Original Article

Quantitative Assessment of Salivary Gland Parenchymal Vascularization Using Power Doppler Ultrasound and Superb Microvascular Imaging: A Potential Tool in the Diagnosis of Sjögren’s Syndrome

Fethi Emre Ustabaşoğlu1, Selçuk Korkmaz2, Ufuk İlgın3, Serdar Solak4, Osman Kula1, Sezin Turan3, Hakan Emmüngil3

1Department of Radiology, Trakya University Faculty of Medicine, Edirne, Turkey
2Department of Biostatistics and Medical Informatics, Trakya University Faculty of Medicine, Edirne, Turkey
3Department of Rheumatology, Trakya University Faculty of Medicine, Edirne, Turkey

Address for Correspondence: Fethi Emre Ustabaşoğlu, Department of Radiology, Trakya University Faculty of Medicine, Edirne, Turkey
+90 284 235 76 53
ustabasioglu@hotmail.com

Received: 17 November 2019
Accepted: 08 April 2020

DOI: 10.4274/balkanmedj.galenos.2020.2019.11.91


Background: Primary Sjögren’s syndrome (pSS) is a chronic inflammatory autoimmune disease. Minor salivary gland biopsy is gold standard for pSS diagnosis. Superb microvascular imaging (SMI), power Doppler ultrasound (PDUS) and color Doppler of salivary gland represent non-invasive, non-irradiating modality for evaluation the vascularization of salivary gland in the diagnosis and follow-up of pSS.

Aims: To evaluate the efficacy of SMI and vascularity index (VI) in salivary glands for sonographic diagnosis of pSS.

Study Design: Prospective case-control study.

Material and Methods: 20 participants with pSS and 20 healthy subjects were included in the study. Both parotid glands (PG) and submandibular glands (SG) were evaluated by SMI, PDUS, color Doppler and diagnostic accuracy of SMI was compared with these techniques.

Results: In patients group VI values of SMI in PG and SG were 3.5 ± 1.66, 5.06 ± 1.94, respectively. While same values were 1.0 ± 0.98, 2.44 ± 1.34 in control group (p = 0.001). In patients group VI values of PDUS in PG and SG were 1.3 ± 1.20, 2.59 ± 1.82, respectively. While same values were 0.3 ± 0.32, 0.85 ± 0.68 in control group (p = 0.001). SMI VI cut-off value for pSS diagnosis in PG that maximizes the accuracy was 1.85 (AUC: 0.906; 95% CI 0.844, 0.968), and its sensitivity and specificity were 87.5 % and 72.5 %, respectively. Whereas SMI VI cut off value for pSS diagnosis in SMG that maximizes the accuracy was 3.35 (AUC: 0.873; 95% CI 0.800, 0.946), its sensitivity and specificity were 82.5%, 70%, respectively.

Conclusions: SMI has higher sensitivity and specificity than PDUS in the diagnosis of pSS. Quantitative SMI VI values, with their high reproducibility, could become a non-invasive useful technique in diagnosis of pSS with clinical parameters, laboratory findings, and other imaging modalities such as gray-scale ultrasound and PDUS.

Keywords: Power Doppler ultrasound salivary glands, primary Sjögren’s syndrome, superb microvascular imaging, ultrasonography

Primary Sjögren's syndrome (pSS) is a systemic disease characterized by xerostomia and keratoconjunctivitis sicca in which the autoimmune response of cellular and humoral mechanisms affects the salivary glands (1). In the parenchyma of the salivary gland, pathologic damage to the acinis secondary to lymphocyte infiltration and fibrosis are seen (2). There should be no other rheumatologic disease for the diagnosis of pSS. In secondary SS, there are diseases such as systemic lupus erythematosus and rheumatoid arthritis. In diagnosis, tests such as sialoscintigraphy, and serologic and pathologic findings are used together with clinical findings (3-5). Salivary gland sonography is a non-invasive method that does not involve ionizing radiation and has a major significance in pSS diagnosis (6,7). Major sonographic findings in patients with pSS are heterogeneous parenchyma with multiple hypoechoic areas and reticular pattern due to hyperechoic stripes (8). Hypoechoic areas usually have a radius of 2-5 mm and are caused by lymphocytic infiltration, whereas echogenic stripes are caused by fibrosis and fatty infiltration (9,10). Recently, salivary gland sonography has been proposed as a
promising, highly specific, and non-invasive modality in the diagnosis of pSS even in the early clinical stage (11). Sonographic scoring systems are under way (12) and parotid ultrasonography was mentioned as an arising diagnostic test in the 2016 American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) pSS classification consensus although not included in the classification criteria currently (13). Color Doppler (CD) and Power Doppler ultrasound (PDUS) are other important techniques used in pSS. Superb microvascular imaging (SMI), on the other hand, is a more sensitive vessel imaging modality than these two methods and can show smaller vessels than CD and PDUS imaging (14). In a recent study (15) performed on parotid glands (PG), SMI values were found significantly higher than PDUS and CD values in healthy children and adolescents.

Our study is the first that measuring salivary gland parenchyma vascularity of patients with pSS using SMI. We aimed to evaluate salivary gland parenchyma using grayscale ultrasound (US) and vascularity degree using SMI and PDUS in patients with pSS.

Materials and Methods
Our study was conducted at XXXXXXXXX between February and April 2019. The local ethics committee of XXXXXXXXXX approved the study protocol. All patients who participated in the study gave informed consent.

Patient population
Our study was designed prospectively, and 20 patients (20 women) with pSS and 20 healthy controls (20 women) were evaluated. Consecutive patients with pSS from Rheumatology Department who met the 2016 ACR/EULAR criteria and who had a focus score of at least one/4 mm² in labial salivary gland biopsy, were included in the study. Activity of disease was assessed using the EULAR SJögren’s syndrome disease activity index (ESSDAI) (16). Antinuclear antibody (ANA) and auto-antibodies against Ro (SS-A) and La (SS-B) were tested using an indirect immunofluorescence assay (Euroimmun, Lübeck, Germany).

Control subjects were chosen from volunteers of a similar age and sex who had no clinical signs or symptoms of pSS. Patients with any systemic disease or drug use which might affect salivary glands were excluded. Participants receiving antidepressant therapy, anticholinergic drugs or drugs that affect the salivary glands, those who were diagnosed as having sarcoidosis, hepatitis C infection, pre-existing lymphoma, or who had a history of salivary gland surgery and head and neck radiation were also excluded.

PGs and submandibular glands (SGs) of patients and controls were examined using US, PDUS, and SMI, and all the measurements were statistically analyzed.

Sonography technique
Two radiologists (XXX and XXX) with 9 years’ radiology experience who were blinded to all clinical information of the patients separately performed US, PDUS, and SMI to all participants (20 patients and 20 controls) on the same day. Both examiners were blinded to each other’s examination results and the patients’ symptoms. Both the patient and control group were evaluated twice for interobserver variability analysis. Before initiation of the study, eight healthy control subjects were selected for PDUS and SMI technique standardization. These examinations were not included in the statistical analysis.

PGs and SGs were examined using an Aplio 500 Platinum ultrasound device (Canon Medical Systems, Japan) with 5-14 MHz linear probe. The US of the salivary glands was performed while participants were in the supine position with extended neck, and they turned their head to the opposite side. After this, patients’ head was tilted back maximally to access the submandibular area.

PGs were evaluated in the retromandibular fossa, anterior to the ear and sternocleidomastoid muscle, and SGs were evaluated in the posterior part of the submandibular triangle. Thyroid was also scanned for comparison with the salivary glands for evaluation of parenchymal echogenicity.

Parenchymal echogenicity, inhomogeneity, presence of hypoechoic areas, and hyperechoic foci in the salivary glands were used as US parameters.

PDUS and SMI Technique
Eight hundred seventy to 966 Hz pulse repetition frequency and 10-15 frame rate was used in PDUS measurements. The SMI examination was performed on the same area as the US with standardized parameters of the ultrasound device, without compression. A color box was placed with a fixed window with 3 x 2 cm dimensions adjusted to the examined gland. After that, observer captured the image, put the standardized (15 x 5 mm) region of interest into the center of the gland, and automatically calculated the vascularity index (VI). VI represents the percentage of color pixels in the total grayscale pixels in a defined region of interest. Same measurement protocol was used for both PDUS and SMI evaluation. The normal large vessels visible within the salivary glands (external carotid artery and retromandibular vein in the PG and facial artery and vein in the SG) were excluded from the PDUS and SMI VI scores. All images were transferred electronically to a picture archiving system (Sectra PACS Linköping-SWEDEN). Figure 1 reveals the SMI and PDUS evaluations of a patient.

Statistical analysis
The Shapiro-Wilk test was used to analyze the normality of data distribution. Quantitative variables were expressed as mean and standard deviation or median and interquartile range based on their distribution. Categorical variables were expressed as frequencies and percentages. The group comparisons were performed using Student’s t test if data follow a normal distribution, whereas Mann-Whitney U test was used when the data distribution is non-normal. Fisher’s exact test was used to assess the relationships between categorical variables. Bonferroni adjustment of p values was used for multiple testing. The receiver operating characteristic (ROC) curve analysis and Youden index were used to detect cut-off values for PDUS and SMI. Interobserver agreement in the measurements was calculated using intra-class correlation coefficients (ICCs). A 95% confidence interval (CI) was constructed for each ICC. A p value less than 0.05 considered as statistically significant. All statistical analyses conducted using SPSS version 16 (Chicago, ILL).

RESULTS
Both PGs and SGs of 20 patients with pSS and 20 control subjects were evaluated. There were no statistically significant difference between mean ages of patient (56.75±14.07) and control (55.55±13.67) groups (p=0.786). All patients included in the study were female. In US imaging, the difference between the pSS patients and control group was statistically significant for heterogeneity (p<0.001), presence of hypoechoic areas (p<0.001), and hyperechoic foci (p = 0.02). Results are summarized in Table I.

The PDUS and SMI VI values of both PGs and SGs of the patient and control groups are revealed in Table II. VI values of SMI in both PGs and SGs were remarkably higher in the patient group than that in the control group (p<0.001). Similarly, the VI values of PDUS in both PGs and SGs were statistically higher in patient group compared to control group (p<0.001).

In our all study population, to investigate the efficacy and specificity of SMI and PDUS, ROC curves were calculated and the VI values acquired by these two methods were compared. VI values acquired using SMI method were significantly higher than PDUS VI values (p<0.001).

In both the patient and control group, values of VI with PDUS or SMI were not significantly different between the right and left side. Figure 2 and 3 show the ROC curve drawn based on SMI and PDUS VI values for pSS diagnosis.

The SMI VI cut-off value for the pSS diagnosis in PG that maximized the Youden index was 1.85 (AUC: 0.906; 95% CI: 0.844-0.968), and its sensitivity and specificity were 87.5% and 72.5%, respectively. The SMI VI cut-off value for pSS diagnosis in SMG that maximized the Youden index was 3.35 (AUC: 0.873; 95% CI: 0.800-0.946), and its sensitivity and specificity were 82.5% and 70%, respectively.

The PDUS VI cut-off value for pSS diagnosis in PG that maximized the accuracy was 0.55 (AUC: 0.817; 95% CI: 0.716-0.918), and its sensitivity and specificity were 82.5% and 70%, respectively. The PDUS VI cut-off value for determining the diagnosis of pSS in SMG that maximized the accuracy was 1.45 (AUC: 0.862; 95% CI: 0.716-0.918), and its sensitivity and specificity were 77.5% and 67.5%, respectively.

Interobserver variability results are shown in Table III. It is found that there was excellent agreement between the two blinded observers for both the patient and control group in SMI and PDUS VI measurements (ICC and 95% CI: 0.994 (0.991-0.996) for SMI VI and 0.945 (0.945-0.977) for PDUS VI).

DISCUSSION
Salivary gland inflammation is a highly frequent clinical entity in patients with pSS and salivary gland destruction can be correlated with disease activity (17). In the diagnosis of pSS, histopathologic confirmation of disease by minor salivary gland biopsy remains the gold standard. However, in recent years, salivary gland sonography has gained an important place in diagnosis (18,19).

CD and PDUS can show increased vascularity caused by inflammation, thus can be used in the diagnosis of pSS. PDUS is an integrated power spectrum that depends on a low angle and has higher sensitivity in detecting low blood flow velocity. On the other hand, CD is based on the mean Doppler frequency shift (20,21). According to the developed filtering techniques in SMI, background noises are eliminated, and small, slow velocity vascular structures can be imaged successfully. Compared with standard PDUS, SMI has high sensitivity and resolution (22). Indeed, in the present study, SMI was found to be superior to PDUS in identifying salivary gland vascularization.

In a study in which SMI properties of PGs were investigated, Caliskan et al. (15) studied the vascularization of PG of healthy children using SMI and PDUS. However, in this study no quantitative method such as VI was used and they only compared vascular dot numbers as a semi-quantitative method. Shimizu et al. (23) stated that vascularization in patients with pSS was increased by finding 4-6 vascular spots in PG by means of CD. However, conventional CD is not capable of detecting very slow blood flow. With the capabilities to detect slow moving blood, SMI can be more effective than CD and even PDUS in the diagnosis of pSS. Our study has some limitations. Main limitation of our study is the small patient population, which composed only female patients. Secondly, the patients were examined using ultrasonography, and salivary glands were evaluated in terms of gland heterogeneity, presence of hypoechoic areas, presence of hyperechoic foci, and clearance of SG posterior borders, but the relationship between these parameters and SMI degree was not analysed. Another limitation was the lack of elastographic evaluation. However, the main goal of this study was
to evaluate the vascularity degrees and emphasize the role of SMI, which is a new diagnostic tool.

In conclusion, our study showed that SMI has higher sensitivity and specificity than PDUS in the diagnosis of pSS. Quantitative SMI VI values, which have high reproducibility, could become a useful non-invasive tool for diagnosis of pSS along with clinical parameters, laboratory findings, and other imaging modalities such as US and PDUS.

REFERENCES

| TABLE 1. General characteristics and sonographic findings of study population |
|----------------------------------|-----------------|-----------------|-----------------|
|                                  | pSS group (n= 20) | Control group (n= 20) | p value     |
| Age (years)                      | 56.75±14.07      | 55.55±13.67       | 0.786        |
| Heterogenity                     | 13 (65.00)       | 0 (0.00)          | <0.001*     |
| Presence of hypoechoic areas     |                 |                 |              |
| Normal                           | 10 (50.00)       | 20 (100.00)       | <0.001*     |
| Slight                           | 5 (25.00)        | 0 (0.00)          |             |
| Moderate                         | 4 (20.00)        | 0 (0.00)          |             |
| Severe                           | 1 (5.00)         | 0 (0.00)          |             |
| Hyperechoic foci                 |                 |                 |              |
| Normal                           | 14 (70.00)       | 20 (100.00)       | 0.02*       |
| Slight                           | 2 (10.00)        | 0 (0.00)          |             |
| Moderate                         | 3 (15.00)        | 0 (0.00)          |             |
| Severe                           | 1 (5.00)         | 0 (0.00)          |             |

Descriptives are expressed as mean±standard deviation and frequency (percent)
* Statistical significance at p<0.05
n: number
pSS primer Sjögren Syndrome

| TABLE 2. Comparison between pSS patients and control group based on SMI and PDUS VI values for both parotid glands (PG) and submandibular glands (SG) |
|----------------------------------|-----------------|-----------------|-----------------|
|                                  | pSS group (n= 20) | Control group (n= 20) | p value     |
| SMI VI in PGs                    | 2.93±1.35       | 1.37±0.68       | <0.001*     |
| PDUS VI in PGs                   | 1.58±0.94       | 0.51±0.23       | <0.001*     |
| SMI VI in SGs                    | 2.28 (0.95)     | 0.94 (0.69)     | <0.001*     |
| PDUS VI in SGs                   | 4.37±1.49       | 2.50±0.93       | <0.001*     |

Descriptives are expressed as mean±standard deviation and median (interquartile range)
* Statistical significance at p<0.05

| TABLE 3. Interobserver variability for SMI VI and PDUS VI values of parotid glands |
|----------------------------------|-----------------|-----------------|-----------------|
|                                  | Reader 1        | Reader 2        | ICC (95% CI)    |
| SMI VI                           |                 |                 |                 |
| pSS patients (n=20)              | 2.99±1.67       | 2.88±1.51       | 0.994 (0.991-0.996) |
| Control group (n=20)             | 1.32±0.98       | 1.42±0.92       |                 |
| PDUS VI                          |                 |                 |                 |
| pSS patients (n=20)              | 1.56±1.20       | 1.59±0.95       |                 |
| Control group (n=20) | 0.44±0.32 | 0.58±0.35 | 0.965 (0.945-0.977) |

Descriptives are expressed as mean ± standard.


**FIG. 1.** (A) Power Doppler (PDUS) and (B) Superb microvascular Imaging (SMI), examination of parotid gland in a 37-year-old patient with primary Sjögren syndrome. SMI image of the patient (B) shows a substantially higher amount of blood flow coding (VI, 4.3%) compared with the PDUS image of the patient (A; VI, 1.4%).

**FIG. 2.** ROC curve for the diagnosis of pSS based on SMI evaluation.
FIG. 3. ROC curve for the diagnosis of pSS based on PDUS evaluation.