Salivary cystatin D is a candidate non-invasive biomarker for primary Sjögren's syndrome diagnosis and salivary gland injury

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Abstract

Objective

To evaluate the role of cystatin D as a non-invasive biomarker for primary Sjögren's syndrome (pSS), salivary cystatin D levels were measured and the association between cystatin D and clinical parameters was analysed.

Methods

A total of 73 patients with pSS, 23 patients with head and neck cancer who had completed radiotherapy (HNCR), and 58 healthy controls (HC) were included in this study. Salivary cystatin D levels were measured via an enzyme-linked immunosorbent assay (ELISA). Salivary gland flow rate, salivary gland ultrasound scores, and disease activity indexes were assessed in patients with pSS. The receiver operating characteristic (ROC) curves was used to assess the potential value of salivary cystatin D as a diagnostic biomarker.

Results

Salivary cystatin D levels were significantly reduced in the patients with pSS, compared with the patients with HNCR ($p<0.001$) and HC ($p<0.001$). Salivary cystatin D level was positively correlated with unstimulated salivary gland flow rate (USFR) and stimulated salivary gland flow rate (SSFR), whereas, negatively correlated with serum IL-6 levels, IgE levels and peripheral blood $CD4^+$ T cell counts. In addition, salivary cystatin D levels were significantly reduced in the pSS patients with parotid or submandibular gland ultrasonography scores ≥2. The diagnostic value of salivary cystatin D was determined by receiver operating curve (ROC) analysis, with an area under the curve of 0.713 (95% CI: 0.632, 0.749, $p<0.001$), a sensitivity of 51.9% and a specificity of 83.6%. In addition, cystatin D improved the accuracy of pSS diagnosis, particularly in the patient with negative anti-SSA.

Conclusion

Salivary cystatin D emerged as a promising biomarker for pSS diagnosis and was correlated with salivary gland dysfunction.

Key words

cystatin D, primary Sjögren's syndrome, saliva, diagnosis, biomarker

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Introduction

Primary Sjögren's syndrome (pSS) is a common systemic autoimmune disease characterised by mononuclear cell and lymphocyte infiltration of the exocrine glands, leading to dryness of the eye and mouth (1, 2). For patients with pSS, delayed diagnosis and treatment invariably contribute to damage to multiple organs and systems, including the kidney, lung, blood and the nervous system, affecting the quality of life, and even resulting in death (3). Currently, no effective biomarkers are used in clinical practice for the diagnosis and assessment of disease activity for pSS. Serum anti Sjögren's syndrome A (anti-SSA) antibodies, a common adjunct marker for the diagnosis of pSS, have low sensitivity and specificity. The diagnosis of pSS is mainly based on classification criteria combined with histopathology, presence of anti-SSA, tear and saliva secretion analysis that are complicated and likely to lead to misdiagnosis and missed diagnosis (4). The assessment of disease activity in pSS is based mainly on the European League against Rheumatism (EULAR) Sjögren's syndrome disease activity index (ESSDAI) score that is complex and includes 12 items (5). Pathological examinations are generally not acceptable to patients because of their invasive nature. However, it is worthy to assess the degree of salivary gland glands damage for diagnosis and prognosis. Therefore, a new, stable, non-invasive biomarker is needed for pSS diagnosis and assessment of disease activity.

Saliva is a complex biological fluid composed of water, diverse salivary proteins, and molecules derived from the bloodstream, and is secreted from the major and minor salivary glands. The components of saliva are similar to those of serum and reflect the physiological and pathological state of the salivary glands (6). Changes in the salivary levels of these molecular constituents can be used not only to assess the function of the salivary glands but also as biomarkers for systemic disease detection and risk assessment, including cardiovascular, cancer, and autoimmune disease (7-9). As a chronic autoimmune disease, pSS requires regular monitoring to assess disease activity for modulation of treatment. The collection of saliva is convenient, non-invasive and preferred over other sample types, especially for repeated testing; therefore, the use of salivary marker as a diagnostic and evaluative tool has clinical advantages (10).

Cystatin D is a natural cysteine protease inhibitor and a secreted protein found in human saliva and tear fluid. Recent studies have shown that cystatin D is involved in innate immunity and inflammation by inhibiting coronavirus replication at physiological concentrations (11, 12). Herein, we selected cystatin D as a biomarker of pSS to explore its value. We detected the expression of cystatin D in the saliva of pSS patients, head and neck cancer patients treated with radiotherapy (HNCR) patients, and healthy controls (HC) and further analysed the correlation between cystatin D and clinical characteristics in patients with pSS to assess the efficacy of cystatin D for salivary gland dysfunction.

Materials and methods

Study population

A total of 73 patients (68 females and 5 males) diagnosed with pSS were recruited from the Department of Rheumatology and Immunology of the First Affiliated Hospital of Xi 'an Jiaotong University from September 2022 to October 2024. The age of the patients ranged from 28 to 72 years, with an average age of 49.74±10.89 years. The patients met the 2016 ACR-EULAR Classification Criteria for primary Sjögren's syndrome (13). The exclusion criteria were as follows: 1. other rheumatic connective tissue diseases; 2. patients recently taking oral cholinergic drugs; 3. diagnosis of HIV, HCV and/or other virus infections; 4. serious complication; 5. patients unwilling to undergo clinical trials or with incomplete data.

In the same period, 58 HC (52 females and 6 males) with no history of dry mouth or dry eyes were selected as the healthy control group, aged from 29 to 68 years, with an average age of 46.95±10.94 years. The 23 HNCR patients (21 females and 2 males) with dry mouth, aged from 35 to 73 years, with an average age of 51.61±9.66 years,

were defined as the disease control group. The cases who were found to have the following antibodies were excluded from the HC and HNCR groups: positive HIV, HCV, rheumatoid factors (RF) or anti-nuclear antibodies. This study was conducted in accordance with the Declaration of Helsinki and approved by the Medical Ethics Committee of the First Affiliated Hospital of Xi'an Jiaotong University, China (Ethical approval no.: 2023-63). All the participants provided written informed consent to participate in this study.

There were no statistically significant differences in age or sex among the three groups. We reviewed cases verified by medical records, follow-up visit by telephone (condition of gingival bleeding, loose teeth and toothache) and diagnosis by dentists, and confirmed the periodontal disease rates of the two patient groups (pSS and HNCR). The proportions of periodontal disease were no statistically significant differences between the HNCP and pSS groups. Their demographic and clinical characteristics are shown in Table I.

Table I. Demographic and clinical characteristics of pSS patients.

Parameters	Healthy controls (n=58)	HNCR patients (n=23)	pSS patients (n=73)
Age, years*	46.95 ± 10.94	51.61 ± 9.66	49.74 ± 10.89
Gender, female (%)	52 (89.66%)	21 (91.30%)	68 (93.15%)
Duration of disease, years*	-	-	5.20 ± 4.19
Periodontal disease (%)	-	6 (26.09%)	22 (27.85%)
Treatment			
Glucocorticoid (%)	-	-	11 (15.07%)
Iguratimod (%)	-	-	24 (32.88%)
Hydroxychloroquine (%)	-	-	61 (83.56%)
Total glucosides of paeony (%)	-	-	64 (87.67%)
Belimumab (%)	-	-	2 (2.74%)
USFR, ml/min*	0.66 ± 0.26	0.25 ± 0.12	0.11 ± 0.06
SSER, ml/min*	1.31 ± 0.33	0.88 ± 0.34	0.73 ± 0.41
SGUS ≥2 in parotid gland (%)	0	6 (26.09%)	46 (63.01%)
SGUS ≥2 in submandibular gland (%)	0	3 (13.04%)	36 (49.32%)
Biomarker			
Anti-SSA/Ro52kD (%)	0	0	55 (75.34%)
Anti-SSA/Ro60kD (%)	0	0	48 (65.75%)
Anti-SSB (%)	0	0	26 (35.62%)
RF (%)	0	0	25 (34.25%)
IgG, g/L*	-	-	17.94 ± 7.35
ESR, mm/h*	-	-	24.75 ± 15.86
CRP, mg/L*	-	-	12.5 ± 6.94
IL-6, pg/mL*	-	-	4.86 ± 3.15
Cystatin D (pg/mL) *	321.6 ± 164.1	311.2 ± 121.3	212.7 ± 117.2

*The values are expressed as the mean ± standard deviation.
CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; IL-6: interleukin 6; SGUS: salivary gland ultrasonography; SSER: stimulated salivary gland flow rates; USFR: unstimulated salivary gland flow rates; pSS: primary Sjögren's syndrome.

Salivary sample collection

Before collecting the saliva sample and measuring salivary flow rate, food was prohibited for 4 hours and the mouth was rinsed with water for 10 minutes. First, the tip of the subject's tongue was pressed against the roof of the mouth to collect saliva for 10 minutes, and then 2 drops of 2% citric acid were applied to the front of the tongue at intervals of 1 minute for 4 times, and saliva was collected again for 10 minutes. The volume of saliva collected twice was measured and the saliva flow rate = saliva volume/collection time was calculated. The saliva collected in the unstimulated state was centrifuged at 4°C at 10000 g for 10 minutes, and the supernatant after centrifugation was frozen at -80°C.

Salivary cystatin D detection

Salivary cystatin D was quantified in unstimulated saliva using a human cystatin D ELISA kit following the manufacturer's instructions (Thermo Fisher Scientific, USA). All salivary samples were analysed concurrently in a single batch, with cystatin D levels being determined by reference to a standardised calibration curve. For each sample, duplicate wells were employed in the assay, and the resultant values were averaged to yield the final measured concentration.

Salivary gland ultrasonography (SGUS)

SGUS was conducted on the bilateral parotid and submandibular glands using a Logic 9 system (General Electric Medical Systems, Milwaukee, WI, USA). A simplified scoring system based on parenchymal inhomogeneity was defined as follows: grade 0, normal parenchyma; grade 1, mild inhomogeneity without anechoic or hypoechoic areas and hyperechogenic bands; grade 2, moderate inhomogeneity with focal anechoic or hypoechoic areas; grade 3, severe inhomogeneity with diffuse anechoic or hypoechoic areas occupying the entire gland or fibrous gland (14). An SGUS score of ≥2 was considered a pathological change.

Statistical analysis

Data were statistically analysed using SPSS 23.0 (SPSS, Inc, Chicago, IL) and presented as mean ± standard deviation (SD). The Shapiro-Wilk test and Levene's test were used to assess normality and homogeneity of variance, respectively. Depending on the situation, the t-test or Mann-Whitney U-test was used to assess the differences between the two groups. Pearson's correlation coefficient or Spearman's correlation coefficient was used to analyse correlations between two variables. The diagnostic value of salivary cystatin D was assessed by constructing receiver operating characteristic (ROC) curves, followed by assessment of overall accuracy using the areas under the curves. The Youden Index, defined as the sensitivity plus the specificity minus one, was used to identify the cut-off value of cystatin D. *p*-values <0.05 were considered statistically significant.

Results

Salivary cystatin D reduction in pSS patients

We compared the unstimulated and stimulated salivary gland flow rates in HC, HNCR and pSS patients. Com-

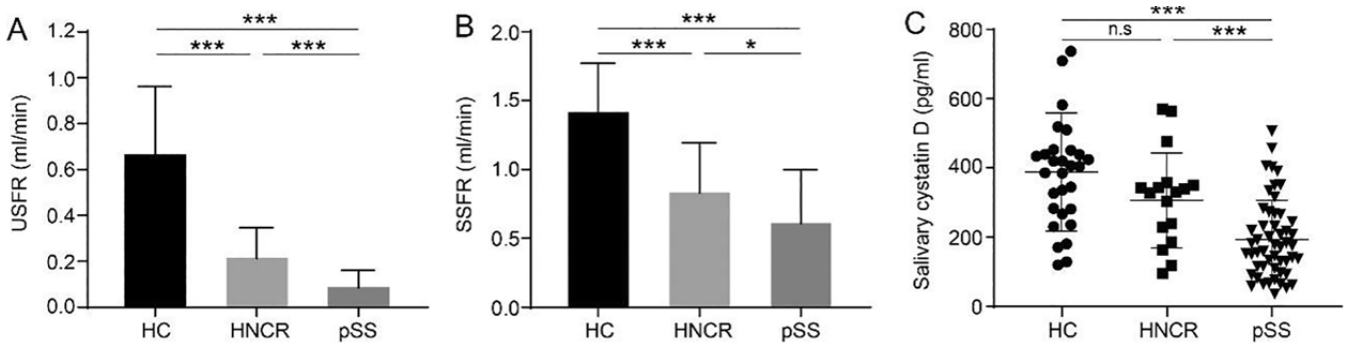

Fig. 1. Salivary cystatin D levels decreased in pSS patients.
A-B: Comparison of USFR and SSFR in HC, HNCR and pSS groups. **C**: Salivary cystatin D levels in HC, HNCR and pSS groups were determined by ELISA. USFR: unstimulated salivary gland flow rates; SSFR: stimulated salivary gland flow rates; HC: healthy controls (n=58); HNCR: head and neck cancer patients who completed radiotherapy (n=23); pSS: Sjögren's syndrome (n=73).
*p<0.05, ***p<0.001.

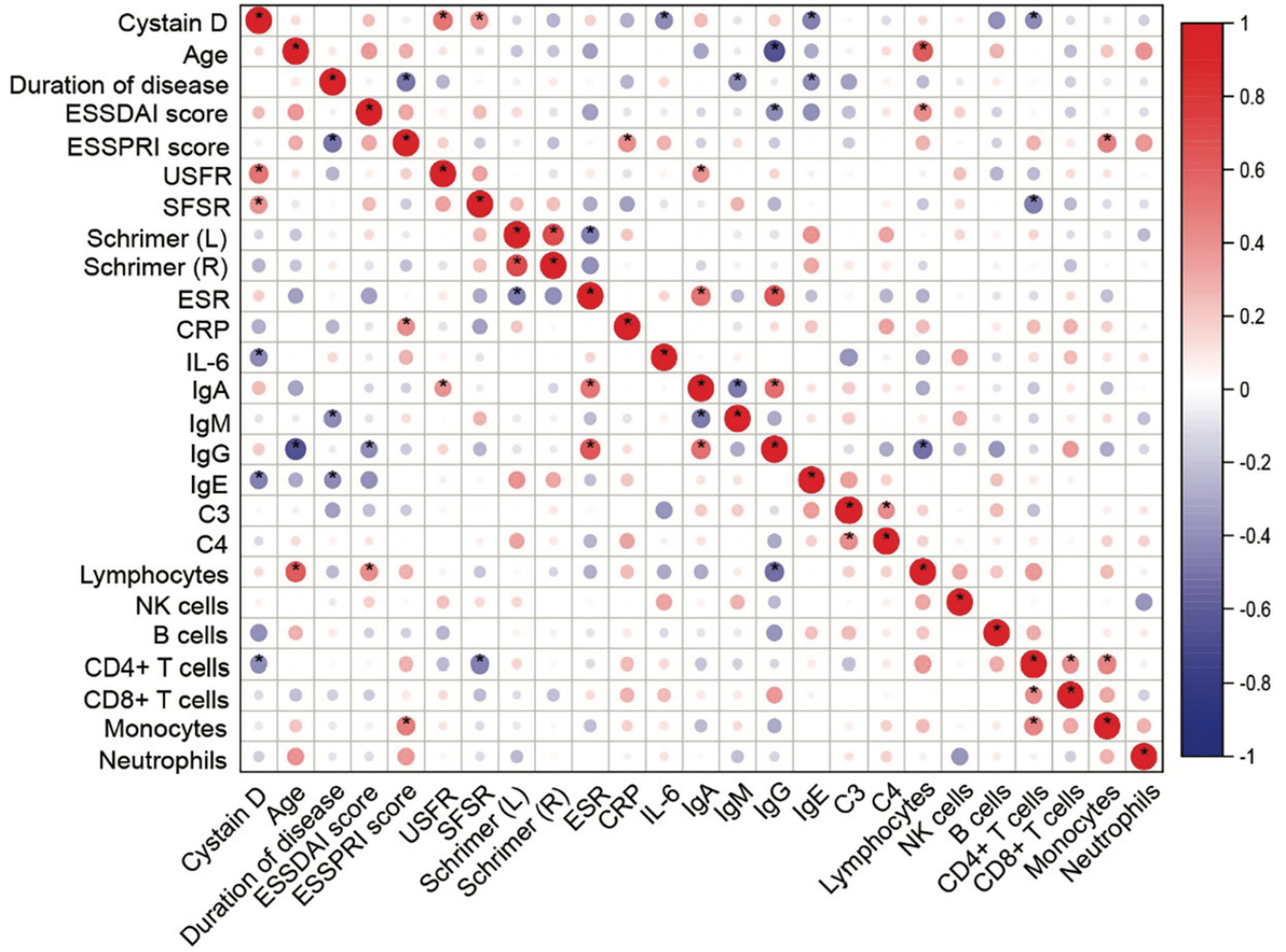

Fig. 2. Correlation between saliva cystatin D and pSS related clinical parameters ascertained by Spearman's correlation analysis. The coloiur red indicates positive correlation between two variables while blue colour indicates negative correlation. Darker shades of blue and red indicate significant correlation, and lighter shades indicate low collinearity between variables. Asterisks represent statistically significant differences.

pared with the HC group, the unstimulated and stimulated salivary gland flow rates were significantly reduced in the HNCR and pSS groups, and this reduction was more significant in the pSS group (Fig. 1A-B). Unlike hypoptyalism caused by physical damage in the HNCR group, the pSS group had the lowest level of cystatin D and the least level of saliva secretion among the 3 groups, and there were no statistically significant differences of cystatin D levels between the HC and HNCR groups (Fig. 1C). Based on the above results, we hypothesised that salivary cystatin D level may be a specific marker for reduced salivary production in the patients with pSS.

The relationship between salivary cystatin D and clinical parameters of pSS patients

We analysed the correlation between salivary cystatin D levels and clinical parameters in the patients with pSS (Fig. 2). Salivary cystatin D was positive associated USFR and SSFR, and negatively associated with serum IL-6 levels, IgE levels and peripheral blood $CD4^+$ T cell counts, whereas, it was not correlated with the EULAR Sjogren's Syndrome Disease Activity Index (ESSDAI), EULAR Sjögren's syndrome Patient Reported Index (ESSPRI), or other clinical indicators.

Cystatin D levels were correlated with salivary gland damage in pSS patients

To determine whether cystatin D can reflect the severity of salivary gland damage in patients with pSS, we analysed the correlation between cystatin D, salivary flow rate, and salivary gland ultrasonography score. The results showed that salivary cystatin D level was positively correlated with unstimulated and stimulated salivary gland flow rates in patients with pSS (Fig. 3A-B), but was not correlated with salivary gland flow rates in HC and HNCR patients (data not shown). Subsequently, we used SGUS to evaluate the degree of injury to the submandibular and parotid glands, and an SGUS score ≥2 was considered as pathological injury. Among the 73 patients with pSS, 46 and 36 patients had SGUS scores ≥2 in the parotid and submandibular glands, respectively. Salivary cystatin D levels were significantly lower in those patients who had an SGUS score ≥2 in both the parotid and submandibular glands, compared with the patients with SGUS scores <2 (Fig. 3C-D), and this finding suggested that salivary cystatin D level was related to the degree of salivary gland damage.

Salivary cystatin D as a diagnostic biomarker

To determine the value of salivary cystatin D as a diagnostic biomarker, the ROC analysis was conducted. The results showed that the area under the ROC curve was 0.713 (95% CI: 0.632, 0.749, $p<0.001$), and the cut-off point of cystatin D was 318.1 pg/mL, with a sensitivity of 51.9%, specificity of 83.6%, and Youden index of 35.5% for the diagnosis of pSS (Fig. 4A). Anti-SSA serves as the signature autoantibody for pSS and poses challenges in diagnosis when it is negative. Herein, we used ROC analysis to assess the diagnostic value of salivary cystatin D in the patients with negative anti-SSA/Ro52 and anti-SSA/Ro60. The results showed that the area under the ROC curve was 0.758 (95% CI: 0.563, 0.625, $p<0.05$), and the cut-off point of cystatin D was 161.3 pg/mL, with a sensitivity of 87.7%, specificity of 62.5%, and Youden index of 50.2% for pSS (Fig. 4B).

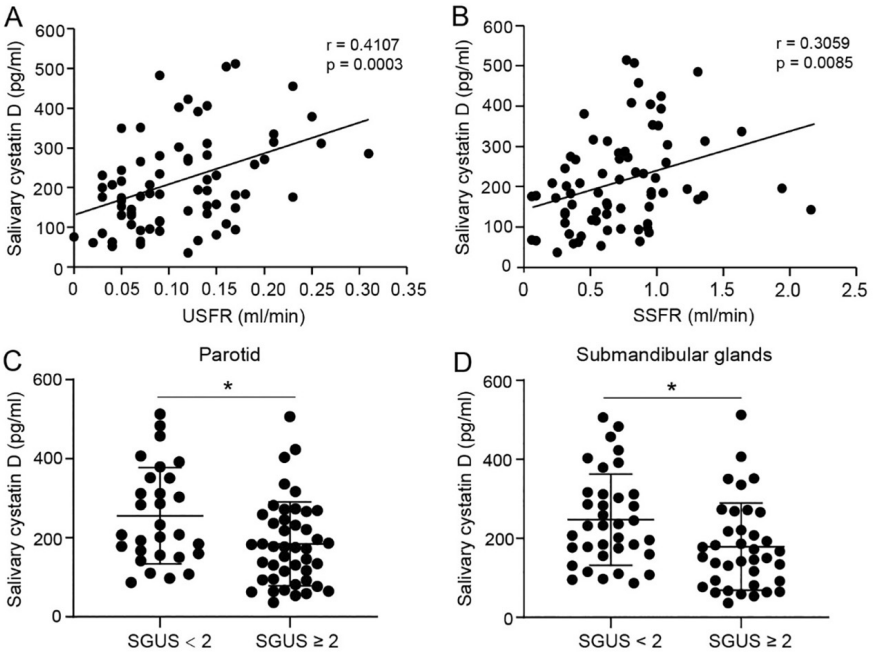

Fig. 3. Cystatin D levels were correlated with salivary gland injury in primary Sjögren's syndrome (pSS) patients.
A-**B**: The relationship between salivary cystatin D levels and salivary gland flow rates in pSS patients. **C**-**D**: Comparison of salivary cystatin D levels in pSS patients with parotid or submandibular glands with different SGUS scores. USFR: Unstimulated salivary gland flow rates; SSFR: Stimulated salivary gland flow rates. SGUS: salivary gland ultrasonography.
*$p<0.05$, **$p<0.01$.

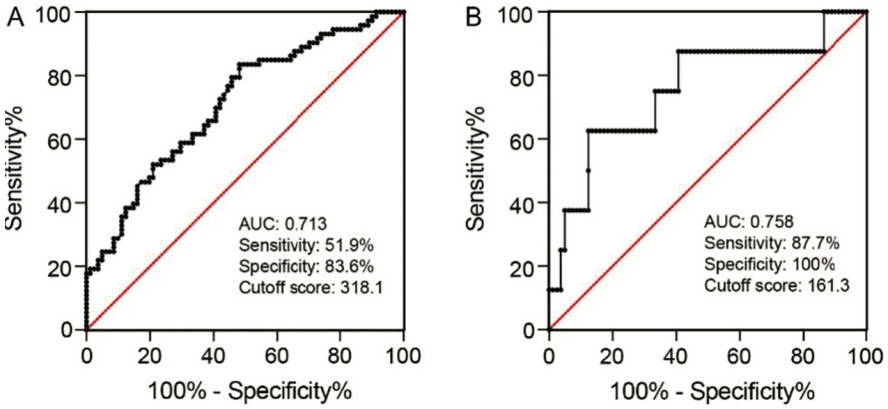

Fig. 4. Receiver operating characteristic (ROC) curve of cystatin D expression levels for diagnosis of primary Sjögren's syndrome (pSS). **A**: ROC curve of cystatin D in all the pSS patients. **B**: ROC curve of cystatin D in the patients with negative anti-SSA/Ro52 and anti-SSA/Ro60.

Discussion

Primary Sjögren's syndrome is a systemic autoimmune disease, where most patients present with dry mouth due to reduced salivation as the primary symptom. In clinical practice, it is difficult to perform the recommended invasive

tests to diagnose pSS. A lip biopsy is not readily available in many clinics because of the lack of experienced personnel to collect samples and interpret the results (pathologists). Moreover, determining the ocular staining score requires experienced ophthalmologists, and salivary rate measurements require time and effort. A wide range of serum and salivary biomarkers have been used to diagnose pSS and assess its disease activity. However, these biomarkers cannot fully reveal the biological characteristics of the disease or indicate clinical course owing to their low sensitivity and specificity (15-17). Therefore, a new, stable, non-invasive biomarker is needed for pSS diagnosis. Cystatin D, as a natural cysteine protease inhibitor, a recent study found that it suppresses osteoclast-mediated bone destruction by blocking the activation of the nuclear factor kappa B mechanism (18). Furthermore, cystatin D is considered a tumour suppressor and exhibits anti-migratory and antiproliferative cellular effects, probably due to a cathepsin-independent mechanism (19, 20). In summary, based on the characteristics of cystatin D secreted in human tears and saliva and its involvement in the immune response, it may be an important molecular marker reflecting the function of salivary glands. The composition of salivary proteins may be affected by a variety of factors, including disease states, medications, oral hygiene and periodontal disease. These factors were taken into account in this study, the patients with other rheumatic connective tissue diseases, recently taking oral cholinergic drugs, diagnosis of HIV, HCV and/or other virus infections or serious complication, were excluded. Furthermore, the proportions of periodontal disease were compared between the HNCP and pSS groups to eliminate the effect of salivary proteins. There were no statistically significant differences. In addition, for oral hygiene, before collecting the saliva sample and measuring salivary flow rate, food was prohibited for 4 hours and the mouth was rinsed with water for 10 minutes to reduce effect of confounding factors and identify the role of cystatin D in pSS.

This study demonstrated that salivary cystatin D levels are lower in patients with pSS, especially in those with a lower salivary flow rate. To confirm whether cystatin D can distinguish between reduced salivary gland flow rate caused by immune injury and non-immune injury, we compared cystatin D levels in the saliva of patients with HNCR and pSS, and found that cystatin D was significantly reduced in the saliva of the patients with pSS compared with the HNCR patients. The continual interaction between the innate and adaptive immune systems plays a crucial role in the initiation of the inflammatory process of pSS, and the amplification as well as perpetuation of the autoimmune process (21). In this study, the correlation analysis confirmed that cystatin D was negatively correlated with the serum IL-6 levels and peripheral blood $CD4^+$ T cell counts of the pSS patients. All of these results indirectly indicated that the reduction of salivary cystatin D in the pSS patients was not simply the result of damage to salivary epithelial cells, because of the different cystatin D expression in the HNCR patients with epithelial cell damage caused by radiotherapy. Our findings in pSS may be the result of infiltration of immune cells, such as $CD4^+$T cells or B cells into the salivary glands. In addition, we evaluated the correlation between cystatin D expression and the presence of pathological damage in the salivary gland and found that it was significantly lower in patients with a higher ultrasonography score in the submandibular and/or parotid glands. ROC curve analysis also confirmed that cystatin D had a potential diagnostic value for pSS. When cystatin D, as an independent diagnostic indicator, the sensitivity was 51.9%, which was dissatisfactory for pSS diagnosis. Whereas, in patients with negative anti-SSA autoantibody, who had more difficulties in diagnosing pSS (13), the sensitivity of cystatin D increased as 87.7%, and the specificity was still high. The cystatin D showed superior diagnostic value, specially combined with anti-SSA autoantibody.

To our knowledge, this is the first report that supported the use of cystatin D as a novel pSS biomarker that can be measured from non-invasively obtainable salivary samples. To translate these findings into clinical practice, future studies with a larger sample size are warranted. In conclusion, salivary cystatin D represents a promising candidate for the diagnosis of pSS and was associated with salivary gland dysfunction.

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References

1. BEN-CHETRIT E, CHAN EK, SULLIVAN KF, TAN EM: A 52-kD protein is a novel component of the SS-A/Ro antigenic particle. *J Exp Med* 1988; 167(5): 1560-71. https://doi.org/10.1084/jem.167.5.1560
2. THORLACIUS GE, BJÖRK A, WAHREN-HERLENIUS M: Genetics and epigenetics of primary Sjögren syndrome: implications for future therapies. *Nat Rev Rheumatol* 2023; 19(5): 288-306. https://doi.org/10.1038/s41584-023-00932-6
3. HUANG H, XIE W, GENG Y, FAN Y, ZHANG Z: Mortality in patients with primary Sjögren's syndrome: a systematic review and meta-analysis. *Rheumatology* (Oxford) 2021; 60(9): 4029-38. https://doi.org/10.1093/rheumatology/keab364
4. STEFANSKI AL, TOMIAK C, PLEYER U, DIETRICH T, BURMESTER GR, DÖRNER T: The diagnosis and treatment of Sjögren's syndrome. *Dtsch Arztebl Int* 2017; 114(20): 354-61. https://doi.org/10.3238/arztebl.2017.0354
5. SEROR R, BOWMAN SJ, BRITO-ZERON P *et al*.: EULAR Sjögren's syndrome disease activity index (ESSDAI): a user guide. *RMD Open* 2015; 1(1): e000022. https://doi.org/10.1136/rmdopen-2014-000022
6. NONAKA T, WONG DTW: Saliva diagnostics. *Annu Rev Anal Chem* (Palo Alto Calif) 2022; 15(1): 107-21. https://doi.org/10.1146/annurev-anchem-061020-123959
7. GOHEL V, JONES JA, WEHLER CJ: Salivary biomarkers and cardiovascular disease: a systematic review. *Clin Chem Lab Med* 2018; 56(9): 1432-42. https://doi.org/10.1515/cclm-2017-1018
8. RAPADO-GONZÁLEZ Ó, MARTÍNEZ-REGLERO C, SALGADO-BARREIRA Á *et al*.: Salivary biomarkers for cancer diagnosis: a meta-analysis. *Ann Med* 2020; 52(3-4): 131-44. https://doi.org/10.1080/07853890.2020.1730431
9. STANESCU II, CALENIC B, DIMA A *et al*.: Salivary biomarkers of inflammation in systemic lupus erythematosus. *Ann Anat* 2018; 219: 89-93. https://doi.org/10.1016/j.aanat.2018.02.012
10. HUANG Z, YANG X, HUANG Y *et al*.: Saliva - a new opportunity for fluid biopsy. *Clin Chem Lab Med* 2022; 61(1): 4-32. https://doi.org/10.1515/cclm-2022-0793
11. COLLINS AR, GRUBB A: Cystatin D, a natural salivary cysteine protease inhibitor, inhibits

coronavirus replication at its physiologic concentration. *Oral Microbiol Immunol* 1998; 13(1): 59-61. https://doi.org/10.1111/j.1399-302x.1998.tb00753.x
12. SOOND SM, KOZHEVNIKOVA MV, TOWNSEND PA, ZAMYATNIN AA JR: Cysteine cathepsin protease inhibition: an update on its diagnostic, prognostic and therapeutic potential in cancer. *Pharmaceuticals* (Basel) 2019; 12(2): 87. https://doi.org/10.3390/ph12020087
13. SHIBOSKI CH, SHIBOSKI SC, SEROR R *et al*.: 2016 American College of Rheumatology/European League Against Rheumatism Classification Criteria for Primary Sjögren's Syndrome: a consensus and data-driven methodology involving three international patient cohorts. *Arthritis Rheumatol* 2017; 69(1): 35-45. https://doi.org/10.1002/art.39859
14. THEANDER E, MANDL T: Primary Sjögren's syndrome: diagnostic and prognostic value of salivary gland ultrasonography using a simplified scoring system. *Arthritis Care Res* (Hoboken) 2014; 66(7): 1102-7. https://doi.org/10.1002/acr.22264
15. JUNG JY, KIM JW, KIM HA, SUH CH: Salivary biomarkers in patients with Sjögren's syndrome – a systematic review. *Int J Mol Sci* 2021; 22(23): 12903. https://doi.org/10.3390/ijms222312903
16. HYNNE H, AQRAWI LA, JENSEN JL *et al*.: Proteomic profiling of saliva and tears in radiated head and neck cancer patients as compared to primary Sjögren's syndrome patients. *Int J Mol Sci* 2022; 23(7): 3714. https://doi.org/10.3390/ijms23073714
17. AQRAWI LA, GALTUNG HK, GUERREIRO EM *et al*.: Proteomic and histopathological characterisation of sicca subjects and primary Sjögren's syndrome patients reveals promising tear, saliva and extracellular vesicle disease biomarkers. *Arthritis Res Ther* 2019; 21(1): 181. https://doi.org/10.1186/s13075-019-1961-4
18. WANG F, ZHANG C, GE W, ZHANG G: Up-regulated CST5 inhibits bone resorption and activation of osteoclasts in rat models of osteoporosis via suppression of the NF-κB pathway. *J Cell Mol Med* 2019; 23(10): 6744-54. https://doi.org/10.1111/jcmm.14552
19. ALVAREZ-DÍAZ S, VALLE N, GARCÍA JM *et al*.: Cystatin D is a candidate tumor suppressor gene induced by vitamin D in human colon cancer cells. *J Clin Invest* 2009; 119(8): 2343-58. https://doi.org/10.1172/jci37205
20. FERRER-MAYORGA G, ALVAREZ-DÍAZ S, VALLE N *et al*.: Cystatin D locates in the nucleus at sites of active transcription and modulates gene and protein expression. *J Biol Chem* 2015; 290(44): 26533-48. https://doi.org/10.1074/jbc.M115.660175
21. BALDINI C, CHATZIS LG, FULVIO G, LA ROCCA G, PONTARINI E, BOMBARDIERI M: Pathogenesis of Sjögren's disease: one year in review 2024. *Clin Exp Rheumatol* 2024; 42(12): 2336-43. https://doi.org/10.55563/clinexprheumatol/i8iszc