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RESEARCH

Surgical advantage of modified labial salivary gland biopsy using chalazion forceps: a prospective randomized controlled study

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Abstract

Labial salivary gland biopsy (LSGB) is one of the specific diagnostic criteria for primary Sjögren's syndrome (pSS). In traditional LSGB, there is no lower lip fixation device, the field of view is unclear due to intraoperative bleeding, and the incision is large, which is unfavourable for healing. The use of auxiliary devices to improve the shortcomings of traditional LSGB technique would be meaningful. Therefore, this case–control study aimed to assess the value of modified LSGB using chalazion forceps as compared with traditional LSGB. After obtaining written informed consent from all participating parents and patients, we randomly assigned 217 eligible participants to undergo LSGB using chalazion forceps (n = 125) or traditional LSGB (n = 92). The outcome variables were surgical time, incision length, intraoperative bleeding, pain score at 24 h after surgery, incision healing status at 7 days after surgery, gland collection, and pathological results. The final diagnostic results of the two surgical methods were compared, and the match rates between the pathological results and the final clinical diagnoses were compared between the two groups. The data were analysed using parametric and nonparametric tests. Compared with the traditional group, the modified group had a smaller incision, shorter operative time, less blood loss, lower 24 h pain score, and better Grade A incision healing at 7 days after surgery ($p < 0.01$). There was no statistically significant difference between the patients in the two surgical-method groups in terms of the positive biopsy results and the final diagnosis based on expert opinions ($p > 0.05$). By multivariable regression analysis, only a focus score (FS) of ≥ 1 ($p < 0.01$), dry eye disease ($p < 0.05$) and anti-nuclear antibodies (ANA) titre ≥ 1:320 ($p < 0.05$) were correlated with the diagnosis of pSS. The positive biopsy results of patients in the different surgical-method groups had a biopsy accuracy of > 80.0% for the diagnosis of pSS. The positive biopsy results in the different surgical-method groups were consistent with the expert opinions and the 2016 ACR-EULAR primary SS classification criteria. The modified LSGB using an auxiliary chalazion forceps offers a good safety with a small incision, shorter operative time, less bleeding, reduced pain and a low incidence of postoperative complications.The match rate of LSGB pathological results of the proposed surgical procedure with the final diagnosis of pSS is high.

Keywords Labial salivary gland biopsy · Sjögren's syndrome · Chalazion forceps

Introduction

Sjögren's syndrome (SS) is the second most common rheumatic autoimmune disease, with an incidence rate of approximately 0.05–0.4% [1]. According to the American–European Group Consensus criteria, the 2012 American College of Rheumatology (ACR) criteria, and the 2016 SS classification criteria proposed by the American College of Rheumatology and the European League Against Rheumatism (ACR-EULAR) [2–4], labial salivary gland biopsy (LSGB) is a specific method to diagnose SS and has high clinical

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significance [5]. The sensitivity and specificity of LSGB in primary Sjögren's syndrome (pSS) are both around 0.8 [6].

The diagnosis of pSS should be made on the basis of clinical manifestations, glandular dysfunction, laboratory tests, and LSGB. The common complications after traditional LSGB are local pain and incision dehiscence, and 6% of patients will experience persistent lip numbness after surgery. In contrast, the combined prevalence of permanent or potentially permanent neurological adverse events using the minimally invasive technique (incision of 2–3 mm) is less than 1/8 that using linear incisions (≥ 5 mm) [7]. The chalazion forceps has a wide clinical application and can be used to treat ophthalmic diseases [8, 9]. It can also assist in oral biopsy, especially for lip lesions [10]. Studies have shown that this device can be used in patients with pSS to simplify minor salivary gland biopsy [11, 12]. In a prospective study of 23 suspected pSS patients, LSGB was performed using scattered granuloma forceps. The results showed that this technique helped to achieve superior yield, ensured adequate glandular sampling for histopathological analysis, and reduced complications associated with traditional techniques [13].

The main objective of this study was to perform LSGB in patients with suspected SS and to assess the value of LSGB conducted with a chalazion forceps compared to traditional LSGB. This study describes the clinical features and glandular dysfunction in patients who underwent biopsy and the effects of modified surgery on LSGB pathological results, incisions, specimens, pain scores, and wound healing. It analyses the factors influencing the diagnosis of pSS and compares the two surgical methods on the match rate between the LSGB pathological results and the final clinical diagnosis.

Methods

Patients

This was a prospective randomized controlled study that included all suspected SS patients over 18 years old who visited the outpatient clinic and were admitted to wards of the Department of Rheumatology of Hospital Affiliated with Zunyi Medical University. Between May 2021 and December 2023, a total of 217 patients treated with LSGB were included in the study. Patients were randomly grouped using a simple randomisation method based on computer-generated random numbers prepared by a statistician not involved in conducting the trial, singularly in the conventional lip gland biopsy group and doubly in the modified lip gland biopsy group, with 92 cases in the conventional lip gland biopsy group and 125 cases in the modified lip gland biopsy group.

LSGB technique and histological parameters

Option 1: Procedure for traditional LSGB

Ninety-two patients with suspected SS were randomly selected for LSGB. This operation was performed by one experienced stomatologist using a linear incision. The procedure was as follows: Routine disinfection was performed, followed by local anaesthesia of the surgical area. The patient's lower lip was immobilized to expose the surgical site, and a surgical blade was used to create a long fusiform incision from the mucosa to the muscle layer of the lower lip. The gland was bluntly dissected, placed in fixative solution, and examined. The surgeon aimed to harvest at least four minor salivary glands. If the minor salivary glands were too small (<2 mm), six glands needed to be harvested, with a minimum glandular surface area of 8 mm^2 [14, 15]. The incision was sutured, the patient was given discharge instructions after the surgery, and the stitches were removed after the patient returned to hospital 1 week later.

Option 2: Procedure for modified LSGB using chalazion forceps

Another 125 patients with suspected SS were randomly selected for LSGB, which was performed by one experienced rheumatologist using a linear incision. The procedure was as follows: Using chalazion forceps (Xinhua Surgical Instrument Co., Ltd., lot number: 249142), the surgeon first turned spiral button upwards and loosened it to select the site for specimen collection. Then, spiral button was turned to slowly compress fixation pressure plate and fixation tray plate to clamp and fix the lower lip. The surgeon gently everted fixation pressure plate to fully expose the surgical site within the scope of ring pressure plate, the excess saliva was wiped away with gauze, and the site of salivary exudation was observed for local anaesthesia and biopsy (Fig. 1). Other treatments were the same as described above.

Histopathological analysis was performed by two experienced pathologists. The grading criteria for pathological manifestations were as follows: the standard for positivity was based on the Chisholm rating, i.e., the degree of lymphocytic infiltration was ≥ 1 focus/4 mm^2 (a focus was defined as an aggregation of at least 50 lymphocytes in the labial gland interstitium within 4 mm^2 of tissues) or the focus score (FS) was ≥ 1. The diagnosis of SS can be considered when the pathology is positive [6].

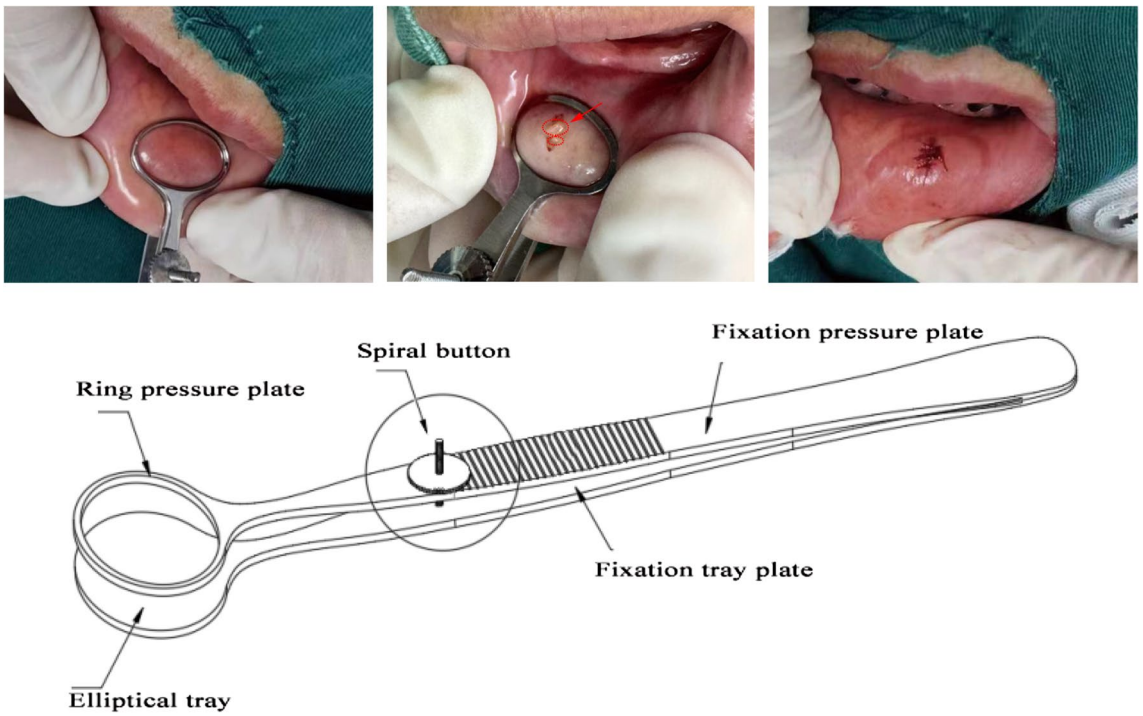

Fig. 1 Schematic of the procedure using chalazion forceps-assisted labial salivary gland biopsy (Red arrow: salivary glands)

Dryness symptoms and glandular dysfunction

At the time of biopsy, the patients were asked whether they had symptoms of dry mouth or dry eyes. According to the inclusion criteria of the 2016 ACR-EULAR: patients with at least one of the symptoms of dry eyes or dry mouth, i.e., those who satisfied at least one of the five descriptions, were considered positive. The Schirmer I test (SIT) and tear film break-up time (BUT) were used to test for dry eyes, and salivary gland emission computed tomography (ECT) was used to evaluation of salivary gland dysfunction. The definition of glandular dysfunction was as follows: SIT <5 mm within 5 min using standardized sterile test paper (Ophtalmos, Sao Paulo, Brazil), and/or tear film rupture occurring within 10 seconds using 0.125% fluorescein solution, and/or salivary glands not sensitive to acidic substance stimulation during dynamic imaging, no radioactivity or only a small amount of radioactivity in the oral cavity, and no decrease in the time–radioactivity curve and no increase in the oral curve after acidic substance stimulation. The above evaluations were performed by a physician specializing in radiology [16].

Clinical parameters

Information on patient demographics, autoantibodies, and clinical manifestations was obtained from medical reports. The autoantibodies evaluated were anti-nuclear antibodies (ANA) (note that ANA ≥1:320 was defined as positive), which were measured using indirect immunofluorescence. Rheumatoid factor (RF) (RF>20 IU/ml was defined as positive) was measured by the turbidimetric method, and anti-Ro/Sjögren syndrome-antigen A (anti-Ro/SSA), anti-La/Sjögren syndrome-antigen B (anti-La/SSB), anti-Ro52 autoantibodies (anti-RO52), anti-major centromere autoantigen (anti-CENP)-B and anti-centromere antibodies (anti-ACA) were detected using an ANA Test Kit. The detections were performed strictly according to the instruction manual, and the results were evaluated using the EUROLineScan software. These antibodies were evaluated using only the European immunoassay. The intensity of each reaction with positive and negative controls was indicated as follows: "-", "+", "++" and "+++" (if the assays all showed a signal of "+", "++" or "+++", autoantibodies were considered to be present).

The surgical duration of traditional LSGB was the time from the start of local anaesthesia to the end of suturing (in minutes). The duration of modified LSGB using the chalazion forceps ranged from the start of chalazion forceps use before local anaesthesia to the end of suturing (in minutes). Only one piece of gauze was used for haemostasis during the operation. Postoperative bleeding was estimated based on the final weight of the gauze (g) minus the starting weight of the gauze. Incision pain at 24 h after surgery was evaluated by an oral interview (such as a telephone interview) using the 0–10 numerical rating scale (NRS). There

were four score strata: no pain: 0; mild pain: 1–3; moderate pain: 4–6; and severe pain: 7–10 [17]. The status of incision healing 7 days after surgery was divided into grades A, B, and C according to the degree of incision healing after suture removal. Grade A healing referred to initial healing with an excellent outcome and no adverse reactions. Grade B healing referred to poor healing; inflammatory reactions, such as swelling, induration, haematoma, and effusion, occurred at the healing site, but there was no suppuration. Grade C healing referred to incision suppuration requiring incision and drainage.

The surface of the glands was evaluated using an Olympus BX50 microscope. The diameter d of the actual field of view (FOV) was 22 (number of FOVs)/10 (magnification of the objective lens) = 2.2 mm, and the area s of the actual FOV was $\pi r^2 = \pi 1/4d^2 \approx 4$ (mm^2) [18]. According to this formula, if 4× (magnification of the objective lens) was used, the diameter d of the actual FOV=5.5 mm, and the area s of the actual FOV ≈ 24 (mm^2).

According to the 2016 ACR-EULAR classification criteria for pSS, any patient meeting the following conditions can be diagnosed with SS: satisfying one or more of the inclusion criteria, not satisfying any exclusion criterion, total score on the five items ≥ 4 points, and an expert opinion of pSS positivity [4]. If the patient met the diagnosis of SS and had underlying disease (such as any connective tissue disease), the patient was classified as having secondary Sjögren syndrome (sSS); otherwise, the patient was classified as having pSS [2].

Statistical analysis and ethical issues

SPSS 27.0 software was applied for statistical analysis. The t test was used for comparison between groups of normally distributed measures, the Mann–Whitney rank sum test was used for comparison between groups of non-normally distributed measures, and the chi-square test and Fisher's exact probability method were used for comparison between groups of qualitative data for statistical analysis. $P < 0.05$ was considered to indicate a statistically significant difference. This study was approved by the Ethics Committee for Clinical Application of Medical Technologies of Zunyi Medical University (approval number: 20210135).

Results

Clinical characterisation of patients with two surgical procedures

The traditional and modified LSGB groups had a similar age distribution, sex, dry mouth and/or dry eye rate, anti-ANA titre, anti-Ro/SSA, anti-La/SSB, anti-RO52, anti-CENP B and/or anti-ACA, systemic involvement (joint pain, nerve, glandular enlargement), combined thyroid disease, RF, immunoglobulin G (IgG), complement C3, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), white blood cell count (WBC), platelet count (PLT), alanine aminotransferase (ALT) and/or aspartate aminotransferase (AST), FS ≥ 1, SIT and/or BUT, salivary gland ECT ($p > 0.05$). The incision length, operative time, amount of blood loss, 24 h pain score, and Grade A incision healing at 7 days after surgery in the modified group were better than those in the traditional group ($p < 0.01$). There was one case of gland collection failure in each of the two groups. The diameter of the glands collected (≥ 2 mm) and the surface area of the glands (≥ 8 mm^2) were not significantly different ($p > 0.05$), but the traditional group had significantly more glands (≥ 3) ($p < 0.01$). The two groups had similar proportions of individuals diagnosed with pSS ($p > 0.05$). The complete data are shown in Table 1.

Clinical characterisation of patients with and without pSS

For the clinical indicators with statistically significant differences shown in Table 1, the influence of sSS needed be excluded. Further analysis of the clinical parameters of the groups with and without pSS revealed that these groups differed in the rate of dry mouth, the rate of dry eyes, the rate of anti-ANA titre ≥ 320, anti-Ro/SSA, anti-La/SSB, anti-RO52, RF, the WBC count, the proportion of patients with FS ≥ 1, incision length, and the number of glands (≥ 3) ($p < 0.05$). There was no significant difference in surgical methods, interstitial lung disease (ILD), Serum creatinine (Scr), 24 h urine protein quantification between the pSS group and the non-pSS group ($p > 0.05$). The complete data are shown in Table 2.

3.3 Analysis of factors influencing primary Sjögren's syndrome

Univariable and multivariable logistic regression analyses revealed that sex, age, and surgical method were not independent predictive factors for the diagnosis of pSS ($p > 0.05$). Univariable analysis showed that the number of glands (≥ 3) (odds ratio (OR) = 2.431, 95% confidence interval (CI) 1.347-4.386, $p < 0.01$), incision length (OR 3.983, 95% CI 1.569-10.112, $p < 0.01$), and anti-ANA titre ≥ 1:320 (OR 8.75, 95% CI 3.4232-22.31, $p < 0.01$) were independent predictors for the diagnosis of pSS. Other variables that were determined to be significantly different above were still associated with the diagnosis of pSS ($p < 0.05$). Multivariable logistic regression analysis revealed that only dry eye (OR 11.233, 95% CI 1.579-79.914, $p < 0.05$) and anti-ANA titre ≥ 1:320 (OR

Table 1 Clinical characterisation of patients with two surgical procedures

	Traditional LSGB group		Modified LSGB group		p value
N total	217				n/a
N (%)	92 (42.4%)		125 (57.6%)		n/a
Female, N (%)	86 (93.5%)		113 (90.4%)		0.417
Age at diagnosis, mean ± sd [y]	50.2 ± 13.94		51.9 ± 10.75		0.269
Dry mouth	60 (65.2%)		89 (71.2%)		0.348
Dry eye	31 (33.7%)		52 (41.6%)		0.236
anti-ANA titre ≥ 1:320	75 (81.5%)		101 (80.8%)		0.893
anti-Ro/SSA (+)	53 (57.6%)		65 (52%)		0.412
anti-La/SSB(+)	20 (21.7%)		29 (23.2%)		0.939
anti-RO52 (+)	51 (55.4%)		72 (57.6%)		0.75
anti-CENP Band/or anti-ACA (+)	14 (15.2%)		29 (23.2%)		0.145
Systemic involvement					
joint pain	30 (32.6%)		46 (36.8%)		0.522
ILD	19 (20.7%)		46 (36.8%)		< 0.01
nerve	2 (2.2%)		8 (6.4%)		0.196
Glandular enlargement	1 (1.1%)		1 (0.8%)		1
combined thyroid disease	18 (19.6%)		28 (22.4%)		0.614
	Available	Pathological, *N* (%)	Available	Pathological, *N* (%)	
RF > 20I u/ml	87	21 (24.1%)	103	40 (38.8%)	0.32
IgG > 15.6 mg/dL	75	25 (33.3%)	118	54 (45.8%)	0.087
Complement C3 < 0.79 g/L	75	25 (33.3%)	118	35 (29.7%)	0.591
ESR > 38 mm/h and/or CRP > 8.2 mg/L	82	25 (30.5%)	113	44 (38.9%)	0.494
WBC < 4.00×10^9/L	87	18 (20.7%)	123	18 (14.6%)	0.251
PLT < 100×10^9/L	87	4 (4.6%)	123	4 (3.3%)	0.616
AST > 35 IU/L and/or ALT > 40 IU/L	87	15 (17.2%)	122	23 (18.9%)	0.766
Scr > 90 umol/L	85	5 (5.9%)	119	22 (18.5%)	< 0.01
urine protein quantification > 0.5 g/24 h	88	7 (8.0%)	123	2 (1.6%)	< 0.05
SIT ≤ 5 mm/5 min and/or BUT < 10 s	20	19 (1.0%)	44	43 (0.98%)	0.561
salivary gland ECT (+)	33	26 (78.8%)	27	24 (88.9%)	0.296
FS ≥ 1	91	52 (56.5%)	124	58 (46.4%)	0.141
gland collection failure	92	1 (1.1%)	125	1 (0.7%)	0.827
incision length, median (IQR) [mm]	92	1 (1,1.2)	125	0.7 (0.4,0.7)	< 0.01
operative time,median (IQR) [m]	92	5.15 (4.47,5.48)	125	4.56 (4.14,5.17)	< 0.01
amount of blood loss,median (IQR) [g]	92	0.18 (0.15,0.22)	125	0.04 (0.03,0.05)	< 0.01
24 h pain score,median (IQR)	92	3 (2,3)	125	2 (1,2)	< 0.01
grade A healing of incisions	92	76 (83.0%)	125	125 (100%)	< 0.01
Number of glands (≥ 3)	91	74 (81.3%)	124	30 (24.2%)	< 0.01
surface area of the glands(≥ 8mm^2)	91	89 (97.8%)	124	114 (91.9%)	0.064
diameter of the glands(≥ 2 mm)	91	89 (97.8%)	124	114 (91.9%)	0.064
Expert opinion final diagnosis					
pSS	51 (55.4%)		53 (42.4%)		0.058
sSS	9 (9.8%)		18 (14.4%)		0.308
Other diseases	32 (34.8%)		54 (43.2%)		0.21

N, number; y, years; mm, millimeter; m, minute; g, Gram weight; IQR, interquartile range; n/a, not applicable; sd, standard deviation

19.783, 95% CI 1.326-295.084, $p < 0.05$) and FS ≥ 1 (OR 1986.159, 95% CI 97.154-40603.688, $p < 0.01$)) were independent predictors for the diagnosis of pSS. The complete analysis is shown in Table 3.

Accuracy analysis of LSGB pathological results in different surgical methods

To analyse the accuracy of the diagnosis of pSS based on the LSGB pathological results from different surgical methods,

Table 2 Clinical characterisation of patients with and without pSS

	pSS		Non pSS		*p* value
N total	190				n/a
N (%)	104		86		n/a
Female, N (%)	99 (95.2%)		76 (88.4%)		0.083
Age at diagnosis, mean ± sd [y]	52.06 ± 13.11		50.42 ± 11.68		0.369
Dry mouth	86 (45.3%)		46 (53.5%)		< 0.01
Dry eye	54 (51.9%)		21 (24.4%)		< 0.01
anti-ANA titre ≥ 1:320	98 (94.2%)		56 (65.1%)		< 0.01
anti-Ro/SSA (+)	71 (68.3%)		32 (37.2%)		< 0.01
anti-La/SSB (+)	36 (34.6%)		8 (9.3%)		< 0.01
anti-RO52 (+)	74 (71.2%)		35 (40.7%)		< 0.01
anti-CENP B and/or anti-ACA (+)	24 (23.1%)		19 (22.1%)		0.872
joint pain	32 (30.8%)		23 (26.7%)		0.543
ILD	28 (26.9%)		28 (32.6%)		0.396
combined thyroid disease	21 (20.2%)		17 (19.8%)		0.942
surgical procedure					
Traditional LSGB group	51 (49.0%)		32 (37.2%)		0.102
Modified LSGB group	53 (51.0%)		54 (62.8%)		
	Available	Pathological, *N* (%)	Available	Pathological, *N* (%)	
RF > 20I u/ml	82	31 (37.8%)	62	12 (19.4%)	< 0.05
IgG > 15.6 mg/dL	99	42 (42.4%)	68	22 (32.4%)	0.188
Complement C3 < 0.79 g/L	98	31 (31.6%)	67	21 (31.3%)	0.997
ESR > 38 mm/h and/or CRP > 8.2 mg/L	98	35 (35.7%)	70	23 (32.9%)	0.701
WBC < 4.00 × 109/L	101	24 (23.8%)	82	9 (11.0%)	0.025
PLT < 100 × 109/L	101	6 (5.9%)	82	1 (1.2%)	0.132
Scr > 90 umol/L	97	12 (12.4%)	80	9 (11.3%)	0.818
urine protein quantification > 0.5 g/24 h	103	4 (3.9%)	82	3 (3.7%)	1
SIT ≤ 5 mm/5 min	33	30 (90.9%)	7	6 (85.7%)	0.552
salivary gland ECT (+)	39	34 (87.2%)	14	10 (71.4%)	0.222
FS ≥ 1	103	87 (84.5%)	85	3 (3.53%)	< 0.01
incision length	104	1 (0.5,1.0)	86	0.5 (0.4,1)	< 0.01
Number of glands (≥ 3)	103	60 (58.3%)	85	31 (36.5%)	< 0.05
surface area of the glands (≥ 8 mm^2)	103	99 (96.1%)	85	77 (90.6%)	0.123
diameter of the glands (≥ 2 mm)	103	99 (96.1%)	85	77 (90.6%)	0.123

after excluding 27/217 patients with sSS, the other 190 patients were studied. Among these 190 patients, two had failed LSGB(1 case of pSS by expert opinion in the traditional surgery group and 1 case without SS in the modified group), and the diagnosis of SS in 85 cases was ruled out by expert assessment. In the traditional surgical group, 44 patients had positive biopsy results (FS ≥ 1) (according to expert opinion, all 44 of them had pSS); 38 patients had negative biopsy results (FS < 1) (according to expert opinion, six of them had pSS and 32 did not have SS). The sensitivity of traditional LSGB pathological results to the diagnosis of pSS was 88.00%, and the specificity was 100%. The positive predictive value (PPV) was 100%, and the negative predictive value (NPV) was 84.21%. The accuracy of the diagnosis was 92.68%. In the modified surgical group, 46 patients had positive biopsy results (FS ≥ 1) (according to expert opinion, 43 of them had pSS, and three patients did not have SS); 60 patients had negative biopsy results (FS < 1) (according to expert opinion, 10 of them had pSS, and 50 did not have SS). The sensitivity of modified LSGB pathological results to the diagnosis of pSS was 81.13%, and the specificity was 94.34%. The PPV was 93.48%, and the NPV was 83.33%. The accuracy of the diagnosis was 87.74%.

3.5 Evaluation of the consistency between LSGB pathological positive results and expert opinions and diagnostic criteria

According to the pSS classification standard in the 2016 ACR-EULAR, complete data for patients with sSS and patients with pSS (final diagnosis) are shown in Table 4. In the traditional

Table 3 Analysis of factors influencing diagnostic results in 190 patients

Characteristic	Univariable analysis OR [95% CI]	*P* value	Multivariable analysis OR [95% CI]	*P* value
Female	2.605 [0.855–7.94]	0.092	0.831 [0.057–12.099]	0.893
Age at diagnosis	1.001 [0.988–1.034]	0.367	0.967 [0.908–1.030]	0.302
Dry mouth	4.155 [2.144–8.051]	< 0.01	1.946 [0.262–14.436]	0.515
Dry eye	3.343 [1.79–6.242]	< 0.01	11.233 [1.579–79.914]	< 0.05
anti-ANA titre ≥ 1:320	8.75 [3.432–22.31]	< 0.01	19.783 [1.326–295.084]	< 0.05
anti-Ro/SSA (+)	3.631 [1.99–6.625]	< 0.01	0.624 [0.096–4.057]	0.621
anti-La/SSB (+)	5.162 [2.246–11.864]	< 0.01	3.748 [0.449–31.261]	0.222
RF (+)	2.533 [1.17–5.482]	< 0.05	n/a	n/a
anti-RO52 (+)	3.594 [1.964–6.577]	< 0.01	1.83 [0.312–10.740]	0.503
WBC < 4.00 × 109/L	2.528 [1.102–5.8]	< 0.05	6.643 [0.854–51.643]	0.07
FS ≥ 1	148.628 [41.76–528.957]	< 0.01	1986.159 [97.154–40,603.688]	< 0.01
Number of glands (≥ 3)	2.431 [1.347–4.386]	< 0.01	0.526 [0.073–3.811]	0.525
incision length	3.983 [1.569–10.112]	< 0.01	25.176 [0.548–1156.590]	0.099
Modified LSGB surgical method	0.616 [0.344–1.102]	0.103	1.778 [0.124–25.597]	0.672

Table 4 Diagnosed with pSS according to the 2016 ACR-EULAR

	Traditional LSGB group	Pathological, N (%)	Modified LSGB group	Pathological, N (%)	*p* value
satisfying one or more of the inclusion criteria	92	61 (66.3)	125	95 (76)	0.116
not satisfying any exclusion criterion	92	92 (100)	125	125 (100)	n/a
SIT ≤ 5 mm/5 min (1 point)	17	16 (94.1)	29	25 (86.2)	0.637
unstimulated whole saliva flow rate ≤ 0.1 ml/min (1 point)	92	0 (0)	125	0 (0)	n/a
anti-Ro/SSA (+) (3 points)	92	53 (57.6)	125	65 (52.0)	0.412
FS ≥ 1 foci/4 mm^2 (3 points)	91	52 (56.5)	124	58 (46.4)	0.141
Average score M(P25, P75)	91	3 (3,6)	124	3 (0,6)	0.213
A total score of ≥ 4 points	92	40 (43.5)	125	51 (40.8)	0.693
Diagnosed with sSS	92	9 (9.8)	125	18 (14.4)	0.308
Diagnosed with pSS	83	33 (40.0)	107	42 (39.3)	0.943
Expert opinion diagnosis pSS	83	51 (55.4)	107	53 (42.4)	0.058

group, the consistencies of positive biopsy results (FS ≥ 1) with both expert opinion and ACR-EULAR diagnostic criteria were high and significant ($p < 0.05$), but the consistency with expert opinion (kappa = 0.851) was higher than that with ACR-EULAR criteria (kappa = 0.591). In the modified group, the consistencies of positive biopsy results (FS ≥ 1) with both expert opinion and ACR-EULAR diagnostic criteria were high and significant ($p < 0.05$), but the consistency with expert opinion (kappa = 0.805) was higher than that with ACR-EULAR criteria (kappa = 0.728).

Discussion

The diagnosis of SS is challenging because of its complicated clinical manifestations and nonspecific signs. LSGB plays an important role in the diagnosis of SS, especially in patients who are negative for anti-SSA and anti-SSB antibodies. The current traditional LSGB surgical methods have the following deficiencies: (1) there is no fixed device, and an assistant is needed to fix the lower lip; due to the slippery mucosa of the lip, the fixation by hand can

easily become unstable; (2) intraoperative bleeding may occur due to the abundant blood supply of the lip, which can affect the surgical field of vision; (3) the incision is large and prone to complications such as infection, suture dehiscence, granulomas, keloids, and lip numbness; (4) in some patients, the lip gland atrophies, which is not conducive to finding the cause of surgical failure. To address the above technical issues, in this study we proposed an improved LSGB technique using a chalazion clamp, which has the following advantages: (1) the fixed pressure plate and fixed tray plate can be inserted and fixed directly from the inner and outer sides of the patient's lower lip, with simple and quick operation and stable fixation [10]; (2) Ring pressure plate is conducive to hemostasis, exposing the gland, and maintaining a clear surgical field; (3) the elliptical tray is conducive to lifting the gland upward and only needs to open the mucosal superficial layer to expose the existing gland; (4) the front part of the fixed pressure plate is equipped with an adjustable spiral button, which can adjust the appropriate pressure and tightness according to the thickness of the patient's lip, with strong controllability. (5) The chalazion forceps have three sizes, and the elliptical hollow metal ring pressure plate at the front end of the device has three sizes (12 mm × 21 mm, 10 mm × 17 mm, 11 mm × 11 mm), which is conveniently chosen based on the size of the incision. In view of the dispersion of the lesions, it is recommended to obtain at least four LSGs; three respondents have advocated the use of fewer glands (two to three), and two respondents recommend using a greater number of glands (five to seven) [15].

This study showed that compared with the traditional surgery group, the modified surgery group had a smaller incision, less bleeding, shorter operative time, milder postoperative pain, and better incision healing at 7 days after surgery. The results also revealed that the LSGB pathological results in the different surgical method groups were similar; there was no significant difference in the diagnostic rate of pSS between the two groups. There were significant differences in some clinical parameters between the modified and traditional groups, such as ILD, Scr, and urine protein quantification, and consequently, these indicators should be excluded to avoid biasing the final diagnosis. This study excluded sSS patients from the follow-up analysis. Among them were 19 patients with rheumatoid arthritis (RA), five patients with systemic lupus erythematosus (SLE), one patient with systemic sclerosis (SSC), one patient with antineutrophil cytoplasm antibody (ANCA)-associated vasculitis, and one patient with Behçet's disease. Any of these diseases may affect some clinical parameters. The prevalence of sSS is 5–30% in RA patients and 14–17.8% in SLE patients [19, 20]. Our results showed that there was no significant difference in ILD, Scr, or 24 h urine protein quantification between the pSS group and the non-pSS group. Moreover, the incision length and the number of glands were significantly increased in the pSS group compared with the non-PSS group ($p < 0.05$), and the univariate analysis showed that the incision length and the number of glands were independent risk factors affecting the diagnosis of pSS; therefore, an incision length of approximately 1 cm and a number of glands ≥ 3 were significantly correlated with the diagnosis of pSS. However, according to multivariable analysis, only FS ≥ 1, dry eyes and an anti-ANA titre ≥ 1:320 were significantly associated with the diagnosis of pSS [21].

The accuracy of LSGB pathological results from different surgical methods for the diagnosis of pSS showed that the sensitivity of LSGB from the two surgical methods was 81.13–88.00%, and the specificity was 94.34–100%. The accuracy of the diagnosis was 87.74–92.68%, which was consistent with earlier results [22]. The LSGB results of the two surgical methods were in good agreement with the expert opinions and the standard SS classification.

None of our enrolled patients experienced major complications, which is consistent with the findings of previous studies, indicating that the modified surgical method is a safe technique [23]. In the traditional surgery group, when the stitches were removed 7 days after surgery, 17.4% (16/92) patients had partial incision dehiscence, local mucosal congestion, swelling, and pain; however, mucosal healing can take up to 3 weeks. In the LSGB technique, the use of either loupes or a small operating microscope to assist in the biopsy has not been reported. Tsesis et al. suggested that the use of a small operating microscope in dentistry can achieve higher accuracy [24]. Whether the use of either loupes or a small operating microscope in the modified LSGB procedure can significantly improve the success rate of biopsies should be studied.

Conclusion

In summary, the small-incision technique of using auxiliary chalazion forceps collects more superficial glands with a small incision, which may explain the low prevalence of incision complications. It is preferable to take ≥ 3 glands to increase the positivity rate while ensuring that the body surface area and diameter of the glands are large enough. The sample of this study was small; therefore, case–control studies with large samples would reduce any bias in the findings.

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Data availability The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Declarations

Conflict of interest The authors declare no conflict of interest.

References

1. Qin B, Wang J, Yang Z, et al. Epidemiology of primary Sjogren's syndrome: a systematic review and meta-analysis. Ann Rheum Dis. 2015;74(11):1983–9. https://doi.org/10.1136/annrheumdis-2014-205375.
2. Vitali C, Bombardieri S, Jonsson R, et al. Classification criteria for Sjögren's syndrome: a revised version of the European criteria proposed by the American-European consensus group. Ann Rheum Dis. 2002;61:554–8. https://doi.org/10.1136/ard.61.6.554.
3. Shiboski SC, Shiboski CH, Criswell L, et al. American college of rheumatology classification criteria for Sjögren's syndrome: a data-driven, expert consensus approach in the Sjögren's international collaborative clinical alliance cohort. Arthr Care Res. 2012;64:475–87. https://doi.org/10.1002/acr.2159.
4. Shiboski CH, Shiboski SC, Seror R, et al. International Sjögren's syndrome criteria working, 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. Arthr Rheumatol. 2017;69:35–45. https://doi.org/10.1002/art.39859.
5. Brito-Zeron P, Baldini C, Bootsma H, et al. Sjogren syndrome Nat Rev Dis Primers. 2016;7(2):16047. https://doi.org/10.1038/nrdp.2016.47.
6. Vitali C, Moutsopoulos HM, Bombardieri S. The European community study group on diagnostic criteria for Sjogren's syndrome. sensitivity and specificity of tests for ocular and oral involvement in Sjogren's syndrome. Ann Rheum Dis. 1994;53:637–47. https://doi.org/10.1136/ard.53.10.637.
7. Varela Centelles P, Sánchez-Sánchez M, Costa-Bouzas J, et al. Neurological adverse events related to lip biopsy in patients suspicious for Sjögren's syndrome: a systematic review and prevalence meta-analysis. Rheumatology (Oxford). 2014;53(7):1208–14. https://doi.org/10.1093/rheumatology/ket485.
8. Jin X, Fan F, Zhang F, et al. A treatment method for chronic suppurative lacrimal canaliculitis using chalazion forceps. Indian J Ophthalmol. 2016;64(8):589–92. https://doi.org/10.4103/0301-4738.191506.
9. He J, Jiang J, Bao L, Gong J, et al. Use of chalazion clamp for excision of the eyelid margin lesion. Eur J Ophthalmol. 2024;34(2):594–7. https://doi.org/10.1177/11206721231218032.
10. Jeng PY, Chang MC, Chiang CP, et al. Oral soft tissue biopsy surgery: Current principles and key tissue stabilization techniques. J Dent Sci. 2024 Jan;19(1):11–20. https://doi.org/10.1016/j.jds.2023.09.015.
11. Varela-Centelles P, Seoane-Romero JM, Sánchez-Sánchez M, et al. Minor salivary gland biopsy in Sjögren's syndrome: a review and introduction of a new tool to ease the procedure. Med Oral Patol Oral Cir Bucal. 2014;19(1):e20–3. https://doi.org/10.4317/medoral.19131.
12. Jeng PY, Chang MC, Chiang CP, et al. Oral soft tissue biopsy surgery: current principles and key tissue stabilization techniques. J Dent Sci. 2024;19(1):11–20. https://doi.org/10.1016/j.jds.
13. Wijaya C, Ramli RR, Khoo SG. Dry surgical field minor salivary gland harvest using a chalazion clamp for sicca syndrome. J Laryngol Otol. 2019;133(5):419–23. https://doi.org/10.1017/S0022215119000077X.
14. Greenspan JS, Daniels TE, Talal N, et al. The histopathology of Sjogren's syndrome in labial salivary gland biopsies. Oral Surg Oral Med Oral Pathol. 1974;37:217–29. https://doi.org/10.1016/0030-4220(74)90417-4.
15. Fisher BA, Jonsson R, Daniels T, et al. Standardisation of labial salivary gland histopathology in clinical trials in primary Sjogren's syndrome. Ann Rheum Dis. 2017;76:1161–8. https://doi.org/10.1136/annrheumdis-2016-210448.
16. Chen YC, Chen HY, Hsu CH. Recent advances in salivary scintigraphic evaluation of salivary gland function. Diagnostics (Basel). 2021;11(7):1173. https://doi.org/10.3390/diagnostics11071173.
17. Farrar JT, Young JP, LaMoreaux L, et al. Clinical importance of changes in chronic pain intensity measured on an 11-point numerical pain rating scale[J]. Pain. 2001;94(2):149–58. https://doi.org/10.1016/S0304-3959(01)00349-9.
18. MiaoLi Z. How to calculate the field of view under a microscope. J Diagn Pathol. 2009;16(1):1007–8096.
19. Park Y, Oh M, Lee YS, et al. Salivary ultrasonography and histopathologic evaluation of secondary Sjögren's syndrome in rheumatoid arthritis patients. Sci Rep. 2023;13(1):11339. https://doi.org/10.1038/s41598-023-38469-z.
20. Yao Q, Altman RD, Wang X. Systemic lupus erythematosus with Sjögren syndrome compared to systemic lupus erythematosus alone: a meta-analysis. J Clin Rheumatol. 2012;18(1):28–32. https://doi.org/10.1097/RHU.0b013e31823ecbdf.
21. Stefanski AL, Tomiak C, Pleyer U, et al. The diagnosis and Treatment of Sjögren's Syndrome. Dtsch Arztebl Int. 2017;114(20):354–61. https://doi.org/10.3238/arztebl.2017.0354.
22. Guellec D, Cornec D, Jousse-Joulin S, et al. Diagnostic value of labial minor salivary gland biopsy for Sjogren's syndrome: a systematic review. Autoimmun Rev. 2013;12:416–20. https://doi.org/10.1016/j.autrev.2012.08.001.
23. Lida Santiago M, Seisdedos MR, García Salinas RN, et al. Frequency of complications and usefulness of the minor salivary gland biopsy. Reumatol Clin. 2012;8(5):255–8. https://doi.org/10.1016/j.reuma.2012.03.006.
24. Bud M, Jitaru S, Lucaciu O, et al. The advantages of the dental operative microscope in restorative dentistry. Med Pharm Rep. 2021 Jan; 94(1):22–27. https://doi.org/10.15386/mpr-1662