Original article

Clinical and biochemical characteristics of patients having general symptoms with increased serum IgG4.

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Abstract

Objective: To differentiate patients with IgG4-related diseases (RD) from patients with other hyper IgG4 conditions who visit general medicine department. **Methods:** Fifty-six patients with high serum IgG4 levels (>135 mg/dL) were classified into three groups based on the final diagnosis: definite and possible IgG4-RD and others. Clinical and laboratory characteristics of the three groups of patients were retrospectively analyzed. **Results:** Major manifestations were renal dysfunction and general malaise, while thirst was the most frequent symptom in the definite group, in which submandibular glands and lymph nodes were likely to be affected. Biopsy of minor salivary glands was the least diagnostic for IgG4-RD despite the high frequency of biopsy. In the definite group, serum levels of IgG4 and IgG, IgG4/IgG ratio and basophil number were increased, while serum levels of CRP, IgA and complements were decreased. A negative correlation between serum levels of IgG4 and IgM was found in the definite group. **Conclusion:** The results indicated that in patients with renal dysfunction, malaise, thirst or weight loss, measurements of the levels of basophils, immunoglobulins and complements are helpful for diagnosing IgG4-RD. Considering distribution of affected tissues and localization of diagnostic biopsies, physical examination and laboratory workup are required for early diagnosis.

Introduction

Immunoglobulin G subclass 4 (IgG4)-related disease (IgG4-RD) was proposed in Japan in 2011 as a novel clinical entity characterized by elevated serum IgG4 concentration and tumefaction or tissue infiltration by IgG4-positive plasma cells (1-3). General practitioners often encounter patients who have ambiguous and unclassified symptoms with an increased serum IgG4 concentration. Since IgG4-RD often involves many organs and tissues, its differential diagnosis at the early phase is clinically important (1-5). However, it is often difficult to make a final diagnosis of IgG4-RD even if the process for diagnosis is carried out according to the IgG4-RD comprehensive diagnostic criteria (1-3).

Serum IgG4 levels can be increased by various disorders including not only IgG4-RD but also autoimmune, inflammatory and hematologic diseases. Actually, an increase in serum IgG4 level can be increased by many syndromes, so-called hyper IgG4 syndromes, including Sjögren's syndrome, sarcoidosis, multicentric Castleman's disease, granulomatosis with polyangiitis, eosinophilic granulomatosis with polyangiitis, eosinophilia, rheumatoid arthritis, chronic hepatitis, liver cirrhosis, malignant lymphoma, cancers, pemphigus and membranous nephritis, all of which need to be distinguished from IgG4-RD (3-5). Symptomatic, physical and laboratory

characteristics for differentiation of IgG4-RD from other hyper IgG4 syndromes are required in the clinical setting of general medicine.

To establish a method for efficient differentiation of IgG4-RD from other hyper IgG4 conditions and to identify serological indicators useful for rapid and accurate identification of IgG4-RD, clinical and biochemical data were retrospectively analyzed in this study. We uncovered several critical features and biochemical trends that would be useful for differential diagnosis of hyper IgG4 syndromes. Our results also indicated that, considering the wide distribution of affected organs and diagnostic tissues for biopsy, detailed physical examination at the time of initial checkup is necessary.

Patients and Methods

Study subjects and protocol

We retrospectively reviewed medical records of 225 patients in whom serum IgG4 levels were measured during the period from April 1, 2014 to August 31, 2017 in the Department of General Medicine of Okayama University Hospital. Fifty-six of the 225 patients had serum IgG4 levels higher than 135 mg/dL. In those 56 patients, serum IgG4 levels were measured once in 7 patients and multiple times in 49 patients during the study period. In patients in whom serum IgG4 levels were measured multiple times during the study period, the first measurement during the study period was used for analysis. The information regarding this retrospective study was disclosed on the website of our department and by poster notice in our hospital with an opportunity for denial about research and we established a contact point for participants' opt-out. This study was approved by the Ethical Committee of Okayama University Hospital (No. 1605-005) and adhered to the Declaration of Helsinki and Ethical Guidelines for Medical and Health Research Involving Human Subjects.

Classification of IgG4-RD

We classified IgG4-RD diagnoses according to the comprehensive diagnostic criteria for IgG4-RD 2011 and consensus statement on the pathology of IgG4-RD and also, if necessary, reassessed by organ-specific diagnostic criteria. The comprehensive diagnostic criteria 2011 include: 1) clinical examination showing characteristic diffuse/localized swelling or masses in single or multiple organs, 2) hematological examination showing elevated serum IgG4 concentration (≥135 mg/dL), and 3) histopathologic examination showing i) marked lymphocyte and plasmacyte infiltration and fibrosis and ii) infiltration of IgG4+ plasma cells with a ratio of IgG4+/IgG+ cells >40% and >10 IgG4+ plasma cells/HPF.

Out of 56 cases having increased serum IgG4 levels higher than 135 mg/dL, 13 cases that fulfilled all of the IgG4-RD comprehensive diagnostic criteria 2011 including items 1), 2) and 3) were defined as a “definite” group. Among 11 cases that underwent lymph node biopsies, none of the cases met item 3) of the comprehensive diagnostic criteria 2011. However, 4 of the 11 cases were included in the “definite” group because they satisfied the consensus statement on the pathology of IgG4-RD in addition to items 1) and 2) of the comprehensive diagnostic criteria 2011. Thus, a total of 17 cases were included in the “definite” group in the present study. Since the

remaining 7 cases out of the 11 lymph-node biopsied cases did not meet the pathological diagnostic criteria of IgG4-RD and they were eventually diagnosed as having disorders other than IgG4-RD, they were classified into an “other” group.

The patients who satisfied items 1) and 2) of the IgG4-RD comprehensive diagnostic criteria 2011 but did not satisfy the diagnostic criteria of the consensus statement on the pathology of IgG4-RD were defined as a “possible” group (17 patients). All of the cases in the possible group had serum IgG4 levels > 135 mg/dL and also had diffuse/local swelling or a characteristic mass in one or more organs. Therefore, in view of clinical aspects, these 17 cases were initially cases of suspected IgG4-RD. However, the cases in the “possible” group could not completely fulfill the pathological diagnostic criteria. On the other hand, the cases fulfilling only item 2) of the IgG4-RD comprehensive diagnostic criteria 2011 were included in the “other” group (22 patients).

In addition, there were 6 cases that showed characteristic diffuse/localized swelling or masses in single or multiple organs but had serum IgG4 levels lower than 135 mg/dL. However, since none of the 6 cases met the histologic diagnostic criteria, they were not classified into a “probable” group based on the comprehensive diagnostic criteria for IgG4-RD. Therefore, there was no patient fulfilling diagnostic

criteria 1) and 3) who belonged to the “probable” group in the present study.

Examination of biochemical markers

White blood cell count (WBC), hemoglobin (Hb), platelets (Plt), eosinophils (Eo), basophils (Baso), immunoglobulin G (IgG), immunoglobulin A (IgA), immunoglobulin M (IgM), immunoglobulin E (IgE), complement component 3 (C3), complement component 4 (C4), 50% hemolytic complement activity (CH50), C-reactive protein (CRP), lactate dehydrogenase (LDH), and erythrocyte sedimentation rate in 1 hour (ESR) were determined by an auto-analyzer system in the Central Laboratory of Okayama University Hospital.

Serum levels of IgG4 were measured by a turbidimetric immunoassay (TIA) using a commercially available kit (Binding Site, Birmingham, UK) and serum levels of soluble interleukin-2 receptor (sIL-2R) were measured by a radioimmunoassay using a commercially available kit (BML, Kawagoe, Japan). Biochemical data obtained within one week from the day when serum IgG4 level was measured were used in the analysis.

Statistical analysis

The data were subjected to ANOVA and linear regression analysis to determine differences. If differences were detected by ANOVA, Tukey-Kramer's post-hoc test was used to determine which means differed. *P* values <0.05 were considered statistically significant. All statistical analyses were performed using EZR (Saitama Medical Center, Jichi University, Saitama, Japan), a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria, ver. 3.1.1) (6).

Results

Patients' characteristics and serum IgG4 levels

Out of total of 225 patients in whom serum IgG4 levels were measured, 56 patients with serum IgG4 levels higher than 135 mg/dL met the selection criteria for this study. Of those 56 patients, 17 patients (30%) were included in the "definite" group (mean ± SEM of serum IgG4: 1334 ± 162 mg/dL), 17 patients (30%) were included in the possible group (serum IgG4: 343 ± 68 mg/dL), and 22 patients (39%) were included in the other group (serum IgG4: 246 ± 24 mg/dL). As shown in **Fig. 1A**, male ratios in the definite and possible groups were 71% and 76%, respectively, and the male ratio in the other group was 45%. The mean ages of patients in the three groups ranged from 64 to 65 years without a significant difference among the three groups (**Fig. 1A**).

The final diagnosis in the other group included the following four categories: 1) collagen disease and vasculitis (n=8) including eosinophilic granulomatosis with polyangiitis (n=2), rapidly progressive glomerulonephritis (n=2), rheumatoid arthritis (n=1), sarcoidosis (n=1), SAPHO syndrome (n=1) and Budd-Chiari syndrome (n=1); 2) hematologic disease (n=8) including multicentric Castleman's disease (n=5),

myelodysplastic syndrome (n=1), diffuse large B-cell lymphoma (n=1) and idiopathic eosinophilia (n=1); 3) neoplasms (n=4) including squamous cell lung cancer (n=2), parotid gland cancer (n=1) and duodenal papilla cancer (n=1); and 4) others including ulcerative colitis (n=1) and fever of unknown origin (n=1).

In the present study, complication of bronchial asthma was seen in 3 cases in the definite group, in one case in the possible group and in one case in the other group, while allergic rhinitis was also seen in one case each in the definite group, possible group and other group. No specific interrelationship between IgG4-RD and accompanying allergic diseases was evident in our study.

Manifestations and affected tissues

In the 56 enrolled patients with serum IgG4 higher than 135 mg/dL, the total number of initial symptoms was 63. The initial manifestations in the order of frequency were as follows: renal dysfunction (14%, 9 out of 63 symptoms), general malaise (11%, 7/63), thirst (10%, 6/63) and weight decrease (10%, 6/63). In the definite group, thirst (24%, in 4 out of 17 symptoms), renal dysfunction (18%, 3/17), weight decrease (18%, 3/17), and submandibular gland swelling (18%, 3/17) were frequent symptoms (**Fig. 1B**). In the possible group, renal dysfunction (21%, 4 out of

19 symptoms) was the most frequent symptom.

The number of affected tissues in all cases was 104. Affected tissues were determined from clinical symptoms, laboratory data and results of contrast-enhanced computed tomography (CE-CT), ^{67}Ga-scintigraphy and ^{18}F-fluorodeoxyglucose-positron emission tomography-computed tomography (FDG/PET-CT). The affected tissues in all cases in the order of frequency included lymph nodes (15%, 16 out of 104 tissues), retroperitoneal tissues (15%, 16/104), submandibular glands (13%, 13/104), kidneys (12%, 12/104), pancreas (11%, 11/104) and lungs (8%, 8/104) (**Fig. 2A**). In the definite group, submandibular glands (19%, 10 out of 53 tissues), lymph nodes (15%, 8/53), retroperitoneum (11%, 6/53) and kidneys (11%, 6/53) were frequently affected. In the possible group, the retroperitoneum (36%, 10 out of 28 tissues) and pancreas (21%, 6/28) tended to be involved. In the other group, lymph nodes (30%, 7 out of 23 tissues) were the most frequently affected tissues (**Fig. 2A**).

There were 50 biopsied tissues in all cases (**Fig. 2B**). The selection of biopsied tissues was discussed and determined by a conference in our department and, consequently, biopsies were actually performed in all of the 17 cases in the definite group. However, biopsies were not performed in 4 cases (3 cases with a

retroperitoneal lesion and one case with a pancreatic lesion) out of the 17 cases in the possible group and in 4 cases out of the 22 cases in the other group.

Among all cases, the major tissues for biopsy were minor salivary glands (24%, 12 out of 50 tissues), lymph nodes (22%, 11/50), submandibular glands (12%, 6/50), and kidneys (10%, 5/50) in that order as shown in **Fig. 2B**. Biopsied tissues that were useful for a definitive diagnosis of IgG4-RD were the retroperitoneum (100%, 3 out of 3 tissues) and submandibular glands (83%, 5/6), whereas biopsies of minor salivary glands (8%, 1/12) were not very useful for a pathological diagnosis (**Fig. 2B**).

Comparison of biochemical markers and correlations with serum IgG4 levels

For the biochemical parameters, there were significant differences among the groups in serum levels of IgG4 and IgG, IgG4/IgG ratio, serum levels of IgA, CRP, C3, C4 and CH50, and basophil number as shown in **Fig. 3**. Regarding the differential markers for the definite group, serum levels of IgG4 and IgG, IgG4/IgG ratio and basophil number were significantly higher and serum levels of IgA, CRP and C3, C4 and CH50 were significantly lower in the definite group than in the other two groups. There was also a significant difference between the possible group and the other two groups in serum CRP levels (**Fig. 3**).

Correlations of serum IgG4 level with biochemical parameters are shown in **Fig. 4**. Serum level of IgG was positively correlated with serum level of IgG4 in the definite group (R = 0.763, P < 0.01) and the possible group (R = 0.686, P < 0.01), while serum level of IgM was negatively correlated with serum level of IgG4 (R = -0.565, P < 0.05) in the definite group. Of note, a positive correlation of serum IgG4 level with serum C3 level (R = 0.607, P < 0.05) was found in the other group (**Fig. 4**).

Discussion

In the present study, among patients who were suspected of having IgG4-RD and in whom serum IgG4 levels were measured, 56 patients (24.9%, out of all of the 225 patients) were found to have serum IgG4 levels higher than 135 mg/dL, and only 17 (7.6%) of all of the patients were classified into the definite group. Regarding their symptoms, the initial manifestations in all cases were varied, with the main symptoms being renal dysfunction (14%), general malaise, thirst and body weight loss. In the definite group, thirst was the most frequent symptom (24%), probably due to the accompanying IgG4-related Mikulicz's disease (7). The possible group of 17 patients (7.6%, out of 225 patients) had characteristic diffuse/localized swelling or masses in single or multiple organs with serum IgG4 levels >135 mg/dL, though this group did not fulfill the pathological criteria. In contrast to the definite group, renal dysfunction was the most frequent symptom (21%) in the possible group. Given that renal dysfunction is often caused by the affected retroperitoneum (8, 9), the difficulty in retroperitoneal biopsy and lack of pathological criteria are likely to be related to the occurrence of renal damage in the possible group.

Affected tissues of hyper IgG4 syndromes were often detected in lymph

nodes (15%), retroperitoneum (15%), submandibular glands, kidneys, pancreas and lungs in the present study, being similar to the results of previous studies (3, 10). The high frequencies of retroperitoneal and renal lesions might be due to the large percentage of patients referred from the urology department with suspicion of IgG4-related retroperitoneal fibrosis. The relatively low frequency of pancreatic and pulmonary lesions in the present study might be due to the tendency for direct referral to gastrointestinal and respiratory departments.

Since pathological findings are essential for a definite diagnosis of IgG4-RD, biopsy should be performed from pathologically active lesions. If the approach for biopsy of the target lesion is difficult and/or invasive, a whole-body investigation using imaging technologies such as CE-CT, ^{67}Ga-scintigraphy and FDG/PET-CT should be discussed (11-13). For instance, instead of retroperitoneal biopsy, a prostate biopsy after confirmation of prostate lesions by MRI may be an alternative and less invasive method for pathological confirmation of IgG4-RD (9). In the present study, the most frequently biopsied tissues were minor salivary glands (24%), lymph nodes (22%), submandibular glands and kidneys. Biopsied tissues that were most useful for a definitive diagnosis of IgG4-RD were the retroperitoneum (100%) and submandibular glands (83%). In contrast, a definite diagnosis of IgG4-RD was made in only 8% of

the patients in whom a minor salivary gland biopsy was performed. A biopsy specimen can be easily obtained from minor salivary glands, and it has been reported that positive rates of a minor salivary gland biopsy in IgG4-RD were approximately 40% to 63% (14). Therefore, the possibility of IgG4-RD should not be excluded even if a diagnosis of IgG4-RD cannot be made from biopsy of minor salivary glands, and investigation of other suspected tissues for IgG4-RD should be considered (7).

In the present study, 4 of the 11 cases of lymph node biopsy were satisfied the consensus statement on the pathology of IgG4-RD in addition to items 1) and 2) of the comprehensive diagnostic criteria 2011. As for the pathology for diagnosing IgG4-related lymphadenopathy (15, 16), distinguishing hyper IL-6 syndromes such as multicentric Castleman's disease (MCD) and rheumatic diseases is critical. Given that the pathological findings of MCD and IgG4-related lymphadenopathy are similar, a diagnosis solely based on the pathology is often difficult (15, 16). Therefore, a comprehensive diagnosis based on clinical, serological, or radiological evidence is necessary (5). In this study, no fibrotic changes were detected in 4 biopsied cases of lymph nodes, while histological type II (reactive follicular hyperplasia-like) and type III (interfollicular expansion and immunoblastosis) were observed in 2 cases each (16). According to the consensus statement on the pathology of IgG4-related

lymphadenopathy, we diagnosed these cases by a comprehensive approach.

Storiform fibrosis and obliterative phlebitis are also considered to be key features for the pathological diagnosis of IgG4-RD (5), though these are not included in the comprehensive diagnostic criteria. However, these features are rarely found in lymph nodes, salivary glands or lacrimal glands (5). Recently, tissue biopsies are mostly performed by a needle biopsy for which only a tiny specimen is obtained, and typical findings of storiform fibrosis and obstructive phlebitis are therefore hardly detected (17, 18). We reexamined tissues of the submandibular glands, pancreas and retroperitoneum, and we detected storiform fibrosis in only 14% of the cases (1 out of 7 cases) and obstructive phlebitis in 43% (3/7) of the cases in the definite group but in no cases in the possible and other groups. Thus, the significance of pathological findings might be different depending on the biopsied tissues and/or the procedure for biopsy.

In the present study, results of analysis of biochemical parameters showed that there were significant differences between the definite group and the possible or other group in serum levels of IgG4 and IgG and IgG4/IgG ratio, which were also reported to be elevated in cases of IgG4-RD (19). In contrast, serum IgA level was significantly lower in the definite group than in the other two groups. It has been

reported that serum levels of IgG4, IgG and IgE, but not serum levels of IgA and IgM, tended to be elevated in cases of IgG4-RD (15, 20). Of interest, basophil levels were also increased in the definite group in our study. In this regard, increased IgG4 production by B cells was reported to be associated with enhanced production of B-cell activating factor (BAFF) and IL-13 via activation of Toll-like receptors in basophils (21).

There were significant differences in serum levels of C3, C4 and CH50 between the definite group and the possible or other group. Complement levels tended to be decreased in cases of IgG4-RD as was observed in previous studies (22, 23). Deposition of C1q is often observed in affected tissues of IgG4-RD, which is linked to activation via the classical complemental pathway leading to decreases in serum levels of C3, C4 and CH50 (24, 25). When IgG4-related kidney diseases were complicated, the decreases in levels of complements tended to be more pronounced (23, 26-28). In addition to the contribution of IgG4 to the consumption of complements (29, 30), involvement of globulin subclasses other than IgG4 has been implicated in IgG4-RD, and deposition of IgG1 was also detected in involved organs in IgG4-RD (25). In the present study, the proportion of patients with hypo-CH50 (<25 U/mL) was 54% (7 out of 13 patients) in the definite group, while hyper-CH50 (>48

U/mL) was seen in 36% (5/14) of the patients in the possible group and in 60% (12/20) of the patients in the other group, implying that detection of hypo-complementemia is a possible predictor for IgG4-RD.

As for inflammatory markers, serum CRP levels showed a significant difference between the other group and the definite or possible group. The other group of 22 patients (10%, out of 225 patients), who had serum IgG4 levels higher than 135 mg/dL, did not have characteristic diffuse/localized findings and also failed to satisfy the pathological diagnostic criteria. In the other group, the levels of inflammatory markers including WBC and CRP were higher than the levels in the other two groups. In the present study, many inflammatory diseases other than IgG4-RD also showed high serum IgG4 levels, due to the activities of collagen disease and vasculitis, hematologic disease and neoplastic diseases. Given that serum CRP levels are not usually elevated in cases of IgG4-RD (15, 31), the inclusion of various inflammatory diseases in the other group might have resulted in the difference in serum CRP levels among the groups. The present study has some limitations including a relatively small sample size and its retrospective nature. In addition, patients who had already received corticosteroid treatment in other hospitals and patients with serum IgG4 levels below 135 mg/dL were eliminated in this study.

Further study including a multi-center database is necessary to identify effective biomarkers for differential diagnosis of hyper IgG4 conditions and to further examine the substantial differences between the definite and possible groups of hyper IgG4 syndrome.

In conclusion, hyper IgG4 syndromes are occasionally detected by nonspecific symptoms such as general malaise and weight decrease as shown in our department. Among patients suspected of having IgG4-RD by considering general symptoms, blood cell counts and levels of serum IgG, IgA, IgM and complements can be diagnostic for IgG4-RD. Given the distribution of affected tissues and the approach for diagnostic biopsies, systemic examination and a detailed whole-body scan should be considered for each case.

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Disclosure

The authors declare no conflicts of interest in association with the present study.

Contributor ship statement:

KH, YH, and FO conceived and designed the study. KH, YH, MO, TM, HO, EK, HK, YS, and FO were involved in the acquisition of data. KH, YH, EK and YS analyzed the data. KH, YH and FO wrote the manuscript.

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Figure Legends:

Fig. 1. Patients' characteristics: A) Patients' profile: Diagnosis of IgG4-RD was classified according to the comprehensive diagnostic criteria for IgG4-RD 2011 and consensus statement on the pathology of IgG4-RD, and also reassessed by organ-specific diagnostic criteria. The ratios of males were 71% (12 males) in the definite group (n=17), 76% (13 males) in the possible group (n=17), and 45% (10 males) in the other group (n=22). Dotted boxes indicate males and hatched boxes indicate females. The average ages of patients in the three groups were 64-65 years. **B) Initial manifestations:** Classification of initial manifestations in each group. Black boxes indiate the definite group, gray boxes indicate the possible group and white boxes indicate the other group.

Fig. 2. Affected tissues and approach for biopsy: A) Classification of affected tissues: Clinically affected tissues are shown in the frequency order. **B) Biopsied tissues:** Tissue sources for biopsy are shown in the frequency order. Black boxes indicate the definite group, gray boxes indicate the possible group and white boxes indicate the other group.

Fig. 3. Comparison of laboratory markers in the three groups: Various laboratory data related to IgG4-RD were compared among the three groups. Black boxes indicate the definite group, gray boxes indicate the possible group and white boxes indicate the other group. $^{**}P < 0.01$ and $^{*}P < 0.05$, significant correlations between the indicated groups.

Fig. 4. Interrelationships between level of serum IgG4 and related markers: Correlations of serum IgG4 level with serum levels of IgG, IgM, C3 and C4 were statistically analyzed by linear regression analysis in each group. *R*: correlation coefficients. $^{**}P < 0.01$ and $^{*}P < 0.05$, significant correlations between the indicated factors and shown by filled dots.