Distinct Clinical, Serological, and Sonographic Characteristics of Hashimoto's Thyroiditis Based with and without IgG4-Positive Plasma Cells

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Context: IgG4-related sclerosing disease is a new syndrome characterized by high serum IgG4 levels and increased IgG4-positive plasma cells in the involved organs. Recently the first description was made by our group of a subsection of Hashimoto's autoimmune thyroiditis (HT) patients showing indistinguishable histopathological features with IgG4-related sclerosing disease, which was termed as IgG4 thyroiditis.

Objective: The objective of the study was analysis of the immunophenotypic features of IgG4 in 70 cases of HT patients and to clarify the histopathological and clinical characteristics of the patients with IgG4 thyroiditis.

Design: Thyroid tissue samples were obtained from 70 patients with HT who were treated surgically. Quantitative analyses of the expression of IgG4 and IgG were performed. Statistical analyses of clinical and histopathological parameters were also conducted.

Results: On the basis of immunohistochemistry of IgG4 and IgG4/IgG ratio, the 70 patients with HT were divided into two groups: IgG4 thyroiditis (19 cases) and non-IgG4 thyroiditis (51 cases). Histopathologically, IgG4 thyroiditis showed higher grade of stromal fibrosis, lymphoplasmacytic infiltration, and follicular cell degeneration than non-IgG4 thyroiditis. Moreover, these two groups were also demonstrated to be related with different clinical features, with IgG4 thyroiditis associated more with male gender, rapid progress, subclinical hypothyroidism, more diffuse low echogenicity, and higher level of circulating antibodies.

Conclusions: From both clinical and histopathological aspects, IgG4 thyroiditis and non-IgG4 thyroiditis were demonstrated to be distinct entities. Measuring serum IgG4 concentration provides a useful method of distinguishing IgG4 thyroiditis from non-IgG4 thyroiditis. **(*J Clin Endocrinol Metab* 95: 1309–1317, 2010)**

Hashimoto's autoimmune thyroiditis (HT), which is characterized by the presence of goiter and serum thyroid autoantibodies, is the most common type of thyroiditis (1, 2). Although the diagnostic criteria describe a well-defined disease entity, HT exhibits various clinical presentations and outcomes and its pathogenesis is poorly understood.

IgG4-related sclerosing disease is a recently recognized syndrome characterized clinically by mass-forming lesions in the exocrine glands and extranodal tissue (most frequently pancreas, biliary duct, and lacrimal gland), elevated serum IgG4 level, and good response to steroid therapy (3–14). It is characterized pathologically by lymphoplasmacytic infiltration and sclerosis as well as IgG4-

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in U.S.A.

doi: 10.1210/jc.2009-1794 Received August 21, 2009. Accepted December 14, 2009.
First Published Online January 22, 2010

Abbreviations: AIP, Autoimmune pancreatitis; ESR, erythrocyte sedimentation rate; FT4, free T4; HPF, high-power field; HT, Hashimoto's autoimmune thyroiditis; L-T4, levothyroxine; Tg-Ab, antithyroglobulin antibody; TPO-Ab, antithyroid peroxidase antibody; TSH, thyroid stimulating hormone.

positive plasma cell infiltration. Recently the first description was made by our group of a portion of patients with HT might have close relationship with IgG4-related sclerosing disease (15). It was found that immunohistochemistry of IgG4 can help subclassify HT into two groups, which were termed as IgG4 thyroiditis (IgG4-positive plasma cell rich group) and non-IgG4 thyroiditis (IgG4-positive plasma cell poor group), respectively, by using our criteria (15). Unfortunately, in the previous study, the case number was very small and some important clinical and laboratory data, especially serum IgG4 concentration, were absent for further analysis.

Herein, to further confirm the previous findings in a larger number of patients and compare detailed clinical manifestations and laboratory data between different groups, the immunohistochemistry of IgG4 and IgG was examined in 70 patients who received total thyroidectomy with a final histopathological diagnosis of HT, and statistical analyses were performed based on histopathological and clinical parameters of these patients.

Patients and Methods

Individuals

Seventy patients with HT from Kuma Hospital (Kobe, Japan) who underwent total thyroidectomy between 1983 and 2006 were studied. These patients were treated surgically for various reasons (16, 17): marked swelling (n = 39), tracheal stenosis (n = 15), suspicious of malignant lymphoma (n = 8), nodular lesion (n = 4), and pain and tenderness (n = 4). According to the Guideline of Japanese Thyroid Society, all patients were diagnosed as HT with the clinical findings: diffuse swelling of the thyroid gland without any other cause (such as Graves' disease) and with any one of the following laboratory findings: 1) positive for antithyroid microsomal antibody or antithyroid peroxidase antibody, 2) positive for antithyroglobulin antibody, and 3) lymphocytic infiltration in the thyroid gland confirmed with cytological examination. Incidental findings of focal (nonspecific) lymphocytic thyroiditis in tumor-bearing thyroid tissue were excluded from this study.

Main clinical data of patients analyzed in the study including age, gender, disease duration of HT, indications for thyroidectomy, weight of resected thyroid gland, thyroid functional status, and ultrasound examination were collected and were summarized in Table 1 and Table 2, respectively.

This study was approved by the Kuma Hospital Bioethical Committee.

Tissue preparation of thyroid glands and blood samples

After surgical resection, thyroid tissues were fixed routinely in 10% neutral buffered formalin and embedded in paraffin. Serial sections (4 μm thick) were cut from each paraffin block. Several sections from each case were stained with hematoxylin and eosin for histological examination, and others were prepared for immunohistochemistry and Masson's trichrome staining.

In addition, peripheral blood samples were obtained from all individuals. Then serum samples were collected for routine laboratory examination. Because serum samples were routinely pre-

TABLE 1. Comparison of clinical features and immunohistochemical and histopathological findings between IgG4 thyroiditis and non-IgG4 thyroiditis

	Hashimoto's autoimmune thyroiditis		
	IgG4 thyroiditis (n = 19)	Non-IgG4 thyroiditis (n = 51)	*P* value
Age (yr)	52.79 ± 10.29	57.73 ± 8.598	0.0775
Gender (male/female)	5/14	3/48	0.0296[a]
Disease duration (yr)	9.647 ± 8.838	17.11 ± 10.34	0.0051
Indications for thyroidectomy (MS/TS/sML/NL/P and T)	10/5/2/0/2	29/10/6/4/2	0.5746[b]
Echogenicity (ultrasound) (diffuse low/coarse)[c]	14/4	15/34	0.0008[a]
ESR (mm/h)[c]	25.72 ± 23.50	21.43 ± 20.84	0.8939
Tg-Ab (fold)	1179 ± 1389	353.3 ± 679.0	0.0108
TPO-Ab (fold)	374.4 ± 335.5	255.3 ± 742.1	0.0088
Serum IgG subclasses[d]			
IgG1 (mg/dl)	1302 ± 297.5	1155 ± 590.4	0.2677
IgG2 (mg/dl)	832.6 ± 304.0	801.1 ± 393.5	0.8763
IgG3 (mg/dl)	78.12 ± 30.20	105.1 ± 98.53	1
IgG4 (mg/dl)	202.4 ± 151.2	55.89 ± 42.56	0.0303
Number of IgG4+ plasma cells per HPF	50.40 ± 22.29	7.773 ± 6.929	<0.0001
Number of IgG+ plasma cells per HPF	101.9 ± 23.83	71.48 ± 32.06	0.0003
IgG4+/IgG+ plasma cells (%)	50.08 ± 17.79	10.63 ± 8.784	<0.0001
Stromal fibrosis (3+, 2+, 1+, −)	12/4/3/0	7/14/24/6	<0.0001
Lymphoplasmacytic infiltration (3+, 2+, 1+, −)	17/2/0/0	19/14/18/0	0.0001
Follicular cell degeneration (3+, 2+, 1+, −)	14/3/2/0	12/17/17/5	0.0003
Lymphoid follicle formation (3+, 2+, 1+, −)	3/7/9/0	9/15/27/0	0.7995

Data are the mean ± SD. MS, Marked swelling; TS, tracheal stenosis; sML, suspicious of malignant lymphoma; NL, nodular lesion; P and T, pain and tenderness.

[a] *P* values were obtained using Fisher's exact test; [b] *P* values were obtained using χ^2 test; [c] not all the patients have complete clinical data; [d] only five cases of IgG4 thyroiditis and seven cases of non-IgG4 thyroiditis are available for serum IgG subclass analysis.

TABLE 2. Weight of thyroid glands at surgery, dose of L-T4, and thyroid functional status before thyroidectomy

	Hashimoto's autoimmune thyroiditis (n = 67)	
	IgG4 thyroiditis (n = 18)	Non-IgG4 thyroiditis (n = 49)
Weight of thyroid glands (g)[a]	202.5 (110.8; 232.0)	188.0 (115.4; 225.5)
L-T4 (μg/d)[a]	75 (50; 100)	50 (0; 100)
Thyroid functional status (subclinical hypo-/eu-/subclinical hyper-)[b]	6/11/1	5/36/8

Weight of thyroid gland, dose of L-T4, and thyroid functional status were correlated using Spearman's ρ test.

[a] The data are expressed as median (25th, 75th percentiles).

[b] Subclinical hypothyroidism, euthyroidism, subclinical hyperthyroidism.

served in a −80 C deep freezer for at most 3 yr in Kuma hospital, only serum samples (before and after thyroidectomy) from 12 patients (12 of 70) who received thyroidectomy in 2005–2006 were available for retrospective measurement of serum IgG4 and the other subclasses of IgG concentrations.

Histopathological evaluation and immunohistochemistry

All hematoxylin and eosin and Masson's trichrome sections were first reviewed by two pathologists (Y.L. and K.K.) to confirm previous histological diagnoses and rule out Riedel's thyroiditis for extrathyroid fibrosis. The degrees of stromal fibrosis, lymphoplasmacytic infiltration, follicular cell degeneration, and lymphoid follicle formation were examined and expressed as 3+, severe; 2+, moderate; 1+, mild; −, negative.

Immunostaining for IgG4 (mouse monoclonal, MC011, 1:500; Binding Site, Birmingham, UK) and IgG (rabbit polyclonal, A0423, 1:8000; Dako Cytomation, Glostrup, Denmark) was performed using the EnVision system (Dako Cytomation). Tonsil tissue served as a positive control (15). For enumeration of IgG4-positive or IgG-positive cells, areas with the highest density of positive cells were evaluated. Five high-power fields (HPFs) in each section were counted and the average number of positive cells per HPF was calculated using image analysis software Win ROOF version 5.8 (Mitani Corp., Tokyo, Japan). One HPF covered an area of 0.034 mm^2 (Olympus AX80T microscope, ×10 eyepiece and ×40 lens; Olympus DP70 camera and DPController software; Tokyo, Japan). The ratio of IgG4-positive plasma cells to IgG-positive plasma cells was also calculated in each case.

Laboratory and ultrasonographic evaluation

Both antithyroglobulin antibodies (Tg-Abs) and antithyroid peroxidase antibodies (TPO-Abs) were determined using commercially available hemagglutination assay kits (Fuji Rebio Inc., Tokyo, Japan; normal ranges less than 100-fold, respectively). Erythrocyte sedimentation rate (ESR) was determined by the method previously described by Ohye *et al.* (18).

Concentrations of serum thyroid stimulating hormone [TSH (normal range 0.30–5.00 mIU/liter)] and free T4 (FT4; normal range 0.70–1.60 ng/dl) before surgery were measured with commercial enzyme immunoassays (AxSYM TSH and AxSYM FT4; Abbott Japan Co., Tokyo, Japan) (19). The results were evaluated and shown as thyroid functional status (subclinical hypothyroidism, euthyroidism, and subclinical hyperthyroidism) in this study. The evaluation criteria are as follows: 1) thyroid function status of patients with levothyroxine (L-T4) therapy at thyroidectomy (n = 53) was evaluated according to thyroid function status, which presented at more than 50% time period from the start of preoperative dose of L-T4 to the day of thyroidectomy; and 2) thyroid function status of patients without L-T4 therapy (n = 17) was evaluated based on their preoperative thyroid function examining data (serum TSH and FT4 levels).

Serum levels of IgG subclasses of the patients were measured with a Behring nephelometer (Dade Behring, Deerfield, IL) using IgG subclasses (BS-NIA IgG1-4; Medical and Biological Laboratory Co., Ltd., Nagoya, Japan) as antibodies for serum samples. The reference ranges were as follows: IgG1, 320–748 mg/dl; IgG2, 208–754 mg/dl; IgG3, 6.6–88.3 mg/dl; IgG4, 4.8–105 mg/dl.

Ultrasonographic examinations were performed as previously described (19, 20). The gain was adjusted to produce an echo-free appearance of the lumen on the internal jugular vein, carotid arteries, and neck-strap muscles. In this setting, a normal thyroid gland has a medium gray-scale homogeneous echo pattern, and the level of echogenicity is higher than that of the surrounding muscles. The diffuse low was defined as a diffuse echo density clearly lower than normal subjects, whereas diffuse coarse was defined as heterogeneous echogenicity or mixed with the signal of normal subjects (20).

Statistical analysis

Data are shown as the arithmetic mean ± SD. Statistical analyses were performed with the Mann-Whitney *U* test to compare the age, disease duration, ESR, and serum concentration of Tg-Abs, TPO-Abs, and each IgG subclass among different groups. The Wilcoxon matched-pairs signed-rank test was used to compare preoperative and postoperative serum IgG4 concentrations in five patients with HT. The significance of differences by gender, indications for thyroidectomy, and echogenicity between different subgroups were determined by χ^2 test or Fisher exact probability test. Weight of thyroid gland, therapy with L-T4, and thyroid functional status before thyroidectomy were correlated using Spearman's ρ test. Analyses were performed using GraphPad Prism version 5.0 (GraphPad Software, Inc., San Diego, CA), and $P < 0.05$ was considered as statistically significant.

Results

Immunohistochemical and histopathological findings

Different investigators have used different immunohistochemical criteria in defining IgG4-related sclerosing disease in various organs with regard to diagnostic threshold, such as greater than 10, greater than 20, greater than 30/HPF IgG4-positive plasma cells (21–24). In contrast, Cheuk *et al.* (25) used a ratio of greater than 40% IgG4/

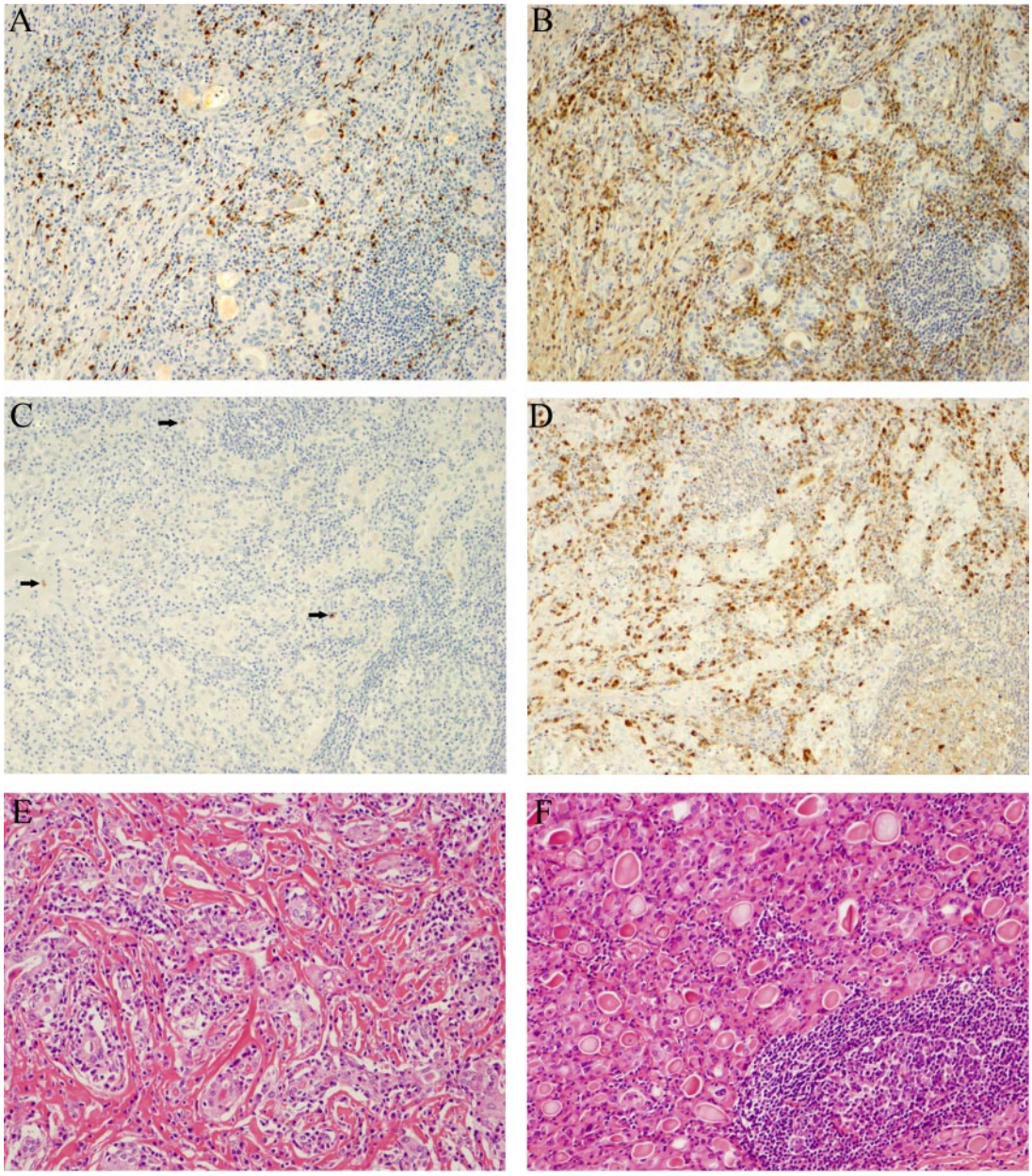

FIG. 1. Immunohistochemistry of IgG4 and IgG. In the IgG4 thyroiditis group, IgG-positive plasma cells were abundantly observed among thyroid follicles (B). Immunostaining of IgG4 also revealed diffuse infiltration of IgG4-positive plasma cells (A). In contrast, in the non-IgG4 thyroiditis group, although many IgG-positive plasma cells were also observed (D), rare IgG4-positive plasma cells were identified (C). Histopathologically, IgG4 thyroiditis (E) showed higher grade of stromal fibrosis, lymphoplasmacytic infiltration, and follicular cell degeneration than non-IgG4 thyroiditis (F). All images, magnification, ×100.

IgG-positive plasma cells as the cutoff. In the previous study, our group proposed greater than 20/HPF IgG4-positive plasma cells and greater than 30% IgG4/IgG ratio as the cutoff because these values gave high specificity and sensitivity for defining a significant increase of IgG4-positive plasma cells in HT.

Using the criteria, 19 cases of IgG4 thyroiditis were identified in the present study (Table 1). Immunohistochemically, all these cases showed diffuse or nodular dense infiltration of IgG4-positive plasma cells (Fig. 1A) (50.40 ± 22.29/HPF) with a high portion of IgG4/IgG-positive plasma cells (50.08 ± 17.79%). In contrast, 51 other cases of HT were defined as non-IgG4 thyroiditis. Only mild to occasional IgG4-positive plasma cell infiltration was present (Fig. 1C) (7.773 ± 6.929/HPF), and the ratio of IgG4 to IgG-positive plasma cells was low (10.63 ± 8.784%). Both the number of IgG4-positive plasma cells per HPF and the ratio of IgG4 to IgG-positive plasma cells were significantly higher in IgG4 thyroiditis than those in non-IgG4 thyroiditis ($P < 0.0001$, respectively). The number of IgG-positive plasma cells per HPF was also elevated significantly in IgG4 thyroiditis compared with non-IgG4 thyroiditis ($P = 0.0003$), which was

TABLE 3. Serum IgG4 concentrations in five patients with IgG4 thyroiditis before and after total thyroidectomy

Case no.	IgG4 concentration before total thyroidectomy (mg/dl)	IgG4 concentration after total thyroidectomy (mg/dl)	*P* value[a]
1	459	97.8	
2	199	64.8	
3	167	25.6	
4	111	125	
5	75.8	66.5	
Mean ± SD	202.4 ± 151.2	75.94 ± 37.52	0.1875

Reference value of serum IgG4 concentration is 4.8–105 mg/dl.

[a] The Wilcoxon matched-pairs signed-rank test was used to calculate two-sided *P* values.

not evident in the previous study. The larger number of cases involved in this study may be an explanation for this difference.

Histopathological characteristics of the two groups were also summarized in Table 1. As we expected, IgG4 thyroiditis showed significantly higher grade of stromal fibrosis, lymphoplasmacytic infiltration and follicular cell degeneration than those of non-IgG4 thyroiditis (Table 1; Fig. 1, E and F). Other typical features, such as lymphoid follicle with germinal center, were both present in the two groups. These results confirmed our previous observations that IgG4-positive plasma cell infiltrate may be a marker of more aggressive fibrotic disease in HT.

Serum IgG4 concentration

Serum samples (before and after thyroidectomy) from five cases of IgG4 thyroiditis and seven cases of non-IgG4 thyroiditis were available for IgG subclasses measurement in this study. The results of serum IgG isoforms concentration were shown in Tables 1 and 3. Before total thyroidectomy, serum IgG4 concentrations were elevated, beyond the reference range (4.8–105 mg/dl), in four of five patients with IgG4 thyroiditis. Only one patient with non-IgG4 thyroiditis (one of seven) showed increased level of serum IgG4 (126 mg/dl) before surgery. Preoperative serum IgG4 concentrations were significantly higher in the IgG4 thyroiditis group than the non-IgG4 thyroiditis group (Fig. 2) ($P = 0.0303$). There were no statistical differences between these two groups with respect to the concentrations of other common IgG subclasses (IgG1, IgG2, IgG3) (Table 1). This finding was consistent with serological examination of IgG4-related sclerosing disease in other organs and suggested the clinical usefulness of circulating IgG4 measurement in differential diagnosis between IgG4 thyroiditis and non-IgG4 thyroiditis.

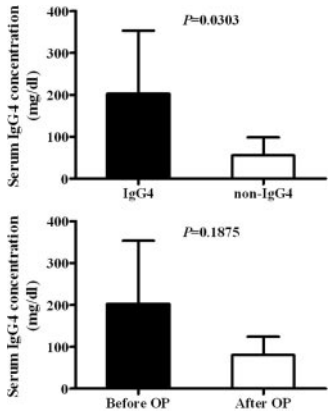

FIG. 2. Comparison of serum IgG4 concentrations. Preoperative serum IgG subclasses concentrations of five patients with IgG4 thyroiditis and seven patients with non-IgG4 thyroiditis were determined. The IgG4 thyroiditis group reveals significantly higher serum IgG4 concentrations than the non-IgG4 thyroiditis group ($P = 0.0303$; Mann-Whitney *U* test; data are mean ± SD). No significant difference of other serum IgG subclasses were observed (data are not shown here). Postoperative serum IgG4 concentrations were also measured in the patients with IgG4 thyroiditis. Although there seemed to be a tendency that after surgery serum IgG4 concentration was decreased, no significant difference was reached by statistical analysis ($P = 0.1875$; Wilcoxon matched pairs signed rank test; data are mean ± SD). OP, Operation.

Postoperative serum IgG4 concentrations of patients with IgG4 thyroiditis were also measured in this study (Table 2). The serum IgG4 concentrations of three patients decreased markedly and were lower than their respective baseline values. One patient (no.5) showed relatively stable IgG4 levels (before: 75.8 mg/dl; after: 66.5 mg/dl). Another patient (no. 4) exhibited an slight elevation of serum IgG4 concentration after operation (before: 111 mg/dl; after: 125 mg/dl). Although there seemed to be a tendency that serum IgG4 concentration will decrease or remain stable after total thyroidectomy, no significant difference was reached by statistical analysis ($P = 0.1875$, Wilcoxon matched-pairs signed-rank test).

Laboratory data and ultrasonographic findings

As shown in Table 1, thyroid autoantibodies, both Tg-Abs and TPO-Abs, were significantly higher in IgG4 thyroiditis patients (Tg-Ab, 1179 ± 1389-fold; TPO-Ab, 374.4 ± 335.5-fold), compared with non-IgG4 thyroiditis patients (Tg-Ab, 353.3 ± 679.0-fold; TPO-Ab, 255.3 ± 742.1-fold) ($P = 0.0108$, $P = 0.0088$, respectively). But no significant difference of ESR between these two groups was found ($P = 0.8939$).

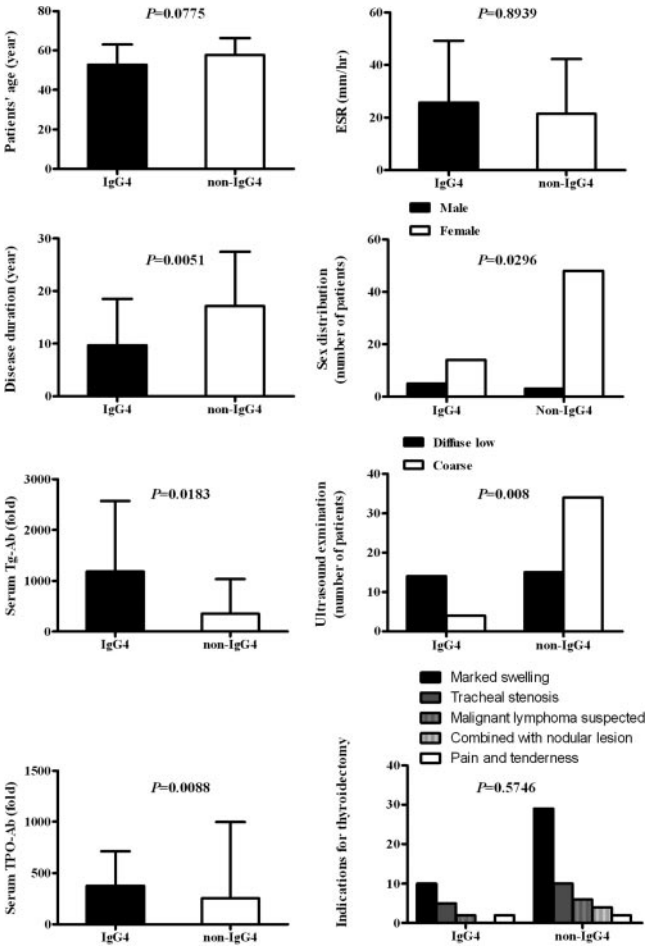

FIG. 3. Comparison of clinical characteristics and laboratory data between the IgG4 thyroiditis group and the non-IgG4 thyroiditis group. Patients with IgG4 thyroiditis showed significantly shorter disease duration and higher serum Tg-Abs and TPO-Abs than those with non-IgG4 thyroiditis (Mann-Whitney *U* test; data are mean ± SD). Moreover, IgG4 thyroiditis was demonstrated to be significantly associated with male gender and diffuse low echogenicity (Fisher's exact test or χ^2 test). No significant difference of patients' age, ESR, and indications for thyroidectomy between the two groups was found.

We then compared the thyroid functional status of IgG4 thyroiditis and non-IgG4 thyroiditis before surgery. The results of different thyroid functional status: subclinical hypothyroidism, euthyroidism, and subclinical hyperthyroidism are listed in Table 2. We found the distribution of the three thyroid functional status was significantly different between the two groups ($P = 0.0243$), and there were more patients with subclinical hypothyroidism in IgG4 thyroiditis than non-IgG4 thyroiditis. The majority of patients involved in the present study also received thyroid hormone therapy with L-T4 at TSH-suppressive dose before surgery, and the IgG4 thyroiditis group received a higher dose of L-T4 than the non-IgG4 thyroiditis group (Table 2). The weight of resected thyroid glands, dose of L-T4, and thyroid function were correlated using Spearman's ρ test. The thyroid functional status was significantly correlated with the dose of L-T4 (Spearman's ρ: r = 0.3634, $P = 0.0025$) but was not significantly associated with weight of thyroid glands (Spearman's ρ: r = 0.1414, $P = 0.2538$). Because L-T4 therapy has a significant impact on thyroid functions, our result suggests that the IgG4 thyroiditis may be more correlated with hypothyroidism than non-IgG4 thyroiditis without L-T4 therapy.

Ultrasound examinations revealed that IgG4 thyroiditis was significantly correlated with diffuse low echogenicity, whereas non-IgG4 thyroiditis showed association with diffuse coarse echogenicity (Fig. 3) ($P = 0.0008$). In addition, although in 11.1% of IgG4 thyroiditis (1/9) and 20.4% of non-IgG4 thyroiditis (10/49), coexisted thyroid nodules were also detected, no significant difference in incidence of nodules was found between the two groups ($P = 0.4903$).

Clinical features of the two groups

The clinical characteristics of IgG4 thyroiditis and non-IgG4 thyroiditis are shown in Table 1. IgG4 thyroiditis group included five males and 14 females with a mean age of 52.79 yr (range 23–67 yr), whereas the non-IgG4 thyroiditis group included three males and 48 females with a mean age of 57.73 yr (range 34–75 yr). Although no significant difference in patients' age was found between these two groups, there were greater proportion of male gender in IgG4 thyroiditis (Fig. 3) ($P = 0.0296$). We then compared disease duration of HT patients before they underwent total thyroidectomy between the two groups. IgG4 thyroiditis group had significantly shorter disease duration (9.647 ± 8.838 yr) than non-IgG4 thyroiditis group (Fig. 3) (17.11 ± 10.34 yr) ($P = 0.0051$). This difference was quite interesting and critical from both clinical and pathological aspects because it refuted a hypothesis that IgG4 thyroiditis is just a late stage of HT.

Usually thyroiditis is treated medically, but thyroidectomy is sometimes indicated (26). As shown in Table 1, patients with HT underwent total thyroidectomy for various reasons in the present series. However, no significant difference of the indications for operation was found between the two groups (Fig. 3) ($P = 0.5746$).

Discussion

Recently much attention has been drawn by IgG4-related sclerosing disease, which is a new disease entity first proposed with regard to autoimmune pancreatitis (AIP) (6, 27, 28). Currently it is recognized as an autoimmune syndrome characterized by tumor-like involvement of exocrine glands or other extranodal tissues (3–5, 7–12, 29) by

lymphoplasmacytic infiltration and sclerosis, accompanied by increased IgG4-positive plasma cells in the tissues and elevated IgG4 titer in the serum and often showing good response to steroid therapy (5). Furthermore, according to the Mayo Clinic experience on diagnosis of AIP, it was proposed that pancreatic immunostaining was diagnostic of AIP when it showed abundant IgG4-postive plasma cells and this was involved into the diagnostic criteria for AIP (the HISORt criteria) (30).

Riedel's thyroiditis (also termed as invasive fibrous thyroiditis) is an extremely rare form of thyroiditis characterized by replacement of the thyroid parenchyma with dense fibrous tissue (31). Riedel's thyroiditis was commonly mentioned as a thyroid involvement of IgG4-related sclerosing disease by some researchers, but until now no serological examination of IgG4 or immunohistochemistry for IgG4 has been performed on Riedel's thyroiditis (9). Furthermore, in a previous study, Harach and Williams (32) performed a characterization of plasma cell subsets in various thyroid diseases by immunohistochemistry. They found that IgA plasma cells but not IgG plasma cells were predominant in Riedel's thyroiditis. Therefore, we suggest that the common hypothesis that Riedel's thyroiditis is linked to IgG4-related sclerosing disease may be questioned.

Recently our group first described a small number of patients with HT showing abundant IgG4-positive plasma cells in the inflamed thyroid tissue and very similar histological features with IgG4-related sclerosing disease. Then according to immunohistochemistry of IgG4 and IgG, a novel subclassification was proposed, by which HT can be subclassified into IgG4 thyroiditis and non-IgG4 thyroiditis (15). It was suggested that IgG4 thyroiditis group might have a close relationship with IgG4-related sclerosing disease. However, 70 cases of HT in the present study could not show a single case of IgG4-related sclerosing disease in other organs.

The previous conclusion that immunohistochemistry of IgG4 could help subclassify HT was confirmed by the present study in larger number of cases. In this study, 70 patients of HT were classified into the two groups: IgG4 thyroiditis (19 cases) and non-IgG4 thyroiditis (51 cases). Histopathologically, IgG4 thyroiditis showed significantly higher grade of stromal fibrosis, lymphoplasmacytic infiltration, and follicular cell degeneration than non-IgG4 thyroiditis. These findings also confirmed and extended our previous observations that IgG4-positive plasma cell infiltrate may be a marker of more fibrotic disease in HT. In addition, detailed clinical characteristics and laboratory data were compared by statistical analysis between the two groups.

According to the present results, several important clinical differences should be pointed out. First, this analysis shows that IgG4 thyroiditis is significantly associated with male gender. This result is consistent with the male predominance of IgG4-related sclerosing diseases in other organs. Although generally the sex ratio of females to males is 8–9:1 for HT (1) and even in IgG4 thyroiditis group female patients is the majority, it was suggested that clinicians should pay more attention to male patients, who might be more easily involved in IgG4 thyroiditis group. Second, IgG4 thyroiditis was proved to be significantly associated with shorter disease duration of HT before surgery than that of non-IgG4 thyroiditis. When we refer to IgG4 thyroiditis as a distinct subgroup of HT, some researchers might wonder whether it is just an end stage of HT. Because in the previous histopathological study of IgG4 thyroiditis (15), this group was reported to present with severe lymphoplasmacytic infiltration, dense fibrosis, and marked follicular cell degeneration, whereas in non-IgG4 thyroiditis, these characteristics were relatively mild or absent. These observations were confirmed by the current study and might give rise to a hypothesis that IgG4 thyroiditis is just a late phase of non-IgG4 thyroiditis. However, this study proved that patients with non-IgG4 thyroiditis showed longer disease duration before thyroidectomy than those with IgG4 thyroiditis. Thus, the alternative hypotheses we proposed are: 1) IgG4 thyroiditis may have more severe symptoms, such as tracheal stenosis, and lead patients to receive operations more quickly; or 2) IgG4 thyroiditis is a rapidly progressive form of HT and as a result its disease duration is shorter; or 3) IgG4 thyroiditis may have a different etiology and pathogenesis from non-IgG4 thyroiditis, and as a result the disease duration before patients undergo surgery is not same. Third, with reference to thyroid function, as shown in Table 2, IgG4 thyroiditis showed significantly more association with subclinical hypothyroidism, and non-IgG4 thyroiditis was correlated with more subclinical hyperthyroidism. This result was also significantly influenced by suppressive dose of L-T4 before thyroidectomy. The above difference of distribution of thyroid functional status between two groups existed even though IgG4 thyroiditis group received higher dose of L-T4, which suggested that the IgG4 thyroiditis may be more correlated with hypothyroidism than non-IgG4 thyroiditis at their natural status. In addition, ultrasound examination revealed that IgG4 thyroiditis was significantly correlated with diffuse low echogenicity, whereas non-IgG4 thyroiditis was associated with diffuse coarse echogenicity, which might reflect the degree of stromal fibrosis and follicular cell degeneration. Furthermore, serologically, preoperative serum concentrations of thyroid autoantibodies, both Tg-Abs and TPO-Abs, were significantly higher in IgG4 thyroiditis than non-IgG4 thyroiditis.

All these points strongly indicate that the novel subclassification does not only have immunohistochemical and pathological value but also shows clinical significance, and it was concluded that IgG4 thyroiditis and non-IgG4 thyroiditis are two distinct clinical entities, which are currently grouped together as one disease in HT.

Usually HT has been treated medically; therefore, performing immunohistochemistry of IgG4 using surgical specimens might not be a practical method for clinicians to recognize IgG4 thyroiditis. Serum IgG4 measurement are now performed routinely in patients with suspected AIP (33). The key strength of the present study is the investigation of preoperative serum IgG4 concentrations in patients with both IgG4 thyroiditis and non-IgG4 thyroiditis. As expected, the preoperative serum level of IgG4 was significantly higher in patients with IgG4 thyroiditis than those with non-IgG4 thyroiditis. Before operation, four of five patients in the IgG4 thyroiditis group showed elevated serum IgG4 concentrations above the reference value, but only one patient (one of seven) with non-IgG4 thyroiditis showed elevated serum IgG4 level (126 mg/dl). Although only a small series of patients were involved in this serological examination of IgG4, the results indicated that serum concentrations of IgG4 could represent the status of IgG4-positive plasma cell infiltration in thyroid tissue, and measuring serum IgG4 concentration might be a sensitive and specific laboratory method for distinguishing IgG4 thyroiditis and non-IgG4 thyroiditis.

Finally, awareness of IgG4 thyroiditis might help clinicians to guide treatment of patients with HT. As is generally known, IgG4-related sclerosing diseases often show good responses to steroid therapy in other organs (5, 6, 34). Because of its close relationship with IgG4-related sclerosing disease, IgG4 thyroiditis might also show effective response to glucocorticoid therapy for releasing troublesome local symptoms and improving clinical outcomes. However, when the initial complaints recur after steroid therapy, surgical treatment is still required to relieve the symptoms completely (15, 18).

In conclusion, the present study confirmed the previous findings that HT can be subclassified into two groups: IgG4 thyroiditis and non-IgG4 thyroiditis, based on immunohistochemistry of IgG4 and IgG4 to IgG ratio using thyroid tissue. Histopathologically, IgG4-positive plasma cell infiltrate may be a marker of more fibrotic disease in HT. Furthermore, from a clinical aspect, these two subgroups also presented significantly different characteristics and were demonstrated to be distinct subtypes of HT. Measuring serum IgG4 concentration provides a practical method to distinguish these new entities. Further studies are needed to determine the role of IgG4 in the pathogenesis of thyroiditis and investigate the appropriate therapeutic approaches.

Acknowledgments

Dr. Yaqiong Li is an exchange Ph.D., student from the Department of Pathology and Pathophysiology, Shandong University School of Medicine and was supported by a scholarship from the Rotary Yoneyama Memorial Foundation, Japan.

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Disclosure Summary: The authors have nothing to declare.

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