



CLINICAL STUDY

A RELIABLE TECHNIQUE FOR MINOR SALIVARY GLAND BIOPSY: A SAFE AND EFFECTIVE METHOD FOR DIAGNOSING SJÖGREN'S SYNDROME IN SERONEGATIVE PATIENTS

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SUMMARY

Aims: To describe a safe and standardized technique for performing minor salivary gland biopsy and to highlight its diagnostic value in patients with seronegative Sjögren's syndrome.

Material and Methods: Medical records of 319 patients who presented to the Rheumatology Clinic at Izmir Bakırçay University Faculty of Medicine Çigli Training and Research Hospital between January 2020 and July 2024 were retrospectively reviewed. These patients had undergone serological testing for suspected SS and were subsequently referred to the ENT Department for minor salivary gland biopsy.

Results: Among the patients, 43 (13.4%) had negative serological results but exhibited MSGB scores of 3 or higher. SS was directly diagnosed in 92 patients (28.8%) based on Chisholm-Mason scores of 3 or 4. Additionally, some of the 167 patients (52.3%) with scores of 1 or 2 were diagnosed with SS based on supplementary clinical findings, leading to the initiation of treatment. MSGB findings contributed to the diagnosis in more than half of the suspected SS cases. Follow-up examinations one month post-biopsy revealed that most patients did not experience adverse symptoms such as pain, numbness, burning, tingling, or swelling.

Conclusion: Our study underscores the critical role of MSGB in diagnosing Sjögren's syndrome, particularly in seronegative patients. Given its safety and importance, MSGB remains a reliable and essential method for identifying SS.

Keywords: Sjögren's syndrome, minor salivary gland biopsy, complications

MİNÖR TÜKÜRK BEZİ BİYOPSİSİ İÇİN GÜVENİLİR BİR TEKNİK: SERONEGATİF HASTALarda SJÖGREN SENDROMUNUN TANISINDA GÜVENLİ VE ETKİLİ BİR YÖNTEM

ÖZET

Amaç: Minör tükürük bezı biyopsisinin (MTBB) güvenli ve standart bir tekniğini tanımlamak ve seronegatif Sjögren sendromu (SS) hastalarında tanışal değerini vurgulamak.

Gereç ve Yöntemler: Ocak 2020 ile Temmuz 2024 arasında İzmir Bakırçay Üniversitesi Tıp Fakültesi Çigli Eğitim ve Araştırma Hastanesi Romatoloji Kliniği'ne başvuran 319 hastanın tıbbi kayıtları retrospektif olarak incelendi. Bu hastalarda SS şüphesiyle serolojik testleri yapılmış ve sonrasında minör tükürük bezı biyopsisi için KBB Kliniği'ne yönlendirilen hastaların dosyaları retrospektif olarak incelenmiştir.

Bulgular: Hastaların 43'ünde (%13,4) serolojik sonuçlar negatif olmasına rağmen MTBB skorları 3 ve üzerindeydi. Chisholm-Mason skorları 3 veya 4 olan 92 hastada (%28,8) doğrudan SS tanısı kondu. Ayrıca skor 1 veya 2 olan 167 hastanın (%52,3) bir kısmına ek klinik bulgulara dayanarak SS tanısı kondu ve tedavi başlatıldı. MTBB bulguları, şüpheli SS olgularının yarısından fazlasının tanısına katkı sağladı. Biyopsi sonrası bir aylık takipterde çoğu hastada ağrı, uyuşma, yanma, karınçalanma veya şişlik gibi advers semptomlar görülmeli.

Sonuç: Çalışmamız, Sjögren sendromunun özellikle seronegatif hastalarda tanısında MTBB'nin kritik rolünü ortaya koymaktadır. Güvenilirliği ve önemi göz önünde bulundurulduğunda, MTBB SS'nin saptanmasında güvenli ve vazgeçilmez bir yöntem olmaya devam etmektedir.

Anahtar Sözcükler: Sjögren Sendromu, minör tükürük bezı biyopsi, komplikasyon

INTRODUCTION

Sjögren syndrome (SS) is an autoimmune disease characterized by inflammation of the exocrine glands, predominantly causing xerostomia and/or xerophthalmia and, less commonly, systemic symptoms. In the United States, an average of 5.8 out of 100,000 people are diagnosed with SS each year, the majority of

whom are women. When the disease is suspected, definitive diagnosis is based on a combination of serologic evidence, oral and ocular secretion studies, and pathologic findings^{1,2}. In addition to the lacrimal and salivary glands, the disease can also affect other exocrine glands: the lungs, kidneys, and blood vessels. The presence of the disease alone is defined as primary SS (pSS), while the presence of another autoimmune disease is defined as secondary SS (sSS)³.

SS is diagnosed based on the criteria accepted by the American-European Consensus Group in 2002. These are the presence of ocular and oral dryness combined with objective evidence of autoimmunity with a positive minor salivary gland biopsy (>1 focus count), positive

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anti-Sjögren syndrome-associated antigen A (anti SS-A/Ro) or anti-Sjögren syndrome-associated antigen B (anti SS-B/La) antibodies, and the presence of keratoconjunctivitis sicca with a positive Schirmer test with <5 mm wetting of the filter paper in 5 minutes^{4,5}.

Since the diagnosis of SS is challenging, multiple diagnostic tools are typically required. Minor salivary gland biopsy (MSGB) plays a crucial role, particularly in diagnosing seronegative SS patients^{6,7}. The MSGB procedure is relatively simple and can be performed with local anesthesia on an outpatient basis⁸. The most common complication after a minor salivary gland biopsy is numbness. In addition, complications such as minor bleeding, hematoma, local infection, premature cessation of sutures, keloid, and granuloma formation may also occur⁹. The MSGB is undoubtedly important for the classification, diagnosis, and prognosis of SS. Three main classification systems, proposed by Chisholm and Mason, Greenspan and Daniels, and Tarpley, are still widely used for interpreting biopsy results⁶. In their original study, Derrick Chisholm and David Mason obtained MSGB samples from 40 patients and 60 cadavers. According to this study, the classification of Chisholm and Mason, defined in 1968, uses a 5-grade classification from 0 to 4 based on the presence of mild or moderate lymphocytic infiltration and/or lymphocytic foci.

The Chisholm and Mason scoring system evaluates the degree of lymphocytic infiltration per 4 mm² of salivary tissue as follows:

- Grade 0: No infiltration
- Grade 1: Mild infiltration
- Grade 2: Moderate infiltration or multiple foci
- Grade 3: A single focus
- Grade 4: Multiple foci

A focus is defined as an accumulation of 50 or more lymphocytes per 4 mm². Chisholm and Mason observed that all patients with salivary duct antibodies had a positive biopsy (grade 3 or 4). They concluded that MSGB could serve as a valuable diagnostic tool for Sjögren's syndrome¹⁰. Since then, this grading system has been widely used by pathologists; it had a sensitivity of 72.1% and a specificity of 80% in a study for SS, respectively¹¹.

In light of this information, this study aims to describe a safe and standardized technique for performing minor salivary gland biopsy and to highlight its diagnostic value in patients with seronegative Sjögren's syndrome.

MATERIAL and METHODS

The medical records of 319 patients who went to the Rheumatology Clinic at the Izmir Bakircay University Hospital, had serological tests that led to a preliminary diagnosis of SS, and were then sent to the ENT Department for a minor salivary gland biopsy between January 2020 and July 2024 were looked at retrospectively. This study was approved by the local clinical research ethics committee in Izmir Bakircay University. This study was conducted according to the World Medical Association Declaration of Helsinki and all the patients signed informed consent forms for inclusion to the study.

From the blood tests, the anti-nuclear antibody (ANA), anti-Sjögren syndrome-associated antigen A (Anti SSA/Ro), anti-Sjögren syndrome-associated antigen B (Anti-SSB), and rheumatoid factor (RF) values were tracked. We recorded the results of the patients who underwent ophthalmology consultation and the Schirmer test. Under local anesthesia, the same technique and procedure were used on all patients for a minor salivary gland biopsy. An incision was made about 3 cm from the lower lip mucosal surface to the vermillion line, as shown in Figure 1 (Lower lip minor salivary gland biopsy procedure). The procedure involved stretching the lower lip outward. Apply 0.5-1 ml of 1% lidocaine and 1/100000 adrenaline to the submucosa. Then, a superficial incision of approximately 2 cm parallel to the vermillion border is made with a number 15 scalpel. We use blunt dissection to separate the minor salivary glands from their fascia, working parallel to the sensitive nerves. After at least 4 minor salivary gland tissues are removed, the incision is closed by primary suturing with 4/0 Vicryl. Tissue samples were placed in formalin and forwarded to pathology, where a single experienced head and neck pathologist examined all specimens to ensure diagnostic consistency. A pathologist with expertise in Sjögren's syndrome evaluated the biopsy specimens, determined the number of



lymphocytic foci, and scored them using the Chisholm-Mason scoring system.



Figure 1: Lower lip minor salivary gland biopsy procedure

All patients' epicrisis reports at the 1st month follow-up were reviewed, and their answers to questions about possible complications asked to minor salivary gland patients in our clinic were examined. The patients' demographic, histopathological, and serological features were looked at. The results were also looked at, along with the percentage rates that showed how often the findings happened.

Statistical Analyses

Only descriptive statistics were reported; no hypothesis testing, confidence intervals, or modeling were performed. Categorical variables were presented as counts (percentages). Histopathology was tabulated by Chisholm-Mason grades and (if available) focus score; serologic results (ANA, anti-SSA/Ro, anti-SSB/La, RF) and Schirmer testing were summarized as n (%). Procedure-related symptoms/complications at 1 month were

reported as prevalence. No imputation was performed for missing data; the number of evaluable cases (denominator) for each variable is indicated in the tables/text. No subgroup or between-group comparisons were conducted. Summaries were generated using the IBM SPSS Statistics version 25.0 software (IBM Corp., Armonk, NY, USA).

RESULTS

A total of 319 MSGB with a preliminary diagnosis of SS were included in the study. Of these patients, 288 (90.3%) were female and 31 (9.7%) were male. Serological evaluation revealed that anti-nuclear antibody (ANA) was negative in 224 patients (70.2%) and positive in 90 patients (28.2%), while ANA results were unavailable for 5 patients. Anti-SSA (Ro) was negative in 236 patients (73.9%) and positive in 10 patients (3.1%), with 73 patients (22.9%) having missing data. Similarly, anti-SSB (La) was negative in 242 patients (75.8%) and positive in 4 patients (1.2%), and the results were not available for 73 patients. Rheumatoid factor (RF) was below 10 in 234 patients (73.3%), between 10?14 in 21 patients (6.5%), and above 14 (considered positive) in 43 patients (13.4%); RF data were missing for 21 patients.

Ophthalmologic examination using the Schirmer test showed values of ≤ 5 mm in 83 patients (26%), 6-14 mm in 37 patients (11.5%), and ≥ 15 mm in 6 patients (1.8%), while the test was not performed or the data were unavailable in 203 patients (63.6%).

The Chisholm-Mason scores obtained from MSGB are summarized in Table 1. A definitive diagnosis of Sjögren's syndrome was made in 92 patients (28.8%) with Chisholm-Mason scores of 3 or 4. Additionally, 43 patients (13.4%) were diagnosed with SS based on MSGB scores of 3 or higher, despite having negative serological results. Among the 167 patients (52.3%) with scores of 1 or 2, some were diagnosed with SS based on additional clinical findings, and treatment was initiated accordingly. In contrast, a diagnosis of SS was excluded in 60 patients (18.8%) who had a score of 0.

Postoperative follow-up data were available for 282 patients who returned for their one-month evaluation. Among these, 241 patients (85%) reported no pain, and only 10



patients (3.5%) experienced pain. Swelling was reported by 11 patients (4%), while 250 patients (88%) had no swelling. Burning or tingling sensations were noted by 15 patients (5%), whereas 251 patients (89%) reported no such complaints. Numbness was reported by 9 patients (3%), and 253 patients (90%) reported no numbness. Importantly, no hematomas were observed in any of the patients.

Table 1: Chisholm-Mason scores and importance of MSGB

Chisholm-Mason	Number of patients	Percentage of patients	Importance of MSGB
Score	patients	patients	
Grade 0	60	%18.8	Exclusion of SS Diagnosis
Grade 1	106	%33.2	When clinical findings are present, a definitive diagnosis may be confirmed.
Grade 2	61	%19.1	When clinical findings are present, a definitive diagnosis may be confirmed.
Grade 3	51	%16	Definitive Diagnosis of SS
Grade 4	41	%12.9	Definitive Diagnosis of SS
Total	319	%100	

Table 2: The 2016 ACR/EULAR Classification Criteria for pSS

Item	Weight/score	Rules for classification
Labial salivary gland with focal lymphocytic sialadenitis and focus score of ≥ 1 foci/ 4 mm^2	3	- To be applied to any individual who meets the inclusion criteria (presence of ocular and/or oral symptoms) with at least one domain of ocular or oral dryness or ESSDAI ≥ 1 .
Presence of anti-SSA/Ro antibodies	3	- Absence of any of the conditions listed below as exclusion criteria.
Ocular Staining Score (OSS) ≥ 5 or van Bijsterveld score ≥ 4	1	- A score of ≥ 4 when the weights from the five criteria
Schirmer's test $\leq 5\text{ mm}/5\text{ min}$ at least one eye	1	
Unstimulated whole saliva	1	



flow rate \leq 0.1 mL/min

items are summed.

DISCUSSION

Sjögren's syndrome is a systemic autoimmune disease characterized by chronic plasmacytic infiltration of all exocrine glands, particularly the salivary and lacrimal glands¹². The diagnosis of SS is challenging due to the lack of highly specific clinical and paraclinical parameters. Therefore, through our study, we aimed to emphasize the importance of this procedure, which is commonly performed in ENT practice but whose clinical value remains underappreciated, and to highlight its relevance in routine clinical decision-making.

Since 1965, various diagnostic criteria have been proposed. The European Classification Criteria were introduced in 1993 and later revised by the American-European Consensus Group (AECG) in 2002. The AECG aimed to enhance diagnostic specificity by requiring evidence of autoimmunity, either through the presence of anti-Ro or anti-La autoantibodies or focal lymphocytic sialadenitis on MSGB⁽⁵⁾. As a result of extensive research, the European League Against Rheumatism (EULAR) and the American College of Rheumatology (ACR) established the EULAR/ACR classification criteria in 2016. These criteria have since become a crucial tool for evaluating and diagnosing Sjögren's syndrome (Table 2)¹³. A distinguishing feature of SS is B-cell hyperreactivity, which leads to the production of autoantibodies such as ANA, including anti-Ro60, anti-Ro52, and anti-La, as well as rheumatoid factors (RF)¹⁴. These autoantibodies are present in only 45% to 75% of patients with Sjögren's syndrome¹⁵. In patients lacking serum anti-Ro52/Ro60 (SSA/Ro) antibodies, the diagnosis of SS is based on internationally established classification criteria. These include reduced tear and/or salivary flow and the presence of focal lymphocytic sialadenitis on MSGB¹³. Patients who meet the diagnostic criteria for SS but do not test positive for conventional serum autoantibodies are classified as seronegative SS. However, there is no universally accepted definition of which autoantibodies should distinguish seropositive

SS from seronegative SS. Consequently, the reported prevalence of seronegative SS in the literature ranges from 8% to 37%.

Due to the absence of specific autoantibody biomarkers, seronegative SS may be underdiagnosed in clinical settings unless further diagnostic evaluations, such as MSGB, are performed¹⁶.

In our study, despite negative serological results in 43 (13.4%) patients, MSGB scores of 3 or higher were observed. SS was directly diagnosed in 92 (28.8%) patients with Chisholm-Mason scores of 3 and 4. Additionally, some of the 167 (52.3%) patients with scores of 1 and 2 were diagnosed with SS based on additional clinical findings, leading to the initiation of treatment. In more than half of the patients suspected of having SS, the diagnosis was established using MSGB findings. These results highlight the critical role of MSGB, particularly in seronegative SS patients.

Follow-up epicrisis evaluations at one month post-biopsy revealed that the majority of patients did not report symptoms such as pain, numbness, burning/tingling, or swelling. We believe this outcome is associated with the superficial biopsy technique employed in our clinic. Our method involves stretching and evertting the lower lip, which enhances the visibility of minor salivary glands. Additionally, local anesthetic infiltration into the submucosa facilitates separation of the epithelium from the glands. Beginning the procedure with a very superficial incision using a scalpel, without damaging underlying structures, is another key aspect. Furthermore, performing glandular dissection parallel to nerve fibers may prevent the development of numbness or sensory loss.

Consistent with our findings, Kim et al.¹⁷ reported similar outcomes using a comparable technique; by contrast, deeper biopsy approaches have been associated with higher complication rates¹⁸.

Complications associated with minor salivary gland biopsy (MSGB) are uncommon. Reported rates include immediate bleeding in



approximately 7?8% of cases and hematoma in 2?3%. Persistent sensory disturbance (numbness or paresthesia) is infrequent, typically 1?6% across published series¹⁹. Consistent with these data, we observed minimal complications in our cohort; we attribute this to maintaining a superficial dissection plane parallel to the course of the sensory nerve fibers.

Based on our findings, we propose that numbness, the most frequently reported post-biopsy complication in the literature, is primarily due to deeper biopsy techniques. We believe that our method minimizes complication rates, but further prospective, well-controlled studies are necessary to obtain more objective data regarding this technique.

In recent years, research has also explored the molecular basis of SS, an autoimmune disease. Salivary gland biopsy materials have been analyzed for various parameters, including DNA microarray profiles, monocyte chemotactic protein-1 receptor presence, IL-22-producing cell levels, expression of IL-17, IL-23, and their respective receptors.

Another indispensable aspect of salivary gland biopsy is its role in assessing lymphoma risk. Patients with SS have an increased risk of lymphoma compared to the general population, making MSGB crucial not only for routine histopathological evaluation but also for detecting ectopic germinal centers, a key indicator of lymphoma development²⁰. Additionally, Jonsson et al. reported that the presence or absence of germinal center-like structures in biopsy samples from patients diagnosed with pSS can be used to classify patient subgroups based on serological profiles, thereby aiding in prognostic prediction²¹. Beyond focus score, histologic/immunohistochemical features such as ectopic germinal center-like structures (identified with markers including BCL6/CD20/CD21) carry prognostic significance?including association with B-cell activation and lymphoma risk?and may refine risk stratification in future cohorts. Circulating and tissue biomarkers (e.g., CXCL13 as a surrogate of lymphoid organization; salivary/serologic panels reflecting B-cell activation) are promising for monitoring

disease activity and could be integrated with LSGB readouts in prospective studies²². These findings highlight just a few of the benefits that MSGB offers in the differential diagnosis of Sjögren's syndrome.

Limitations

The most significant limitation of our study is its retrospective design, which resulted in the inaccessibility of some data. Based on our findings, it is evident that more objective and robust conclusions could be achieved through prospective studies involving larger patient cohorts, specifically designed to calculate the sensitivity, specificity, and positive predictive value (PPV) of MSGB, thereby more accurately determining its diagnostic utility.

CONCLUSION

Minor salivary gland biopsy is undeniably significant in diagnosing Sjögren's syndrome. Our study highlights the significance of minor salivary biopsy in diagnosing Sjögren's disease, particularly in seronegative patients. MSGB, utilizing the approach we employed, is a secure and significant way to diagnose Sjögren's syndrome.

We aimed to underscore the significance of this evaluation, commonly conducted in ENT practice, whose usefulness we believe remains inadequately recognized, through our study. We believe that minor salivary gland biopsy will assume an increasingly significant role in the diagnosis and treatment of the disease due to advancements in immunohistochemistry techniques and biomarkers.

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