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Original Article

IgG4-related sialadenitis in Uygur and Han population: clinical and pathological studies

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Abstract: Aims: To observe the clinical and pathological features of the IgG4-related Sialadenitis and to evaluate the clinical and pathological differences between Uygur and Han population. Materials and methods: We perform a retrospective analysis of data from Uygur patients (n=38) and Han patients (n=55) with the chronic nonspecific salivary gland inflammations which were infiltrated by multiply plasma cells. All the samples had been resected during treatment for oral lesion during 2004-2014. Formalin fixed paraffin-embedded specimens were used for immunohistochemistry, the primary antibodies were used CD38, IgG, IgG4, Kappa, Lambda. The numbers of IgG positive and IgG4 positive cells were estimated. Results: 30.91% (11 men and 6 women) Han and 26.32% (9 men and 1 woman) Uighur samples strongly suggested to be the IgG4-related sialadenitis. The main pathological findings were: diffuse lymph plasma cells infiltration through the lesion area, extensive salivary glands atrophy, The storiform-arranged type pattern resembles the spokes of a cartwheel with spindle cells radiating form a center. Even lymphoid follicle formations were commonly observed. Besides, the numbers of IgG4+ plasma cells and the IgG4+ plasma cells/IgG+ plasma cells between Han and Uygur people had significant difference in IgG4-related sialadenitis. Conclusions: IgG4-related sialadenitis is a systemic disease and may have racial variation between Uygur and Han people.

Keywords: IgG4-related sialadenitis, clinical and pathological features, Han, Uygur, racial variation

Introduction

IgG (immunoglobulin G) has four subclasses: IgG1, IgG2, IgG3 and IgG4, IgG4 is the least abundant IgG subclass and accounts only for less than 5% of the total IgG in healthy people but IgG4 concentrations within individual persons are very stable [1]. IgG4-related disease is a recently recognized unique fibro-inflammatory systematic condition characterized by dense lymph plasma cells infiltrates rich in IgG4-positive plasma cells, storiform-arranged fibrosis, and, often but not always, elevated serum IgG4 concentrations [2]. The disease was first recognized in the pancreas and known as autoimmune pancreatitis. It was in 2001 that autoimmune pancreatitis was first linked with the presence of elevated levels of serum IgG4 [3], but until 2003, when extrapancreatic manifestations were founded in patients with autoimmune pancreatitis, the IgG4-related disease was first recognized as a systemic condition [4].

IgG4-related disease is a systemic disorder and can affect in virtually various organs such as the biliary tree, salivary glands, periorbital tissues, kidneys, lungs, lymph nodes, meninges, aorta, breast, prostate, thyroid, pericardium, and skin [2]. It has been attracted much attention including the pathologist, clinical doctors, rheumatologist, immunologist, otolaryngologist, ophthalmologist since the IgG4-related disease has been proposed. With the development of the modern pathology, more and more diseases are considered to be IgG4 related diseases. Mikulicz's diseases is a chronic inflammatory disease, which character facture are diffuse enlargement of the lacrimal and submandibular glands, elevated levels of

serum immunoglobulin G4 (IgG4), and multiply IgG4-positive plasma cells infiltration and fibrosis in both glands. Mikulicz's disease is considered as an IgG4-related disease [5]. Küttner's tumor, or sclerosing sialadenitis, the histopathological manifestation is peri-ductal fibrosis, dense lymphocytic infiltration with lymphoid follicles, loss of the acinus and, marked sclerosis of the salivary glands [6]. Recently, Küttner's tumor has been regarded to fall within the IgG4-related disease spectrum [7]. Mikulicz's diseases, Küttner's tumor, and Riedel's thyroiditis, names have been used in medical literature for more than a century in some cases, recently maybe replaced by another new name: IgG4-related disease [2].

Recently Nicholas Andrew et al took a meta-analysis of all published cases of IgG4-related orbital disease found that not only the frequency but also the number of the positive IgG4 in IgG4-related orbital disease may have racial variation [8]. The Han nationality belongs to the Asian race is the largest population of Chinese people, while the Uighur people almost all living in Xinjiang Uygur Autonomous Region in China is Caucasian race. The Uighur and the Han people have different genetic polymorphism. We proposed a view that whether the racial variation exist in the IgG4-related sialadenitis between Uygur and Han in China?

We take this retrospective research to observe the clinical and pathology feature of the IgG4-related sialadenitis and then evaluate the differences between Uygur and Han population.

Materials and methods

Samples

We obtained 55 Han samples (30 men and 25 women) from the department of Pathology in Urumqi General Hospital of Lanzhou Military Area Command in China and 38 Uighur samples (22 men and 16 women) from the department of Pathology in Xinjiang Uygur Autonomous Region People's Hospital in China from 2004 to 2014. All the Han samples and all the Uygur samples had chronic nonspecific salivary gland inflammations which were infiltrated with multiply plasma cells. All the tissue samples were obtained from salivary glands. Formalin fixed paraffin-embedded specimens were used for immunohistochemistry.

Laboratory data

We retrospectively analyzed the anti-SSA antibody and anti-SSB antibody.

Histological examination and immunohistochemistry

The specimens were fixed in 10% formaldehyde and embedded in paraffin. Serial 4-mm-thick sections were cut from the block of paraffin-embedded tissue and stained with hematoxylin and eosin (H&E). The sections were immunohistochemically stained using an automated Bond Max stainer (Leica Biosystems, Leica Autostainer XL, Germany). The following primary antibodies were used: IgG (Rabbit Anti-Human IgG4 Polyclonal Antibody; 1:6000 dilution; Maixin, Fujian, China), IgG4 (Mouse Anti-Human IgG4 Monoclonal Antibody, HP6025; ready-to-use, Maixin, Fujian, China), CD38 (Mouse Anti-human CD38 Monoclonal Antibody, ready-to-use, Maixin, Fujian, China), Kappa (Rabbit Anti-Human Kappa Light Chain Polyclonal Antibody, ready-to-use, Maixin, Fujian, China), Lambda (Mouse Anti-Human Lambda Light Chain Monoclonal Antibody, ready-to-use, Maixin, Fujian, China). Following immunostaining, the number of IgG4-positive and IgG-positive plasma cells was estimated in three areas with the highest density of IgG4-positive plasma cells by two different pathologists who were blinded to analyze the slides. In accordance with the consensus statement on the pathological features of IgG4-related disease published in 2012 [9], three different high-power fields (HPFS) (eyepiece, ×10; lens, ×20) were examined under an Olympus microscope to calculate the average number of IgG4-positive plasma cells per HPFS and the IgG4-positive/IgG-positive plasma cell ratio. The mean number of IgG4-positive plasma cells in 3 high-power fields was calculate for each specimen and scored as follows: less than 50 positive cells/high-power field as negative and more than 50 cells as positive. In addition, the mean ratio of IgG4-positive plasma cells/IgG-positive plasma cells were calculate for 3 high-power fields in each specimen, and a ratio over 40% was interpreted as positive.

Statistical analysis

Data are presented as mean ± sd. Differences between groups were examined for statistical

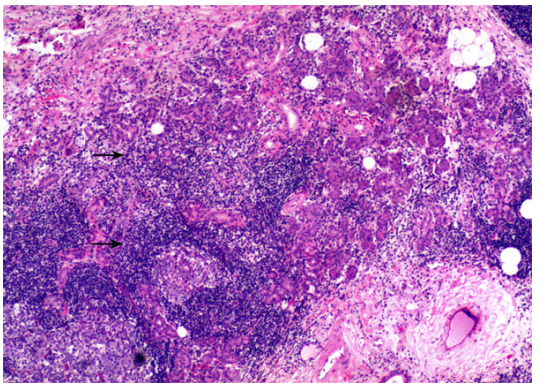

Figure 1. Widespread atrophy of salivary glands and a dense lymphocyte infiltrated the dilated ducts, germinal centers are observed. (hematoxylin and eosin, H&E ×40).

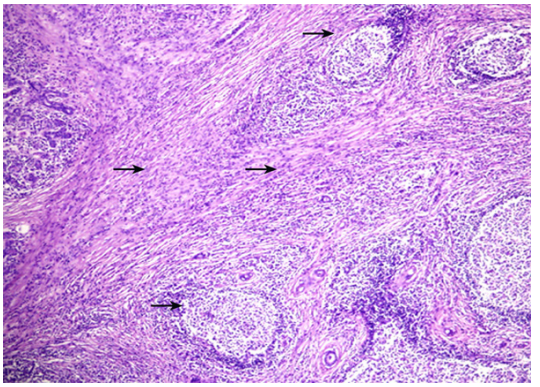

Figure 2. Storiform-arranged type fibrosis and germinal centers are observed. (hematoxylin and eosin, H&E ×100).

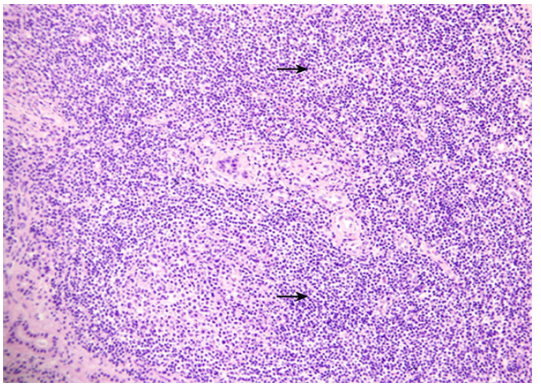

Figure 3. The salivary glands are widely infiltrated by lymphocytes and plasma cells, collagen sheaths around the ducts can be seen. (hematoxylin and eosin, H&E ×100).

significance by using the independent-samples T test or Rank sum test, whereas differences in

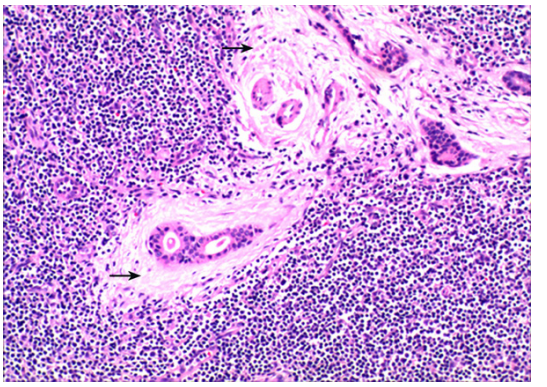

Figure 4. The salivary glands are widely infiltrated by lymphocytes and plasma cells, collagen sheaths around the ducts can be seen. (hematoxylin and eosin, H&E ×100).

frequencies were analyzed with the Fisher Exact probability test. A *P* value less than 0.05 denoted the presence of a statistically significant difference.

Results

Confirmation of histological diagnosis in IgG4-related sialadenitis

According to the histological examination and immunohistochemistry, we confirmed that 17 (11 men and 6 women, mean age 54.3 years) of the 55 Han and 10 (9 men and 1 women, mean age 47.3 years) of the 38 Uighur samples strongly suggested to be the IgG4-related sialadenitis. There are 3 most obvious characteristics of the IgG4-related sialadenitis: diffuse lymph plasma cells infiltration through the lesion area, widespread salivary glands atrophy, the storiform-arranged type pattern resembles the spokes of a cartwheel with spindle cells radiating form a center, even lymphoid follicle formations were commonly observed. (**Figures 1** and **2**). Obliterative phlebitis was not seen in our samples, but the fibrosis was remarkable, we can find the collagen sheath around the ducts (**Figures 3** and **4**).

Many IgG4+ plasma cells were scattered in the periphery of the follicles. The concentration of IgG4+ plasma cells was greater than 50 per high-power field. The ratio of IgG4+ plasma cells to IgG+ plasma cells was >40%. There were more IgG and IgG4+ plasma cells in Han people than in Uighur people (**Figures 5** and **6**). The kappa and lambda antibodies were posi-

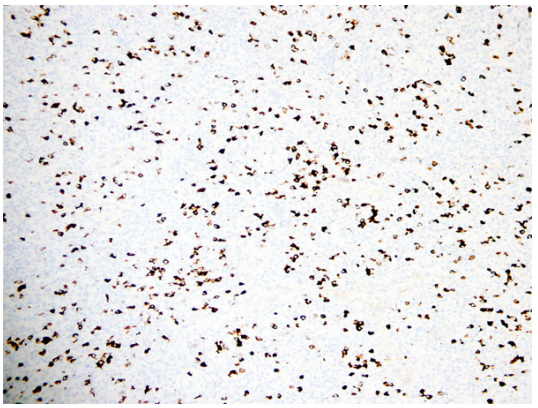

Figure 5. Immunostaining indicating diffuse infiltration of immunoglobulin G4 (IgG4) positive cells in the Han samples. (immunohistochemical stains , ×200).

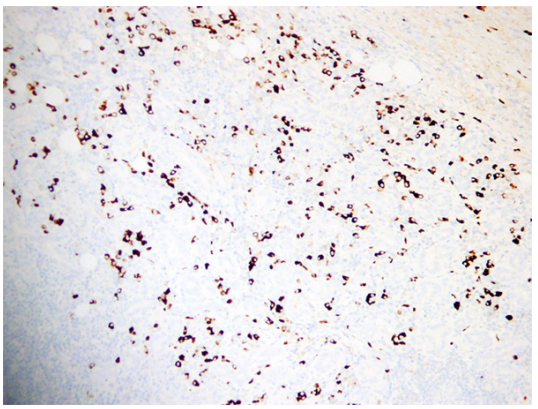

Figure 6. Immunostaining indicating many infiltration of immunoglobulin G4 (IgG4) positive cells in the Uighur samples. (immunohistochemical stains, ×200).

tive in the plasma cells in all the positive samples and it suggested that plasma cells polyclonal hyperplasia. The morbidity of IgG4-related sialadenitist has no significant difference between the Han and the Uighur (There was no figure). There were only two samples had other organs affected in Han but none in Uighur people and there was no significant difference between the Han and the Uighur population. The clinical and laboratory findings between Han and the Uighur in IgG4-related sialadenitis as follows (**Table 1**).

We observed that the mean numbers of IgG4+ plasma cells in Han people is much more than that in Uighur people. There are 270.18 IgG4+ plasma cells in Han people while only 105.10 in Uighur people on average, the *P* value was 0.00, the rate of IgG4+ cells/IgG+ cells has a significant difference between Han peoples and Uighur peoples (**Table 1**). It has statistically significant differences between the two groups. Besides, the numbers of IgG4-positive/IgG-positive plasma cell ratio were 59.66% ± 7.31% and 53.58% ± 5.94% and there were also statistically significant differences between the two groups.

Discussion

IgG4-related disease is a recently recognized unique fibroinflammatory systematic condition characterized by a dense lymphoplasmacytic infiltrate rich in IgG4-positive plasma cells, storiform-arranged type fibrosis, and, often but not always, elevated serum IgG4 concentrations.

Since the histopathological of Mikulicz's disease (MD) is very similar to the Sjögren's syndrome (SS), Mikulicz's disease has been considered as a subtype of Sjögren's syndrome (SS) for a long time. But now MD is distinguished from the SS. Recently, Tsuboi and colleagues has showed that the gene expression pattern in labial salivary glands from IgG4-related sialadenitis patients is not the same as that in labial salivary glands from SS patients. It suggested that the pathogenic mechanisms of IgG4-related disease and SS is different [10], It was reported that MD patients showed high immunoglobulin G4 (IgG4) concentrations and abundant infiltration of IgG4-positive cells as well as lymphocytes and fibrosis was detected in the lacrimal and salivary glands of MD patients, and these manifestation suggested that MD is an IgG4-related disease [11]. In our study we found that the 4 MD which we diagnosed at the first time strongly suggested to be the IgG4-related sialadenitis according to the clinical and the histological examination and immunohistochemistry later.

Küttner's tumor, first described in 1896 by H. Küttner lateral, is a benign tumor-like lesion most affecting the submandibular gland [12] and often known as chronic sclerosing sialadenitis, is a chronic inflammatory disease of the salivary gland, which characterized manifestations are: progressive periductal fibrosis, a dense lymphocyte infiltrated the dilated ducts and lymphoid follicles formation and acinus atrophy [13]. Recently it was reported that some samples of sclerosing sialadenitis from Japan or America have abundant IgG4-positive

Table 1. Comparison of clinical and laboratory findings between Han and Uyghur people in IgG4-related Sialadenitis

	Han	Uyghur	*P* value
Sex (male:female)	11:06	9:01	0.148
Mean age	54.29 ± 15.15	47.3 ± 12.44	0.238
Sites (right:left)	9:08	6:04	0.722
SSA/SSB	None	None	
IgG4+ cells (HPF)	270.18 ± 100.08	105.10 ± 19.23	0.000*
IgG4+ cells/IgG+ cells (%)	59.66% ± 7.31%	53.58% ± 5.94%	0.035*
Complicated of other organs	11.8% (2/17)	0% (0/10)	0.288

*: $P<0.05$. From this table we can easily find that the number of IgG4+ cells and the IgG4-positive/IgG-positive plasma cell ratio were different between the Han and Uyghur people, there were statistically significant differences between the two groups.

plasma cells, so Küttner's tumor has been considered to the spectrum of IgG4-RD [7], Kitagawa et al also suggested that dense infiltration of IgG4-positive plasma cells detected in the salivary glands with sclerosing sialadenitis is indicative of Küttner's tumor [13], Masaki Y, Dong L, Kurose N, et al analyze 4 cases among Japanese patients and proposed that Mikulicz diseases, Küttner's tumor, autoimmune pancreatitis, and other conditions belong to the IgG4-related systemic diseases [14]. In our study 2 cases have been diagnosed chronic sclerosing sialadenitis before were proved to be the IgG4-related sialadenitis finally.

Histopathology analysis of biopsy specimens remains the cornerstone in the diagnosis of IgG4-related disease [2]. Wallace et al had drawn the conclusions that it was not required to remove the whole submandibular gland to biopsy, incisional submandibular gland biopsies appear to be perfectly adequate for the purpose of establishing the diagnosis of IgG4-RD. Besides, biopsy of minor salivary glands (such as lip biopsy) may also be adequate to establish the diagnosis in many cases, even though a negative result would not necessary rule out the diagnosis of IgG4-RD [15]. There is another article reported by Doe et al strongly suggested that minor salivary gland biopsy is useful for IgG4-RD diagnosis in patients if it is difficult to obtain tissue biopsy samples from target organs [16]. Whether the minor salivary gland biopsy is effective or not, another experiment including more patients is needed to assess, and the diagnostic criteria will be determined at the same time.

Harrision et al investigated 129 cases of chronic submandibular sialadenitis collected from 1969 to 1989 and drew a conclusion that IgG4-realated sialadenitis is rare, and the IgG4 plasma cells that were present in the glands were part of a nonspecific chronic inflammatory infiltrate, maybe is related to the sialoliths [17]. But in our investigation we found that the IgG4-realated sialadenitis is not rare and we found 27 cases of the 93 total cases of chronic sialadenitis which were infiltrated by multiply plasma cells were diagnosed IgG4-realated sialadenitis. Besides, we also found that not only the numbers of IgG4+ plasma cells but also the IgG4+ plasma cells/IgG+ plasma cells between Han and Uighur people are different in IgG4-related sialadenitis. It probably supports that the racial variation is existed among the two different races.

IgG4-related disease may confer an increased risk of a range of malignancy [18]. The association between IgG4-related sialadenitis and malignancy is not clear. Ohta et al reported that a patient with IgG4-related sclerosing sialadenitis had salivary duct carcinoma and this result suggested that progression from longstanding IgG4-related sclerosing diseases to malignancy can occur [19]. The data from the Sapporo Medical University and Related Institutions Database for Investigation and Best Treatments of IgG4-related Diseases (SMART) according to analysis the 122 patients with IgG4-related dacryoadenitis and/or sialadenitis (IgG4-DS) concluded that complications of malignancy were observed in 7.4% of cases. Malignancy and IgG4-DS were diagnosed simultaneously in the patients with MALT lymphoma, colon cancer, and GIST [20]. Another prospective cohort study is necessary to evaluate the risk of malignancy in a large population of patients with IgG4-related sialadenitis in future.

As we know, IgG4-related disease is very sensitive to the glucocorticoid and glucocorticoid typically the first line of therapy [2]. But when is the best time to use the glucocorticoid to give treatment and which dose is the most appropriate is still not very clear. Shimizu et al found that in IgG4-RD early therapeutic intervention

is very useful for the retention of salivary glandular function and will achieve the best outcomes in patients [21]. The data from the Sapporo Medical University and Related Institutions Database for Investigation and Best Treatments of IgG4-related Diseases (SMART) to use glucocorticoid is that the mean maintenance dose of prednisolone was 4.8 mg/day, the clinical emission rate was 73.8%, and the annual relapse rate was 11.5% [20]. If the minor salivary gland biopsy is effective, we can not only make the correct diagnose earlier and give the earliest intervention to get the best result, but also can avoid some unnecessary operations for the patients.

Conclusions

IgG4-related sialadenitis is a systematic disease and may have racial variation between Uighur and Han people. With more and more in-depth understanding and research in IgG4-related sialadenitis, there will have more reports of IgG4 related cases of salivary gland inflammation and infection situation and its pathogenesis, diagnostic criteria and standard treatment also will be more and more perfect.

Disclosure of conflict of interest

None.

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