Diagnostic performance of dry eye tests, serology and labial salivary gland biopsy in primary Sjogren's syndrome in an Indian setting

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ABSTRACT

Purpose: To evaluate the diagnostic performance of dry eye tests, serology and labial salivary gland biopsy in Primary Sjogren's syndrome (pSS).

Methods: Prospective cross-sectional study spanning 7-months of patients referred from rheumatology for evaluation of dry eyes. Severity of ocular symptoms was assessed using the Ocular Surface Disease Index (OSDI) questionnaire. The sensitivity, specificity and positive likelihood ratio (+LR) of Schirmer-I (Sch-I) test, Tear Break-up time (TBUT) and Ocular Staining Score (OSS) were assessed and Receiver Operating characteristic (ROC) curves were analysed. The American College of Rheumatology (ACR) criteria was used to diagnose pSS.

Results: Of the 95 patients, 31 (32.6%) fulfilled the ACR criteria for pSS. OSDI score was significantly ($p < 0.001$) higher (24.6 ± 9.4) in pSS than non-pSS (17.8 ± 5.4) patients. The sensitivity and specificity were 3.2% and 100% for Sch-I ≤ 5 mm and 61.3% and 90.6% for OSS ≥ 3 respectively. TBUT of < 10 s had poor specificity (12.5%); however at lower threshold (< 8 s), sensitivity (80.6%) and specificity (60.9%) were optimal. The + LR was 2.07 for TBUT < 8 s and 6.54 for OSS ≥ 3 . Twenty-six (83.9%) patients diagnosed as pSS had anti-SSA or anti-SSB antibodies. Anti-SSA, when compared with anti-SSB, had better sensitivity (76.7% vs. 52.4%) than specificity (88.7% vs. 100%). Labial biopsy had the best diagnostic performance (sensitivity 96.8%; specificity 88.9%; + LR 8.71).

Conclusion: In patients with high OSDI scores, dry eye tests in combination with serology will identify the subset of patients with a high probability of pSS. Labial biopsy may be considered in patients with negative serology.

1. Introduction

Sjogren syndrome (SS), a chronic autoimmune disease of unknown etiology affecting salivary and lacrimal glands, is characterized by marked mononuclear cell infiltration leading to progressive impairment of gland function.¹ The peak incidence is in the fourth to fifth decade. It generally affects women and presents as xerostomia and xerophthalmia.¹ The pathogenesis is multifactorial and includes environmental, viral and genetic factors.¹

Numerous criteria have been proposed to diagnosis SS; the modified American-European Consensus Group (AECG) criteria² and the American College of Rheumatology (ACR) classification criteria are widely used.³ The ACR criteria, used in the current study, requires at least 2 of the 3 criteria of Ocular Staining Score (OSS) of ≥ 3 , positive auto-

antibodies to Ro (SSA) and/or La (SSB) antigens or (positive rheumatoid factor and ANA $\geq 1:320$) and evidence of focal lymphocytic sialadenitis.³ Although several tests are available for the evaluation of dry eyes,⁴ the AECG criteria² incorporated the Schirmer I (Sch-I) test and Rose Bengal score whereas the ACR criteria³ is based on the OSS. The performance of these tests and the optimal threshold has not been evaluated in the Indian population. This study was undertaken to evaluate the diagnostic performance of Sch-I test, Tear Break-Up Time (TBUT), OSS, auto-antibodies and histopathology in patients diagnosed as primary SS (pSS) using the ACR criteria, in an Indian setting.

2. Materials and methods

This cross-sectional, observational study was done in a tertiary care

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university affiliated teaching hospital in India over 7-months. Adult patients (> 18 years) evaluated in the rheumatology department for systemic rheumatological symptoms and manifesting symptoms of dry eyes were referred to the eye department. Patients were excluded if there was history of radiation or ocular surgery, were pregnant or mentally challenged or were on topical medications or wearing contact lens. The study was approved (IRB No 8671 dated 19.2.14) by the Institutional Review Board and informed consent was obtained from patients willing to participate in the study.

All patients were asked to stop artificial tear eye drops 1-day prior to eye examination. Patients were asked to fill the Ocular surface disease index (OSDI) questionnaire that includes 12 questions (scores 0 to 4).⁵ The total score was noted (0–100). Sch-I test was done using sterile Schirmer strip (Contracare Ophthalmic) bent at the notch and placed carefully at the junction of the outer and middle one-third of the inferior fornix of both eyes.⁴ Wetting of the strip was measured in millimetres (mm) after 5-min. A value of ≤ 5 mm was considered abnormal.

TBUT was done after 2-min of placing a fluorescein dye strip moistened with a drop of saline in that inferior fornix. With $10\times$ magnification, high illumination and a cobalt blue filter over the light source, patient was asked to blink and then keep the eyes open.⁴ TBUT was defined as the time in seconds between patient's last blink and first appearance of random dry spot on the corneal surface. It was measured thrice and the mean value was recorded. TBUT < 10 s was considered abnormal.⁴

Anterior segment evaluation was conducted with a slit lamp biomicroscope. Grading of conjunctival lissamine green staining pattern (Step 2 of Ocular SICCA) was done separately for each eye and the scores were recorded on the SICCA ocular staining form.⁴ The total OSS for each eye (maximum 12) was calculated as the summation of the fluorescein score for the cornea (maximum score 6) and the lissamine score for nasal and temporal bulbar conjunctiva (maximum score 3 each). A score of 3 or more was considered abnormal.⁴

Patients underwent serological tests that included anti-SSA and anti-SSB. A titre of > 20 Ru/ml was considered as positive. All patients were also advised to undergo minor salivary gland biopsy (labial salivary gland). The presence of focal lymphocytic sialadenitis with a focus score > 1 focus/4 mm² was considered as positive.³

Data was analysed using Statistical Package for the Social Science (SPSS) for windows 17.0. The ACR criterion was used as the gold standard for the diagnosis of pSS.³ The sensitivity, specificity and positive likelihood ratio (+LR) of Sch-I, TBUT and OSS in diagnosing pSS was assessed. Receiver Operating characteristic (ROC) curves were generated and optimal thresholds were identified.

3. Results

During the study period, 95 patients (90 female), aged 43.7 (Standard deviation (SD) 11.5) years were referred from rheumatology for assessment of dry eyes. The majority were housewives (69.4%) and students (18.9%). Common symptoms were itching (56.8%) and foreign body sensation (56.8%). The mean (SD) symptom duration was 2.6 ± 1.8 years prior to presentation.

Thirty-one (32.6%) patients (29 female) fulfilled the ACR criteria for pSS. The mean age of patients diagnosed with pSS (43.5 ± 13.2 years) was not different ($p = 0.43$) to those ($n = 64$) with other diagnoses (Table 1). The diagnosis in these 64 patients included inflammatory and non-specific arthritis ($n = 20$), sicca syndrome ($n = 19$), connective tissue disorders ($n = 18$) and Vitamin D deficiency ($n = 7$). Symptoms of itching and foreign body sensation were more frequent ($p = 0.002$) and present for longer ($p = 0.0009$) duration (3.4 ± 2.2 years) in pSS than those without pSS (2.1 ± 1.3 years). The use of anti-cholinergic drugs was similar in both groups (Table 1).

Anti-SSA and anti-SSB were done on 92 and 49 patients

Table 1
Characteristics of the study patients.

Variables	All Patients (n = 95)		Primary Sjogren's (n = 31)		Other diagnosis (n = 64)		p value*
	n	%	n	%	n	%	
	Mean (SD)		Mean (SD)		Mean (SD)		
Age (in years)	43.7 (11.5)		43.5 (13.2)		43.9 (10.7)		0.43
Gender							
Male	5	5.3	2	6.5	3	4.7	0.66
Female	90	94.7	29	93.5	61	95.3	
Occupation							
Office worker with AC	3	3.2	2	6.5	1	1.6	0.07
Office worker without AC	7	7.4	5	16.1	2	3.1	
House wife	66	69.4	19	61.3	47	73.4	
Student	18	18.9	5	16.1	13	20.3	
Others	1	1.1	0	0	1	1.6	
Itching							
Yes	54	56.8	25	80.7	29	45.3	0.002
No	41	43.2	6	19.3	35	54.7	
Foreign body sensation							
Yes	54	56.8	25	80.7	29	45.3	0.002
No	41	43.2	6	19.3	35	54.7	
Visual disturbance							
Yes	10	10.5	4	12.9	6	9.4	0.72
No	85	89.5	27	87.1	58	90.6	
Symptom duration (years)†	2.6 (1.8)		3.4 (2.2)		2.1 (1.3)		0.0009
Use of systemic anti-cholinergic drugs ‡							
Yes	3	4.2	2	9.1	1	2.0	0.23
No	68	95.8	20	90.9	48	98.0	
Positive anti-SSA titre							
Yes	30	32.6	23	76.7	7	11.3	< 0.001
No	62	67.4	7	23.3	55	88.7	
Positive anti-SSB titre							
Yes	11	22.5	11	52.4	0	0	< 0.001
No	38	77.5	10	47.6	28	100	
Biopsy							
Positive biopsy	37	39.4	30	96.8	7	11.1	< 0.001
Negative biopsy	57	60.6	1	3.2	56	88.9	

SD – Standard deviation; AC – Air conditioning; * Comparison between patients diagnosed to have Primary Sjogren's syndrome (pSS) versus other diagnoses; † Data available for 76 patients; ‡ Data available on 71 patients.

respectively. A significantly higher ($p < 0.001$) proportion of patients with pSS had a positive anti-SSA (23/30; 76.7%) than anti-SSB (11/21; 52.4%). In patients not diagnosed to have pSS, anti-SSA and anti-SSB were positive in 7/62 (11.3%) and 0/28 (0%) patients respectively. Ninety-four patients underwent labial biopsy; focal sialadenitis was seen in more patients ($p < 0.001$) with pSS (Table 1).

Table 2 summarizes the results of the dry eye tests. The mean (SD) OSDI, TBUT, OSS and Schirmer-I were significantly ($p < 0.001$) different in patients with pSS than those without pSS. However when absolute thresholds were used, the tests were not discriminatory for Sch-I at a threshold of ≤ 5 mm and TBUT at a threshold of < 10. However a significantly higher proportion of patients with pSS had an OSS of ≥ 3 (Table 2).

The predictive ability of the diagnostic tests is summarized in

Table 2
Results of the dry eye tests.

Test	Primary Sjogren's (n = 31)	Non- Sjogren's (n = 64)	p value
OSDI Score; Mean (SD)	24.6 (9.4)	17.8 (5.4)	< 0.001
TBUT value; Mean (SD)	7.0 (1.0)	8.0 (1.2)	< 0.001
< 10 s	30	56	
≥ 10 s	1	8	0.26
Ocular staining score; Mean (SD)	1.4 (0.5)	1.9 (0.3)	< 0.001
≥ 3	19	6	
< 3	12	58	< 0.001
Schirmer-I; Mean (SD)	13.7 (5.4)	20.1 (5.1)	< 0.001
≤ 5 mm	1	0	
> 5 mm	30	64	0.33

SD – Standard deviation; Values are actual numbers unless specified; OSDI – Ocular surface disease index; TBUT – Tear Break-up time.

Table 3
Predictive ability of the diagnostic tests.

Test	Sensitivity (%)	Specificity (%)	Positive likelihood ratio
Anti-SSA titre	76.7	88.7	6.79 (3.29–14.02)
Anti-SSB titre	52.4	100	Infinity
Biopsy	96.8	88.9	8.71 (4.32–17.56)
TBUT < 10 s	96.8	12.5	1.11 (0.99–1.24)
TBUT < 8 s	80.6	60.9	2.07 (1.45–2.93)
OSS ≥ 3	61.3	90.6	6.54 (2.90–14.72)
Schirmer-I ≤ 5 mm	3.2	100	Infinity
Schirmer-I ≤ 15 mm	71	82.8	4.13 (2.31–7.40)

American College of Rheumatology (ACR) criteria was used to diagnose primary Sjogren's syndrome; values in parentheses indicate 95% confidence interval; OSS – Ocular staining score; TBUT – Tear Break-up time.

Fig. 1. Receiver Operating Characteristic (ROC) curve for the different diagnostic tests used for diagnosis of primary Sjogren's syndrome. The area under the curve (AUC) is 0.94 for labial biopsy, 0.76 for anti-SSA, 0.75 for anti-SSB, 0.83 for Schirmer-I test, 0.78 for Tear Break-up time and 0.79 for ocular staining score.

Table 3 and the ROC curves in Fig. 1. Labial biopsy had the best diagnostic performance (sensitivity 96.8%; specificity 88.9%; + LR 8.71). Of the serological tests, anti-SSA had a higher sensitivity than anti-SSB while anti-SSB had a very high specificity. TBUT and Sch-I had poor specificity and sensitivity respectively for the diagnosis of pSS at the thresholds suggested in the criteria. Analysis of the ROC curves suggested that the optimal threshold for TBUT was < 8 and for Sch-I it was

≤ 15 mm (Table 3). The positive likelihood ratio for Sch-I ≤ 5 mm, Sch-I ≤ 15 mm TBUT < 10 s, TBUT < 8 s and OSS ≥ 3 were infinity, 4.13, 1.11, 2.07 and 6.54, respectively. Sensitivity and specificity were highest for TBUT (80.6%) and OSS (90.6%), respectively.

4. Discussion

In this cohort of 95 patients referred to ophthalmology for evaluation of dry eyes, 32.6% were diagnosed as pSS. Patients with pSS, when compared with those with other diagnoses, had a higher frequency ($p = 0.002$) of itching and foreign body sensation for a longer duration ($p = 0.0009$). The mean OSDI score was significantly higher and the mean TBUT, OSS and Sch-I values were significantly lower in pSS than in other diagnoses. The predictive ability of the dry eye tests were optimal at thresholds of ≤ 15 mm, < 8 s and ≥ 3 for Sch-I, TBUT and OSS respectively. Anti-SSA had better sensitivity than anti-SSB, while anti-SSB had better specificity. Labial biopsy had the best diagnostic performance.

In other published series, 10.9%–39% of patients were diagnosed to have pSS.^{6–9} Although this appears consistent with our data (32.6%), our patients with systemic complaints evaluated by rheumatologists were referred for ophthalmic evaluation in contrast with other series where patients with dry eye symptoms were referred from ophthalmology to rheumatology for possible pSS. This may have influenced the performance of the diagnostic tests. The age at diagnosis (43.5 years) and female preponderance in our study (93.5%) is similar to published literature.¹⁰

The diagnostic performance of tear functions tests has been assessed in an earlier publication involving 177 patients that included 66 patients with pSS.⁷ The authors observed that the diagnostic performance of the Sch-I test was poor (sensitivity 42%; specificity 76%). In our study, the sensitivity of the Sch-I test was also poor at a threshold of ≤ 5 mm; however although sensitivity and specificity improved to 71% and 82.8% respectively at a higher threshold of ≤ 15 mm, in clinical practice Sch-I values of > 5 mm do not qualify for the diagnosis of dry eye. The same group⁷ also observed that TBUT had a high sensitivity (92%) and very low specificity (17%). At a threshold of < 10 s, our data also showed similar sensitivity (96.8%) and specificity (12.5%). However when we changed the threshold to < 8 s, although sensitivity dropped (80.6%), specificity improved to 60.9%. Since the initial threshold for TBUT was derived from a study of 33 patients (65 eyes) with keratoconjunctivitis sicca,^{4,11} the optimal threshold may be assessed in prospective studies of patients with pSS. The sensitivity and specificity of the Lissamine green staining in our study was similar to that of the previous study.⁷

Serological tests and biopsy have been widely studied in pSS. In an early study ($n = 16$) that used the AECG criteria, 12 (75%) were tested positive for anti-SSA or anti-SSB.⁸ In another study of 24 patients diagnosed to have pSS during the initial evaluation,⁶ 66.6% were anti-SSA or anti-SSB positive. In a recent retrospective study of 290 patients, of whom 65 patients were diagnosed to have pSS,⁹ 21 (32.3%) tested positive for anti-SSA and/or anti-SSB. In our study, 26 patients (83.9%) diagnosed as pSS had anti-SSA or anti-SSB antibodies; 9 patients (29%) with pSS were positive for both anti-SSA and anti-SSB. The high sensitivity of anti-SSA (76.7%) and high specificity of anti-SSA (88.7%) and anti-SSB (100%) in our study supports the observations⁸ that positive serology may obviate the need for biopsy.

Several studies have assessed the role of salivary gland biopsy in the diagnosis of pSS.^{8,9,12–14} In one of the earlier studies,¹² minor salivary gland biopsy was considered to be “most necessary” in patients with partial San Diego criteria for sicca and a positive anti-SSA or anti-SSB antibody. However, more recent studies recommend that salivary gland biopsy may be considered for those with a negative result for the antibodies.^{8,9,11–14} In another study,¹³ the authors suggested that salivary gland biopsy was “redundant” when using the ACR classification when patients had a positive serologic test and positive ocular tests. In our

study, all but one patient who was tested positive for anti-SSA and/or anti-SSB had a positive biopsy. Five patients with negative anti-SSA and anti-SSB had a positive biopsy. These results strengthen the recommendation that salivary gland biopsy may be reserved for those patients who have a positive ocular test and negative anti-SSA and anti-SSB.

The following limitations merit mention. Although anti-SSA was done on most patients (92/95), anti-SSB was done only on 49 patients. This could have influenced the diagnostic accuracy of anti-SSB. The simpler ACR criteria was chosen for the diagnosis of pSS over the AECG criteria. However this is unlikely to have influenced the diagnosis of pSS since a recent study has demonstrated good concordance of both criteria.¹⁵ Our cohort of patients with systemic symptoms were referred for ophthalmic evaluation rather than a cohort of patients presenting with dry eyes who were evaluated for an underlying pSS. The better performance of the diagnostic tests in this setting may be explained by selection bias of a different cohort. The strength of the study was the performance of biopsy is most (99%) patients and the comprehensive evaluation of all diagnostic tests for pSS.

In conclusion, our data suggests that in patients with systemic symptoms and dry eyes and possible pSS, those with high OSDI scores and positive dry eye tests with the modified TBUT threshold identified in this study, in combination with anti-SSA/anti-SSB will identify the subset of patients with a high probability of pSS. Labial biopsy may be indicated only in those with positive ocular tests and negative serology.

Conflicts of interest

There is no conflict of interest for any of the authors listed in the submission or any financial interests to disclose all the authors.

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