Cut-off levels of salivary beta$_2$-microglobulin and sodium differentiating patients with Sjögren's syndrome from those without it and healthy controls

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Abstract

Objectives

For the diagnosis of Sjögren's syndrome (SS), cut-off levels of β_2-microglobulin (β_2MG) and sodium (Na^+) in unstimulated whole saliva have not yet been shown. We aimed to determine the cut-off levels of salivary β_2MG and Na^+ which differentiate SS patients from non-SS patients and healthy controls.

Methods

Seventy-one patients of primary SS (pSS, 69 females/2 males, 60.0±16.8 years old), 50 of secondary SS (sSS, 49/1, 55.8±17.4), 54 of connective tissue diseases other than SS (non-SS-CTD, 43/11, 60.0±16.0), and 75 healthy volunteers (HC, 43/32, 50.7±15.6) were included. Unstimulated whole saliva were examined for levels of β_2MG, Na^+, potassium (K^+), and chloride (Cl^-). Receiver-operating characteristic curve analysis was carried out.

Results

β_2MG, Na^+, and Cl^- levels in the SS group (pSS and sSS) were significantly higher than those in the non-SS group (non-SS-CTD and HC). The salivary β_2MG level was 5.3±4.6 mg/L in pSS, 5.1±2.0 in sSS, 2.5±2.1 in non-SS-CTD, and 1.2±0.7 in HC, respectively. The Na^+ level was 39.2±25.2 mEq/L, 36.4±26.1, 19.6±16.8, and 16.5±7.3, and the Cl^- level was 51.1±25.0, 47.8±24.3, 32.1±16.6, and 27.0±7.9 in the same order. The K^+ level in the SS group was significantly higher than that in HC. The optimal cut-off β_2MG and Na^+ levels that differentiate the SS group from the non-SS group were 2.3 mg/L and 23 mEq/L.

Conclusion

Salivary β_2MG and Na^+ levels are useful markers for differentiating SS patients from non-SS-CTD patients and HC.

Key words
Sjögren's syndrome, sialometry, β_2-microglobulin

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Introduction

Sjögren's syndrome (SS) is an autoimmune disorder characterised by lymphocytic infiltration into exocrine glands, causing their progressive destruction and dysfunction (1). To evaluate salivary gland involvement, the flow rate of unstimulated whole saliva is considered on the basis of 2002 American-European Consensus Group criteria, and the flow rate of stimulated saliva on the basis of the Japanese SS criteria (2, 3). A report showed a negative correlation of the flow rate of stimulated whole saliva with the focus score of the minor salivary gland (4).

Moreover, the levels of salivary components (sialochemistry) are also considered to be useful for SS evaluation (5-9). Some reports showed the increased expression of salivary beta$_2$-microglobulin (β_2MG) and sodium (Na^+) levels in SS patients (7, 10-14). In particular, Hu *et al*. indicated that β_2MG is a high sensitive and specific marker for distinguishing primary SS (11). These components might be useful, however, no report indicated the cut-off levels differentiating patients with SS, including secondary SS, from non-SS patients and healthy controls (HC). The aim of this study is to determine the cut-off levels of salivary components, particularly β_2MG and Na^+, by collecting unstimulated whole saliva.

Patients and methods

Patients and healthy controls

Patients with connective tissue diseases (CTDs), who visited the Japanese Red Cross Medical Centre, Tokyo, Japan, between April 2008 and October 2010, underwent the sialometry and sialochemistry if they agreed collecting unstimulated saliva with or without any attention to their dry complaints. Patients having a history of head or neck radiation therapy, graft versus host disease, sarcoidosis, human immunodeficiency virus infection, hepatitis C infection, lymphoma, amyloidosis, IgG4-related diseases, and acute sialoadenitis were excluded. The patients were diagnosed as SS on the basis of 2002 American-European Consensus Group criteria (2). They were classified into three groups of primary SS (pSS), secondary SS (sSS), and CTDs without SS (non-SS-CTD) patients. The use of steroids, immunosuppressive drugs, and xerogenic drugs, which included antihypertensive, antihistamine, and psychotropic drugs, was examined. As is the case with patients, healthy volunteers who gave their informed consent to offering their unstimulated whole saliva collected were included as HC. This study was approved by the institutional Human Ethics Committee of the Japanese Red Cross Medical Centre.

Saliva collection and salivary component measurements

Patients and volunteers were instructed not to eat, drink, or smoke for at least 2 hours before the collection in the morning. The time necessary for collecting unstimulated whole saliva of 2 mL was measured; in case that 2 mL of saliva could not be collected within 30 minutes, the sample volume collected was measured. The collected salivary sample was centrifuged for 5 minutes at 3500 rpm, and the supernatant was assayed for β_2MG level using a latex agglutination assay kit (Eiken β_2-M-2, Tokyo, Japan), and for Na^+, potassium (K^+), and chloride (Cl^-) levels using the same kit that for human serum.

Statistical analysis

The obtained data were statistically analysed using JMP, version 5.1. Tukey's Honestly Significant Difference method was used to evaluate the significance of differences among the groups. A p-value of <0.05 was considered statistically significant. The correlation coefficient between two variables is shown as r^2. The cut-off levels that differentiate the groups were elucidated using receiver-operating characteristic curves.

Results

Background

Table I shows the background features of the patients and HC. The sSS and non-SS-CTD patients used steroids ($p<0.05$) or immunomodulatory or immunosuppressive drugs ($p<0.05$) significantly more frequently than the pSS patients. The CTDs underlying sSS were rheumatoid arthritis in 18, sys-

Table I. Background features of patients and healthy controls.

	pSS (n=71)	sSS (n=50)	non-SS-CTD (n=54)	HC (n=75)
Age (mean±SD, years)	60.0±16.8	55.8±17.4	60.0±16.0	50.7±15.6
Sex (female/male)	69/2	49/1	43/11	43/32
Xerogenic drugs	31 (43.7%)	18 (36.0%)	23 (52.6%)	–
Steroids	16 (22.5%)	24 (48.0%)	30 (54.5%)	–
Immunomodulatory or immunosuppressive drugs	0 (0.0%)	12 (24.0%)	22 (40.7%)	–
Anti-SSA-antibody positive	46 (64.8%)	28 (56.0%)	4 (7.4%)	–
Anti-SSB-antibody positive	12 (16.9%)	9 (16.7%)	0 (0.0%)	–
Positive eye tests*	43/51 (84.3%)	30/39 (76.9%)	5/32 (15.6%)	–
Positive parotid sialography*	30/38 (78.9%)	17/24 (70.8%)	1/6 (16.7%)	–
Positive salivary gland schintigraphy*	54/64 (84.4%)	40/47 (85.1%)	9/35 (25.7%)	–
Positive salivary gland biopsy*	30/39 (76.9%)	31/39 (80.0%)	0/7 (0.0%)	–

*According to the 2002 American-European Consensus Group criteria. (2)

temic lupus erythematosus in 15, systemic sclerosis in 7, mixed connective tissue diseases in 5, polymyositis in 2, polyarteritis nodosa in 2, and polymyalgia rheumatica in 1. Those underlying non-SS-CTD were rheumatoid arthritis in 31, systemic lupus erythematosus in 13, unclassified connective tissue diseases in 3, and systemic sclerosis, microscopic polyangiitis, eosinophilic granulomatosis with polyangiitis, Behçet's disease, dermatomyositis, mixed connective tissue disease, and polymyalgia rheumatica in 1, each.

HC were significantly younger than pSS ($p<0.05$) and non-SS-CTD patients ($p<0.05$). With regard to age, there was no significant difference among patients with each disease.

Salivary flow rate

Unstimulated salivary flow rate is shown in Table II. In HC, the salivary flow rate did not significantly differ between females and males, and also did not correlate with age ($r^2=0.0037$). In patients with each disease, the flow rate did not correlate with age as well ($r^2=0.0002$ for pSS, 0.030 for sSS, and 0.030 for non-SS-CTD).

The flow rate in patients with each disease was significantly lower than that in HC ($p<0.05$, for each). The flow rate in pSS patients did not differ from that in sSS, but lower than that in non-SS-CTD. No difference was observed between sSS and non-SS-CTD patients.

Sialochemistry

The levels of salivary components are also listed in Table II. Sialochemical measurements could not be performed in 6 pSS and 3 sSS patients because of the very limited amount of saliva collected. As for correlation between age and the level of each salivary component except for K^+, no correlation was observed in all of the patients and HC ($r^2=0.002$ for β_2MG, 0.0001 for Na^+, 0.01 for Cl^-). K^+ level showed slightly possible correlation with age ($r^2=0.17$). Little correlation was also observed between salivary flow rate and the levels of salivary components ($r^2=0.08$ for β_2MG, 0.08 for Na^+, 0.05 for K^+, 0.10 for Cl^-).

The salivary β_2MG level in pSS was significantly higher than either in non-SS-CTD patients or HC, but not different from that in sSS. Between non-SS-CTD patients and HC, no difference was observed. Thus, the level was significantly

Table II. Flow rate and sialochemistry of unstimulated whole saliva in patients and heathy controls.

Flow rate and sialochemistry of unstimulated whole saliva in patients and healthy controls		pSS (n=71)	sSS (n=50)	non-SS-CTD (n=54)	HC (n=75)	Statistical difference					
						pSS		sSS		non-SS-CTD	
Salivary flow rate (mL/min)		0.06±0.14	0.09±0.30	0.16±0.29	0.38±0.42	*vs.* sSS	NS	–		–	
						vs. non-SS-CTD	*	*vs.* non-SS-CTD	NS	–	
						vs. HC	*	*vs.* HC	*	*vs.* HC	*
Sialochemistry		(n=65)	(n=47)	(n=54)	(n=75)						
	β_2MG (mg/L)	5.3±4.6	5.1±2.0	2.5±2.1	1.2±0.7	*vs.* sSS	NS	–		–	
						vs. non-SS-CTD	*	*vs.* non-SS-CTD	*	–	
						vs. HC	*	*vs.* HC	*	*vs.* HC	NS
	Na^+ (mEq/L)	39.2±25.2	36.4±26.1	19.8±16.8	16.5±7.3	*vs.* sSS	NS	–		–	
						vs. non-SS-CTD	*	*vs.* non-SS-CTD	*	–	
						vs. HC	*	*vs.* HC	*	*vs.* HC	NS
	Cl^- (mEq/L)	51.1±25.0	47.8±24.3	32.1±16.6	27.0±7.9	*vs.* sSS	NS	–		–	
						vs. non-SS-CTD	*	*vs.* non-SS-CTD	*	–	
						vs. HC	*	*vs.* HC	*	*vs.* HC	NS
	K^+ (mEq/L)	31.0±11.2	28.0±9.0	26.8±9.2	23.2±6.7	*vs.* sSS	NS	–		–	
						vs. non-SS-CTD	NS	*vs.* non-SS-CTD	NS	–	
						vs. HC	*	*vs.* HC	*	*vs.* HC	NS

pSS: primary Sjögren's syndrome; sSS: secondary Sjögren's syndrome; non-SS-CTD: connective tissue diseases without Sjögren's syndrome; HC: healthy controls.
The data are mean ± standard deviation. NS: no significance; * $p<0.05$.

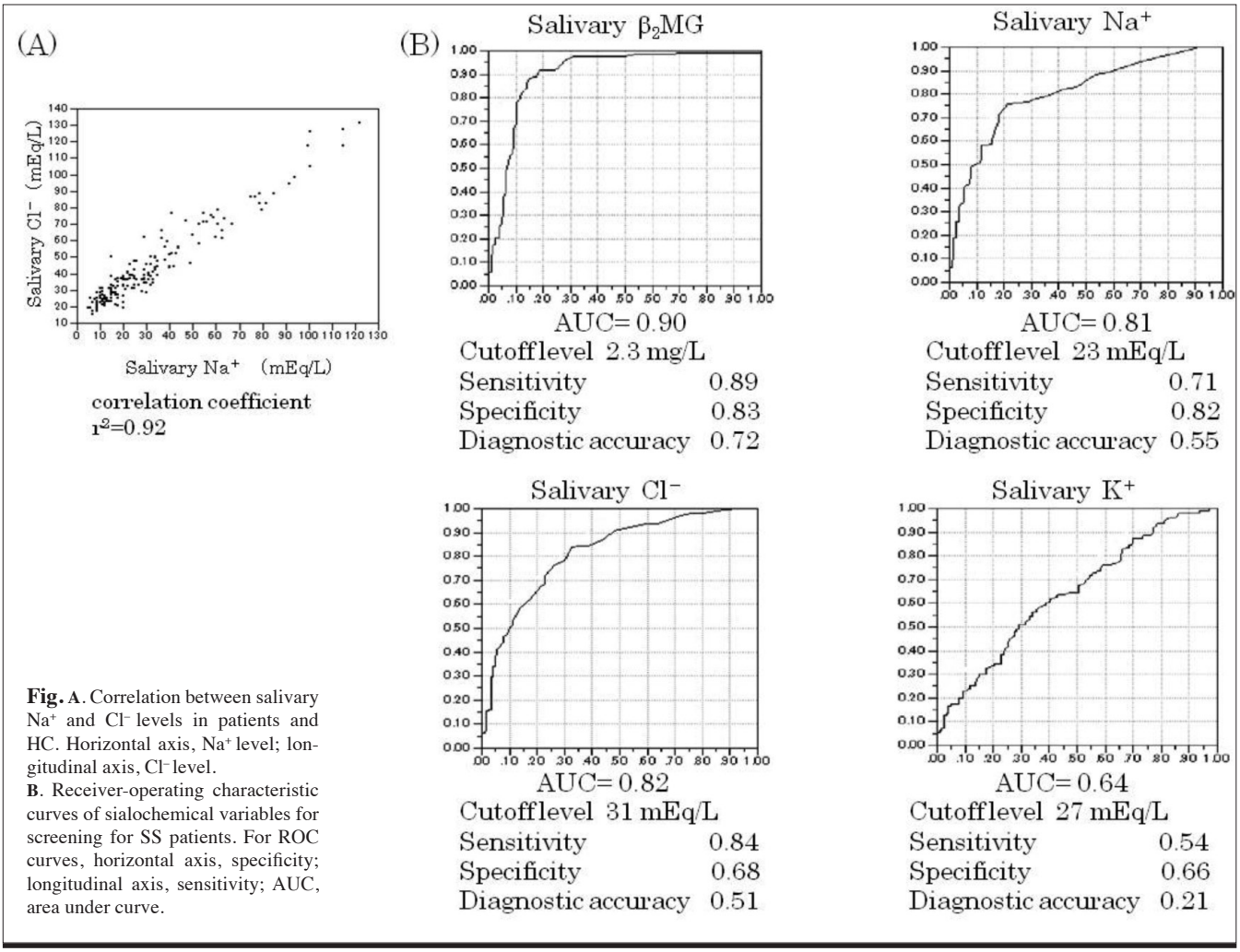

Fig. A. Correlation between salivary Na^+ and Cl^- levels in patients and HC. Horizontal axis, Na^+ level; longitudinal axis, Cl^- level.
B. Receiver-operating characteristic curves of sialochemical variables for screening for SS patients. For ROC curves, horizontal axis, specificity; longitudinal axis, sensitivity; AUC, area under curve.

higher in the SS group (pSS and sSS) than in the non-SS group (non-SS-CTD and HC) ($p<0.05$). The trends of Na^+ and Cl^- levels were just the same as that observed in β_2MG. Between the Na^+ and Cl^- levels, a high positive correlation was observed in patients with each disease and HC (Fig. 1A).

The cut-off levels that differentiate the SS group from the non-SS group are shown in Figure 1B. Their sensitivities, specificities, and diagnostic accuracies, in the case of adopting the cut-off levels of 2.3 mg/L for β_2MG, 23 mEq/L for Na^+, and 31 mEq/L for Cl^-, are also shown. When adopting a cut-off level of 27 mEq/L for K^+ level, all of its sensitivity, specificity, and diagnostic accuracy were lower than those for β_2MG, Na^+, and Cl^-.

Discussion

Recently salivary components has been reviewed as the useful diagnostic, prognostic, and moreover pathogenic biomarkers of SS for the advances of genomic and proteomic technologies (14-16). We also focused on the saliva, which might closely draw influence from salivary glands, mainly involved organs of SS, and showed the cut-off levels of β_2MG and Na^+ for differentiating SS patients.

β_2MG is a non-glycosylated protein staying on the surface of nearly all nucleated cells, particularly lymphocytes and monocytes (17). Interferon (IFN) pathway activation is thought to be related to the disease progression of SS and certainly β_2MG is regulated by IFN (14-16,18). As a correlation of salivary β_2MG level with the grade of inflammation in biopsied minor salivary gland specimens in SS patients are shown, the high salivary β_2MG level in our SS patients indicated an active glandular mononuclear cell infiltration, which would represent disease activity (10). Adopting the β_2MG cut-off level of 2.3 mg/L, the sensitivity and specificity to differentiate the SS group from the non-SS group were sufficiently high.

Primary saliva secreted into the acinar lumen is transported to the oral cavity by hydrostatic forces (7). During this transport, active Na^+ reabsorption occurs. Damaged ducts in SS reabsorb less Na^+. Thus, the final saliva in SS patients has a high level of Na^+ (6, 7). The high level of salivary Na^+ implies a dysfunction of reabsorption, which would suggest a disease progression. Salivary Cl^- has been considered to be transported passively along with the transport of Na^+; therefore, a high level of Cl^- would simply reflect the dysfunction of Na^+ reabsorption (Fig. 1A).

Our study showed a significant difference in salivary K^+ level between the SS group and HC, as was indicated in a previous study (19). K^+ is an intracellular electrolyte and its high level

would reflect the severe damage of acinar cells. In this study, the K+ level might not very clearly differentiate the SS group from the non-SS group. Other factors including age, which showed a slightly positive correlation with the K+ level (r^2=0.17), which might make it complicated to set a sharper cut-off value.

Interestingly, although the salivary flow rate in non-SS-CTD patients was significantly higher than that in pSS patients, it did not differ from that in the sSS patients. Many factors would determine salivary flow rate, and some studies have demonstrated the improvement of salivary flow by steroids (5, 11, 20). In this study, many sSS and non-SS-CTD patients used steroids, which might have affected it.

Some reports indicated that examining saliva from each gland would be better (6, 7). However, the collection of such salivary samples requires specific tools. Examining unstimulated whole saliva is a rapid, simple, inexpensive, and non-invasive technique, and even children or anxious patients can be easily tested.

Our study had some limitations. First, although collection was performed with an effort to minimise fluctuations related to the circadian rhythm and feeding, salivary flow rate and composition would vary in natural daily life. Second, sialochemistry requires a certain amount of saliva. Some patients with progressive destruction of salivary glands could not provide any amount of saliva, although it is enough at least to see a disturbed secretion. Third, this was a retrospective study performed in a single centre. Nevertheless, as far as we know, this is the first report evaluating four groups of pSS, sSS, non-SS-CTDs, and HCs at a time and determining the cut-off levels differentiating SS patients from non-SS patients and healthy controls (HC) in the clinical settings.

In conclusion, the cut-off levels of β_2MG and Na^+ in unstimulated whole saliva for differentiating SS patients from non-SS patients and HC would be 2.3 mg/L for β_2MG and 23 mEq/L for Na^+.

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