ABSTRACT

Objective:

We examined the symptoms in the oral and maxillofacial regions of patients with IgG4-related disease (IgG4-RD) and considered the meaning of a labial minor salivary gland (LMSG) biopsy in such patients.

Methods:

Sixty-three patients diagnosed with IgG4-RD in our hospital, were examined for symptoms in the oral and maxillofacial regions. Out of the 24 patients who were referred to our department, 20 underwent immunohistochemical analysis of the LMSG for IgG and IgG4.

Results:

Symptoms in the oral and maxillofacial region were observed in 18 of the 63 patients. Out of the 18 patients, 15 had swelling of the submandibular glands, 5 had severe xerostomia, 4 had swelling of the parotid glands, and 2 experienced irritation and pain while eating. IgG4-positive plasma cells in the LMSG biopsy samples were observed in 19 of the 20 patients examined (95%). In 6 patients, more than 40% of the infiltrated plasma cells were positive for IgG4; in 2 patients, more than 30% of the plasma cells were positive; in 3 patients, more than 20% of the plasma cells were positive; in 7 patients, more than 10% of the plasma cells were positive; in 1 patient, more than 5% of the plasma cells were positive.

Conclusions:

Out of the 63 patients, only 18 (29%) had symptoms in the oral and maxillofacial regions. Infiltration of IgG4-positive plasma cells, regardless of their intensity in the non-symptomatic LMSG, might be considered as one of the important symptoms of IgG4-RD.

Keywords

IgG4-related disease

Labial minor salivary gland biopsy

Sjögren syndrome

1. Introduction

IgG4-related disease (IgG4-RD) is a newly recognized fibro-inflammatory condition, characterized by tumefactive lesions, a dense lymphoplasmacytic infiltration containing rich IgG4-positive plasma cells, storiform fibrosis, and often elevated serum IgG4 concentrations [1,2]. In the field of oral and maxillofacial surgery and oral medicine, Mikulicz's disease (MD) [3] and Küttner's tumor [4] are now considered to be a part of the IgG4-RD spectrum. MD had been previously considered as one of the phenotypic variants of Sjögren syndrome (SS) based on the histopathological similarities [5]. However, after universal recognition of the disease entity of IgG4-RD, MD is now recognized as a different disease from SS [6].

Two Japanese IgG4-RD study groups, which were organized by the Ministry of Health, Labor and Welfare Japan, had released the comprehensive diagnostic criteria for IgG4-RD in 2011 [7]. They divided the situation of the disease into three categories- definitive, probable, and possible according to the results from the clinical, hematological, and histopathological examinations. If a definitive or probable diagnosis of this disease seems warranted, biopsy samples must be taken from the inflammatory regions with a highly invasive surgery. To avoid such a surgery for the diagnosis of this disease, alternative histopathological examinations should be considered. Takano *et al*. [8] and Moriyama *et al.* [9] reported that a labial minor salivary gland (LMSG) biopsy showed high specificity but low sensitivity for the diagnosis of IgG4-RD. Therefore, in this study, we carefully examined the symptoms in the oral and maxillofacial regions (face, maxilla, mandible, oral cavity, and neck) of the patients with IgG4-RD and considered the meaning of LMSG biopsy.

2. Patients, materials, and methods

2.1. Patients

The selection of patients with IgG4-RD has been shown in Fig. 1. First, we selected all the patients with serum IgG4 level >135 mg/dl, who were examined in Dokkyo Medical University Hospital from 2005 to 2014. Out of the 480 patients with serum IgG4 level >135 mg/dl, 63 patients (44 men and 19 women; median age 68 years) were diagnosed with IgG4-RD in other departments of our hospital with the diagnosis criteria described below, which is based on the comprehensive diagnostic criteria for IgG4-RD as established in 2011 [7]. Out of these 63 patients (21 Definite cases and 42 Possible cases) with IgG4-RD, 24 were referred to our department (Department of Oral and Maxillofacial Surgery) to perform a differential diagnosis of these patients from those diagnosed with SS and/or to examine their oral symptoms. Out of these 24 patients, 20 underwent a LMSG biopsy according to the criteria of SS and IgG/IgG4 immunostaining.

2.2. Diagnosis of the patients as IgG4-RD

The patients were examined for IgG4-RD based on the following: (1) clinical examination showing characteristic diffuse/localized swelling or masses in single or multiple organs, (2) hematological examination showing elevated serum IgG4 concentrations (≥135 mg/dl), and (3) histopathological examination showing [a] marked lymphocyte and plasmacyte infiltration and fibrosis, and [b] infiltration of IgG4-positive plasma cells in a ratio of IgG4/IgG-positive cells greater than 40% and IgG4-positive plasma cells/ high-power field (HPF) of greater than 10. Subsequently, the diagnosis of IgG4-RD was established based on a combination of the abovementioned criteria: Definite (1) + (2) + (3); Probable (1) + (3); Possible (1) + (2). However, it is important to differentiate IgG4-RD from the malignant tumors of each organ (e.g., cancer, lymphoma) and similar diseases (e.g., SS, primary sclerosing cholangitis, Castleman's disease,

secondary retroperitoneal fibrosis, Wegener's granulomatosis, sarcoidosis, and Churg-Strauss syndrome) by additional histopathological examinations. In situations, where the patient cannot be diagnosed using the above-mentioned criteria, they may be diagnosed using organ-specific diagnostic criteria for IgG4-RD [9].

2.3. Differential diagnosis of the patients from SS and/or examination of the oral and maxillofacial regions

Twenty-four patients were referred to our department (Department of Oral and Maxillofacial Surgery) to perform a differential diagnosis of the patients from those with SS and/or to examine their oral symptoms. The patients were examined for oral dryness, salivary flow (gum test or Saxon test), and eye dryness (Schirmer test or the fluorescein test); $^{99m}TcO_4^-$ salivary scintigraphy, MRI sialography, anti-SS-A/B in serum, and labial minor salivary gland biopsy results were also analyzed [10]. The swelling of the submandibular glands, sublingual glands, parotid glands, minor salivary glands, and lachrymal glands; and the appearance of fibro-inflammatory lesions in the oral cavity and the facial skin, was also evaluated by careful palpation. Furthermore, the oral functions of the patients, such as speaking, mastication, tasting, and swallowing was assessed. Since no patient had a swelling in their lachrymal glands, no biopsy material was taken from the lachrymal glands.

2.4. Immunohistochemistry for IgG and IgG4 in the biopsy samples

Sections (4-μm thick) were mounted on silane-coated glass slides, deparaffinized, and rinsed. Following antigen retrieval with 0.05% protease (Type XXIV, Sigma, St. Louis, MO) treatment in phosphate buffered saline (PBS; pH 7.6) for 15 minutes at room temperature, the sections were immersed in 0.3% hydrogen peroxide to block the endogenous peroxidase activity. Subsequently, the sections were probed with or without the anti-IgG (pre-diluted, The Binding

Site Group, Birmingham, UK) and anti-IgG4 (1:2000, DAKO, Carpinteria, CA) primary antibody for 1 hour at room temperature. The sections were then incubated with the peroxidase-labeled secondary antibody (MAX-PO [MULTI] kit, NICHIREI, Tokyo, Japan) for 30 minutes at room temperature. Subsequently, the sections were washed with cold PBS and then allowed to react with 3,3'-diaminobenzidine tetrahydrochloride solution and 0.03% hydrogen peroxide for 3 minutes at room temperature. The final step was counterstaining the sections with hematoxylin. It is important to note that the clear staining in all the cells had completely disappeared, upon replacing the primary antibody solution with PBS.

2.5. Evaluation of the immunohistochemical staining

The evaluation of the immunohistochemistry was performed by two oral surgeons (ST and HK) based on the IgG4-RD diagnosis criteria mentioned above [10].

3. Results

3.1. Characteristics of the patients with IgG4-RD

Among the 63 patients with IgG4-RD, 37 patients first visited the Department of Pulmonary Medicine and Clinical Immunology to be examined for any auto-immune or allergic diseases; 22 patients first visited the Department of Gastroenterology or Gastroenterological Surgery to be examined for pancreatic disease (auto-immune pancreatitis or pancreatic cancer), bile duct disease (cholangitis or cholangiocarcinoma), or esophageal cancer; and 4 patients first visited Otolaryngology or Hematology to be examined for their painless neck mass. Among the 63 patients with IgG4-RD, 21 patients were classified as Definite IgG4-RD cases by the positive result of immunohistochemistry (IgG4+/IgG: more than 40%) in the symptomatic organs. On the other hand, 42 patients were classified as Possible IgG4-RD cases, because histopathological examination was not performed, or the results of immunohistochemistry did not match the criteria.

Based on their medical charts, out of the 63 patients with IgG4-RD, 45 patients (71%) showed no symptoms in the oral and maxillofacial regions whereas 18 patients (29%) demonstrated oral and maxillofacial symptoms. Among these 18 patients, because some patients had more than two symptoms, 15 patients showed swelling of the submandibular glands, 5 patients had severe xerostomia, 4 patients revealed swelling of the parotid glands, and 2 patients experienced irritation and pain while eating.

3.2 Characteristics of the patients with IgG4-RD who visited our department

Among the 63 patients with IgG4-RD, 24 patients were referred to our department from other departments in the hospital for the following reasons: (1) examination of the symptoms of the oral and maxillofacial regions as IgG4-RD (17 patients) and (2) differential diagnosis of the patients from SS (2 patients). Five patients routinely visited our department

for oral examinations independent of any concern about IgG4-RD.

3.3 Examinations of the patients with IgG4-RD who visited our department

Nine patients (3, 5, 7, 12, 16, 17, 20, 22, and 23) were already diagnosed as Definite, and 15 patients (1, 2, 4, 6, 8, 9, 10, 11, 13, 14, 15, 18, 19, 21, and 24) were diagnosed as Possible with IgG4-RD (Table 1). None of the patients were diagnosed as Probable with IgG4-RD (Table 1). Out of the 24 patients, 15 (1, 2, 4, 6, 7, 8, 11, 12, 13, 15, 16, 17, 19, 21, and 23) showed swelling of submandibular glands and 2 patients (1 and 4) revealed swelling of submandibular glands and parotid glands (Table 1). Salivary flow was examined by a gum test and Saxon test. Out of the 18 patients examined, 10 (1, 6, 7, 11, 13, 16, 17, 18, 19, and 20) manifested decreased salivary flow (less than 10 ml/10 min by gum test) and 2 (17 and 23) out of the 11 patients examined revealed decreased salivary flow (less than 2 g/2 min by Saxon test) (Table 1). Six patients (2, 4, 6, 9, 11, and 18) among the 15 patients examined, had dry eyes (Table 1). Function of the salivary glands was assessed by 99mTcO4-salivary scintigraphy according to the criteria of SS [11]. Eight (1, 2, 4, 7, 11, 19, 20, and 22) out of the 17 patients examined, had dysfunction of the salivary glands in the salivary scintigraphy (Table 1). Morphology of the salivary glands (parotid glands) was assessed by MRI sialography instead of the contrast media infused–sialography according to the criteria of SS [11]. Two (7 and 9) out of the 15 patients examined showed an abnormal view of the parotid gland in the MRI sialography (Table 1). None of the patients examined obtained positive results for serum anti-SS-A/SS-B (Table 1). Out of the 15 patients examined, 9 (2, 4, 7, 8, 11, 13, 18, 19, and 20) obtained positive results (Focus score $\geq$1) in the LMSG biopsy according to the criteria of SS [11] (Table 1). Seven patients (2, 4, 7, 11, 18, 19, and 20) were matched to the Japanese criteria for SS.

LMSG biopsy revealed IgG4-positive plasma cells in most of the patients tested (19 positive out of 20 examined). Moreover, in 6 patients (7, 8, 13, 17, 19, and 20), LMSG biopsy

revealed that more than 40% of IgG-positive plasma cells were IgG4-positive (Table 1, Fig. 2: Case 19). Case No. 8, 13, and 19 were upgraded to “Definite” from “Possible” based on the results of LMSG biopsy (Table 1). We detected the infiltration of IgG4-positive plasma cells with hyperplastic ectopic germinal centers in four patients (7, 8, 17, and 19), but the destruction of acinus was not observed in either of them, as described by Moriyama *et al.* [12]. Serum IgG4 levels varied among the patients from 175 to 2790 mg/dl (Table 1).

3.4 Association of the symptoms and function of the oral and maxillofacial regions with the ratio of IgG4-positive cells

We examined the association of the symptoms and function of the oral and maxillofacial regions with the ratio of IgG4-positive cells. There was no apparent relationship between swelling of the salivary gland and the ratio of IgG4-positive cells (30.9% ± 20.5 in the swelling group vs. 23.4% ± 14.2 in the non-swelling group, $p = 0.47$) (Fig. 3a), dry mouth and the ratio of IgG4-positive cells (32.8% ± 32.1 in the dry mouth group vs. 18.2% ± 20.0 in the non-dry mouth group, $p = 0.09$) (Fig. 3b), or the function of the salivary glands (assessed by 99mTcO4-salivary scintigraphy according to the criteria of SS) and the ratio of IgG4-positive cells (37.4% ± 21.0 in the dysfunction group vs. 30.0% ± 17.1 in the normal function group, $p = 0.20$) (Fig. 3c). In addition, there was no apparent relationship between the serum IgG4 levels and either the staining of IgG4-positive cells or the clinical symptoms in the oral and maxillofacial regions (data not shown).

4. Discussion

Diagnosis of IgG4-RD was established using the following criteria (1) clinical examination showing characteristic diffuse/localized swelling or masses in single or multiple organs, (2) hematological examination showing elevated serum IgG4 concentrations (≥135 mg/dl), and (3) histopathologic examination of the lesions with inflammation. Clinical examination and blood test might have been non-invasive; however, the histopathological examination was invasive, especially for the organs in the deeper regions of the body, such as the liver, kidney, pancreas, retroperitoneal tissue, and lungs. Subsequently, we examined the oral and maxillofacial symptoms of the patients with IgG4-RD and considered the meaning of the LMSG biopsy, because the symptoms of IgG4-RD were reported to appear more frequently in the oral and maxillofacial regions [13].

In this study, none of the patients first visited our department with IgG4-RD as their chief complaint in the oral and maxillofacial regions. The patients visited the departments of Pulmonary Medicine and Clinical Immunology, Gastroenterology, Gastroenterological Surgery, Otolaryngology, or Hematology. Subsequently, 38% of the patients were referred to our department for physical examination of the oral and maxillofacial regions and/or differential diagnosis of SS. Unexpectedly, only 29% of the patients showed some symptoms in the oral and maxillofacial regions.

Contrary to the clinical symptoms, IgG4-positive plasma cell infiltrations at various intensities were observed in the LMSG biopsy samples obtained from the patients. In six patients among 20 examined, more than 40% of the infiltrated plasma cells were positive for IgG4; in two patients, more than 30% of the plasma cells were positive; in three patients, more than 20% of the plasma cells were positive; in seven patients, more than 10% of the plasma cells were positive; and in one patient, more than 5% of the plasma cells were positive. Only one patient obtained a negative result for IgG4 immunohistochemistry in the LMSG biopsy

sample. Thus, we observed the characteristic histopathological changes (infiltration of the IgG4-positive plasma cells) for IgG4-RD in the LMSG biopsy sample without any clinical symptoms, such as swelling and sclerosis. Only 8 patients, who showed massive IgG4-positive plasma cells (more than 30%) in the LMSG, complained of xerostomia.

Histopathological criteria for the diagnosis of IgG4-RD were as follows: (1) marked lymphocyte and plasmacyte infiltration and fibrosis and (2) infiltration of IgG4-positive plasma cells in a ratio of IgG4/IgG-positive cells greater than 40% and IgG4-positive plasma cells/HPF of greater than 10 [3]. These criteria were for the organs with inflammation, which showed swelling and/or fluorodeoxyglucose (FDG) accumulation on positron emission tomography (PET) examination. Therefore, it was not a good idea to apply these criteria to minor salivary glands without clinical symptoms and/or inflammation. In fact, several investigators had reported that biopsy specimens obtained from the submandibular glands with swelling and inflammation, were extremely useful in the diagnosis of IgG4-RD; however, biopsy specimens obtained from the LMSG without any clinical symptoms might be less informative for establishing an IgG4-RD diagnosis [8, 9, 12]. We interpreted our results as follows: infiltration of IgG4-positive plasma cells in the LMSG without any clinical symptom and/or inflammation might have a significant meaning towards the diagnosis of IgG4-RD. To confirm our interpretation, we examined the infiltration of IgG4-positive plasma cells in the LMSG biopsy samples with non-specific inflammation such as mucocele, or the LMSG taken from patients with SS. As expected, these LMSGs rarely showed positive results in IgG4 immunohistochemistry (data not shown).

In this study, we clearly showed that the infiltration of the IgG4-positive plasma cells, regardless of the intensity in the non-symptomatic LMSG, might be considered as one of the important symptoms for IgG4-RD. Recently, a heightened interest in IgG4-RD, has resulted in increased diagnosis without biopsy of local lesions, causing IgG4-RD to be often misdiagnosed

as malignant lymphoma or a similar disease [14, 15]. Moreover, Sato and colleagues reported a case of IgG4-producing B-cell lymphoma presenting with high serum IgG4 and strong infiltration of IgG4-positive cells in the affected organ [16]. Thus, we should be careful while considering the possibility of malignant lymphoma if the LMSG biopsy is positive. We are currently conducting a clinical investigation to show whether the histopathological changes in the LMSG can reflect the progression of this disease and the effect of treatment in the IgG4-RD patients receiving steroid therapy.

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Figure legends

Fig. 1 Selection of patients with IgG4-RD

We selected all the patients with serum IgG4 level >135 mg/dl, examined in our hospital from 2005 to 2014. Among the 480 patients with serum IgG4 level >135 mg/dl, 63 patients (44 men and 19 women; median age 68 years) were diagnosed with IgG4-RD in other departments of our hospital based on the diagnosis criteria. Out of these 63 patients (21 Definite cases and 42 Possible cases) with IgG4-RD, 24 were referred to our department.

Fig. 2 Immunohistochemistry for IgG and IgG4

Sectioned LMSG samples were stained with anti-IgG primary antibody (A) and anti-IgG4 primary antibody (B). A: IgG immunostaining for Case 19. B: IgG4 immunostaining for Case 19. More than half of the IgG-positive plasma cells showed IgG4 immunostaining. Scale bar, 50 μm.

Fig. 3 Association of the symptoms and the function of the oral and maxillofacial regions with the staining of IgG4-positive cells

There was no apparent relationship between the staining of IgG4-positive cells and swelling (A), dry mouth (B), or dysfunction of salivary glands (C).