. Thyroid function is strongly associated with obesity. The aim of this study is to investigate whether serum free thyroxine (FT4) and/or thyrotropin (TSH) levels are associated with the efficacy of weight reduction therapy in obese patients. We enrolled a total of 283 obese patients and cross-sectionally investigated the association of serum FT4 and/or TSH levels with metabolic features. Furthermore, in 97 obese patients who received 6-month weight reduction therapy, we assessed the relationship of serum FT4 and/or TSH levels to the efficacy of weight reduction therapy. Neither baseline serum FT4 nor TSH levels showed any correlations with body weight (BW) and body mass index (BMI) in these obese patients. However, in 57 obese female patients who underwent weight reduction therapy for six months, serum FT4 levels prior to the therapy was negatively correlated with the degrees of reduction of BW ($r = -0.354$, $p = 0.007$) and BMI ($r = -0.373$, $p = 0.004$). The correlation between baseline serum FT4 levels with the efficacy of weight reduction therapy was not observed in obese male or postmenopausal female patients. This study demonstrates that baseline serum FT4 levels are associated with weight reduction in obese female premenopausal patients. Therefore, baseline FT4 levels can be used as a clinical, noninvasive, hormonal predictor of weight reduction efficacy in obese patients.

Key words: Thyroid function, Free thyroxine, TSH, Obesity, Weight reduction therapy

OBESITY leads to several metabolic diseases, such as type 2 diabetes, hypertension, and dyslipidemia. The incidence of obesity is increasing, especially in developed countries. Thus, obesity is currently a major global threat to health [1].

Weight reduction therapy is the first step in the management of obesity, followed by a decrease of obesity-related metabolic sequelae and cardiovascular disease (CVD) complications [2-4]. However, to date, no useful method of predicting outcomes of weight reduction therapy in obese patients has been established.

It is well known that thyroid hormone is related to systemic energy expenditure, thereby influencing body weight (BW) [5, 6]. Recently, there have been an increasing number of reports regarding the relationship between thyroid function and BW [7-9]. For instance, it was reported that serum thyrotropin (TSH) levels are positively correlated to body mass index (BMI), which is calculated as weight in kilograms divided by the square of the height in meters as an index of obesity, and that serum free thyroxine (FT4) levels are negatively correlated to BMI [6, 10]. Moreover, higher TSH levels are found in severely obese subjects compared with those in nonobese ones [11].

As mentioned above, although there have been sev-

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eral studies on cross-sectional and longitudinal associations among BW, BMI, and thyroid function, no prospective studies regarding the association between thyroid function and weight changes through lifestyle intervention in obese patients have been reported. Against this background, observation of the association of thyroid function with the efficacy of weight reduction therapy may provide important evidence influencing the treatment for obesity.

In this study, we focused on the clinical significance of baseline thyroid function in weight reduction therapy. Therefore, the present study was performed as a prospective observational study in order to examine the relationship of the baseline thyroid function with weight loss during weight reduction therapy in obese Japanese patients.

Methods

Subjects

A total of 283 obese Japanese patients (Table 1) were consecutively enrolled in the outpatient clinic at the National Hospital Organization Kyoto Medical Center during the period from 2010 to 2014. We defined and recruited obese subjects with a BMI of $\geq$ 25 kg/m^2 as previously reported in Japan [12].

The exclusion criteria were a previous history of thyroid disorders including overt hypothyroidism (TSH$\geq$10μIU/mL); renal disease; severe liver dysfunction; or secondary obesity due to endocrine disorders, such as Cushing syndrome, polycystic ovary syndrome, and acromegaly. None of the patients had received anti-obesity drugs or had recently been treated with glucocorticoids, estrogen, or L-thyroxine therapy. This study protocol was approved by the ethics committee for human research at Kyoto Medical Center (#15-014) and at Tokyo Medical and Dental University (#2079). All participants provided written informed consent.

Data collection and laboratory measurements

The systolic and diastolic blood pressure (SBP and DBP) levels were measured after a 15-minute rest in the sitting position twice with an automatic electronic sphygmomanometer (BP-103i II; Nippon Colin, Komaki, Japan). Blood was taken in the morning after an overnight fast and used to determine fasting plasma glucose (FPG), glycosylated hemoglobin A1c (HbA1c), immunoreactive insulin (IRI), low-density-lipoprotein cholesterol (LDL-C), high-density-lipoprotein cholesterol (HDL-C), triglycerides (TG), FT4 and TSH according to standard procedures [4]. The value for HbA1c is estimated as a National Glycohemoglobin Standardization Program (NGSP) equivalent value (%). Serum levels of high-sensitivity C-reactive protein (hs-CRP), adiponectin, and leptin were also determined, as previously described [4, 13].

Weight reduction therapy

Ninety-seven out of 283 obese patients (40 men and 57 women, Supplemental Table 1) underwent weight reduction therapy involving lifestyle modification for 6 months and they were instructed to maintain the same

Table 1 Baseline clinical and biochemical characteristics of the study cohort

	Total	Male	Female	*p*
n	283	103	180	
Age (years)	49.1 ± 0.9	49.4 ± 1.4	49.0 ± 1.1	0.807
BW (kg)	87.0 ± 1.2	95.8 ± 2.1	81.9 ± 1.4	<0.001
BMI (kg/m^2)	33 ± 0.4	33 ± 0.6	33 ± 0.5	0.892
Waist (cm)	105 ± 0.9	108 ± 1.4	103 ± 1.1	0.011
FBS (mg/dL)	110 ± 1.9	116 ± 3.5	107 ± 2.1	0.039
HbA1c (NGSP) (%)	6.1 ± 0.1	6.2 ± 0.1	6.1 ± 0.1	0.407
IRI (μU/mL)	28.1 ± 2.3	40.5 ± 5.4	21.0 ± 1.8	<0.001
HOMA-R	8.6 ± 0.9	13.0 ± 2.2	6.0 ± 0.6	0.002
FT4 (ng/dL)	1.16 ± 0.01	1.20 ± 0.02	1.15 ± 0.01	0.025
TSH (μIU/mL)	2.15 ± 0.09	2.02 ± 0.14	2.22 ± 0.11	0.275

Data are expressed as mean ± SE. *p*-value: *t*-testing: Male *vs* Female

levels of energy intake and physical activity for the entire period, as recommended by the Japan Atherosclerosis Society's Guidelines for the Diagnosis and Treatment of Atherosclerotic Cardiovascular Diseases, as previously reported [4, 14, 15]. Before and after the therapy, we measured anthropometric and metabolic parameters for each patient. For the assessment of weight reduction, we analyzed the change in weight (post-intervention BW – baseline BW) during the therapy [16]. We defined the patients who reduced their BW by more than 5% from their baseline BW as being successful at weight reduction for 6 months, as reported previously [17]. We measured baseline FT4 and TSH in the 97 obese patients who enrolled, in order to analyze the association of baseline FT4 and TSH with the change in weight during lifestyle intervention. We also measured serum FT4 and FT3 (triiodo-thyronine) before and after the reduction therapy in some patients.

Statistical analysis

Data are presented as the mean ± SE, and $p < 0.05$ was considered statistically significant. Data for all quantitative variables were normally distributed. A two-tailed, unpaired *t*-test was used to assess differences between the two groups before and after treatment for continuous and categorical variables. Pearson's correlation coefficients were used to investigate the correlations between the baseline thyroid function and baseline metabolic parameter values, and the correlations between the change in weight and the baseline thyroid function and baseline metabolic parameter values. All statistical analyses were performed using SPSS 12.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results

Baseline clinical characteristics of obese patients

Table 1 summarizes the characteristics of the study cohort of 283 obese patients. There was no significant difference in the baseline serum TSH levels between the male and the female patients. However, BW, Waist, FBS, IRI, HOMA-R and FT4 levels in the male patients were significantly higher than those in the female patients.

Baseline correlation among body weight, BMI, and thyroid function

Neither BW nor BMI showed significant correlations with serum baseline FT4 and TSH levels in the patients irrespective of sex (Table 2).

Effect of body weight reduction on metabolic parameters in obese patients

Ninety-seven out of 283 obese patients had received weight reduction therapy for 6 months, according to the protocol as previously reported [4, 14]. In those, BW, BMI, Waist, SBP, DBP, FBS, HbA1c (NGSP), IRI, HOMA-R, AST, ALT, and eGFR were significantly decreased after the 6-month weight reduction therapy (Supplemental Table 1). In addition, γ-GTP, TC, LDL-C, BUN, and hs-CRP in the male patients and TG, HDL-C, Cre, and serum leptin levels in the female patients were significantly decreased after the 6-month weight reduction therapy (Supplemental Table 1). However, serum adiponectin levels were not significantly different after the therapy both in the male and the female patients (Supplemental Table 1).

Table 2 Correlation of baseline clinical and metabolic parameters with baseline serum FT4 and TSH levels

	Male				Female			
	FT4		TSH		FT4		TSH	
	r	*p*	r	*p*	r	*p*	r	*p*
Age (years)	-0.212	0.032	-0.014	0.891	-0.119	0.111	-0.042	0.575
BW (kg)	0.041	0.683	0.146	0.140	0.109	0.147	0.043	0.563
BMI (kg/m^2)	0.072	0.473	0.161	0.104	0.087	0.248	0.097	0.199
Waist (cm)	0.005	0.961	0.149	0.135	0.107	0.158	-0.004	0.953
FBS (mg/dL)	-0.102	0.306	-0.115	0.247	0.094	0.207	0.000	1.000
HbA1c (NGSP) (%)	-0.122	0.219	-0.099	0.318	0.114	0.129	0.005	0.946
IRI (μU/mL)	-0.108	0.276	0.058	0.563	0.078	0.297	0.048	0.525
HOMA-R	-0.100	0.316	0.017	0.866	0.100	0.182	0.025	0.736

Serum FT4 and FT3 levels before and after the 6-month weight reduction therapy

We measured serum FT4 and FT3 levels in some patients who underwent the 6-month weight reduction therapy before and after the tharapy. Serum FT4 levels were not significantly different before and after the therapy both in the male and the female patients (Table 3A). On the other hand, serum FT3 levels were significantly different in the female but not in the male patients before and after the therapy (Table 3B).

Correlations of changes of body weight and BMI with baseline serum FT4 levels

The changes in BW, BMI and Waist after the 6-month weight reduction therapy showed significant negative correlations with baseline serum FT4 levels in the female, but not in the male patients (Table 4). We categorized the fifty-seven female patients into 6 groups according to the baseline serum FT4 levels with 0.1pg/mL increments and the trend test by analysis of variance (ANOVA) revealed that the changes in BW and BMI were significantly correlated with the baseline serum FT4 levels among the groups, which validated the correlation (Supplemental Table 2).

Furthermore, in the female patients who were successful at achieving weight reduction, baseline serum FT4 levels but not TSH levels were significantly correlated to the changes in BW, BMI, and Waist (Table 5).

On the other hand, the baseline FT3 levels were not correlated with the changes in BW, BMI and Waist after the 6-month weight reduction therapy both in the male and the female patients (Supplemental Table 3).

Since it is well known that menopause is associated the thyroid function in women [18], we categorized the fifty-seven female patients into two groups, one is premenopausal (n=29) and the other one is postmenopausal (n=28). Interestingly, significant negative correlations of the changes in BW, BMI and Waist after the 6-month weight reduction therapy with baseline serum FT4 levels were found in the premenopausal, but not in the postmenopausal patients, suggesting that female sex hormone might be related to the correlation (Table 6). In the female patients who were successful at achieving weight reduction, the changes in BW, BMI and Waist after the 6-month weight reduction therapy showed significant negative correlations with baseline serum FT4 levels only in the premenopausal patients (Supplemental Table 4).

Table 3 Serum FT4 (ng/dL) (A) and FT3 (pg/mL) (B) levels in some patients who underwent the 6-month weight reduction therapy.

A	Baseline	After 6 months	*p*
Total (n=41)	1.30 ± 0.03	1.33 ± 0.04	0.503
Male (n=14)	1.29 ± 0.05	1.38 ± 0.08	0.334
Female (n=27)	1.31 ± 0.04	1.30 ± 0.04	0.638
B	**Baseline**	**After 6 months**	***p***
Total (n=43)	3.32 ± 0.07	3.25 ± 0.08	0.354
Male (n=14)	3.43 ± 0.14	3.54 ± 0.21	0.557
Female (n=29)	3.27 ± 0.08	3.10 ± 0.06	0.036

Data are expressed as mean ± SE. *p*-value: *t*-testing: Baseline *vs* After 6 months

Table 4 Correlations of changes of clinical and metabolic parameters with baseline serum FT4 and TSH levels after the 6-month weight reduction therapy

	Male (n=40)				Female (n=57)			
	FT4		TSH		FT4		TSH	
	r	*p*	r	*p*	r	*p*	r	*p*
ΔBW	0.104	0.524	-0.131	0.421	-0.354	0.007	0.139	0.304
ΔBMI	0.082	0.617	-0.141	0.386	-0.373	0.004	0.163	0.227
ΔWaist	0.143	0.379	-0.071	0.664	-0.265	0.046	0.100	0.459
ΔFBS	0.014	0.932	0.008	0.961	0.092	0.496	0.017	0.901
ΔHbA1c (NGSP)	0.129	0.427	0.042	0.797	-0.032	0.813	0.138	0.307
ΔIRI	-0.020	0.913	-0.165	0.350	-0.304	0.033	-0.230	0.112
ΔHOMA-R	-0.043	0.811	-0.165	0.352	-0.288	0.045	-0.158	0.280

Table 5 Correlations of changes of clinical and metabolic parameters with baseline serum FT4 and TSH levels In those who reduced their BW by more than 5% from their baseline BW after the 6-month weight reduction therapy

	Male (n=23)				Female (n=36)			
	FT4		TSH		FT4		TSH	
	r	*p*	r	*p*	r	*p*	r	*p*
ΔBW	0.210	0.337	0.176	0.422	-0.501	0.002	0.096	0.579
ΔBMI	0.177	0.419	0.186	0.396	-0.546	0.002	0.099	0.565
ΔWaist	0.013	0.954	0.013	0.954	-0.378	0.023	0.168	0.328
ΔFBS	0.211	0.335	0.118	0.590	0.138	0.421	-0.030	0.864
ΔHbA1c (NGSP)	0.124	0.572	0.131	0.550	-0.007	0.966	0.094	0.587
ΔIRI	0.105	0.670	-0.127	0.606	-0.365	0.037	-0.232	0.194
ΔHOMA-R	0.146	0.551	-0.125	0.610	-0.349	0.047	-0.154	0.392

Table 6 Correlations of changes of clinical and metabolic parameters with baseline serum FT4 and TSH levels in pre- and post-menopausal females after the 6-month weight reduction therapy

	Premenopausal (n=29)				Postmenopausal (n=28)			
	FT4		TSH		FT4		TSH	
	r	*p*	r	*p*	r	*p*	r	*p*
ΔBW	-0.434	0.019	0.141	0.467	-0.133	0.501	0.210	0.283
ΔBMI	-0.458	0.012	0.139	0.472	-0.160	0.415	0.261	0.179
ΔWt	-0.510	0.005	-0.105	0.588	-0.039	0.845	0.290	0.134
ΔFBS	0.355	0.059	0.126	0.516	-0.300	0.121	-0.140	0.476
ΔHbA1c	0.037	0.848	0.146	0.449	-0.219	0.264	0.111	0.575
ΔIRI	-0.223	0.307	-0.377	0.076	-0.354	0.076	0.034	0.868
ΔHOMA-R	-0.158	0.471	-0.228	0.295	-0.403	0.041	-0.018	0.932

Correlations of changes of metabolic parameters with thyroid function

The changes in IRI and HOMA-R during the therapy showed significant negative correlations to baseline serum FT4 levels in the female patients, but not in the male patients (Table 4). Furthermore, in those who were successful at weight reduction, baseline serum FT4 levels were significantly correlated to the changes in IRI and HOMA-R (Table 5).

In addition, the changes in metabolic parameters examined in the current study such as, HbA1c, IRI and HOMA-R showed no correlations to baseline serum FT3 levels both in the male and the female patients. FBS showed a significant positive correlation to baseline serum FT3 levels in the female but not in the male patients (Supplemental Table 3).

Discussion

We demonstrated for the first time that baseline serum FT4 levels are associated with the efficacy of weight reduction therapy in obese female patients. In the current study, we performed weight reduction therapy for 6 months for 97 obese patients, as previously reported [19]. Numerous anthropometric and metabolic parameters were improved after the 6-month weight reduction therapy, confirming that our intervention for weight reduction was successful (Supplemental Table 1). Moreover, when focusing on the obese patients who were successful at weight reduction, only in the female patients were serum FT4 levels in prior to the therapy significantly correlated to the changes in body weight and BMI.

It is of note that only in the female patients, baseline