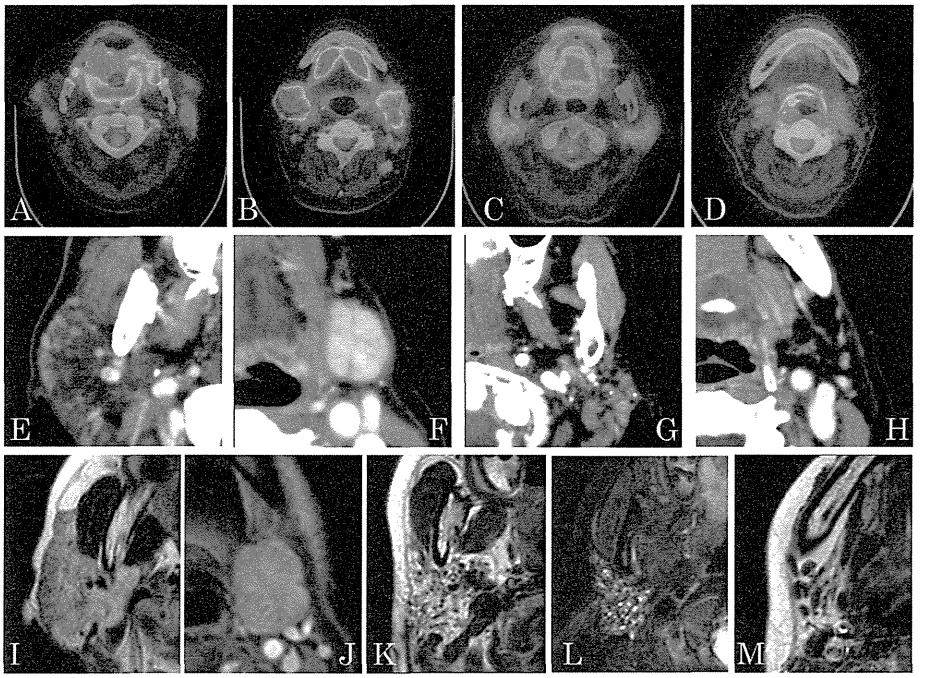

Fig. 2 Characteristic findings on FDG-PET/CT, CT and MRI. **a-d** FDG-PET/CT, **e-h** CT and **i-m** MRI. **a, e, i** Parotid glands of a patient with IgG4-DS. **b, f, j** Submandibular glands of a patient with IgG4-DS. **c, g, k, l** Parotid glands of a patient with SS. **d, h, m** Submandibular glands of a patient with SS. FDG-PET/CT shows abnormal ^{18}F-FDG accumulation in the parotid (**a, c**) and submandibular glands (**b**). A patient with IgG4-DS showing parotid (**e, i**) and submandibular gland swelling (**f, j**), superficial enhancement of the parotid glands (**e**), and a septum-like structure in the submandibular glands (**f**). A patient with SS showing atrophic parotid (**g**) and submandibular glands (**h, m**), and a salt-and-pepper appearance (**g, k, l**). It also shows dot-like calcifications on CT (**g**), and small multiple cystic areas on T2-weighted images (**l**). *FDG-PET/CT* 2-[^{18}F]-fluoro-2-deoxy-D-glucose positron emission tomography/computed tomography, *CT* computed tomography, *IgG4-DS* IgG4-related dacryoadenitis and sialadenitis, *MRI* magnetic resonance imaging, *SS* Sjögren's syndrome

dot-like calcifications on CT [20]; and small multiple cystic areas, which show hyperintensity on T2-weighted images.

Statistical analysis

We calculated the sensitivity, specificity and accuracy using the following equations: sensitivity for IgG4-DS = a/(a+d+g), sensitivity for SS = e/(b+e+h), specificity = i/(c+f+i), accuracy = (a+e+i)/(a+b+c+d+e+f+g+h+i), where a: number of patients we diagnosed with IgG4-DS and who actually had IgG4-DS, b: number of patients we diagnosed with IgG4-DS but who had SS, c: number of patients we diagnosed with IgG4-DS but who were normal, d: number of patients we diagnosed with SS but who had IgG4-DS, e: number of patients we diagnosed with SS and who actually had SS, f: number of patients we diagnosed with SS but who were normal, g: number of patients we diagnosed as normal but who had IgG4-DS, h: number of patients we diagnosed as normal but who had SS, and i: number of patients we diagnosed as normal and who were actually normal. We performed the Bartlett test for the analysis of equal variance. To determine whether or not there were significant differences between two given groups among three, we performed the Steel-Dwass test in case of unequal variance, and the Tukey-Kramer test in case of equal variance using the statistical software JMP Pro 11.0 (SAS Institute, Cary, NC, USA). p values <0.05 were considered significant. Intra-observer agreement rates between the repeat diagnoses (diagnosis carried out under blinded conditions and repeated after 3 weeks) were assessed with kappa values using 3 grades: 1 or 2, 3, and 4 or 5. Values <0.20 indicated poor agreement, 0.21–0.40 fair agreement, 0.41–0.60 moderate agreement, 0.61–0.80 good agreement and 0.81–1.00 excellent agreement.

Results

Intra-observer agreement rates between the repeat diagnoses were very high. Kappa values for selecting the same diagnosis from normal, IgG4-DS or SS were 0.83 (range, 0.76–0.91) on sonography, 0.69 (range, 0.5–0.90) on FDG-PET/CT, 0.70 (range, 0.49–0.80) on CT and 0.80 (range, 0.64–0.87) on MRI. Moreover, average kappa values for each finding were 0.62 for sonography, 0.61 for FDG-PET/CT, 0.51 for CT and 0.54 for MRI; no findings showed an inverted order of scores the second time. Therefore, we have shown the results of the first diagnosis in the relevant figures.

Analysis of sonographic findings

Figure 3 shows the results of sonographic analysis. In all findings, significant differences ($p \leq 0.0093$) were observed between any two of the three diagnoses. The parotid glands of patients with SS mainly exhibited multiple hypoechoic areas (median score 4) and hyperechoic lines and/or spots (median score 4) (Fig. 3a and b), while the reticular pattern in the parotid glands showed overlap between IgG4-DS (median score 1) and SS (median score 2) (Fig. 3c). In the submandibular glands, overlaps were seen between IgG4-DS and SS regarding the findings of multiple hypoechoic areas (median score 4 for both IgG4-DS and SS) and hyperechoic lines and/or spots (median score 3 for IgG4-DS and 4 for SS), although significant differences ($p = 0.0093$, $p < 0.0001$, respectively) were seen between the two (Fig. 3d and e). Obscuration of the submandibular gland configuration was mainly observed in SS (median score 3) (Fig. 3f). In contrast, IgG4-DS mainly exhibited reticular and nodal patterns (median score 5), and separation between IgG4-DS and the other conditions was especially good concerning the nodal pattern (median score 1 for normal glands and 2 for SS) (Fig. 3g and h). Intra-observer agreement rates between the repeat diagnoses were very high (kappa values, 0.67–0.87), which showed the nodal pattern could be easily detected.

Each case was diagnosed as normal, IgG4-DS or SS based on all of the sonographic findings. Sonographic

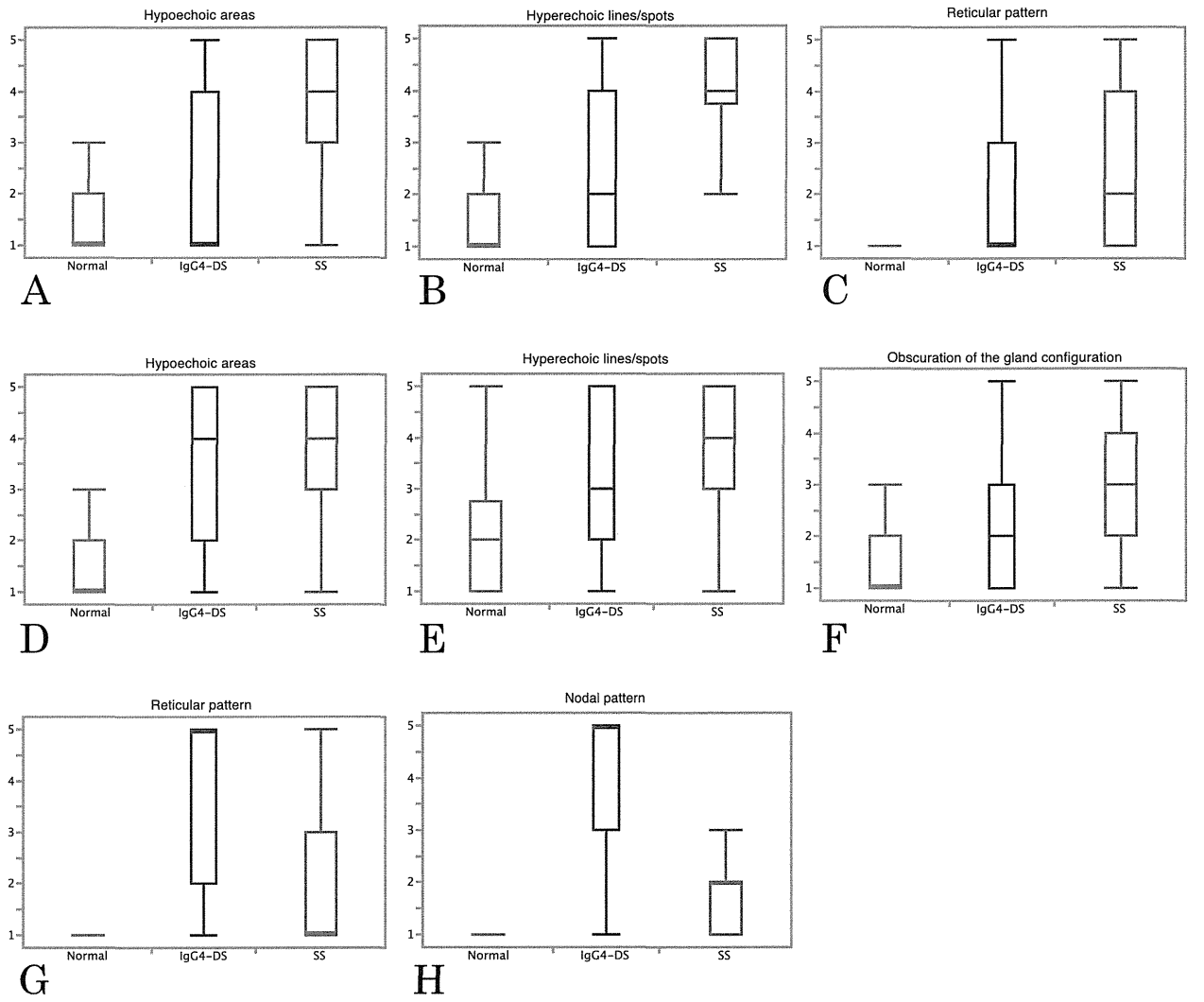

Fig. 3 Results of sonographic analysis. Values are shown with median and quartile points for **a-c** the parotid glands and **d-h** the submandibular glands. **a** Multiple hypoechoic areas, **b** hyperechoic lines and/or spots and **c** reticular pattern in the parotid glands. **d** Multiple hypoechoic areas, **e** hyperechoic lines and/or spots, **f** obscuration of the gland configuration, **g** reticular pattern and **h** nodal pattern in the submandibular glands. Multiple hypoechoic areas (**a**) and hyperechoic lines and/or spots (**b**) in the parotid glands and obscuration of submandibular gland configuration (**f**) were primarily observed in patients with SS. Reticular and nodal patterns observed mainly in patients with IgG4-DS (**g, h**). *IgG4-DS* IgG4-related dacryoadenitis and sialadenitis, *SS* Sjögren's syndrome

sensitivity for detection of IgG4-DS and of SS, specificity and accuracy were 0.85, 0.80, 0.84 and 0.83, respectively.

Analysis of FDG-PET/CT findings

FDG-PET/CT involving patients with IgG4-DS showed a tendency for abnormal accumulation of ^{18}F-FDG and swelling of both the parotid (median scores 2 and 4, respectively) and submandibular glands (median scores 5 and 4, respectively) (Fig. 4a–d). Separation between IgG4-DS and the other conditions was particularly good regarding the abnormal accumulation of ^{18}F-FDG in the submandibular glands (median score 2 for normal glands and 1 for SS) (Fig. 4c). In relation to this finding, significant differences were observed between IgG4-DS and SS (p <0.0001), and between patients with IgG4-DS and those with normal glands (p <0.0001); however, this was not seen between patients with normal glands and SS (p = 0.1180). Intra-observer agreement rates between the repeat diagnoses were high (kappa values, 0.51–0.89). Regarding all other findings, significant differences (p ≤0.0049) were observed between any two of three diagnoses.

Each case was diagnosed as normal, IgG4-DS or SS based on all of the findings from FDG-PET/CT. The sensitivity for detection of IgG4-DS and of SS, specificity and accuracy using FDG-PET/CT were 0.79, 0.59, 0.81 and 0.73, respectively.

Analysis of CT findings

Figure 5 shows the results of CT findings concerning the parotid glands (Fig. 5a–c), and the submandibular glands (Fig. 5d and e). Patients with IgG4-DS had large parotid (median score 4) and submandibular glands (median score 4) (Fig. 5a and d). Although there were significant differences between normal glands (parotid glands, median score 3, p = 0.0255; submandibular glands, median score 3, p <0.0001), there seemed to be a similar tendency between these two. Conversely, patients with SS had small parotid (median score 3) and submandibular glands (median score 2), especially submandibular glands. Patients with SS had a high score regarding the finding of a salt-and-pepper appearance and/or dot-like calcification (median score 4) (Fig. 5b). Regarding this finding, significant differences were observed between the other two

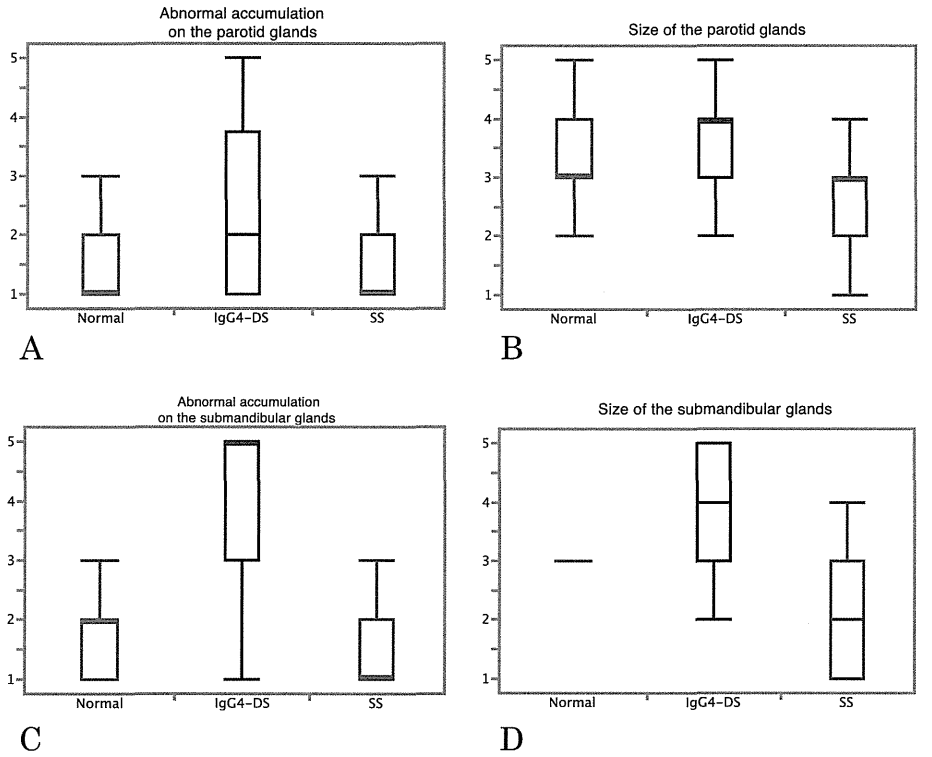

Fig. 4 Results of FDG-PET/CT. Values are shown with median and quartile points for **a, b** the parotid glands and **c, d** the submandibular glands. **a** Abnormal accumulation of ^{18}F-FDG in the parotid glands, **b** size of the parotid glands, **c** abnormal accumulation of ^{18}F-FDG in the submandibular glands and **d** size of the submandibular glands. Abnormal accumulation of ^{18}F-FDG and swelling of the glands in both the parotid and submandibular glands of a patient with IgG4-DS (**a-d**). Separation of IgG4-DS from the other conditions is especially good regarding the abnormal accumulation of ^{18}F-FDG in the submandibular glands (**c**), and significant differences can be observed between IgG4-DS and SS (p <0.0001), and between IgG4-DS and normal glands (p <0.0001). *FDG-PET/CT* 2-[^{18}F]-fluoro-2-deoxy-D-glucose positron emission tomography/computed tomography, *IgG4-DS* IgG4-related dacryoadenitis and sialadenitis, *SS* Sjögren's syndrome

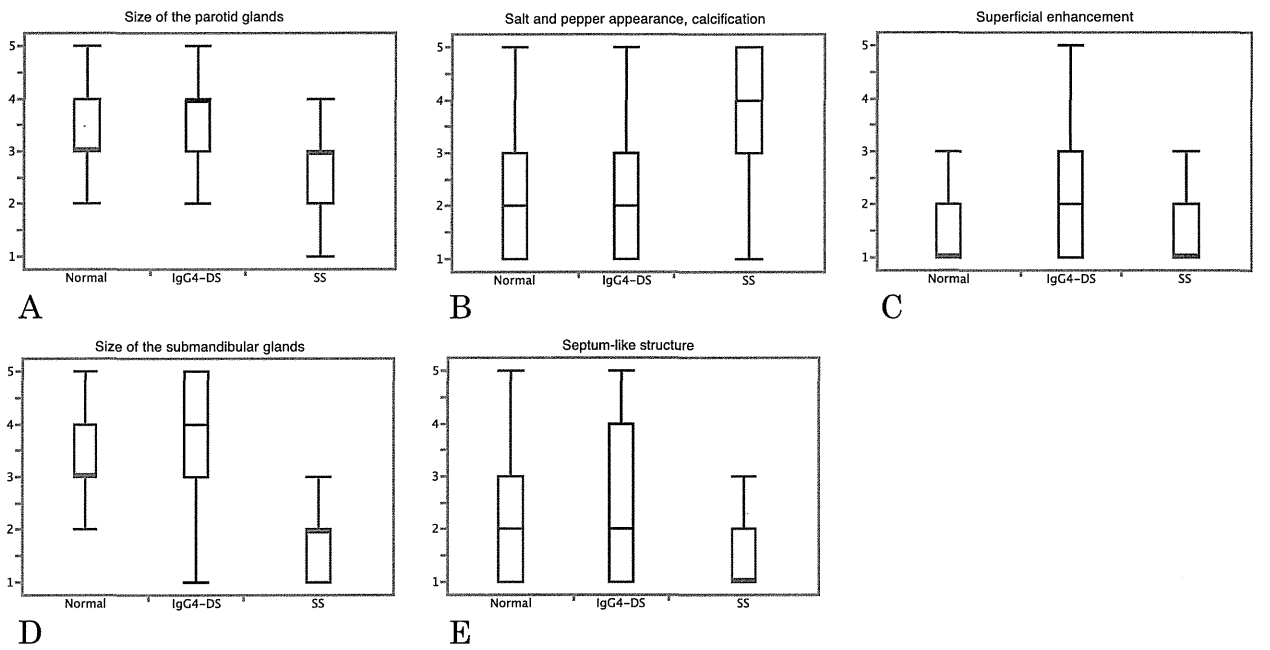

Fig. 5 Results of CT. Values are shown with median and quartile points for **a-c** the parotid glands and **d, e** the submandibular glands. **a** Size of the parotid glands, **b** salt-and-pepper appearance and/or dot-like calcification in the parotid glands, **c** superficial enhancement of the parotid glands, **d** size of the submandibular glands and **e** septum-like structure in the submandibular glands. Patient with IgG4-DS exhibiting large parotid and submandibular glands (**a, d**). SS shows a high score in the finding of a salt-and-pepper appearance and/or dot-like calcification (**b**). *CT* computed tomography, *IgG4-DS* IgG4-related dacryoadenitis and sialadenitis, *SS* Sjögren's syndrome

conditions (median score 2 for both normal glands and IgG4-DS) (p <0.0001), although this was not the case for between patients with normal glands and IgG4-DS (p = 0.7945). Superficial enhancement of the parotid glands (median score 2 for IgG4-DS) (Fig. 5c) and the presence of a septum-like structure in the submandibular glands (median score 2 for IgG4-DS) (Fig. 5e), which we considered were some of the characteristic findings of IgG4-DS, showed significant differences between the other two conditions (p <=0.0316); however, overlap was also observed and separation was not so good.

Each case was diagnosed as normal, IgG4-DS or SS based on all of the findings from CT. The sensitivity for detection of IgG4-DS and of SS, specificity and accuracy by CT were 0.64, 0.73, 0.70 and 0.70, respectively.

Analysis of MRI findings

Figure 6 shows the results of MRI evaluation of the parotid glands (Fig. 6a–c), and the submandibular glands (Fig. 6d). Patients with IgG4-DS had large parotid (median score 4) and submandibular glands (median score 5) (Fig. 6a and d). These findings differed significantly between the other two conditions for both the parotid and submandibular glands (p <0.0001). Conversely, patients with SS had small parotid (median score 3) and submandibular glands (median score 2), especially submandibular glands; there was a very clear separation between the other two conditions. Patients with SS had a high score regarding the finding of a salt-and-pepper appearance and/or multiple cystic areas in the parotid glands (median score 4.5) (Fig. 6b); there was also a very clear separation between the other two conditions (median score 1.5 for normal glands and 2 for IgG4-DS). Intra-observer agreement rates between the repeat diagnoses were high (kappa values, 0.54–0.79) regarding this finding. In relation to this finding, significant differences were observed between the other two conditions (p <0.0001), although a significant difference was not seen between patients with normal glands and IgG4-DS (p = 0.4163). Fatty degeneration in the parotid glands, which we considered to be one of the characteristic findings of SS, showed significant differences between the other two conditions (p <=0.0061); however, overlap was also observed and separation was not so good.

Each case was diagnosed as normal, IgG4-DS or SS based on all of the findings. The sensitivity for detection of IgG4-DS and of SS, specificity and accuracy using MRI were 0.80, 0.67, 0.81 and 0.76, respectively.

Comparison of the four imaging modalities

Figure 7 shows the sensitivity, specificity and accuracy of the four imaging modalities; sonography showed the highest levels. Regarding the sensitivity of IgG4-DS, there were significant differences between CT and the

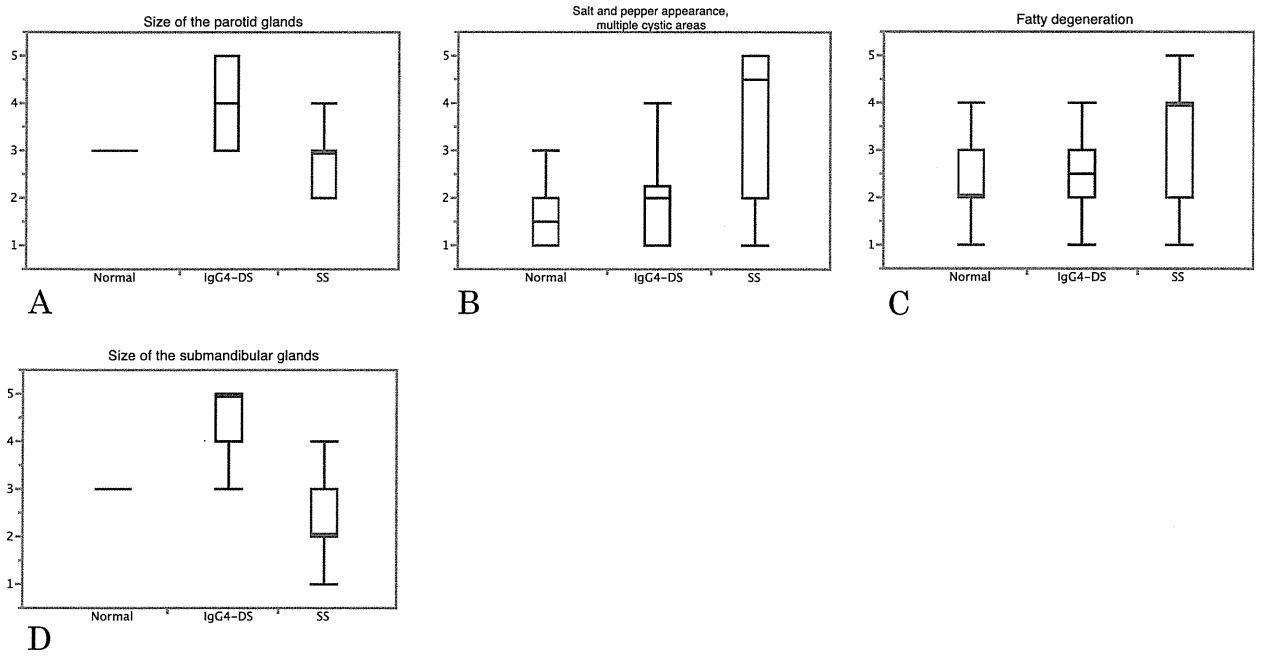

Fig. 6 Results of MRI. Values are shown with median and quartile points for (**a-c**) the parotid glands and (**d**) the submandibular glands. **a** Size of the parotid glands, **b** salt-and-pepper appearance and/or multiple cystic areas in the parotid glands, **c** fatty degeneration of the parotid glands and **d** size of the submandibular glands. Patient with IgG4-DS showing large parotid and submandibular glands (**a, d**). SS shows small parotid and submandibular glands (**a, d**), and a high score in the finding of a salt-and-pepper appearance and/or multiple cystic areas in the parotid glands (**b**). *IgG4-DS* IgG4-related dacryoadenitis and sialadenitis, *MRI* magnetic resonance imaging, *SS* Sjögren's syndrome

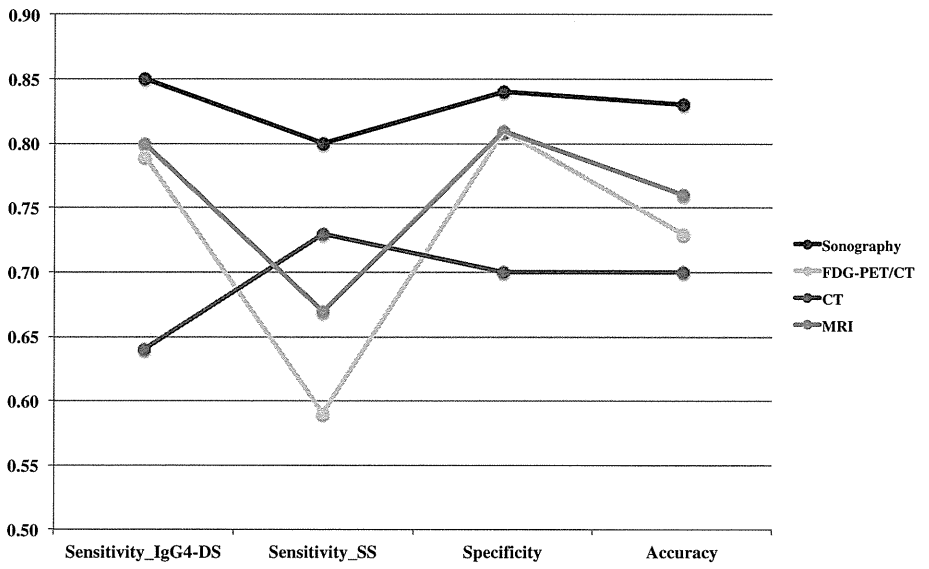

Fig. 7 Sensitivity, specificity and accuracy of the four imaging modalities. Values are the average for all observers of each modality. Sonography showed not only the highest accuracy, but also the highest sensitivity for both IgG4-DS and SS, and the highest specificity among the modalities. There were significant differences between sonography and CT ($p = 0.0001$) and between sonography and FDG-PET/CT ($p = 0.0058$) regarding accuracy. *CT* computed tomography, *FDG-PET/CT* 2-[^{18}F]-fluoro-2-deoxy-D-glucose positron emission tomography/computed tomography, *IgG4-DS* IgG4-related dacryoadenitis and sialadenitis, *MRI* magnetic resonance imaging, *SS* Sjögren's syndrome

other three modalities (p <0.001). Concerning the sensitivity of SS, there were significant differences between sonography and FDG-PET/CT (p = 0.0004) and between CT and FDG-PET/CT (p = 0.0180). In relation to specificity, there were significant differences between sonography and CT (p = 0.0463). Regarding the accuracy, there were significant differences between sonography and CT (p = 0.0001) and between sonography and FDG-PET/CT (p = 0.0058); however, there were no significant differences between sonography and MRI (p = 0.0796).

Discussion

For screening IgG4-DS, the nodal pattern of the submandibular glands on sonograms and the abnormal accumulation of ^{18}F-FDG in the submandibular glands on FDG-PET/CT were very effective. However, the effective findings regarding the screening of SS were a salt-and-pepper appearance and/or multiple cystic areas in the parotid glands on MRI. Taking into consideration high sensitivity for both IgG4-DS and SS, and high specificity in addition to high accuracy, the most effective imaging modality was sonography.

We did not include images of the lacrimal glands. Even if they were included, we could detect the affected lacrimal glands as a nodal pattern on sonograms, as abnormal ^{18}F-FDG accumulation on FDG-PET/CT, and as abnormal swelling on CT and MRI. Consequently, the abnormal findings of images of the lacrimal glands would raise the accuracy of all modalities equally, and the effectiveness of each modality would not change appreciably.

Sonography

Sonography provided useful information regarding the screening of IgG4-DS. The nodal and reticular patterns, often with normal parenchyma surrounding the affected region and located bilaterally, were easily detected in the submandibular glands even by inexperienced observers. When the sublingual glands are affected, they also show a nodal and/or reticular pattern on sonograms (data not shown); therefore, it would be easier to make an accurate diagnosis. Using sonography, not only IgG4-DS but also SS can be detected. Multiple hypoechoic areas and hyperechoic lines and/or spots in the parotid glands achieved high scores. These findings might be misleading, because IgG4-DS sometimes exhibits similar findings. However, hypoechoic areas of IgG4-DS were observed in the normal parotid parenchyma without a reduction in the echo intensity level and heterogeneity. Moreover, IgG4-DS mainly affects the submandibular glands, and most of the cases present with normal parotid glands [9]. Conversely, SS shows atrophic changes in both the parotid and submandibular glands. Therefore, the combination of parotid and submandibular gland findings could lead to accurate diagnosis. In the present study we only analyzed B-mode sonograms. If Doppler images are added, it can make differentiation between IgG4-DS and SS much easier. The nodal and reticular patterns of IgG4-DS show high vascularity [9], while SS exhibits small dot-like vascularity in the parotid glands [22]. When the IgG4-DS patients had shown submandibular gland swelling and salivary gland tumors had been suspected, sonography revealed nodal and/or reticular changes on both sides, and tumorous lesions were easily ruled out.

FDG-PET/CT

FDG-PET/CT showed abnormal ^{18}F-FDG accumulation in the glands affected with IgG4-DS. However, this imaging modality could not differentiate between normal glands and SS. Patients with SS do not undergo FDG-PET/CT, unless they have other malignant diseases. Our cases involving FDG-PET/CT were patients with SS who were partly suspected of having malignant lymphoma. When patients actually had lymphoma, the SUV was high and we could not differentiate the disease from IgG4-DS. Conversely, very severe SS with atrophic submandibular glands exhibited a very low SUV both in the parotid and submandibular glands. In contrast, SS patients in the lower stages (their sialography showed punctuate or globular patterns) had a rather high SUV (SUV; 3–4). This finding was in accordance with a report by Cohen et al. [23]. Normal salivary, sublingual, submandibular and parotid glands sometimes show high SUVs. Because Nakamoto et al. have reported that the intensity of ^{18}F-FDG uptake in the salivary glands is variable [24], we need to carefully differentiate IgG4-DS from normal variances.

CT and MRI

Nodal changes in IgG4-DS detected using sonography were not clearly observed on CT or MRI. In some cases CT and MRI displayed superficial enhancement in the parotid glands of IgG4-DS. However, unspecific swelling of the parotid and submandibular glands was observed in general. Most of the patients with normal salivary glands, that were large in size, were misdiagnosed as IgG4-DS. Some of our cases showed slightly low signal intensity on T2-weighted images on MRI; however, they were not significant. These results were not in accordance with the very low signal intensity on T2-weighted images [12, 13]. This may be because of the limited number of our MRI of the IgG4-DS patients. Diffusion-weighted images might differentiate nodal changes regarding IgG4-DS more clearly. SS, however, showed a characteristic salt-and-pepper appearance on CT and MRI. When this appearance was not pronounced, inexperienced observers could not detect it on CT.

Two things are important in diagnosing IgG4-DS. The first is to differentiate malignant lymphoma. Malignant lymphoma sometimes affects salivary glands, and can appear in bilateral glands, which mimic IgG4-DS [25]. To make a final diagnosis, biopsy is recommended. Second, IgG4-DS does not always occur simultaneously in both the lacrimal and salivary glands. Even if the findings are indefinite at the time of examination, follow-up is necessary when IgG4-DS is suspected.

Conclusions

Changes in the submandibular glands affected by IgG4-DS, which often occur bilaterally, could be easily detected using sonography as a result of characteristic bilateral nodal/reticular changes and by FDG-PET/CT because of abnormal ^{18}F-FDG accumulation. Even inexperienced observers could detect these findings. In addition to IgG4-DS, sonography could also differentiate SS. Therefore, we recommend sonography as a modality for the screening of IgG4-DS, because it is easy to use, does not involve radiation exposure and is an effective imaging modality.

Abbreviations

AIP: autoimmune pancreatitis; CT: computed tomography; FDG-PET/CT: 2-[^{18}F]-fluoro-2-deoxy-D-glucose positron emission tomography/computed tomography; IgG4: immunoglobulin G4; IgG4-DS: IgG4-related dacryoadenitis and sialadenitis; IgG4-RD: IgG4-related disease; MHz: megahertz; MRI: magnetic resonance imaging; SS: Sjögren's syndrome; SUV: standardized uptake value.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

MS was responsible for study design, literature review, imaging data collection, data analysis, manuscript writing and editing. KO, YK, YT, HF and WW participated in imaging data collection, data interpretation, data analysis and critical revision of the manuscript. MM, YO and SF were responsible for clinical data collection and critical revision of the manuscript. SN and KY were responsible for study design, supervised the study and critical revision of the manuscript. All the authors read and approved the final manuscript.

Acknowledgements

This study was supported in part by grants from the Ministry of Education, Culture, Sports, Science and Technology of Japan (24592835).

Author details

[1]Department of Oral and Maxillofacial Radiology, Kyushu University Hospital, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan. [2]Department of Oral and Maxillofacial Radiology, Faculty of Dental Science, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan. [3]Department of Oral and Maxillofacial Radiology, School of Dentistry, Aichi Gakuin University, 1-100 Kusumoto-cho, Chikusa-ku, Nagoya 464-8650, Japan. [4]Section of Oral and Maxillofacial Oncology, Division of Maxillofacial Diagnostic and Surgical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan. [5]Section of Oral and Maxillofacial Surgery, Division of Maxillofacial Diagnostic and Surgical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan.

Received: 15 April 2015 Accepted: 10 August 2015
Published online: 23 August 2015

References

1. Tsubota K, Fujita H, Tsuzaka K, Takeuchi T. Mikulicz's disease and Sjögren's syndrome. Invest Ophthalmol Vis Sci. 2000;41:1666–73.
2. Yamamoto M, Harada S, Ohara M, Suzuki C, Naishiro Y, Yamamoto H, et al. Clinical and pathological differences between Mikulicz's disease and Sjögren's syndrome. Rheumatology. 2005;44:227–34.
3. Yamamoto M, Takahashi H, Sugai S, Imai K. Clinical and pathological characteristics of Mikulicz's disease (IgG4-related plasmacytic exocrinopathy). Autoimmunity Rev. 2005;4:195–200.
4. Wallace ZS, Deshpande V, Mattoo H, Mahajan VS, Kulikova M, Pillai S, et al. IgG4-related disease: clinical and laboratory features in 125 patients. Arthritis Rheumatol. 2015; doi: 10.1002/art.39205. Epub 2015 May 18.
5. Ishii S, Shishido F, Miyajima M, Sakuma K, Shigihara T, Kikuchi K. Whole-body gallium-67 scintigraphic findings in IgG4-related disease. Clin Nucl Med. 2011;36:542–5.
6. Ishii S, Miyajima M, Sakuma K, Kikuchi K, Shishido F. Comparison between sarcoidosis and IgG4-related disease by whole-body 67Ga scintigraphy. Nucl Med Commun. 2013;34:13–8.
7. Ebbo M, Grados A, Guedj E, Gobert D, Colavolpe C, Zaidan M, et al. Usefulness of 2-[18F]-fluoro-2-deoxy-d-glucose-positron emission tomography/computed tomography for staging and evaluation of treatment response in IgG4-related disease: a retrospective multicenter study. Arthritis Care Res (Hoboken). 2014;66:86–96.
8. Zhang J, Chen H, Ma Y, Xiao Y, Niu N, Lin W, et al. Characterizing IgG4-related disease with 18F-FDG PET/CT: a prospective cohort study. Eur J Nucl Med Mol Imaging. 2014;41:1624–34.
9. Shimizu M, Moriyama M, Okamura K, Kawazu T, Chikui T, Goto TK, et al. Sonographic diagnosis for Mikulicz disease. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2009;108:105–13.
10. Asai S, Okami K, Nakamura N, Shiraishi S, Sugimoto R, Anar D, et al. Localized or diffuse lesions of the submandibular glands in immunoglobulin g4-related disease in association with differential organ involvement. J Ultrasound Med. 2013;32:731–6.
11. Takagi Y, Nakamura H, Origuchi T, Miyashita T, Kawakami A, Sumi M, et al. IgG4-related Mikulicz's disease: ultrasonography of the salivary and lacrimal glands for monitoring the efficacy of corticosteroid therapy. Clin Exp Rheumatol. 2013;31:773–5.
12. Katsura M, Mori H, Kunimatsu A, Sasaki H, Abe O, Machida T, et al. Radiological features of IgG4-related disease in the head, neck, and brain. Neuroradiology. 2012;54:873–82.
13. Fujita A, Sakai O, Chapman MN, Sugimoto H. IgG4-related disease of the head and neck: CT and MR imaging manifestations. Radiographics. 2012;32:1945–58.
14. Okazaki K, Kawa S, Kamisawa T, Shimosegawa T, Tanaka M. Research Committee for Intractable Pancreatic Disease and Japan Pancreas Society. Japanese consensus guidelines for management of autoimmune pancreatitis: I. Concept and diagnosis of autoimmune pancreatitis. J Gastroenterol. 2010;45:249–65.
15. Yamamoto M, Takahashi H, Ohara M, Suzuki C, Naishiro Y, Yamamoto H, et al. A new conceptualization for Mikulicz's disease as an IgG4-related plasmacytic disease. Mod Rheumatol. 2006;16:335–40.
16. Fujibayashi T, Sugai S, Miyasaka N, Hayashi Y, Tsubota K. Revised Japanese criteria for Sjögren's syndrome (1999): availability and validity. Mod Rheumatol. 2004;14:425–34.
17. Vitali C, Bombardieri S, Jonsson R, Moutsopoulos HM, Alexander EL, Carsons SE, et al. Classification criteria for Sjogren's syndrome: a revised version of the European criteria proposed by the American–European Consensus Group. Ann Rheum Dis. 2002;61:554–8.
18. Takagi Y, Kimura Y, Nakamura H, Sasaki M, Eguchi K, Nakamura T. Salivary gland ultrasonography: can it be an alternative to sialography as an imaging modality for Sjogren's syndrome? Ann Rheum Dis. 2010;69:1321–4.
19. Shimizu M, Okamura K, Yoshiura K, Ohyama Y, Nakamura S, Kinukawa N. Sonographic diagnostic criteria for screening Sjögren's syndrome. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2006;102:85–93.
20. Sun Z, Zhang Z, Fu K, Zhao Y, Liu D, Ma X. Diagnostic accuracy of parotid CT for identifying Sjögren's syndrome. Eur J Radiol. 2012;81:2702–9.
21. Takashima S, Takeuchi N, Morimoto S, Tomiyama N, Ikezoe J, Shogen K, et al. MR imaging of Sjögren syndrome: correlation with sialography and pathology. J Comput Assist Tomogr. 1991;15:393–400.
22. Shimizu M, Okamura K, Yoshiura K, Ohyama Y, Nakamura S. Sonographic diagnosis of Sjögren syndrome: evaluation of parotid gland vascularity as a diagnostic tool. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2008;106:587–94.
23. Cohen C, Mekinian A, Uzunhan Y, Fauchais AL, Dhote R, Pop G, et al. 18F-fluorodeoxyglucose positron emission tomography/computer tomography

as an objective tool for assessing disease activity in Sjögren's syndrome. Autoimmun Rev. 2013;12:1109–14.
24. Nakamoto Y, Tatsumi M, Hammoud D, Cohade C, Osman MM, Wahl RL. Normal FDG distribution patterns in the head and neck: PET/CT evaluation. Radiology. 2005;234:879–85.
25. Ohta M, Moriyama M, Goto Y, Kawano S, Tanaka A, Maehara T, et al. A case of marginal zone B cell lymphoma mimicking IgG4-related dacryoadenitis and sialoadenitis. World J Surg Oncol. 2015;13:459. doi:10.1186/s12957-015-0459-z. Epub 2015 Feb 21.

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Journal of Oral and Maxillofacial Surgery, Medicine, and Pathology 27 (2015) 96–101

Contents lists available at ScienceDirect

Journal of Oral and Maxillofacial Surgery, Medicine, and Pathology

journal homepage: www.elsevier.com/locate/jomsmp

Oral Medicine/Original Research

Differences of stimulated and unstimulated salivary flow rates in patients with dry mouth

Jun-Nosuke Hayashida*, Sakae Minami, Masafumi Moriyama, Takeshi Toyoshima, Shouichi Shinozaki, Akihiko Tanaka, Takashi Maehara, Seiji Nakamura

Section of Oral and Maxillofacial Oncology, Division of Maxillofacial Diagnostic and Surgical Sciences, Faculty of Dental Science, Kyushu University, Fukuoka, Japan

ARTICLE INFO

Article history:
Received 28 July 2013
Received in revised form 18 April 2014
Accepted 28 April 2014
Available online 26 June 2014

Keywords:
Dry mouth
Sjögren's syndrome
Stimulated salivary flow rate
Unstimulated salivary flow rate
Gum test, Saxon test

ABSTRACT

Purpose: The purpose of this study was to clarify the usefulness of noninvasive examination items such as sialometry and Visual Analog Scale (VAS) in distinguishing Sjögren's syndrome (SS) in dry mouth patients from neurogenic/neuropsychiatric disorders and drugs (DND).
Patients and methods: The study cohort comprised 50 patients with SS and 28 patients with DND. The gum test and Saxon test for stimulated salivary flow rate (SSFR), the spitting test for unstimulated salivary flow rate (USFR) and VAS were performed in all the patients with dry mouth.
Results: In SS patients, the SSFR (mean: gum test, 6.34 mL/10 min; Saxon test, 1.19 g/2 min) and USFR (0.61 mL/15 min) were decreased. In DND patients, the SSFR (gum test, 16.35 mL/10 min; Saxon test, 3.58 g/2 min) was within the normal range, but the USFR (0.90 mL/15 min) was decreased. In VAS, SS patients scored significantly higher in the items of "water-drinking at meals", "difficulty in swallowing", and "taste abnormality", while significantly lower in the item of "oral pain".
Conclusion: These results suggest that the SSFR, USFR and VAS could be useful in distinguishing DND from SS.

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1. Introduction

The number of patients complaining of dry mouth has increased recently, because of raised awareness of oral health [1–4]. Xerostomia is defined as a subjective complaint of dry mouth, and is caused by the evaporation and/or hyposalivation of saliva. Evaporation of saliva is mainly caused by mouth opening or mouth breathing, which often occurs during the night without an apparent decrease in the salivary flow. Hyposalivation occurs due to various causes, including radiation therapy to the head and neck, the use of medications, and certain systemic conditions and diseases such as diarrhea, dehydration, hyperthyroidism, diabetes mellitus, kidney malfunction, anemia, and Sjögren's syndrome [5–10]. Hyposalivation can be divided into two groups according to the mechanism of disorder: a destruction of the secretary cells of the salivary glands and a dysfunction of the autonomic nervous system which stimulates saliva secretion. One of the causes of the former disorder is Sjögren's syndrome (SS), and the latter is caused by anxiety, depression, and medications such as antidepressant, antiemetic, antihistamine, and antihypertensive. The term "dry mouth associated with neurogenic/neuropsychiatric disorders and drugs (DND)" is proposed for the latter disorder by the Japanese Society of Oral Medicine in 2008 [11]. SS and DND compose a majority of the patients with dry mouth.

SS is diagnosed based on the diagnostic criteria including the 1999 revised diagnostic criteria of the Ministry of Health, Labor and Welfare (MHLW) and those of the American-European Consensus Group, which are generally used in Japan [12–14]. However, a limitation of the criteria is that many patients go untested and do not receive a confirmed diagnosis. The reasons for this may include the large number of tests required to fulfill the diagnostic criteria, the fact that some tests are invasive (especially lip biopsy and sialography), and the fact that some patients do not want to be tested. On the other hand, there are no diagnostic criteria for DND. Thus, it is

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* Corresponding author at: Section of Oral and Maxillofacial Oncology, Division of Maxillofacial Diagnostic and Surgical Sciences, Faculty of Dental Science, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan.
Tel.: +81 92 642 6447; fax: +81 92 642 6386.
E-mail address: junno@dent.kyushu-u.ac.jp (J. Hayashida).

http://dx.doi.org/10.1016/j.ajoms.2014.04.011
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necessary to ascertain how best to carry out the tests for diagnosis of SS and DND without imposing an excessive burden on patients. In this context, the purpose of this study was to clarify the usefulness of noninvasive examination items such as sialometry and Visual Analog Scale (VAS) in distinguishing DND from SS. Differences of stimulated and unstimulated salivary flow rates and score of the VAS were compared between the patients with SS and DND.

2. Patients and methods

2.1. Patients

Fifty patients with SS (48 women and 2 men; mean age, 62.6 ± 10.5 years) and 30 patients with DND (25 women and 5 men; mean age, 53.9 ± 8.8 years) referred to the Department of Oral and Maxillofacial Surgery, Kyushu University Hospital from 2009 to 2013 were included in the study. All the patients presented with subjective complaint of dry mouth and decreased USFR flow rate (<1.5 mL/15 min) or SWS flow rate (<2.00 g/2 min). SS was diagnosed according to both the Research Committee on SS of the MHLW of the Japanese Government (1999) [12] and the American–European Consensus Group criteria for SS [14]. None of the patients had other autoimmune diseases and were treated with steroids or any other immunodepressants. DND was diagnosed according to the following criteria: (1) fail to fulfill each of the above-mentioned diagnostic criteria for SS and (2) taking drug or diagnosed with depression in Department of Psychosomatic Medicine. The patients with DND were taking antidepressant drug ($n = 8$), sleeping-inducing drug ($n = 8$), antihypertension drug ($n = 6$), and other oral medicines with side effect of dry mouth ($n = 10$). In contrast, three patients with DND were diagnosed with depression by the physicians in our hospital but were not taking any drugs. Informed consent, which was approved by the Ethics Committee of Kyushu University, Japan, was obtained from all the patients, and healthy controls were included in the study (IRB serial number: 25-287).

2.2. Measurements of salivary flow rates

The gum test was carried out by asking the subjects to chew gum for 10 min. The saliva secreted during that time was collected in a cap and its volume measured. If the volume of the saliva was <10 mL, the subject was classified as "decreased" [12–14]. The Saxon test was undertaken by having the subjects chew Surgeon® Type IV Gauze Sponge (Hakuzo Medical Corporation, Osaka, Japan) once a second for 2 min and measuring the weight of the gauze. If the increase in weight of the gauze was <2 g, the subject was classified as "decreased" [12–14]. The spitting test was carried out by asking subjects to spit saliva into a cup for 15 min. The amount of saliva in the unstimulated condition (sitting on a chair and not moving) was measured. If the volume of saliva was <1.5 mL, the subject was classified as "decreased" [13,14].

2.3. Subjective symptoms of dry mouth

The subjective symptoms and major complaints of dry mouth were ascertained from the medical interview. Additionally, a VAS was used for quantifying the subjective symptoms of dry mouth. The scale was from 0 mm to 100 mm. A reading of 0 mm was designated as "do not feel any symptoms" and that of 100 mm as "feel significant symptoms". Patients were asked to mark their subjective feeling between these two points arbitrarily, and the distance from 0 mm to their mark was measured. With this VAS method, six items of the symptoms of dry mouth (xerostomia, feeling hyposalivation, oral pain, water-drinking at meals, difficulty in swallowing, and taste abnormality) were assessed [13,14].

2.4. Statistical analyses

The Mann–Whitney *U*-test, chi-square test, and Pearson's product–moment correlation coefficient were used for statistical assessments. $p < 0.05$ was considered significant.

3. Results

3.1. Differences in subjective symptoms between SS patients and DND patients

In terms of major complaints, SS patients complained of "dryness of eyes" significantly more often than DND patients, whereas DND patients complained of "feeling oral pain" significantly more often than SS patients (Table 1). According to the comparisons between patients with SS and DND in the VAS, SS patients scored significantly higher in the items of "water-drinking at meals", "difficulty in swallowing", and "taste abnormality", while significantly lower in the item of "oral pain". There was no significant difference in the items of "xerostomia" and "feeling hyposalivation" between SS and DND patients (Fig. 1).

3.2. Salivary flow rates of patients with dry mouth

The SSFR of SS patients (mean: gum test, 6.34 mL/10 min; Saxon test, 1.19 g/2 min) was decreased significantly compared

Table 1
Frequency of major complaints with dry mouth patients.

	SS ($n = 50$)	DND ($n = 26$)
Xerostomia (%)	84	100
Hyposalivation	42	58
Dryness of eyes	60*	27
Feeling oral pain	18	65**
Drinking excess water at meals	52	77
Difficulty in swallowing	44	38
Abnormality of tasting	60	62
No complaint	12	0

χ^2 test.
SS, Sjögren's syndrome; DND, dry mouth associated with neurogenic/neuropsychiatric disorders and drugs.
* $p < 0.05$
** $p < 0.01$.

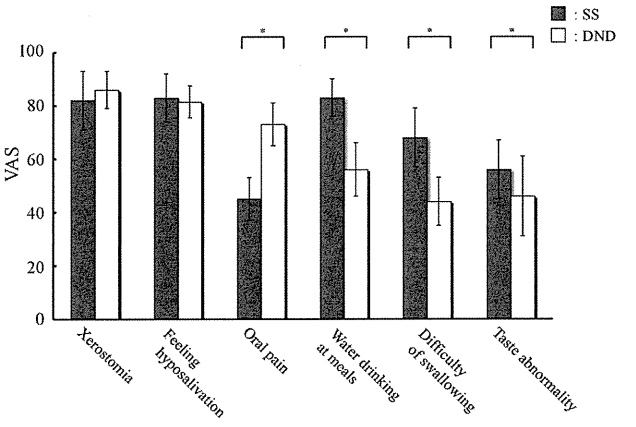

Fig. 1. Visual analog scale value of patients with dry mouth. Results of the visual analog scale (VAS) values of patients with Sjögren's syndrome (SS) and dry mouth associated with neurogenic/neuropsychiatric disorders and drugs (DND). The patients of both groups complained strongly in the items of xerostomia, feeling hyposalivation and oral pain. Additionally, the VAS values of water-drinking at meals difficulty in swallowing, and taste abnormality of DND patients were lower than those of SS patients (Mann–Whitney *U*-test, *$p < 0.05$).

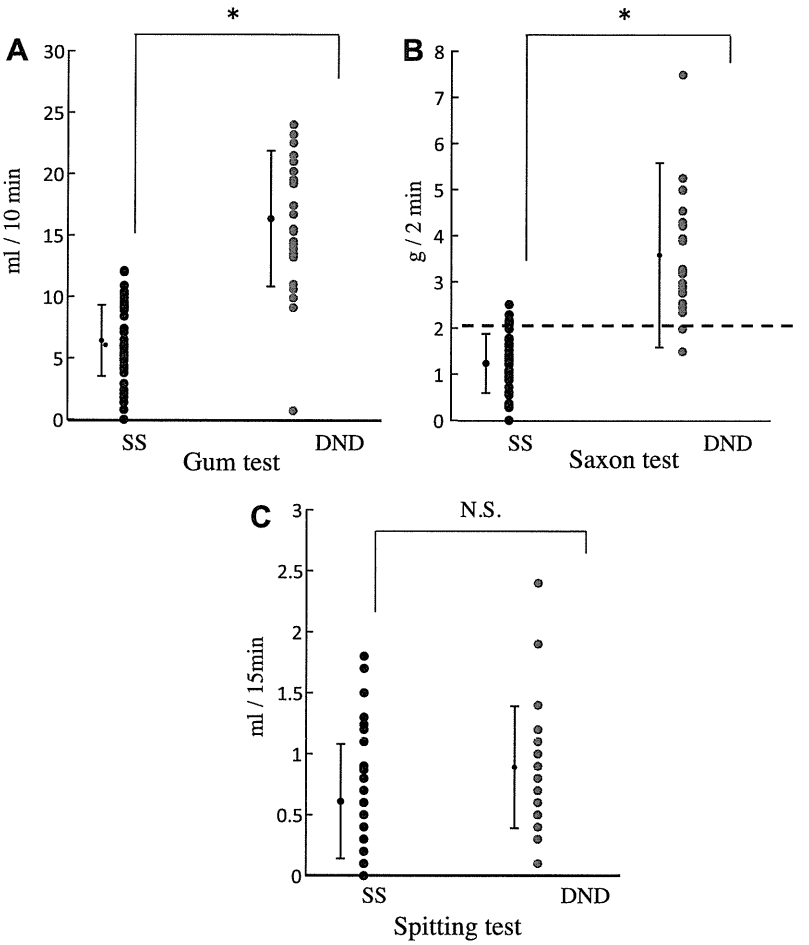

Fig. 2. (A–C) Stimulated and unstimulated salivary flow rates of patients with dry mouth. Results of the stimulated salivary flow rate (SSFR) and unstimulated salivary flow rate (USFR) of SS patients and DND patients. The decrease in SSFR of SS patients was only slightly more than that of DND patients (Mann–Whitney *U*-test, $^{*}p < 0.01$).

Table 2
The frequency of classifications as "decreased" in SSFR and USFR.

	SSFR		USFR
	Gum test	Saxon test	Spitting test
SS	44/50 (88.0%)*	43/50 (86.0%)*	47/50 (94.0%)
DND	3/26 (11.5%)	2/26 (7.7%)	24/26 (92.3%)

χ^2 test.
The definitions of "decreased" by each test is indicated in Section 2. SSFR, stimulated salivary flow rate; USFR, unstimulated salivary flow rate.
* $p < 0.01$.

with that of DND patients (mean: gum test, 16.4 mL/10 min; Saxon test, 3.58 g/2 min) (Mann–Whitney *U*-test, $p < 0.05$, Fig. 2). There was no significant difference in the USFR between SS patients (mean: 0.61 mL/15 min) and DND patients (mean: 0.90 mL/15 min). According to the frequency of the classifications of "decreased" in SS patients and DND patients, the SSFR of SS patients (gum test, 88.0%; Saxon test, 86.0%) was significantly higher than that of DND patients (gum test, 11.5%; Saxon test, 7.7%), but there was no significant difference in the USFR (Tables 2 and 3).

3.3. *Correlations among the measurement methods of salivary flow rate*

The correlations among the measurement methods of the gum test, Saxon test, and spitting test for patients with dry mouth were investigated. In SS patients, there was a positive correlation between the gum test and Saxon test (Pearson's product–moment correlation coefficient, $p < 0.01$, Fig. 3); there were also positive correlations between the spitting test and gum test, as well as between the spitting test and Saxon test (Pearson's product–moment correlation coefficient, $p < 0.05$, Fig. 3). In DND patients, there was a positive correlation between the gum test and Saxon test (Pearson's product–moment correlation coefficient, $p < 0.05$, Fig. 4). However, a clear correlation was not found between the spitting test and

Table 3
Classification of xerostomia (dry mouth).

Dry mouth caused by salivary gland dysfunction
(1) Sjögren's syndrome
(2) Radiation-induced dry mouth
(3) Dry mouth associated with aging
(4) Graft-versus-host disease (GVHD)
(5) Sarcoidosis
(6) Acquired immunodeficiency syndrome (AIDS)
(7) Malignant lymphoma
(8) Idiopathic xerostomia
Dry mouth associated with neurogenic or neuropsychiatric disorders and drugs
(1) Dry mouth associated with neurogenic or neuropsychiatric disorders
(2) Drug-induced dry mouth
Dry mouth associated with systemic diseases or metabolic disorders
(1) Dry mouth associated with systemic and metabolic diseases
(2) Dry mouth induced by excessive oral vaporization

J. Jpn. Soc. Oral Mucous Membr. 2008;14:86–8.

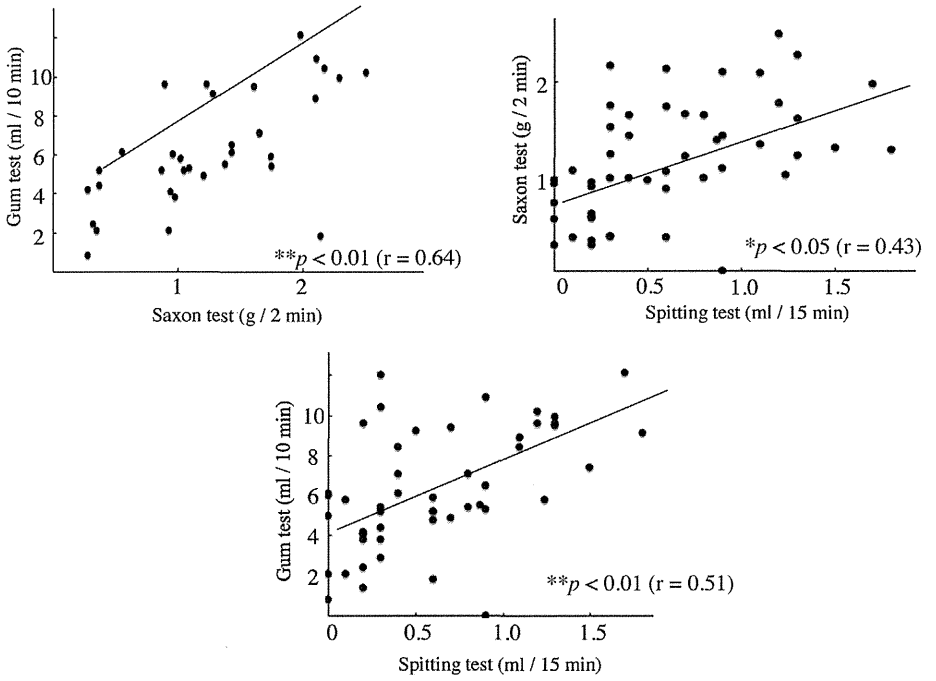

Fig. 3. Correlations among the measurement methods of the salivary flow rate in SS patients. Correlations among the gum test, Saxon test and spitting test in SS patients. Positive correlations were shown between the gum test and Saxon test (Pearson's product–moment correlation coefficient $r = 0.64$, $^{**}p < 0.01$), between the gum test and spitting test ($r = 0.43$, $^{*}p < 0.05$) and between the Saxon test and spitting test ($r = 0.51$, $^{**}p < 0.01$).

gum test, or between the spitting test and Saxon test (Pearson's product–moment correlation coefficient, N.S., Fig. 4).

4. Discussion

Recently, the number of patients with dry mouth has increased. Additionally, it has been reported that increases in social stress and drug use, an aging society, and changes in chewing habits can cause dry mouth [1–10,15–17]. SS is caused by salivary-gland dysfunction, but radiotherapy of the head and neck as well as atrophy due to aging is also important [5,6]. DND is caused mainly by depression, stress and drugs (e.g., antianxiety, antidepressant, antihypertensive). This mechanism of action is by suppression of the salivary secretory nerve system such as the central nervous system (CNS)

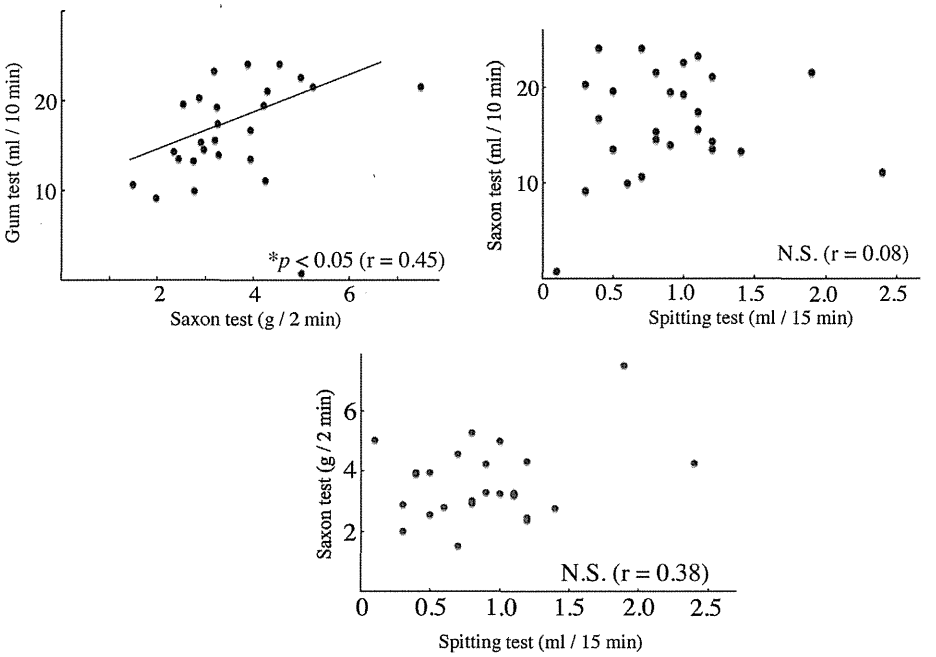

Fig. 4. Correlations among measurement methods of the salivary flow rate in DND patients. Correlations among the gum test, Saxon test and spitting test in DND patients. A positive correlation was shown between the gum test and Saxon test (Pearson's product–moment correlation coefficient $r = 0.45$, $^{*}p < 0.05$), but a correlation was not recognized between the gum test and spitting test ($r = 0.08$, no significant difference) or between the Saxon test and spitting test ($r = 0.38$, no significant difference).

or the salivary nucleus of the facial nervous system [7–9,18,19]. In addition, evaporation of saliva associated with breathing through the mouth, hyperventilation, opening the mouth, and swallowing disorders can also result in dry mouth [20]. These patients are considered to present with xerostomia in the absence of hyposalivation, because the salivary flow rate is less than the rate of fluid loss from the mouth by evaporation and by the absorption of water through the oral mucosa [1]. Therefore, in the present study, we focused on patients with SS and DND who composed a majority of the population with dry mouth.

Another problem is that a definitive test for the diagnosis of dry mouth is lacking. Although it is possible to distinguish SS from DND by all the examinations in the criteria of SS, these examinations can be painful and complicated for patients. For the increasing number of patients with dry mouth, simple methods were needed to understand clinical conditions and help a rapid and more precise diagnosis.

With respect to major complaints, "dryness of eyes" was observed more frequently in SS patients, and "feeling oral pain" was observed more frequently in DND patients. This difference could be used to distinguish DND patients from SS patients, but it is difficult to distinguish them using this difference alone because all patients with dry mouth complained of "xerostomia". In the comparison of the VAS between SS patients and DND patients, three items associated with the diet of DND patients were milder than those of SS patients. These results suggested that the saliva of DND patients was secreted normally at meal times. Thus, a VAS seemed to be useful also in distinguishing DND patients from SS patients.

According to salivary flow rates, the mean volume of the SSFR in SS patients was reduced significantly compared with that of DND patients. Moreover, the comparison between SS patients and DND patients showed that the prevalence of the classification of "decreased" in the SSFR in SS patients was higher than in DND patients, also there was no significant difference in the USFR. Positive correlations among the gum test, Saxon test, and spitting test were found in SS patients, as well as the gum test and Saxon test in DND patients. These results suggest that the decreases in the SSFR and USFR in SS patients were caused by salivary-gland dysfunctions, and that decrease in the USFR in DND patients was by suppression of the CNS and the nervous system in the salivary glands [7–9]. Upon consideration of the normal SSFR in DND patients, these suppressions in DND patients might be broken down by stimulation such as food intake. However, it is still necessary to elucidate the mechanisms underlying drug-induced hyposalivation because different results might be derived depending on the inhibition strength or period of CNS or administration period of the drugs.

Upon perusal of the results of the VAS, SSFR, and USFR, it appears that the decrease in the USFR caused chronic symptoms such as xerostomia and hyposalivation, and that the decrease in the SSFR caused all the complaints except oral pain itemized in the VAS. Oral pain was not correlated with the decrease in the SSFR and USFR, because DND patients might have increased sensitivity for the pain. These findings suggest that the VAS, SSFR, and USFR are available for distinction between DND and SS despite simple methods that can be undertaken readily by general dentists. However, measurements of salivary flow rates often vary depending upon the measurement conditions, and often lack accuracy. Moreover, the gum test is sometimes difficult for patients wearing dentures, and the Saxon test often causes nausea. Considering the positive correlation between the gum test and Saxon test observed in the present study, it is preferable to carry out only one test or the other. Newer test methods include measurement of the USFR by the cotton roll method, measurement of water content in the oral mucosa using a moisture-checking device, and a saliva wetness test using test papers [21]. Previously, we confirmed the correlation and consistency in measurement of the moisture of the tongue surface [21,22] using an oral-moisture checking device (Moisture Checker for Mucus®), of which the accuracy has been established. The test of water content in the oral mucosa using an oral moisture-checking device is simple and could be used in the criteria for the diagnosis of SS. According to our studies of SS [5,23–30], soluble substances such as cytokines and chemokines are produced in the salivary glands and can be detected in saliva. Hence, these substances could be used to diagnose SS in the future.

In order to distinguish DND from SS, the SSFR, USFR, and VAS are useful and simple methods for taking into account both criteria for SS. These tests can be carried out even in general dental clinics. This is very important as the first step to distinguish DND from SS. Subsequently, if a patient is suspected of having SS attends a general dental clinic, he/she can be referred to special facilities to undergo lip biopsy, scintigraphy of the salivary glands or sialography.

Conflict of interest

None declared.

Funding

None.

Author contributions

All authors were involved in drafting the article or revising it, and all authors approved the final version. Dr. Hayashida and Dr. Minami had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analyses. Conception and design of the study was done by Hayashida and Nakamura. Hayashida, Minami and Moriyama took care of data acquisition. Minami, Hayashida and Toyoshima did the analysis and interpretation of the data.

Acknowledgements

This work was supported by a Grant-in-Aid (numbers: 23792354 and 26463013) from the Ministry of Education, Culture, Sports, Science, and Technology in Japan.

References

[1] Guggenheimer J, Moore PA. Xerostomia: etiology, recognition and treatment. J Am Dent Assoc 2003;134:61–9, quiz 118–9.
[2] Fox PC. Xerostomia: recognition and management. Dent Assist 2008;77, 18, 20, 44–8; quiz 50–1.
[3] Greenspan D. Xerostomia: diagnosis and management. Oncology (Williston Park) 1996;10:7–11.
[4] Billings RJ, Proskin HM, Moss ME. Xerostomia and associated factors in a community-dwelling adult population. Commun Dent Oral Epidemiol 1996;24:312–6.
[5] Nakamura S, Ikebe-Hiroki A, Shinohara M, Ohyama Y, Mouri T, Sasaki M, et al. An association between salivary gland disease and serological abnormalities in Sjogren's syndrome. J Oral Pathol Med 1997;26:426–30.
[6] Rahman S, Maillou P, Barker D, Donachie M. Radiotherapy and the oral environment the effects of radiotherapy on the hard and soft tissues of the mouth and its management. Eur J Prosthodont Restor Dent 2013;21:80–7.
[7] Nonzee V, Manopatanakul S, Khovidhunkit SO. Xerostomia, hyposalivation and oral microbiota in patients using antihypertensive medications. J Med Assoc Thai 2012;95:96–104.
[8] Scully C, Felix DH. Oral medicine – update for the dental practitioner: dry mouth and disorders of salivation. Br Dent J 2005;199:423–7.
[9] Matear DW, Barbaro J. Effectiveness of saliva substitute products in the treatment of dry mouth in the elderly: a pilot study. J R Soc Promot Health 2005;125:35–41.
[10] Ivanovski K, Naumovski V, Kostadinova M, Pesevska S, Drijanska K, Filipce V. Xerostomia and salivary levels of glucose and urea in patients with diabetes. Prilozi 2012;33:219–29.
[11] Nakamura S. Classification idea of xerostomia at Japanese Society for Oral Mucous Membrane. J Oral Maxillofac Surg Med Pathol 2008;14:86–8.
[12] Fujibayashi T, Sugai S, Miyake N, Hayashi Y, Tsubota K. Revised Japanese criteria for Sjögren's syndrome (1999): availability and validity. Mod Rheumatol 2004;14:425–34.

[13] Kohler P, Winter M. A quantitative test for xerostomia: the Saxon test, an oral equivalent of the Schirmer test. Arthritis Rheum 1985;25:1128–32.
[14] Vitali C, Bombardieri S, Jonsson R, Moutsopoulos HM, Alexander EL, Carsons SE, et al. Classification criteria for Sjogren's syndrome: a revised version of the European criteria proposed by the American–European Consensus Group. Ann Rheum Dis 2002;61:554–8.
[15] Ettinger R. Xerostomia—a complication of ageing. Aust Dent J 1981;26:365–71.
[16] Navazesh M, Ship II. Xerostomia: diagnosis and treatment. Am J Otolaryngol 1983;4:283–92.
[17] Spielman A, Ben-Aryeh H, Gutman D, Szargel R, Deutsch E. Xerostomia—diagnosis and treatment. Oral Surg Oral Med Oral Pathol 1981;51:144–7.
[18] Scully C. Drug effects on salivary glands: dry mouth. Oral Dis 2003;9:165–76.
[19] Wynn RL. New drug approvals in 2000. Gen Dent 2001;49:341–5.
[20] Dawes C. How much saliva is enough for avoidance of xerostomia. Caries Res 2004;38:236–40.
[21] Murakami M, Nishi Y, Kamashita Y, Nagaoka E. Relationship between medical treatment and oral dryness diagnosed by oral moisture-checking device in patients with maxillofacial prostheses. J Prosthodont Res 2009;53:67–71.
[22] Bahn S, Ccnn W.Workshop on diagnostic criteria for Sjogren's syndrome. Clin Exp Rheumatol 1989;7:111–219.
[23] Hiroki A, Nakamura S, Shinohara M, Gondo H, Ohyama Y, Hayashi S, et al. A comparison of glandular involvement between chronic graft-versus-host disease and Sjogren's syndrome. Int J Oral Maxillofac Surg 1996;25:298–307.
[24] Ohyama Y, Nakamura S, Hara H, Shinohara M, Sasaki M, Ikebe-Hiroki A, et al. Accumulation of human T lymphotropic virus type I-infected T cells in the salivary glands of patients with human T lymphotropic virus type I-associated Sjogren's syndrome. Arthritis Rheum 1998;41:1972–8.
[25] Nakamura S, Ohyama Y. Cytokine expression in the salivary glands of Sjögren's patients according to the tissue infiltrate and lymphoepithelial lesions (Letters). Arthritis Rheum 1997;40:989–90.
[26] Ohyama Y, Nakamura S, Matsuzaki G, Shinohara M, Hiroki A, Fujimura T, et al. Cytokine messenger RNA expression in the labial salivary glands of patients with Sjogren's syndrome. Arthritis Rheum 1996;39:1376–84.
[27] Tsunawaki S, Nakamura S, Ohyama Y, Sasaki M, Ikebe-Hiroki A, Hiraki A, et al. Possible function of salivary gland epithelial cells as nonprofessional antigen-presenting cells in the development of Sjogren's syndrome. J Rheumatol 2002;29:1884–96.
[28] Maehara T, Moriyama M, Hayashida JN, Tanaka A, Shinozaki S, Kubo Y, et al. Selective localization of T helper subsets in labial salivary glands from primary Sjogren's syndrome patients. Clin Exp Immunol 2012;169:89–99.
[29] Shinozaki S, Moriyama M, Hayashida JN, Tanaka A, Maehara T, Ieda S, et al. Close association between oral Candida species and oral mucosal disorders in patients with xerostomia. Oral Dis 2012;18:667–72.
[30] Moriyama M, Hayashida JN, Toyoshima T, Ohyama Y, Shinozaki S, Tanaka A, et al. Cytokine/chemokine profiles contribute to understanding the pathogenesis and diagnosis of primary Sjogren's syndrome. Clin Exp Immunol 2012;169:17–26.

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MODERN RHEUMATOLOGY

http://informahealthcare.com/mor
ISSN 1439-7595 (print), 1439-7609 (online)

Mod Rheumatol, 2015; Early Online: 1–7
© 2015 Japan College of Rheumatology
DOI: 10.3109/14397595.2015.1106661

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ORIGINAL ARTICLE

Maternal predictive factors for fetal congenital heart block in pregnant mothers positive for anti-SS-A antibodies

Hiroto Tsuboi[1,13], Takayuki Sumida[1,13], Hisashi Noma[2,13], Kazumasa Yamagishi[3,13], Ai Anami[4,13], Kotaro Fukushima[4,13], Hitoshi Horigome[5,13], Yasuki Maeno[6,13], Mitsumasa Kishimoto[7,13], Yoshinari Takasaki[8,13], Masahiro Nakayama[9,13], Masako Waguri[10,13], Haruhiko Sago[11,13], and Atsuko Murashima[12,13]

[1]*Department of Internal Medicine, Faculty of Medicine, University of Tsukuba, Ibaraki, Japan,* [2]*Department of Data Science, The Institute of Statistical Mathematics, Tokyo, Japan,* [3]*Department of Public Health Medicine, Faculty of Medicine, University of Tsukuba, Ibaraki, Japan,* [4]*Department of Obstetrics and Gynecology, Kyushu University Hospital, Fukuoka, Japan,* [5]*Department of Child Health, Faculty of Medicine, University of Tsukuba, Ibaraki, Japan,* [6]*The Department of Pediatrics and Child Health, Kurume University School of Medicine, Fukuoka, Japan,* [7]*Immuno-Rheumatology Center, St Luke's International Hospital, Tokyo, Japan,* [8]*Department of Rheumatology, School of Medicine, Juntendo University, Tokyo, Japan,* [9]*Department of Laboratory Medicine, Osaka Medical Center and Research Institute for Maternal and Child Health, Osaka, Japan,* [10]*Department of Maternal Medicine, Osaka Medical Center and Research Institute for Maternal and Child Health, Osaka, Japan,* [11]*Center of Maternal-Fetal, Neonatal and Reproductive Medicine, National Center for Child Health and Development, Tokyo, Japan,* [12]*Department of Maternal Medicine, National Center for Child Health and Development, Tokyo, Japan, and* [13]*The Research Team for Surveillance of Autoantibody-Exposed Fetuses and Treatment of Neonatal Lupus Erythematosus, the Research Program of the Japan Ministry of Health, Labor and Welfare (MHLW) (H22-Jisedai-Ippan-007), Tokyo, Japan*

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Abstract

Objective: To determine the maternal predictive factors for fetal congenital heart block (CHB) in pregnancy in mothers positive for anti-SS-A antibodies.

Methods: The Research Team for Surveillance of Autoantibody-Exposed Fetuses and Treatment of Neonatal Lupus Erythematosus, the Research Program of the Japan Ministry of Health, Labor and Welfare, performed a national survey on pregnancy of mothers positive for anti-SS-A antibodies. We analyzed 635 pregnant mothers who tested positive for anti-SS-A antibodies before conception but had no previous history of fetal CHB. We performed univariate and multivariate analysis (models 1, 2, and 3 using different set of independent variables) investigated the relation between risk of fetal CHB and maternal clinical features.

Results: Of the 635 pregnant mothers, fetal CHB was detected in 16. Univariate analysis showed that fetal CHB associated with use of corticosteroids before conception (OR 3.72, $p = 0.04$), and negatively with use of corticosteroids (equivalent doses of prednisolone (PSL), at $\geq$10 mg/day) after conception before 16-week gestation (OR 0.17, $p = 0.03$). In multivariate analysis, model 1 identified the use of corticosteroids before conception (OR 4.28, $p = 0.04$) and high titer of anti-SS-A antibodies (OR 3.58, $p = 0.02$) as independent and significant risk factors, and model 3 identified use of corticosteroids (equivalent doses of PSL, at $\geq$10 mg/day) after conception before 16-week gestation as independent protective factor against the development of fetal CHB (OR 0.16, $p = 0.03$). Other maternal clinical features did not influence the development of fetal CHB.

Conclusion: The results identified high titers of anti-SS-A antibodies and use of corticosteroids before conception as independent risk factors, and use of corticosteroids (equivalent doses of PSL, at $\geq$10 mg/day) after conception before 16-week gestation as an independent protective factor for fetal CHB.

Keywords

Anti-SS-A antibodies, Congenital heart block, Risk factor

History

Received 19 March 2015
Accepted 4 October 2015
Published online 17 November 2015

Introduction

Anti-SS-A/Ro antibodies are associated with neonatal lupus erythematosus (NLE), which presents clinically with fetal congenital heart block (CHB), transient skin rash, and hematological and hepatic abnormalities [1]. Anti-SS-A antibodies are also commonly detected in patients with autoimmune diseases, such as Sjögren's syndrome (SS) (60–90%), systemic lupus erythematosus (SLE) (30–50%), and rheumatoid arthritis (RA) (11%) [1]. Importantly, anti-SS-A antibodies are also detected in between 1% and 2% of randomly tested pregnant women [2]. Child birth in Japan is about 1,000,000 per year; therefore, child birth from mothers with anti-SS-A antibodies is estimated at 10,000 per year. The prevalence of neonatal lupus rash was reported to be 10–20% in the offspring of anti-SS-A antibodies-positive women, while laboratory abnormalities in asymptomatic babies can be detected in up to 27% of anti-SS-A antibodies

Correspondence to: Prof. Takayuki Sumida, Department of Internal Medicine, Faculty of Medicine, University of Tsukuba, 1-1-1 Tennodai, Tsukuba-city, Ibaraki 305-8575, Japan. Tel: +81-29-853-7388. Fax: +81-29-853-7388. E-mail: tsumida@md.tsukuba.ac.jp

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positive mothers [1]. Although fetal CHB is a rare manifestation among NLE features and the prevalence of fetal CHB was reported to be 1–2% in pregnant women with anti-SS-A antibodies [1,3–10], fetal CHB is associated with significant mortality (20–30%, primarily fetal/neonatal) and morbidity (67% require permanent placement of a pacemaker before adulthood) [5]. Because of the rarity of fetal CHB in pregnant women with anti-SS-A antibodies, neither randomized prospective clinical trials nor meta-analysis has yet been reported [9]. Previous fetal CHB has been confirmed as a definite maternal risk factor for fetal CHB in pregnant women with anti-SS-A antibodies, and the recurrence rate of fetal CHB in subsequent pregnancies after preceding offspring complicated with fetal CHB is reported to be approximately 12–20%, or 8- to 9-fold risk than in without previous fetal CHB [4,6,7,10]. Recently, Anami et al. [10] reported that anti-SS-A antibody titer of 1:32 or higher in the maternal sera by double immune-diffusion (DID) was an independent risk factor for fetal CHB, and that treatment with either prednisolone (PSL) or betamethasone during pregnancy lowered the risk of fetal CHB in these mothers. On the other hand, Tunks et al. [8] reported that fetal CHB was not associated with higher levels of maternal anti-SS-A antibodies but with that of anti-SS-B/La antibodies. Moreover, Ambrosi et al. [7] identified maternal older age and summer season of child birth as novel risk factors for fetal CHB. Observational studies suggested the possible effectiveness of intravenous gamma globulin (IVIG) and hydroxychloroquine (HCQ) in reducing fetal CHB-risk [9]. Other maternal factors such as health status and use of corticosteroids have not been confirmed as related factors to occurrence of fetal CHB in pregnant women positive for anti-SS-A antibodies [4,6].

Currently, there are no standard guidelines for surveillance, prevention, and treatment on fetal CHB associated with anti-SS-A antibodies. Because the presence of anti-SS-A antibodies can be detected after the diagnosis of fetal CHB or NLE in some cases, screening and management for asymptomatic women with anti-SS-A antibodies seem to be an important clinical issue. Thus, the Research Team for Surveillance of Autoantibody-Exposed Fetuses and Treatment of Neonatal Lupus Erythematosus, a Research Program sponsored by the Japan Ministry of Health, Labor and Welfare (MHLW) invited rheumatologists, obstetricians and gynecologists, pediatric cardiologists, epidemiologists, and biostatisticians to conduct a national survey on pregnancy of mothers positive for anti-SS-A antibodies to clarify the maternal predictive factors for fetal CHB. The present study documents this survey and reports the results.

Patients and methods

The national survey on pregnancy of mothers positive for anti-SS-A antibodies

The Research Team for Surveillance of Autoantibody-Exposed Fetuses and Treatment of Neonatal Lupus Erythematosus, the Research Program of the Japan MHLW conducted the national survey on pregnancy of mothers positive for anti-SS-A antibodies at the institutions affiliated with members of the research team (from 2008 to 2009), as well as 60 Departments of Rheumatology and Perinatal Medical Centers across Japan (from 2010 to 2011). The retrospective survey investigated maternal information such as age at delivery, history of conceptions, including history of NLE and fetal CHB, rheumatologic symptoms, clinical diagnosis, autoantibodies status, including anti-SS-A antibodies, anti-SS-B antibodies (DID and/or ELISA), anti-SS-A 52 kDa antibodies, and anti-SS-A 60 kDa antibodies (detected by western blotting and/or ELISA), other antibodies, treatment before and after conceptions, and interventions for CHB after diagnosis, as well as child-related information, such as gestational week of delivery, gender, birth weight, APGAR score, and presence or absence of NLE and fetal CHB. Approval for this study was obtained from the local ethics committees of the participating institutions.

Selection of cases for analysis among collected cases

Clinical data on 732 cases were collected. First, we excluded the cases with unknown fetal CHB status, as well as cases with previous fetal CHB. Next, we also excluded cases who tested positive for anti-SS-A antibodies after conception, and one case with ± status of anti-SS-A antibody by DID and unknown by ELISA. Thus, the study subjects were 635 pregnant mothers who tested positive for anti-SS-A antibodies before conception, and who had no past history of fetal CHB, including 16 with fetal CHB and 619 without fetal CHB (Figure 1).

Comparison of clinical features between cases with fetal CHB and without fetal CHB

We compared various maternal clinical features, including age at delivery, history of conceptions, rheumatologic symptoms, clinical diagnosis, anti-SS-A antibodies status, and treatment before and after conceptions, between 16 cases with fetal CHB and 619 cases without fetal CHB.

We divided the cases into those with high titer and low titer of anti-SS-A antibodies by DID using a cut-off value of 1:32. The selection of the cut-off value was based on the finding by Anami et al. [10] who reported that a titer of 1:32 or higher in the maternal serum by DID was an independent risk factor for fetal CHB. In cases with unknown DID status, 1:32 by DID was converted into equivalent titers by each ELISA kit [Miyano et al., Clin Rheumatol 24:247–259, 2012, in Japanese], and we regarded cases with high titer by ELISA equivalent to $\geq$1:32 by DID as high titer, and cases with low titer by ELISA equivalent to <1:32 by DID as low titer. Thus, 114 cases were classified as the high titer group (6 with CHB and 108 without CHB), 506 cases as the low titer group (10 with CHB and 496 without CHB), and 15 cases as the unknown titer group (15 without CHB).

Statistical analysis

Comparisons between groups were conducted using the Student's *t*-test for continuous variables and Fisher's exact test for binary variables. Univariate analysis investigated the relation between risk of fetal CHB and the following variables: maternal clinical features, such as age at delivery, history of conceptions, rheumatologic symptoms, clinical diagnosis, anti-SS-A antibodies status, and treatment before and after conceptions. We also performed multivariate analysis with fetal CHB as the dependent variable, and maternal clinical features that were indicated to have associations in the univariate analyses ($p<0.1$), and some maternal factors which could affect corticosteroids therapy considering their clinical relevancies as in the independent variables. We also performed logistic regression analysis, and calculated odds ratio (OR) for CHB, 95% confidence interval (CI), and p value. For missing data, we used the multiple imputation method; 200 imputed datasets were generated using the MICE (multiple imputation by chained equations) method and their results were synthesized by the Rubin's rule [11]. SPSS for Windows, version 18.0 (IBM Japan Inc., Tokyo, Japan) and R version 3.0.3 (R Foundation for Statistical Computing, Vienna, Austria) were used for statistical analyses. All quoted p values are two-sided and the significance level was set to 0.05.

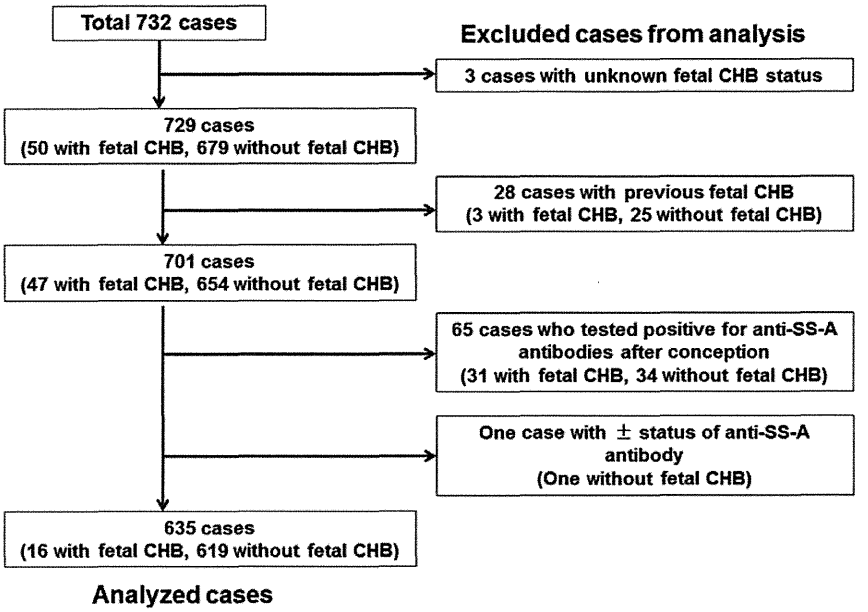

Figure 1. Flow chart showing selection of cases for analysis among 732 pregnant women who tested positive for anti-SS-A antibodies.

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Results

Selected cases for analysis among collected cases

Clinical data on 732 cases (50 cases with fetal CHB, 679 cases without fetal CHB, and 3 cases with unknown fetal CHB status) were collected. First, we excluded the 3 cases with unknown fetal CHB status, as well as 28 cases with previous fetal CHB, which is a definite maternal risk factor for fetal CHB (3 with fetal CHB and 25 without fetal CHB) from analysis. Next, among the residual 701 cases, we also excluded 65 cases who tested positive for anti-SS-A antibodies after conception (31 with fetal CHB and 34 without fetal CHB), and one case with ± status of anti-SS-A antibody by DID and unknown by ELISA (without fetal CHB). Thus, the study subjects were 635 pregnant mothers who tested positive for anti-SS-A antibodies before conception, and who had no past history of fetal CHB, including 16 with fetal CHB and 619 without fetal CHB (Figure 1).

In these analyzed 635 cases, many cases (82.2%, 522/635 cases) had diagnosis of maternal connective tissue disease (CTD). We could speculate that these cases were tested for anti-SS-A antibodies in clinical practice before conception. However, many fetal CHB cases (31/50 cases) were tested positive for anti-SS-A antibodies after conception as described above. These fetal CHB cases had no opportunity to receive systemic examinations and corticosteroids therapy before conception or after conception before 16-week gestation (before onset of fetal CHB). Thus, we supposed that if we included these fetal CHB cases in the present analysis, these cases caused a serious selection bias for corticosteroids therapy.

Comparison of clinical features of mothers with and without fetal CHB

There was no significant difference in age at delivery, history of conception, rheumatologic symptoms, clinical diagnosis, and anti-SS-A antibodies status between patients with fetal CHB and without fetal CHB (Table 1). The frequency of treatment with corticosteroids (equivalent doses of PSL, at $\geq$10 mg/day) during pregnancy before 16 weeks of gestation was significantly lower in patients with fetal CHB (20.0%) than without fetal CHB (60.2%, $p=0.03$). The frequency of treatment with corticosteroids before conception was higher (81.3%), and use of corticosteroids (equivalent doses of PSL, at $\geq$10 mg/day) before and after conception tended to be lower (11.1%) in patients with fetal CHB than without fetal CHB (54.2% and 49.5%, respectively), albeit statistically insignificant ($p=0.06$ and $p=0.053$, respectively) (Table 1).

Table 2 provides details on the number of patients who received corticosteroids treatment stratified according to the type of corticosteroids and time of administration in relation to conception. PSL was the most commonly used by many patients with and without fetal CHB before conception, while other types of corticosteroids were administered in only a few cases. In comparison, betamethasone was administered in 4 patients with fetal CHB and 28 cases without fetal CHB after conception. Regarding before conception, populations treated with PSL or other corticosteroids were comparable between cases with and without fetal CHB ($p=0.135$). On the other hand, after conception, population treated with other corticosteroids than PSL was significantly larger in cases with fetal CHB than in without fetal CHB ($p=0.0002$). We supposed that fetuses with CHB might be treated with placental transferable corticosteroids such as betamethasone and dexamethasone.

Because we did not investigate the reasons why the mothers took corticosteroids before and after conception in this retrospective survey, we further analyzed the association of the timing of corticosteroids use with onset of fetal CHB and diagnosis of maternal CTD. We divided the timing of corticosteroids use into three phases, such as before conception, after conception before 16-week gestation, and after 16-week gestation. We focused on 16-week gestation, because it has been reported that between 16 and 24 weeks of gestation was the period during which the fetus was at the highest risk of developing CHB [2]. Actually, in the present study, every fetal CHB developed between 18 and 30 weeks of gestation. Therefore, the period after conception before 16-week gestation might be a window of opportunity to modify the risk of fetal CHB.

Table 1. Comparison of clinical features of patients with and without fetal CHB.

Clinical features	With CHB (N = 16)	Without CHB (N = 619)	*p* value*
Age at delivery, mean (SD)	30.63 (4.08)	31.88 (4.35)	0.972
History of conception	6 (37.5%)	238 (38.8%)	1.000
Neonatal lupus erythematosus	1 (7.1%)	21 (3.6%)	1.000
Rheumatologic symptoms	14 (87.5%)	463 (77.8%)	0.537
Sicca symptom	4 (25.0%)	157 (25.4%)	1.000
Dry eye	4 (25%)	126 (20.4%)	0.888
Dry mouth	4 (25%)	125 (20.2%)	0.875
Erythema	6 (37.5%)	174 (28.1%)	0.588
Purpura	1 (6.3%)	16 (2.6%)	0.911
Raynaud's phenomenon	1 (6.3%)	84 (13.6%)	0.633
Fever	6 (37.5%)	127 (20.5%)	0.181
Arthralgia	5 (31.3%)	205 (33.1%)	1.000
Meningitis	0 (0%)	6 (1.0%)	1.000
Interstitial nephritis	0 (0%)	13 (2.1%)	1.000
Interstitial pneumonia	0 (0%)	13 (2.1%)	1.000
Pulmonary hypertension	0 (0%)	1 (0.2%)	1.000
Thrombosis	0 (0%)	5 (0.8%)	1.000
Clinical diagnosis of connective tissue disease	14 (87.5%)	508 (82.1%)	0.818
Sjögren's syndrome	7 (43.8%)	238 (38.4%)	0.865
Systemic lupus erythematosus	5 (31.3%)	259 (41.8%)	0.554
Mixed connective tissue disease	1 (6.3%)	37 (6.0%)	1.000
Rheumatoid arthritis	1 (6.3%)	23 (3.7%)	1.000
Anti-phospholipid antibody syndrome	0 (0%)	28 (4.5%)	0.800
High titer of anti-SS-A antibodies	6 (37.5%)	108 (17.9%)	0.094
Corticosteroids therapy before conception	13 (81.3%)	333 (54.2%)	0.059
Use of immunosuppressants before conception	1 (6.3%)	64 (10.4%)	0.900
Use of corticosteroids after conception	12 (75.0%)	369 (59.6%)	0.326
Use of immunosuppressants after conception	0 (0%)	11 (2.5%)	1.000
Use of anti-platelet drugs and/or anti-coagulants after conception	2 (15.4%)	162 (33.3%)	0.289
Plasma exchange after conception	1 (7.1%)	11 (2.4%)	0.815
Use of intravenous immunoglobulins after conception	0 (0%)	0 (0%)	1.000
Use of corticosteroids before and after conception	9 (56.3%)	318 (51.5%)	0.905
Use of corticosteroids (equivalent doses of PSL, at ≥10 mg/day) before and after conception	1 (11.1%)	157 (49.5%)	0.053
Use of corticosteroids after conception before 16-week gestation	10 (62.5%)	355 (57.5%)	0.888
Use of corticosteroids (equivalent doses of PSL, at ≥10 mg/day) after conception before 16-week gestation	2 (20.0%)	210 (60.2%)	0.026

CHB, congenital heart block; PSL, prednisolone.
*Student's *t*-test for continuous variables and Fisher's exact test for binary variables.

Table 2. Type of corticosteroids administered before and after conception.

Type of corticosteroids	With CHB (N = 16)	Without CHB (N = 619)
Before conception		
Total corticosteroids use	13 cases	333 cases
PSL	9	282
mPSL	0	8
Bet	0	2
Dex	0	0
Unknown	4	41
After conception		
Total corticosteroids use	12 cases	369 cases
PSL	5	328
PSL–Bet	2	10
PSL–Bet–Dex	1	0
PSL–Dex	2	2
PSL–Dex–Bet	0	1
mPSL	0	7
mPSL–PSL	0	1
Bet	1	15
Bet–PSL	0	2
Dex	1	1
Unknown	0	2

PSL, prednisolone; mPSL, methylprednisolone; Bet, betamethasone; Dex, dexamethasone.
Data are number of patients.

We identified seven groups according to the timing of corticosteroids use, as followings. Group A, only before conception (N = 27); Group B, continuous use before and after conception (N = 295); Group C, before conception and resumption after conception before 16-week gestation (N = 20); Group D, before conception and resumption after 16-week gestation (N = 4); Group E, only after conception before 16-week gestation (N=50); Group F, only after 16-week gestation (N = 12); and Group G, without use of corticosteroids (N = 227) (Figure 2). Among these seven groups, Groups B, C, and E (total 365 cases) took corticosteroids after conception before 16-week gestation. Of these, 80.8% (295/365 cases, Group B) had continuous use before and after conception, and many cases of Group B (95.6%, 282/295 cases) had diagnosis of CTD. Thus, in Group B, almost cases seemed to take corticosteroids for maternal rheumatologic manifestations. Similarly, in Group C, many cases had diagnosis of CTD (70%, 14/20 cases) and seemed to resume taking of corticosteroids for deterioration of maternal rheumatologic manifestations after conception. In Group C, only one case had developed a fetal CHB at 21-week gestation, in this fetal CHB case, corticosteroids might be resumed not for a fetal CHB but for maternal rheumatologic manifestations. On the other hand, in Group E, some cases did not have diagnosis of CTD (24%, 12/50 cases). We could speculate that corticosteroids might be added after conception before 16-week gestation for prevention of fetal CHB in some cases of Group E. Interestingly, only in Group E, there was no fetal CHB case (Figure 2).

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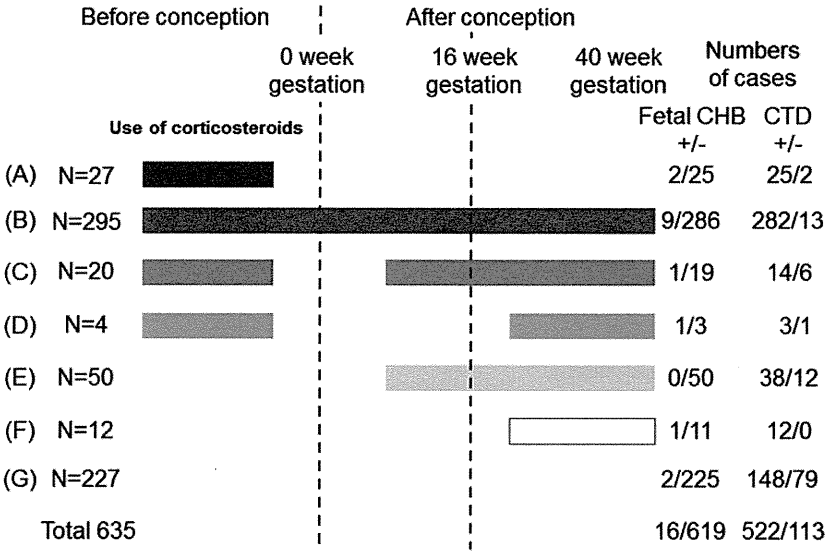

Figure 2. Association of the timing of corticosteroids use with onset of fetal CHB and diagnosis of maternal connective tissue disease.
Group A, only before conception (N = 27); Group B, continuous use before and after conception (N = 295); Group C, before conception and resumption after conception before 16-week gestation (N = 20); Group D, before conception and resumption after 16-week gestation (N = 4); Group E, only after conception before 16-week gestation (N = 50); Group F, only after 16-week gestation (N = 12); and Group G, without use of corticosteroids (N = 227); corticosteroids therapy before conception, Groups A, B, C, and D (N = 346); use of corticosteroids after conception, Groups B, C, D, E, and F (N = 381); use of corticosteroids after conception before 16-week gestation, Group B, C, E (N = 365); CHB, congenital heart block; CTD, connective tissue disease.

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Table 3. Results of univariate analysis of fetal CHB.

Parameters	Odds ratio for fetal CHB	95% CI	*p* value
Age at delivery	0.935	0.833, 1.050	0.256
History of conception	0.950	0.340, 2.654	0.923
Neonatal lupus erythematosus	1.943	0.243, 15.512	0.530
Rheumatologic symptoms	2.105	0.471, 9.407	0.329
Sicca symptom	0.981	0.311, 3.092	0.974
Dry eye	1.304	0.413, 4.121	0.650
Dry mouth	1.317	0.417, 4.163	0.638
Erythema	1.534	0.548, 4.295	0.414
Purpura	2.512	0.311, 20.278	0.387
Raynaud's phenomenon	0.425	0.055, 3.269	0.410
Fever	2.324	0.828, 6.528	0.109
Arthralgia	0.918	0.314, 2.682	0.875
Meningitis	-	-	-
Interstitial nephritis	-	-	-
Interstitial pneumonia	-	-	-
Pulmonary hypertension	-	-	-
Thrombosis	-	-	-
Clinical diagnosis of connective tissue disease	1.530	0.342, 6.844	0.578
Sjögren's syndrome	1.245	0.457, 3.394	0.668
Systemic lupus erythematosus	0.632	0.216, 1.844	0.400
Mixed connective tissue disease	1.049	0.134, 8.189	0.964
Rheumatoid arthritis	1.728	0.218, 13.699	0.604
Anti-phospholipid antibody syndrome	-	-	-
High titer of anti-SS-A antibodies	2.783	0.989, 7.837	0.053
Use of corticosteroids before conception	3.717	1.046, 13.206	0.042
Use of immunosuppressant before conception	0.571	0.074, 4.407	0.590
Use of corticosteroids after conception	2.033	0.647, 6.386	0.224
Use of immunosuppressants after conception	-	-	-
Use of anti-platelet drugs and/or anti-coagulants after conception	0.514	0.136, 1.944	0.326
Plasma exchange after conception	0.994	0.181, 5.474	0.995
Use of corticosteroids before and after conception	1.202	0.441, 3.273	0.719
Use of corticosteroids (equivalent doses of PSL, at ≥10 mg/day) before and after conception	0.268	0.036, 2.012	0.119
Use of corticosteroids after conception before 16-week gestation	1.223	0.438, 3.415	0.700
Use of corticosteroids (equivalent doses of PSL, at ≥10 mg/day) after conception before 16-week gestation	0.170	0.033, 0.878	0.034

CHB, congenital heart block; NLE, neonatal lupus erythematosus; PSL, prednisolone; CI, confidence interval.

Table 4. Results of multivariate analysis of predictive factors for fetal CHB.

Predictor variables	Odds ratio for fetal CHB	95% CI	*p* value
Analysis 1 (N = 635)			
Age at delivery	0.922	0.816, 1.042	0.194
History of conception	1.063	0.368, 3.071	0.910
Rheumatologic symptoms	1.238	0.240, 6.389	0.799
Clinical diagnosis of connective tissue disease	0.626	0.117, 3.348	0.583
High titer of anti-SS-A antibodies	3.581	1.214, 10.561	0.021
Use of corticosteroids before conception	4.284	1.097, 16.730	0.036
Analysis 2 (N = 635)			
Age at delivery	0.922	0.816, 1.041	0.191
History of conception	1.068	0.373, 3.059	0.902
Rheumatologic symptoms	1.746	0.349, 8.737	0.497
Clinical diagnosis of connective tissue disease	0.976	0.184, 5.180	0.977
High titer of anti-SS-A antibodies	2.950	1.022, 8.516	0.045
Use of corticosteroids before and after conception	1.038	0.360, 2.999	0.944
Analysis 3 (N = 635)			
Age at delivery	0.923	0.814, 1.047	0.212
History of conception	1.105	0.373, 3.276	0.857
Rheumatologic symptoms	1.430	0.272, 7.501	0.672
Clinical diagnosis of connective tissue disease	0.835	0.153, 4.553	0.835
High titer of anti-SS-A antibodies	3.591	1.193, 10.806	0.023
Use of corticosteroids (equivalent doses of PSL, at ≥10 mg/day) after conception before 16-week gestation	0.156	0.028, 0.859	0.033

CHB, congenital heart block; PSL, prednisolone; CI, confidence interval.

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Relationship between fetal CHB and various clinical factors

Fetal CHB did not associate with age at delivery, history of conceptions, presence of rheumatologic symptoms, and clinical diagnosis (Table 3). On the other hand, the development of fetal CHB associated significantly with corticosteroid use before conception (OR 3.72, $p = 0.04$), whereas corticosteroids use (equivalent doses of PSL, at ≥10 mg/day) after conception before 16-week gestation was associated with significantly lower risk of fetal CHB (OR 0.17, $p = 0.03$). Further univariate analysis showed that fetal CHB tended to associate with high titer of anti-SS-A antibodies (OR 2.78, $p = 0.053$), albeit with border statistical significance (Table 3).

Multivariate analysis of predictive factors for fetal CHB

We considered three multivariate models in this analysis. First, the variables (1) age at delivery, (2) history of conception, (3) presence of rheumatologic symptoms, (4) clinical diagnosis of CTD, (5) high titer of anti-SS-A antibodies, and (6) corticosteroid use before conception were entered into multivariate analysis. The results identified high titer of anti-SS-A antibodies (OR 3.58, $p = 0.02$) and corticosteroid use before conception (OR 4.28, $p = 0.04$) as independent and significant risk factors for fetal CHB (Table 4, Analysis 1).

Next, variables 1–5 above and the use of corticosteroids before and after conception were entered into model 2 of multivariate analysis. The results identified high titer of anti-SS-A antibodies (OR 2.95, $p = 0.045$) as the only independent and significant risk factor for fetal CHB, but not the use of corticosteroids before and after conception (OR 1.04, $p = 0.94$) (Table 4, Analysis 2).

The last model (model 3) included variables 1–5 above and corticosteroids use (equivalent doses of PSL, at ≥10 mg/day) after conception before 16-week gestation. The results identified high titer of anti-SS-A antibodies as a significant and independent risk factor for fetal CHB (OR 3.59, $p = 0.02$), while corticosteroids use (equivalent doses of PSL, at ≥10 mg/day) after conception before 16-week gestation was an independent protective factor against the development of fetal CHB (OR 0.16, $p = 0.03$) (Table 4, Analysis 3).

Discussion

Our retrospective study of pregnant women with anti-SS-A antibodies reported three clinically important findings. First, univariate and multivariate analyses showed no significant association between the development of fetal CHB and various maternal parameters, such as age at delivery, history of conceptions, the presence of rheumatologic symptoms, and clinical diagnosis, in pregnant mothers who tested positive for anti-SS-A antibodies before conception and had no history of fetal CHB. These findings are somewhat in agreement with those of previous reports [4,6,12,13]. At the present time, only past history of fetal CHB seems to be a definite maternal risk factor for fetal CHB [4,6,7,10], while other maternal clinical features, such as high age [7,10], history of fetal cutaneous NLE [5], and summer season of child birth [7] are possible risk factors for fetal CHB. Considered together, the above results suggest that one cannot predict the development of fetal CHB based on maternal clinical features other than past history of fetal CHB in pregnant women positive for anti-SS-A antibodies.

Second, our multivariate analyses identified high titer of anti-SS-A antibodies (titer ≥1:32 by DID or equivalent by ELISA) as a significant and independent risk factor for fetal CHB. Although this finding was in agreement with previous report by Anami et al. [10], another study showed that fetal CHB did not associate with high levels of maternal anti-SS-A antibodies but with that of anti-SS-B antibodies [8]. These discrepancies could reflect differences in patient population and methods used for detection of antibodies. Moreover, anti-SS-B antibodies are usually detected together with anti-SS-A antibodies. Thus, anti-SS-A and SS-B antibodies might confound with each other. Standardization for antibodies assays and large cohort study are needed to determine the role of anti-SS-A/B antibodies in CHB.

Third, multivariate analyses identified corticosteroid before conception as a significant independent risk factor for fetal CHB, and that the use of corticosteroids (equivalent doses of PSL, at ≥10 mg/day) after conceptions before 16-week gestation was a significant independent protective factor against the development of fetal CHB, though the use of corticosteroid before and after conception had no effect on the development of fetal CHB.