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HTLV-I infection results in resistance toward salivary gland destruction of Sjögren's syndrome

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Abbreviations:

CREB:	cAMP response element-binding
	protein
FS:	focus score
HAM:	HTLV-I-associated myelopathy
SS:	Sjögren's syndrome
NF-ĸB:	nuclear factor kappa B
TLR:	Toll-like receptor

Competing interests: none declared.

ABSTRACT

Objective. The role of HTLV-1 infection in Sjögren's syndrome (SS) remains unclear. In this study, we clinically compared radiographic imaging with histological cellular infiltration between HTLV-1-seropositive and HTLV-1-seronegative SS.

Methods. Sixty primary SS patients were divided into two age-matched groups based on the seropositivity of the anti-HTLV-I antibody. We evaluated the two groups through labial salivary gland biopsy-proven cellular infiltration and sialography-proven radiographic gland destruction.

Results. In these 60 pSS patients, the incidence of abnormalities as determined by salivary gland biopsy and sialography was 51.7% (31/60) and 76.7% (46/60), respectively. Although there was no difference in the prevalence of abnormal findings between salivary gland biopsy and sialography in the whole 60 patients, there were significantly fewer abnormalities determined by sialography in HTLV-Iseropositive SS patients in comparison with HTLV-I-seronegative SS patients. Also, these findings were strengthened by the results that none of HTLV-I-seropositive SS patients with focus score 0 had abnormal sialography findings.

Conclusion. Our results suggest that HTLV-I infection results in resistance toward salivary gland destruction of Sjögren's syndrome.

Introduction

The pathogenesis of Sjögren's syndrome (SS), which is characterized by sicca symptoms or autoantibody production, has been discussed from the perspective of both clinical and molecular mechanisms. Among them, viral infection has been speculated to be a possible trigger of SS (1, 2). We have been investigating the relationship between HTLV-I infection and SS based on our previous epidemiological findings (3-5), in which a high prevalence of HTLV-I infection in patients with SS was found in Nagasaki city, the endemic area of HTLV-I infection. Our previous observations also showed that SS complicated with HTLV-I-associated myelopathy (HAM) might occur by a distinctive mechanism compared with HTLV-I-seronegative SS (6), as indicated by salivary gland destruction having been found to be less in SS patients with HAM. In the present study, we have confirmed the characteristics of salivary gland destruction in HTLV-I-seropositive SS patients.

Patients and methods

Sixty primary SS patients who fulfilled the American-European Consensus Group criteria for the diagnosis of SS (7) were divided into two age-matched groups based on the presence or absence of anti-HTLV-I antibody (Table I). Sera from 60 primary SS patients were examined for the seropositivity of anti-HTLV-I antibody by enzymelinked immunosorbent assay (ELISA; Eitest-ATL kit; Esai, Tokyo, Japan) or the particle agglutination assay (Serodia-ATL Kit; Fujirebio, Tokyo, Japan). The prevalence of anti-nuclear antibody (Fluoro Hep Ana Test; Medical & Biological Laboratories, Nagoya, Japan), anti-SS-A and anti-SS-B antibodies (Mesacup SS-A/Ro test and SS-B/La Test; Medical & Biological Laboratories, Nagoya, Japan), and serum IgG levels are also described in Table I. For evaluation of mononuclear cell infiltration into the labial salivary glands, we used focus score (FS) that was determined by the method of Greenspan et al. for SS criteria (8). The score was defined as the number of foci in 4mm² of salivary gland tissue. Minor salivary gland biopsy was performed for all SS patients. The minor salivary glands were obtained from the lower lips under local anesthesia. Sialography, also carried out in all patients, was performed by inserting into Stensen's duct. For the evaluation of lip biopsy and sialography, we adopted the definition of Chisholm & Mason for lip biopsy specimens (9) and FS. We used positive radiographic grading, as defined by Rubin & Holt (10). For statistical analysis, the Chi-square test was used (p < 0.05; considered as statistically significant). Informed consent was obtained from the participating subjects, and the study was conducted in accordance with the human experimental guidelines of our institution.

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Results

In total, the incidence of abnormalities, as determined by salivary gland biopsy and sialography, were 51.7% (31/60) and 76.7% (46/60) in 60 SS patients, respectively (Table II). Eight HAM patients were included in 23 HTLV-I-seropositive SS patients. All 8 of the HAM patients were negative for sialography, while 4 were positive for salivary gland biopsy. Our study also included 15 HTLV-I-seropositive SS patients who were not complicated with HAM, with 46.7% (7/15) of these patients being positive for sialography. Although none of the HTLV-I-seropositive SS patients were classified in the patients subset of those positive for sialography and negative for salivary gland biopsy, 6 HTLV-I-seronegative SS patients were classified in the sialography-positive and salivary gland biopsy-negative subset (Table II). The chi-square test suggested that HTLV-I infection results in resistance toward salivary gland destruction of Sjögren's syndrome (Table II; *p*-value was 0.035). Additionally, in total comparison based on seropositivity to HTLV-I, abnormality for sialography in HTLV-I-seropositive SS patients was significantly less than that in HTLV-I-seronegative SS patients (HTLV-I-seronegative; 24/37 (64.9%), HTLV-I-seropositive; 7/23 (30.4%), p-value; 0.0095 by Chi-square test). For further assessment of sialography findings in HTLV-I-seronegative and HTLV-I-seropositive SS patients, we divided the patients in both groups according to FS (Table III). Although there was no difference between the patients with FS 1 and FS \geq 1 in HTLV-I-seronegative and HTLV-I-seropositive SS patients, we found a statistical significance in the patients with FS 0 (p=0.016).

Discussion

Both radiographic salivary gland destruction and mononuclear cell infiltration into labial salivary glands are representative clinical characteristics of SS, and they are usually thought to coexist (11). Our previous findings have shown that mononuclear cell infiltration tends to be prominent in HTLV-Iseropositive SS patients as compared

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Table I. Background of SS patients with or without anti-HTLV-I antibody in this study.

	Anti-HTLV	<i>p</i> -value	
	(-)	(+)	*
N (M/F)	37 (3/34)	23 (5/18)	
Age	55.3 ± 12.9	60.3 ± 11.5	0.13*
ANA	30/36 (83.3%)	15/20 (75.0%)	0.45**
Anti-SS-A antibody	17/37 (45.9%)	9/23 (39.1%)	0.60^{**}
Anti-SS-B antibody	4/37 (10.8%)	3/23 (13.0%)	0.79^{**}
IgG	2473.2 ± 1268.5	2210.4 ± 1144.4	0.45^{*}
Number of FS 0	9/37 (24.3%)	5/23 (21.7%)	0.82**
Number of FS 1	8/37 (21.6%)	7/23 (30.4%)	0.44**
Number of FS ≥1	20/37 (54.1%)	11/23 (47.8%)	0.64**
Average number of FS ≥1	2.7 ± 0.9	2.7 ± 0.7	0.42^{*}

Features of the SS patients are shown. The focus sore (FS) were determined by the method of Greenspan *et al.* for Sjögren's syndrome criteria (8). There was no statistical difference between anti-HTLV-I-seronegative and seropositive groups regarding age, the prevalence of anti-nuclear antibody (ANA), anti-SS-A antibody, anti-SS-B antibody, IgG levels, and average number of FS $\geq 1^{*}$ and ^{**} calculated by student *t*-test and Chi-square test, respectively. (p < 0.05 = statistically significant)

 Table II. Results of sialography and lip biopsy in HTLV-I-seronegative and HTLV-I-seropositive SS patients.

		Anti-HTLV-I antibody		
		(-)	(+)	
Sialography	Lip biopsy			
(-)	(-)	3	5	8
(+)	(-)	6	0	6
(-)	(+)	10	11	21
(+)	(+)	18	7	25
		37	23	60

Chi-square test was performed to demonstrate the statistical significance (p<0.05 = statistically significant), resulting in the *p*-value being 0.035. These results suggest that the labial salivary glands of HTLV-I seropositive SS patients were less destructive compared to those of HTLV-I seronegative SS patients.

Table III. Prevalence of positive sialography findings in HTLV-I-seronegative and HTLV-I-seropositive SS patients.

		Anti-HTLV-I antibody			<i>p</i> -value
		(-)		(+)	
FS 0	6/9	(66.7%)	0/5	(0.0%)	0.016^{*}
FS 1	3/8	(37.5%)	1/7	(14.3%)	0.31
FS ≥1	15/20	(75.0%)	6/11	(54.5%)	0.24

The focus sore (FS) were determined by the method of Greenspan *et al.* for Sjögren's syndrome criteria (8). The prevalence of FS was calculated by Chi-square test. (p<0.05 = statistically significant)

with HTLV-I-seronegative SS patients (5); the present data, however, show that major salivary gland destruction is less determined in HTLV-I-seropositive SS patients, which could be more prominent in SS patients complicated with HAM. This discrepancy could depend on specific characteristics induced by HTLV-I infection. In addition,

we found that no patients with FS 0 in HTLV-I-seropositive SS have positive sialography findings compared to the FS 0 patients in the HTLV-I-seronegative SS patients, which might explain the characteristics of HTLV-I-associated SS. It is well known that transactivators such as NF- κ B and CREB, activated by HTLV-I infection, stimulate the

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expression of varying genes (12, 13). HTLV-I could infect salivary epithelial cells of HTLV-I-seropositive patients, which may promote cellular survival or proliferating cell signals. This phenomenon may explain the relatively less destructive features of HTLV-I infection in HTLV-I-seropositive SS patients.

We have previously demonstrated that there is no significant difference with regard to expression of Fas/Fas ligand and Bcl-2 family proteins between HTLV-I-seronegative and HTLV-I-seropositive SS patients (14, 15). Nevertheless, we have recently found that toll-like receptor (TLR) 3, which can bind to the viral RNA sequence, is expressed in the salivary epithelial cells of SS (16). Further investigation, especially the activation of salivary gland cells by HTLV-I through TLRs, may explain the theoretical reasons of our present data.

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