Comparison and clinical analysis of tissue-specific autoantibodies levels in primary Sjögren's disease and other connective tissue diseases

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Abstract

Objective

This study investigates serum levels of anti-parotid secretory protein (PSP), anti-salivary gland protein 1 (SP1), and anti-carbonic anhydrase 6(CA6) antibodies in primary Sjögren's disease (pSjD) and other connective tissue diseases (CTDs), further to evaluate their clinical relevance.

Methods

The study enrolled 60 patients diagnosed with pSjD, along with 30 disease controls (DC) suffering from various CTDs and 30 healthy controls (HC) for comparison. The serum levels of anti-PSP, anti-SP1, and anti-CA6 antibodies were measured using chemiluminescent immunoassays. Statistical analyses were performed using SPSS 27.0, including ANOVA, nonparametric tests (Kruskal-Wallis H and Mann-Whitney U), and Spearman correlation.

Results

The Patients with pSjD showed significantly higher serum levels of anti-CA6 immunoglobulin G (IgG), anti-PSP IgG/IgA, and anti-SP1 IgG than the DC and HC groups ($p<0.05$). These antibodies have a certain predictive accuracy in pSjD. The IgG subtype of anti-CA6, anti-PSP and anti-SP1 had a positive correlation with the erythrocyte sedimentation rate (ESR) and IgG in clinical correlations aspect. The levels of anti-CA6 IgG and anti-PSP IgG increased significantly with the severity of labial gland pathology ($p<0.05$). The subgroups that were positive for anti-SSA52KD(Ro52)/SSA60KD(Ro60)/SSB(La) exhibited higher levels of anti-CA6 IgG and anti-PSP IgG than their seronegative counterparts ($p<0.05$), while positivity for anti-centromere antibody (ACA)was linked to lower levels of anti-CA6 and anti-PSP IgG.

Conclusion

Anti-CA6, anti-PSP and anti-SP1 antibodies show diagnostic value in pSjD, with elevated IgG levels reflecting disease progression, histopathological damage, and distinct autoantibody interactions, implicating their pathogenic contributions.

Key words

primary Sjögren's disease, anti-salivary gland protein 1, anti-carbonic anhydrase 6, anti-parotid secretory protein, anti-centromere antibody

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Introduction

Primary Sjögren's disease (pSjD) is a chronic autoimmune disease characterised by lymphocytic infiltration of the salivary and lacrimal glands, leading to dry mouth and dry eyes, as well as increased secretion of autoantibodies (1, 2). It can manifest as interstitial lung disease, interstitial nephritis, and cytopenias, with some patients developing lymphoma (3-6), and an increased risk of cerebrovascular events and myocardial infarction (7). It is more common in females, typically presenting between the ages of 40 and 50, with significant clinical heterogeneity. The prevalence of pSS ranges from 0.01% to 0.05% (6). Its aetiology includes epigenetic, environmental factors, neuroendocrine influences, and immune dysregulation (8-10).

Autoantibodies play a critical role in the diagnosis of pSjD. The generation of autoantibodies in pSjD is complex, and its clinical heterogeneity may be related to various autoantibodies. Common autoantibodies include anti-SSA(Ro), anti-SSB (La), rheumatoid factor (RF), and antinuclear antibodies (ANA) (11). Anti-Ro and anti-La antibodies are found in approximately 50-70% of pSjD patients (12). Among serum biomarkers, the anti-Ro antibody stands out as the only significant one included in the most recent pSjD criteria from 2016 (13). However, 4.5% to 18% of patients with pSjD are seronegative for conventional autoantibodies (14, 15), diagnosis with conventional antibodies alone may result in missed diagnoses. New biomarkers are required to identify pSjD subsets in anti-Ro-negative patients and to predict their response to immunomodulatory therapy (16).

Novel biomarkers, especially autoantibodies, are crucial for pSjD of diagnosing and identifying its various subgroups. Several novel autoantibodies have been reported in patients with pSjD recently, including anti-α-fodrin, anti-muscarinic receptor 3, anti-carbonic anhydrase II, anti-tissue kallikrein, anti-deoxyribonuclease I, anti-ganglionic acetylcholine receptor, anti-aquaporin-5, anti-parotid secretory protein (PSP), anti-salivary protein 1 (SP1), and anti-carbonic anhydrase 6(CA6). As described above, these autoantibodies are not included in the diagnostic criteria for pSjD (5, 17-20). Among these autoantibodies, the presence of novel tissue specific autoantibodies (TSAs), including anti-SP1, anti-CA6, and anti-PSP, had gradually attracted attention (21), and they could be detected in the early stages of pSjD (22). Despite growing interest in TSAs, the research of their expression situation and clinical value in pSjD is still inadequate. This study evaluates the expression levels of different subtype of anti-PSP, anti-SP1 and anti-CA6 antibodies across three groups: patients with pSjD, those with other connective tissue diseases (CTDs), and healthy controls (HC). Additionally, it will examine the correlation between these biomarkers and the clinical features of pSjD. We hope this analysis will offer new insights and a foundation for diagnosing and treating pSjD.

Methods and materials

Patients and control groups

We collected plasma samples from 60 diagnosed pSjD patients in the Rheumatology and Immunology Department of the Second Hospital of Hebei Medical University from January 2022 to June 2024. pSjD patients met the 2002 international classification/diagnostic criteria or the 2016 ACR/EULAR classification criteria for SjD. A total of 30 other CTDs patients were selected as the disease control (DC) group, including 11 cases of vasculitis, 8 cases of rheumatoid arthritis (RA), 5 cases of systemic lupus erythematosus (SLE), 4 cases of undifferentiated connective tissue disease (UCTD), 1 case of dermatomyositis (DM), and 1 case of systemic sclerosis (SSc). All these CTDs patients complained of xerostomia and xerophthalmia, but did not meet the diagnostic criteria for SjD. In addition, 30 healthy individuals with no autoimmune diseases, matched for age and sex, were chosen as the HC group. This study was reviewed and approved by the Scientific Research Ethics Committee of the Second Hospital of Hebei Medical University, all the enrolled subjects have signed the informed con-

sent form, and the privacy of their personal data has been protected.

Serological testing and laboratory evaluation

Serum samples from 60 pSjD patients, 30 CTDs patients and 30 HC were collected, stored at -80°C before testing, the general data, clinical manifestations, laboratory indicators and other auxiliary examinations were collected. The IgA, IgG and IgM of anti-SP1, anti-PSP, anti-CA6 antibodies were determined by Kessler Biopharmaceuticals using chemiluminescence method. The normal reference value ≥20U/mL is reported as positive according to the instructions provided in the kit.

Statistical analysis

Data were analysed using SPSS 27.0 software. Data were expressed in the form of mean and standard deviation, median and interquartile range (IQR), or number and percentage as appropriate. T-tests, Mann-Whitney U tests, Kruskal-Wallis H tests, ANOVA, chi-square tests were evaluated for statistical analysis when appropriate, Spearman correlation analysis was used for the correlation analysis. Graphpad Prism 8.0 was used for plotting graphs. *P*-values<0.05 were considered statistically significant.

Results

Basic characteristics statistics

As shown in Table I, the research subjects included three groups, the pSjD group was consist of 58 females and 2 males, with a mean age of 50.65±13.25 years. There were 27 females and 3 males in the DC group, the mean age ± SD among CTDs patients was 54.20 years old ± 15.03 years. The HC group included 28 females and 2 males; the average age was 51.43±11.72 years. The gender and age were no significant differences among the three groups (p>0.05).

Comparison of levels of TSAs among the three groups

Figure 1 illustrated the serum levels of different subtypes of anti-PSP, anti-SP1 and anti-CA6 in different groups. The serum IgG levels of anti-CA6, anti-PSP and anti-SP1 in pSjD patients were remarkably higher than those of DC and HC (p<0.05) and the IgA levels of anti-PSP in patients with pSjD group were also higher than those in HC and DC groups (p<0.05). The serum IgM levels of anti-CA6 were elevated in the pSjD and DC groups compared to the HC group (p<0.05). Additionally, the serum IgA levels of anti-SP1 in the pSjD group were significantly elevated compared to those in the DC group (p<0.001). The serum levels of anti-CA6 IgA, anti-PSP IgM, and anti-SP1 IgM showed no statistically significant differences between patients with pSjD and other groups (p>0.05).

Table I. The general information of study subjects.

	pSS group	DC group	HC group	χ^2/F	*p*
Gender (Female/Male)	58/2	27/3	28/2	1.910	0.454
Age(years)	50.65±13.25	54.20±15.03	51.43±11.72	0.716	0.491

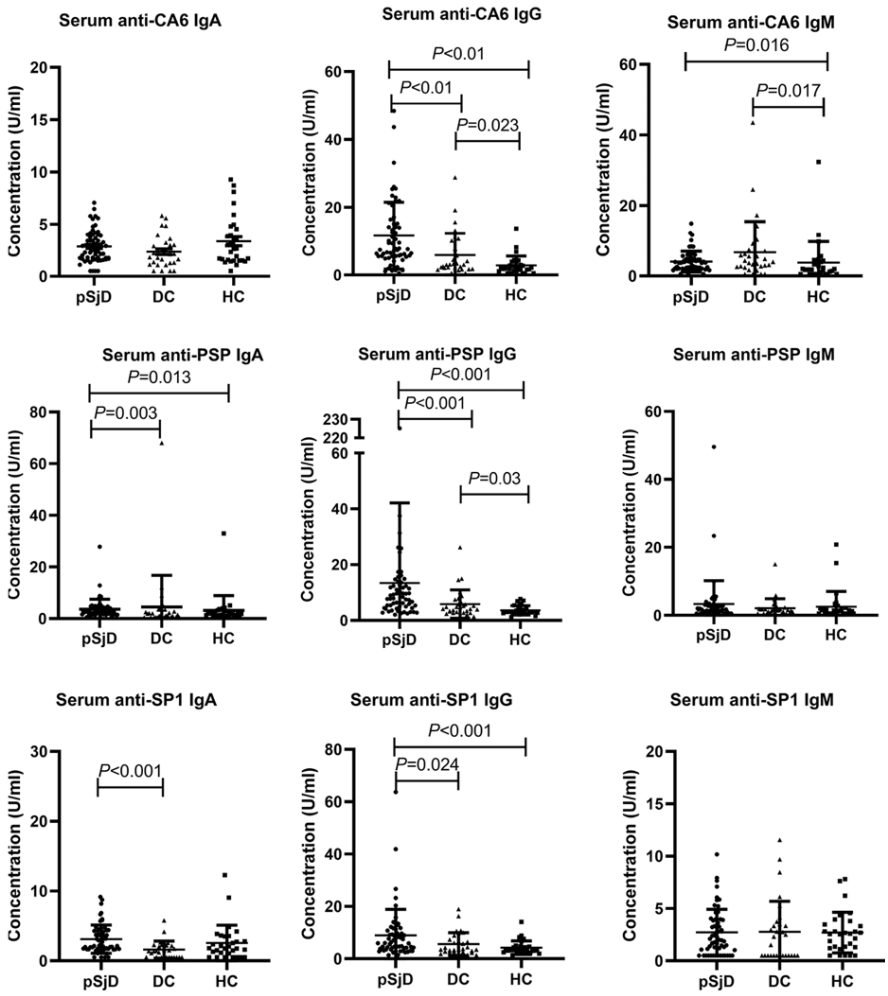

Fig. 1. Comparison of serum TSAs levels in patients with pSjD with DC and HC. The serum levels of anti-CA6 IgG, anti-PSP IgG, anti-SP1 IgG, and anti-PSP IgA were significantly elevated in pSjD compared to both DC and HC (p<0.05).

The diagnostic value of TSAs in diagnosing pSjD

Figure 2 showed the receiver operating characteristic (ROC) curve based on TSAs with statistically significant differences among the groups mentioned above. The areas under the curve of anti-CA6 IgG, anti-PSP IgG, anti-SP1 IgG, and anti-PSP IgA were 0.7953 (95% CI 0.7119–0.8787, p<0.0001), 0.7796 (95% CI 0.6962–0.8630, p<0.0001), 0.6897 (95% CI 0.5952–

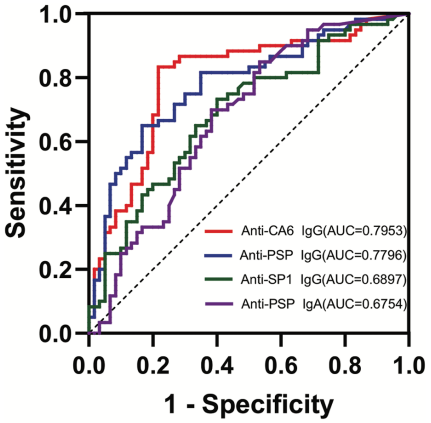

Fig. 2. ROC curve of TSAs in diagnosis of pSjD.

0.7842, p=0.0003), and 0.6754 (95% CI 0.5787–0.7722, p=0.0009), respectively. Among them, the sensitivity of the anti-CA6 IgG was 20% (12/60), specificity 98.3% (59/60), positive predictive value 92.3% (12/13), and negative predictive value 55.1% (59/107); the sensitivity of the anti-PSP IgG was 10% (6/60), specificity 98.3% (59/60), positive predictive value 85.7% (6/7), and negative predictive value 52.2% (59/113); and the sensitivity of the anti-SP1 IgG was 8.3% (5/60), specificity 100% (60/60), positive predictive value 100% (5/5), and negative predictive value 52.2% (60/115).

The correlation analysis of TSAs with clinical data

The correlation analysis between the statistical difference of TSAs and the clinical data was carried out (Table II). Figure 3 showed the correlation of anti-CA6 IgG and clinical data. The serum levels of anti-CA6 IgG antibodies were positively correlated with the erythrocyte sedimentation rate (ESR) (r=0.425, p<0.001) and IgG levels (r=0.438, p<0.001), while they were negatively correlated with serum albumin (ALB) (r=-0.299, p=0.020). The correlation between anti-PSP IgG and clinical data was showed in Figure 4. The anti-PSP IgG level was exhibited a positive correlation with ESR (r=0.566, p<0.001) and IgG levels (r=0.574, p<0.001), and there was a negative correlation with haemoglobin (HGB) (r=-0.343, p=0.007). Figure 5 indicated the correlation between anti-SP1 IgG and clinical data. The serum levels of anti-SP1 IgG antibody positively correlated with ESR (r=0.533, p<0.001) and IgG levels (r=0.423, p<0.001), but negatively correlated with HGB (r=-0.377, p=0.003). Figure 6 indicated the correlation between anti-PSP IgA and clinical data. The serum levels of anti-PSP IgA antibody showed a positive correlation with the ESR (r=0.316, p=0.014), while showed a negative correlation with HGB (r=-0.274, p=0.034).

Table II Correlation of TSAs levels with clinical data in pSjD patients.

Items	Anti-CA6 IgG		Anti-PSP IgG		Anti-SP1 IgG		Anti-PSP IgA	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*
ESSDAI	0.122	0.353	0.214	0.100	0.212	0.105	0.155	0.238
CRP (mg/L)	0.180	0.169	0.232	0.075	0.193	0.139	0.092	0.486
ESR (mm/h)	0.425	<0.001*	0.566	<0.001*	0.533	<0.001*	0.316	0.014*
IgG (g/L)	0.438	<0.001*	0.574	<0.001*	0.423	<0.001*	0.107	0.417
WBC (×10^9/L)	-0.130	0.322	-0.134	0.306	-0.142	0.278	0.013	0.924
PLT (×10^9/L)	-0.182	0.165	-0.216	0.098	-0.183	0.162	-0.102	0.437
HGB (g/L)	-0.246	0.058	-0.343	0.007*	-0.377	0.003*	-0.274	0.034*
ALB (g/L)	-0.299	0.020*	-0.238	0.067	-0.206	0.115	-0.127	0.333
AST (U/L)	0.123	0.349	0.052	0.693	0.026	0.846	0.066	0.618
ALT (U/L)	0.189	0.148	0.028	0.831	0.019	0.886	0.062	0.638
ALP (U/L)	0.168	0.200	0.094	0.477	0.131	0.320	0.142	0.277
GGT (U/L)	0.211	0.105	0.152	0.248	0.172	0.188	0.170	0.193
Serum potassium (mmol/L)	-0.164	0.211	-0.121	0.359	-0.091	0.488	-0.224	0.085
RF (IU/ml)	0.178	0.173	0.219	0.092	0.167	0.201	0.010	0.938
SFR (ml)	-0.056	0.673	0.027	0.836	0.015	0.911	0.076	0.562
Duration (month)	0.158	0.229	0.205	0.117	0.168	0.198	0.034	0.799
Age (year)	-0.184	0.159	-0.239	0.066	-0.224	0.086	-0.093	0.479

ESSDAI: EULAR Sjögren's Syndrome Disease Activity Index; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; IgG: immunoglobulin G; WBC: white blood cell count; PLT: platelet count; HGB, haemoglobin; ALB: albumin; AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase; GGT: gamma-glutamyl transferase; RF: rheumatoid factor; SFR: saliva flow rate. (*p<0 05).

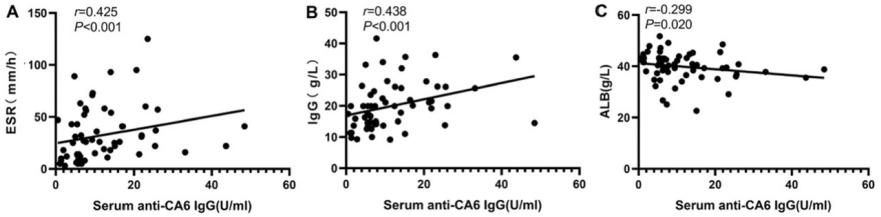

Fig. 3. (**A**) The relationship between serum levels of anti-CA6 IgG and ESR. (**B**) The relationship between serum IgG levels of anti-CA6 and IgG. (**C**) The relationship between serum levels of anti-CA6 IgG and ALB.

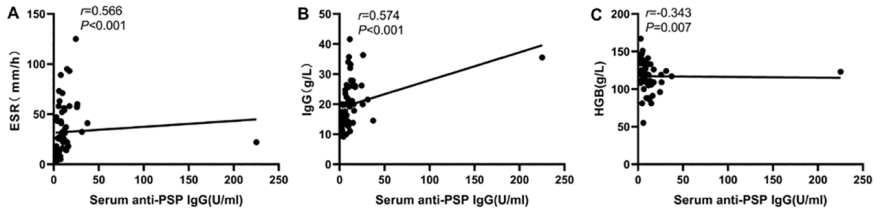

Fig. 4. (**A**) The relationship between serum levels of anti-PSP IgG and ESR. (**B**) The relationship between serum IgG levels of anti-PSP and IgG. (**C**) The relationship between serum levels of anti-PSP IgG and HGB.

The relationship between TSAs and autoantibodies

A comparative analysis of the autoantibody profiles in pSjD patients was conducted in Table III, focusing on ANA titres, those who were negative and positive for anti-Ro52/Ro60 antibodies, anti-La antibodies and anticentromere antibodies (ACA).

Patients with pSjD were divided into two subgroups based on ANA titres us-

ing a cut-off of 1:1000. The low titre positive group (ANA titre <1:1000) included 36 cases, while the high titre positive group (ANA titre ≥1:1000) included 24 cases. Table III showed that there were no statistically significant differences in serum IgG levels of anti-CA6, anti-PSP and anti-SP1 between the two groups, anti-PSP IgA also showed similar result (p>0.05).

In the anti-Ro52 subgroups, the negative group (n=15) had anti-CA6 IgG levels of 5.51 (5.50) U/mL, while the positive group (n=45) had levels of 9.51 (12.63) U/mL (p=0.002). Similarly, anti-PSP IgG levels were 4.46 (4.91) U/mL in the negative group compared to 9.62 (8.69) U/mL in the positive group (p=0.005). However, there were no significant differences in anti-SP1 IgG or anti-PSP IgA levels between the negative and positive anti-Ro52 groups (p>0.05).

In the anti-Ro60 subgroups, the negative group (n=20) had anti-CA6 IgG levels of 5.16 (5.72) U/mL, while the positive group (n=40) had levels of 10.68 (13.37) U/mL (p<0.001). Similarly, anti-PSP IgG levels were 5.20 (4.87) U/mL in the negative group and 9.66 (7.90) U/mL in the positive group (p=0.009), indicated significant elevations in the positive subgroup. Similarly, there were no significant differences in anti-SP1 IgG or anti-PSP IgA levels between the anti-Ro60 negative group and the positive group (p>0.05).

In the anti-La subgroups, the negative group (n=38) had anti-CA6 IgG levels of 6.23 (5.04) U/mL, while the positive group (n=22) had elevated levels of 17.04 (7.32) U/mL (p<0.001). Similarly, anti-PSP IgG levels were 6.42 (6.69) U/mL in the negative group and 11.40 (0.54) U/mL in the positive group (p=0.009). In contrast, no significant differences were observed in anti-SP1 IgG or anti-PSP IgA levels between the anti-La negative and positive groups (p>0.05).

In the ACA subgroups, the negative group (n=50) had anti-CA6 IgG levels of 5.00 (3.38) U/mL, while the positive group (n=10) had significantly higher levels of 9.38 (11.87) U/mL (p=0.003). Similarly, anti-PSP IgG levels were 5.44 (3.11) U/mL in the negative group and elevated to 9.44 (8.84) U/mL in the positive group (p=0.011). In contrast, there were no significant differences in anti-SP1 IgG or anti-PSP IgA levels between the negative and positive ACA groups (p>0.05).

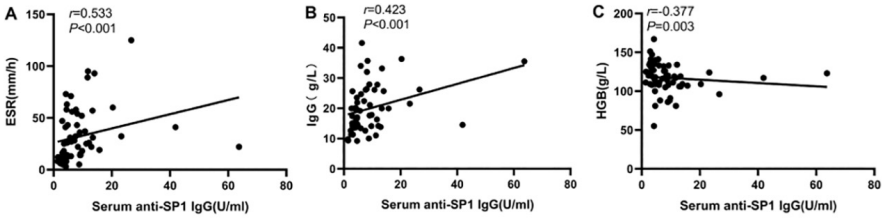

Fig. 5. (**A**) The relationship between serum levels of anti-SP1 IgG and ESR. (**B**) The relationship between serum IgG levels of anti-SP1 and IgG. (**C**) The relationship between serum levels of anti-SP1 IgG and HGB.

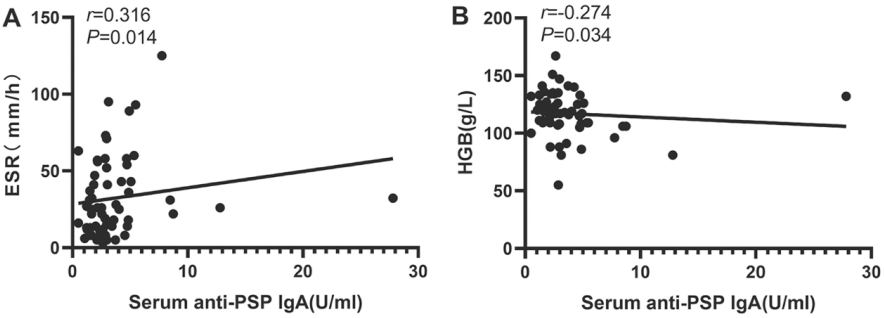

Fig. 6. (**A**) The relationship between serum levels of anti-PSP IgA and ESR. (**B**) The relationship between serum levels of anti-PSP IgA and HGB.

Preliminary exploration of the correlation between TSAs levels and minor salivary gland pathology in pSjD patients

48 cases in 60 pSjD patients underwent labial salivary gland biopsy (LSGB). According to the Chisholm grading system, the pathology in labial salivary gland tissue was classified into grades I-IV. A focus is defined as an infiltration of 50 or more lymphocytes per 4 mm^2, where a small number of lymphocytes corresponds to grade I, moderate infiltration or less than one focus is classified as grade II, one focus as grade III, and more than one focus as grade IV (23). Among the 48 patients, 10 met the criteria for Chisholm grade I, 6 for grade II, 10 for grade III, and 22 for grade IV. The distribution of serum anti-CA6, anti-PSP, anti-SP1 IgG levels, and anti-PSP IgA levels across different pathological grades was presented in Table IV below. The serum IgG levels of anti-CA6 IgG (r=0.356, p=0.013) and anti-PSP antibody (r=0.312, p=0.031) showed a positive correlation with LSGB pathological grades.

The relationship between tear secretion and the levels of TSAs

Among 60 pSjD patients, 34 underwent tear secretion function tests, of which 27 had abnormal tear secretion and 7 had normal secretion. Compared to patients with normal tear secretion (Table V), there were significant difference in the levels of anti-PSP IgG (p=0.011) and SP1 IgG antibodies (p=0.018), respectively.

Discussion

pSjD is a systemic and progressive autoimmune disorder characterised by hyperactivation of B-cells and cytokine production (9). The aetiology and pathogenesis of pSjD remain elusive, but it is clear that B cells play a very pivotal role in the pathogenesis of pSjD, and most of the current diagnosis and treatment are carried out around this target (24, 25). B cells are the primary source of autoantibody production, while the updated diagnosis of pSjD is still limited to traditional autoantibodies such as anti-Ro and anti-La antibodies. Moreover, anti-Ro antibodies are more commonly seen in

Table III. The association between TSAs and autoantibodies.

	ANA low-titre group (n=36)	ANA high-titre group (n=24)	Z	p	Anti-Ro52(-) (n=15)	Anti-Ro52(+) (n=45)	Z	P	Anti-Ro60(-) (n=20)	Anti-Ro60(+) (n=40)	Z	P	Anti-La(-) (n=38)	Anti-La(+) (n=22)	Z	P	ACA (-) (n=50)	ACA (+) (n=10)	Z	p
Anti-CA6 IgG (U/ml)	7.69 (9.59)	8.03 (13.89)	-0.151	0.880	5.51 (5.50)	9.51 (12.63)	-3.081	0.002*	5.16 (5.72)	10.68 (13.37)	-3.56	<0.001*	6.23 (5.04)	17.04 (7.32)	-4.525	<0.001*	9.38 (11.87)	5.00 (3.38)	-2.995	0.003*
Anti-PSP IgG	8.08 (7.22)	7.83 (9.62)	-0.732	0.464	4.46 (4.91)	9.62 (8.69)	-2.808	0.005*	5.20 (4.87)	9.66 (7.90)	-2.603	0.009*	6.42 (6.69)	11.40 (7.54)	-2.60	0.009*	9.44 (8.84)	5.44 (3.11)	-2.559	0.011*
Anti-SP1 IgG	5.17 (5.61)	7.84 (9.26)	-0.973	0.330	4.32 (5.00)	7.52 (7.59)	-1.81	0.070	4.26 (5.88)	7.32 (6.72)	-1.443	0.149	5.37 (5.65)	8.22 (7.29)	-1.48	0.139	6.72 (7.40)	5.64 (3.18)	-1.25	0.211
Anti-PSP IgA	2.64 (2.35)	2.86 (2.55)	-0.792	0.428	4.04 (2.64)	2.54 (2.00)	-1.579	0.114	2.84 (2.32)	2.73 (2.82)	-0.533	0.594	2.87 (2.33)	2.45 (3.06)	-0.545	0.586	2.73 (2.56)	3.26 (1.37)	-0.526	0.599

*p<0 05.

Table IV. The relationship of TSAs and LSGB in pSjD patients.

	Grade I (n=10)	Grade II (n=6)	Grade III (n=10)	Grade IV (n=22)	r	p
Anti-CA6 IgG (U/ml)	5.55 (6.72)	10.11 (7.83)	7.00 (7.14)	12.94 (16.06)	0.356	0.013*
Anti-PSP IgG (U/ml)	5.41 (6.18)	10.15 (11.78)	8.44 (3.98)	11.80 (11.54)	0.312	0.031*
Anti-SP1 IgG (U/ml)	4.07 (7.99)	6.93 (9.19)	7.77 (3.49)	8.53 (8.14)	0.218	0.137
Anti-PSP IgA (U/ml)	3.11 (1.40)	2.41 (11.27)	2.92 (1.74)	2.89 (2.71)	-0.019	0.898

*$p<0.05$.

Table V. The comparison of TSAs in pSjD patients with abnormal *versus* normal tear secretion.

	Abnormal tear secretion (n=27)	Normal tear secretion (n=7)	Z	p
Anti-CA6 IgG (U/ml)	9.51 (11.41)	6.17 (3.89)	-1.682	0.092
Anti-PSP IgG (U/ml)	5.02 (2.33)	4.97 (3.34)	-2.534	0.011*
Anti-SP1 IgG (U/ml)	6.00 (5.07)	4.04 (1.69)	-2.364	0.018*
Anti-PSP-IgA (U/ml)	2.82 (2.56)	2.64 (2.16)	-0.298	0.771

*$p<0.05$.

the late stage of the disease, and some patients are negative for anti-Ro antibodies (11, 26). So, we attempt to find supplementary and alternative indicators to make up for this deficiency.

TSAs, including anti-SP1, anti-CA6 and anti-PSP, are a group of autoantibodies discovered in recent years that may be valuable for the diagnosis of pSjD, so we deeply analysed the serum levels of TSAs in the serum of patients with pSjD, and explored their relationship with clinical data, pathological level of labial gland and tear secretion function. Our data showed that the novel autoantibodies TSAs were different in pSjD patients compared to HC, consistent with previous studies (26-28). Additionally, certain studies have shown that these autoantibodies were expressed in long-term pSjD patients and were more frequently found in those with early or less severe disease (29-31). By plotting the ROC curve, we found that TSAs have moderate accuracy in diagnosing pSjD. Although the sensitivity is relatively low, the specificity of TSAs IgG antibodies exceeds 95%, which is higher than that of anti-Ro antibodies and consistent with literature reports (30, 32). However, limited research examined on the differences of TSAs between pSjD and other CTDs, few researchers found that anti-SP1 was more elevated in pSjD patients than in RA patients in the United States and Greece (29, 30). Chinese researchers reported that anti-SP1 levels were significantly higher in patients with pSjD than in those with RA and SLE (5). Unlike previous studies, our analysis systematically examined the differences in TSAs between pSjD and other CTDs, revealing a significant increase in anti-CA6, anti-PSP, anti-SP1 IgG, and anti-PSP IgA in pSjD patients compared to those with other CTDs, which has important pathophysiological implications. The rise in these antibodies suggests that pSjD patients may have a stronger autoimmune response in their salivary glands, where IgG antibody subtypes are crucial.

Different antibodies in pSjD may have different clinical significance. Anti-Ro antibodies is linked to a higher risk of extra glandular manifestations such as anaemia, cryoglobulinemia, leukopenia, vasculitis, and thrombocytopenia (33). However, the exact role of TSAs in pSjD is still up for debate. pSjD itself is a chronic autoimmune inflammatory condition, the inflammatory processes play a role in the pathogenesis of pSjD (34). Autoantibodies are closely related to the inflammatory state. The literatures reported that the presence of ANA showed higher levels of inflammation in pSjD, anti-Ro and anti-La antibodies also help to increase inflammation levels (34). Similarly, our study also found that these TSAs were closely related to the inflammatory markers, especially the IgG subtype, and confirmed its diagnostic value. This is consistent with previous studies (35), which confirmed that anti-PSP suggested a worse prognosis for pSjD patients. Anti-PSP antibodies can often be detected before lymphocytic infiltration and damage to the salivary and lacrimal glands (36). We found the levels of anti-CA6 IgG, anti-PSP IgG and

anti-SP1 IgG antibodies were not only related to ESR, but also closely associated with blood albumin or haemoglobin levels. Meanwhile, they reflected the imbalance of the body's nutritional and metabolism status under the inflammatory state. On the other hand, there was also a certain correlation between these TSAs and elevated IgG. The elevation of IgG reflected disease activity and the abnormal activation of B cells (24, 37), indirectly suggesting a correlation between these antibodies and the disease activity of pSjD. This may provide reference value for future disease treatment. Subsequently, we will monitor the changes in these antibodies before and after treatment and conduct relevant research.

The relationship between TSAs and traditional autoantibodies remains unclear, our results showed a preliminary exploration of the relationship between TSAs and traditional antibodies, yielding notable findings. The levels of anti-CA6 and PSP IgG increased in the groups positive for anti-Ro52, anti-Ro60, and anti-La patients. The underlying mechanism may be due to shared cross-reactive epitopes between Ro/La antigens, which are targeted by anti-Ro/La antibodies, and proteins like CA6 and PSP. This interaction may drive B-cell epitope spreading and lead to a diversification of autoantibody production (38). At the same time, our results also showed that anti-CA6 and PSP IgG had a positively correlated with serum IgG level. This indicated that the presence of multiple autoantibodies was linked to an active humoral immune response and hypergammaglobulinemia (39). Conversely, our study was the first to showed that pSjD patients positive for ACA had significantly lower levels of anti-CA6 and anti-PSP antibodies than ACA-negative patients. This indicated a negative correlation between these autoantibody profiles. This finding is consistent with the diverse characteristics of pSjD antibody subtypes reported in previous literature(40), offering new insights into disease subclassification and mechanisms. ACA positivity in pSjD is often linked to lower rates of anti-Ro and anti-La antibodies (41). The features of ACA positive pSjD had lower disease activity and less likely to involve salivary gland (41), while the characteristics of patients with positive TSAs are just the opposite. Salivary gland shear wave elastography showed that ACA-positive patients had higher elasticity modulus values, indicating more significant fibrotic changes (42). Importantly, our data revealed significant associations between anti-PSP/anti-CA6 antibodies and the histopathological severity in labial gland biopsies, especially concerning the scores of focal lymphocyte infiltration. Anti-CA6 IgG and anti-PSP IgG may be helpful in disease stratification and monitoring of treatment response.

Patients with positive ANA in pSjD typically exhibit higher levels of inflammation and an increased likelihood of experiencing multi-organ damage. Furthermore, as the ANA titres increase, both inflammation levels and the risk of multi-organ damage also escalate (34). Additionally, the presence of anti-Ro and anti-La antibodies may contribute to an elevated risk of increased inflammation levels, but does not increase the risk of organ damage (34). Moreover, TSAs may be related to dry eye symptoms. Karakus *et al*. found that individuals positive for anti-CA6 antibodies had more severe signs and symptoms of dry eye (28). A cross-sectional study indicated that anti-CA6 antibodies could be seen in patients with severe dry eye (35), and anti-CA6 was significantly correlated with corneal and conjunctival staining scores. It was found that anti-SP1 antibodies were mainly detected in patients with Schirmer test measurements between 3 and 6 mm, while anti-CA6 antibodies were mainly detected in patients with measurements less than 3 mm (43). Previously literatures reported that the prevalence of anti-SP1 was higher in SjD-related dry eye patients compared to non-SjD dry eye patients (33% *vs*. 19%) (22). Similarly, the prevalence of anti-SP1 IgM and anti-PSP IgA antibodies was higher in patients with SjD than in patients without SjD dry eye (31). Our result revealed that pSjD patients with abnormal tear secretion function had higher levels of anti-PSP IgG and anti-SP1 IgG antibodies than those with normal tear secretion function. This suggested these antibodies might play a role in the lacrimal gland dysfunction and affect tear secretion function in pSjD patients. By detecting the concentrations of these antibodies, it may help predict abnormalities in tear secretion function in pSjD patients, providing a basis for early clinical intervention.

LSGB holds an equally important position in the diagnosis of pSjD (44), especially in patients with negative traditional autoantibodies. However, LSGB is invasive, and due to the distribution and heterogeneity of the glands, it cannot reflect the overall picture of the disease. Regarding the relationship between TSAs levels in pSjD patients and LSGB, current research conclusions are controversial. Some studies found that the expression of SP1 mRNA increased with age in IL-14α TG mice. SP1 mRNA was also found in labial biopsies of pSjD patients, and the expression of anti-SP1 antibodies was related to lymphocytic infiltration (5). Previous research indicated (29) that anti-SP1, anti-CA6, and anti-PSP antibodies were found in patients with dry eye and xerostomia with lower labial biopsy focus scores, while anti-Ro and anti-La antibodies were more frequently found in patients with higher labial biopsy focus scores. We found that the level of anti-CA6 IgG and anti-PSP IgG gradually elevated as the Chisholm pathology grade of the LSGB increased. The LSGB grade reflected the degree of glandular damage to some extent, and this result indicated that the titres of anti-CA6 IgG and anti-PSP IgG antibodies were closely related to the degree of glandular damage in pSjD. Salivary gland epithelial cells interact with T/B cells through surface MHC-II and immunomodulatory molecules, thereby promoting local inflammation.(10).The increase in antibody titres may reflect the immune system's ongoing attack on labial gland tissue, with further increases in antibody production as the disease progresses, thereby exacerbating glandular damage and forming a vicious cycle.

Our study has several limitations. It is

a single-centre study with a relatively small sample size, which limits its ability to reflect the national situation of pSjD patients. In the following research, we will gradually increase the sample size and conduct a longitudinal comparison of TSAs in pre- and post-treatment. And we plan to investigate the precise mechanistic roles of TSAs in future studies.

In summary, our study indicated TSAs showed diagnostic potential value as new serological markers in pSjD, demonstrating strong correlations with systemic inflammation (ESR/IgG), labial gland histopathological severity, and tear dysfunction. Elevated levels of anti-CA6 IgG and PSP IgG in subgroups positive for anti-Ro/La, along with inverse associations with ACA, indicated distinct roles in autoimmune stratification. These findings highlight the involvement of targeted antibody pathways in glandular injury, which calls for longitudinal studies to confirm their diagnosis and prognosis potential.

Take home messages

- In primary Sjögren's disease (pSjD), antibodies against carbonic anhydrase 6(CA6), parotid secretory protein (PSP), and salivary gland protein 1 (SP1) were elevated and showed stronger diagnostic potential as novel serological markers.
- The correlation between anti-CA6/PSP IgG subtypes and systemic inflammation as well as labial gland histopathological severity reflects the progression of the disease.
- Anti-Ro/La positive subgroups exhibited higher anti-CA6/PSP IgG levels, while anti-centromere antibody (ACA) positivity predicted reduced levels, suggesting autoimmune stratification.
- Novel tissue specific autoantibodies (TSAs) targeted pathways may drive glandular injury, highlighting therapeutic opportunities for validation in longitudinal cohorts.

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