The Diagnostic Role of Anti-Human Salivary Gland Protein-1 (anti-SP1) in the Early Detection of Primary Sjogren's Syndrome in Some Iraqi Patients

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Abstract

Background: Primary Sjogren's syndrome (pSS) is a chronic disorder described by its immune devastation to the salivary and lacrimal glands. The detection of pSS is complicated especially at the early stage. Thus, the emergence of novel markers preceding the conventional diagnostics for early detection of pSS before its aggravation is required.Objective: This study purposed to reveal the possible use of anti-SP1 as a beneficial indicator for early revelation of pSS in some Iraqi patients.Methods: Eighty-seven Iraqi patients (42 and 45 patients with newly diagnosed pSS and idiopathic sicca symptoms, respectively) enrolled in this study between February 2018 to July 2019. The diagnosis of pSS reliant on AECG and ACR criteria. All patients were assessed for RF, ANA, anti-Ro/SSA, and anti-SP1 by ELISA.Results: A greater prevalence of anti-SP1 was shown in pSS patients than control patients. Anti-SP1-positive pSS patients revealed significant shorter sicca duration with lowered Schirmer's test and USFR, and predominantANA and anti-Ro. Anti-SP1 evinced positive correlations with disease duration, ANA, and anti-Ro,alsonegative correlations with Schirmer's test and USFR. Higher sensitivity (92.9%) and specificity (95.6%) of anti-SP1 in pSS patients than other diagnostic parameters.Conclusion: Despite small sample size, anti-SP1 still be a dependable marker for diagnosis of pSS at the early stage and discrimination of pSS patients than those with idiopathic sicca symptoms.

Keywords: *primary Sjogren's syndrome, anti-salivary gland protein-1 (anti-SP1), sicca symptoms*

Introduction

Primary Sjögren's syndrome (pSS) is a chronic autoimmune disorder that distinguish oneself by lymphocytic recruitment and devastation of the lacrimal and salivary glands with autoantibodies production, leading to sicca symptoms (eyes and mouth dryness) [1]. However, when the disease occurs alongside other autoimmune disorders like systemic lupus erythematosus (SLE), rheumatoid arthritis (RA) and systemic sclerosis, it is called secondary SS (sSS) [2].

The identification of pSS is complicated due to the lack of biomarkers with high efficiency and precision to prophesied the disease at the preliminary phase [3]. In addition, another diagnostic obstacle is that the sicca symptoms with limb pain and fatigue are very common in the people and may be accompanied with other pain syndromes [4].

Alternatively, the American-European Consensus Group (AECG) and American College of Rheumatology (ACR)created criteriato recognize pSS dependent on the utilization of clinical manifestations, measurements of sicca symptoms, histopathology of salivary/ lacrimal glands, and autoantibodies production [5]. Several autoantibodies presently offer assistance to the identification of pSS includeantinuclear autoantibody (ANA), rheumatoid factor (RF), anti-Ro/SSA, and anti-La/SSBwhom may emerge with the disease's exacerbation [6].

Consequently, alternate, non-invasive and dependable markers with high sensitivity and specificity are required to improve and simplify the early diagnostic process of pSS. Hence, novel biomarkers likeanti-salivary gland protein-1 (SP1) were studied in mice and in patients with SS, which established to be manifested earlier in the disease phase [7].

The apparition of SP1 was originally in mice and Shen *et al.*,five years later, evidenced identical proteins appeared in human salivary glandsand the more significantly antibodies to SP1 were existent in pSS patients[8]. Besides, these autoantibodies were observed to be infrequently detected in healthy persons or patients with other autoimmune diseases [3].

The occurrence of anti-SP1, in several studies, has been detected in the early phase of pSS preceding anti-Ro/-La [9]. Notwithstanding, Theander and her colleagues study proclaimed anti-Ro/La might manifest a long time preceding the exhibition of pSSclinical features in particular patients [10] .Whilst the timing of anti-SP1 autoantibodies emergence in pSSpatients is controversial, this study aims to disclose the bearable utilization of anti-SP1 as susceptible indicator for early identification of pSS.

Materials and Method

Patients: A cross-sectionalstudy of 87 Iraqi patients (42 and 45 patients with newly diagnosed pSS and persistent idiopathic sicca symptoms (iSS), respectively) attended the Department of Rheumatology/Baghdad Teaching Hospital during the period from February 2018 to July 2019. The detection of pSS was dependent on the AECG and ACR classification **criteria**[5]. Patients that unachieved these **criteria** were classified as iSS. Patients with sSS or other rheumatic diseases, hepatitis viruses, acquired immuno-deficiency syndrome, IgG4-related disease, head/neck radiation, graft versus host disease, amyloidosis, and medications that may affect salivary/lacrimal gland function were excluded from the study.

Materials and Method

Patients'clinical evaluation was performed by the specialized rheumatologist, and the patients' medical history was recorded. All patients were tested for Schirmer's test and unstimulated salivary flow rate (USFR) [5]. Patients' blood specimens were collected and divided into two aliquots after centrifugation. Whole blood was used for estimation of ESR (erythrocyte sedimentation rate), whereas the serum was divided into two other aliquots. First aliquot was used for detection of CRP (C-reactive protein), hepatitis viruses, and human immunodeficiency virus using Ichroma-II Immunoassay (boditech, Korea). The second aliquot was frozen at -20C° for subsequent estimation of RF, ANA, anti-Ro, and anti-SP1 by ELISA technique (MyBiosource, USA) [11,12]. The current study'sresults were analyzed statistically using MedCalc software (version 19.0.1). The differences between parameters were tested by paired, independent student's t-test and Chi-square, as appropriate. Cramer's V or Phi coefficient, as appropriate, was used for the correlationbetween parameters. The diagnostic performancewaseffectuated for the study parameters. $P \leq 0.05$ was deemed significant statistically.

Result

Significant differences amidst pSS patients and those with iSSregardingsicca duration, Schirmer's test, USFR, ESR, and CRP were illustrated in Table (1). In contrary, non-significant differences were noted between the studygroups for age and gender. However, RF, ANA, anti-Ro/SSA, and anti-SP1 were significantly prevalentin pSS patients.

Table (1): Demographic distribution of the study groups.

Parameter	pSS (n=42)	iSS (n=45)	P-value
Age (years), mean±SD	47.0±10.2	50.1±10.6	0.180
Gender, male: female	11:31	13:29	0.286
Sicca duration (years), mean±SD	2.7±0.5	3.2±0.8	0.011
Schirmer's test (mm/5 min), mean±SD	4.6±0.5	5.3±0.6	<0.001
USFR (ml/15 min), mean±SD	0.09±0.03	0.13±0.04	<0.001
ESR (mm/hr), mean±SD	25.8±6.1	23.7±6.0	0.025

Cont... **Table (1): Demographic distribution of the study groups.**

CRP (mg/l), mean±SD	10.5±2.0	12.4±2.6	0.018
Positive RF, No.(%)	21(50.0%)	17(37.7%)	0.028
Positive ANA, No.(%)	26(61.9%)	18(40.0%)	0.024
Positive Anti- Ro/SSA, No.(%)	30(71.4%)	4(8.9%)	0.031
Positive Anti-SP1, No.(%)	39(92.9%)	2(4.4%)	0.016

pSS (primary Sjogren's syndrome), iSS (idiopathic sicca symptoms), USFR (unstimulated salivary flow rate), ESR (erythrocyte sedimentation rate), CRP (C-reactive protein), RF (rheumatoid factor), ANA (anti-nuclear antibody), Anti-SP1 (anti-salivary gland protein 1).

Table (2) showed significant differences among positive- and negative- anti-SP1 patients with pSS for sicca duration, Schirmer's test, USFR, CRP, ANA and anti-Ro/SSA. Whilst, insignificant variances observed amidst those patients with pSS regarding age, gender, ESR, and RF.

Table (2): The clinical significance of anti-SP1 among patients with pSS.

Parameter **Positive**		**Anti-SP1**		**P-value**
		Negative		
Age (year), mean±SD		47.3±10.4	43.3±5.8	0.216
Gender, male: female		9:30	2:1	0.098
Sicca duration(year), mean±SD		2.6±0.5	2.9±0.2	0.019
Schirmer's test (mm/5 min), mean±SD		4.6±0.4	5.1±0.3	0.032
USFR (ml/15 min), mean±SD		0.09±0.03	0.14±0.01	0.021
ESR (mm/hr), mean±SD		25.7±6.2	18.6±1.2	0.063
CRP (mg/l), mean±SD		10.7±2.5	6.0±1.0	0.003
RF, No.(%)	Positive	21(50.0%)	0(0.0%)	0.072
	negative	18(42.9%)	3(7.1%)	
ANA, No.(%)	Positive	26(61.9%)	1(2.4%)	0.022
	negative	13(31.0%)	2(4.8%)	
Anti-Ro/SSA, No.(%)	Positive	30(71.4%)	0(0.0%)	0.004
	negative	9(21.4%)	3(7.1%)	

A significant positive correlation of sicca duration, CRP, ESR, ANA, and anti-Ro/SSA, additionally, negative correlation of Schirmer's test and USFR with anti-SP1 manifested in pSSpatients. In contrast, non-significant correlation between study groups were noticed for age, gender, and RF with anti-SP1 (data viewed in Table 3).

Table (3): Correlation of anti-SP1 with other parameters among patients with pSS and iSS.

Parameter	pSS		iSS	
	φ	P-value	φ	P-value
Age	0.227*	0.540	0.241*	0.485
Gender	0.255	0.098	0.014	0.926
Sicca duration	0.336	0.029	0.040	0.794
Schirmer's test	-0.806	<0.001	-0.184	0.234
USFR	-0.469	0.002	-0.124	0.421
CRP	0.305	0.048	-0.155	0.315
ESR	0.439	0.004	-0.252	0.102
RF	0.277	0.072	0.040	0.794
ANA	0.354	0.022	-0.258	0.099
Anti-Ro/SSA	0.641	<0.001	-0.282	0.070

*Cramer's V coefficient. φ (phi correlation coefficient)

Table (4) represented the diagnostic performance and accuracy of the studied parameters. The findings evidenced a higher sensitivity (92.9%), specificity (95.6%), PPV (95.1%), NPV (93.5%), PLR (20.9) and NLR (0.1) of anti-SP1 in pSS patients contrasting other diagnostic parameters.

Table (4): The diagnostic performance of the study parameters among pSS patients.

Parameter	**Sensitivity**	**Specificity**	**PPV**	**NPV**	**PLR**	**NLR**
Schirmer's test	92.0%	17.8%	51.3%	72.7%	1.1	0.4
USFR	88.1%	22.2%	51.4%	66.7%	1.1	0.5
RF	50.0%	62.2%	55.3%	57.1%	1.3	0.8
ANA	62.0%	60.0%	59.1%	62.8%	1.5	0.6
Anti-Ro/SSA	71.4%	91.1%	88.2%	77.4%	8.0	0.3
Anti-SP1	92.9%	95.6%	95.1%	93.5%	20.9	0.1

P (positive), N (negative), PV (predictive value) LR (likelihood ratio).

Discussion

The most debatable things is the time of autoantibodies appearance that may precede the clinical onset in pSS patients. The current study revealed significant elevationin patients with newly diagnosed pSS and those with persistent iSS for diagnostic parameters (Schirmer's test, USFR, RF, ANA, anti-Ro, and anti-SP1). These results were consistent with findings by Lee *et al*., which showed a raised predominance of these diagnostic parameters in pSS patients comparing those iSS ones [13], suggesting the superior inflammatory pathways in patients with pSS than with sustained iSS. Nevertheless, the marker(s) that undoubtedly confirm suspected patients with pSS was needed, so significant higher prevalence of positive anti-SP1 was demonstrated in pSS than iSS patients comparing other diagnostic markers. Besides,positive anti-SP1 patients with pSS having elevated CRP and prevalentRF, ANA and anti-Ro conflicting those with negative anti-SP1.Moreover, sicca duration, Schirmer's test, and USRF were noticed markedly minimized in pSS patients with positive anti-SP1, suggesting the appearance of anti-SP1 in pSS patients with shorter duration and acute-phase.A study by Everett *et al.* was in harmony with our findings, they found thatanti-SP1 was disclosed in pSS patients with ≤ 2 years duration and abnormal Schirmer's test, and anti-Ro/La antibodies might not be detected before two-years of disease onset [14].

Referring to the possible replacement of anti-Ro/La with anti-SP1 in the early detection of pSS.Vishwanath*et al*. study reported iSSpatientswith negative Ro/La antibodies exhibited positive anti-SP;hence, tissue-specific autoantibodies (e.g. anti-SP1) are reliable markers for early identification of pSS [15].In contrary, the findings by Suresh *et al.* were discrepancy with our results as they found (based on their study design) that anti-Ro/La, in contrast to anti-SP1, might be linked with the disease's acuteness and exhibited higher sensitivity in the discrimination between normal and diseased individuals [16].

Several explanations may clarify the earlier emergence of anti-SP1, contrasting anti-Ro/La, during the early stage of pSS. Among them the early immunogenic inflammatory processes that may occur initially in the salivary and lacrimal glands destroying the epithelial cells with activation of B cells via the recruitment of follicular T-helper cells [17]. Besides, patients with pSS may develop hyperglobulinemia that leads to the formation and potentially deposition of immune-complexes in the secretory glands causing irreversible damage with the expression of epithelial cells contents and stimulation of chronic immune responses [18].

Another explanation, both Ro and La are parts of extractable nuclear antigens that existed in many cell types, therefore anti-Ro/-La can be found in several autoimmune disorders resulting in decreased their specificity to pSS particularly at the early phase [19]. In return, the tissue specificity of SP1 (salivary gland secretory protein) making anti-SP1 potentially tissue-specific antibodies that may correlate with the early diagnosis of pSS due to the initial inflammatory devastation of the salivary and lacrimal glands [20].

The study findings displayed that anti-SP1 antibodies were correlated positively with the sicca duration, the inflammatory markers, ANA, and anti-Ro, as well as, correlated negatively with the clinical signs' measurements in pSS patients. Therefore, this relationship of anti-SP1 with the shorter disease duration, and the inflammatory as well as immunological markers indicating the involvement of immunoinflammatory reactions in the salivary glands with early emergence of these autoantibodies during the disease onset. Phung *et al.* [21] referred that anti-SP1 was more specific than anti-Ro/La for early identification of SS since SP1 was located particularly in the salivary glands contrasting Ro/La that existed in any nucleated cells. Likewise, the anti-SP1 was rarely detected in healthy persons (<5%), and the patients suffering early SS have >60% of novel autoantibodies (e.g. anti-SP1) and 20-30% of anti-Ro/La, whereas in patients with late SS these proportions were inverted. Karakus *et al.* declaredthat anti-SP1 discovered in >20% of SS patients contrasting 13% ofiSS patients, also anti-SP1 was the only autoantibody correlated significantly in those with ≤5 mm of Schirmer's test [22].

The diagnostic performance of the studied parameters elucidated excellent performance of anti-SP1 with higher sensitivity (92.9%) and specificity (95.6%) in the pSS detection comparing other parameters, suggesting the possible utilization of anti-SP1 antibody for early detection of pSS due to its higher specificity to the salivary glands, and eventually more sensitive to the pSS especially at the early stage. Jin*et al.* stated that anti-SP1 elevated obviously in patients suffering early pSS with shorter disease duration and negative anti-Ro/-La antibodies; indicating the effective use of these

autoantibodies as a diagnostic marker particularly during the early stage at which anti-Ro/La antibodies were negative[23]. In conclusion, notwithstanding small sample size, the current findings are still worthy to proclaim the beneficial use of anti-SP1 as a marker for pSSdetection especially at the early-phase and discrimination patients suffering pSS from those with iSS. Molecular studies with a larger cohort are required to estimate the time of SP1 expression in human salivary gland for possible use of anti-SP1 as a predictor of pSS.

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Conflict of Interest: non

Source of Findings: Self findings.

Ethical Clearance: Non

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