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Sonoelastography and S100 proteins in the differential diagnosis of IgG4-related disease (IgG4-RD)

IgG4 ilişkili hastalığın (IgG4-RD) ayırıcı tanısında sonoelastografi ve S100 proteinleri

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Abstract

Objective: In the present study, we wanted the investigate the reliability and validity of S100 biomarkers in the differential diagnosis of IgG4-related disease (IgG4-RD) and to examine the relationship between two-dimensional shear wave elastography (2D-SWE) scores of parotid/lacrimal glands, which is used as an effective auxiliary tool in disease prognosis.

Methods: Twenty-seven amyloidosis, 17 sarcoidosis, 13 IgG4-RD, and 21 healthy controls (HCs) presenting to rheumatology outpatient clinic were included in this cross-sectional study. S100A8, S100A9, S100A12, and S100A8/A9 (calprotectin) protein levels were detected with the ELISA test. Evaluation of all groups of the parotid and lacrimal glands were performed using 2D-SWE.

Results: There was no significant difference between patient and healthy control groups with respect to age, sex, and body mass index. The mean levels of S100A9 and S100A12 proteins were significantly higher in amyloidosis (S100A9; 25.09±7.92 vs. 12.91±8.74, S100A12; 627.87±643.83 vs. 344.03±118.02, p=0.001) and sarcoidosis groups (S100A9; 23.67±12.34 vs. 12.91±8.74, S100A12; 600.4±381.26 vs. 344.03±118.02 p=0.001) compared to HCs. The calprotectin levels were significantly higher in amyloidosis than IgG-RD and HCs. For the parotid and lacrimal glands, the mean shear wave elasticity mode values were significantly higher in the IgG4-RD patient compared with HCs, amyloidosis, and sarcoidosis groups (all p=0.001), respectively. 2D SWE scores in parotid glands, reflecting loss of elasticity, had a positive correlation with S100A9 level in IgG4-RD (r=0.948, p=0.014).

Conclusion: Results of this study suggest that S100A9, S100A12, and calprotectin are promising biomarkers and might facilitate differential diagnosis of IgG4-RD, sarcoidosis, and amyloidosis. S100A9 and parotid elasticity could be novel biomarker and tool for the assessment of activity status in patients with IgG4-RD.

Keywords: S100A8, S100A9, S100A12, calprotectin, sonoelastography

Öz

Amaç: Bu çalışmada, IgG4-RD hastalığının ayırıcı tanısında S100 biyobelirteçlerinin güvenilirliğini ve geçerliliğini araştırmak ve hastalık prognozunda etkili bir yardımcı araç olarak kullanılan parotis/lakrimal bezlerin iki boyutlu shear wave elastografi (2D-SWE) skorları ile arasındaki ilişkiyi incelemek istedik.

Yöntem: Bu kesitsel çalışmaya romatoloji polikliniğine başvuran 27 amiloidoz, 17 sarkoidoz, 13 IgG4-RD ve 21 sağlıklı kontrol (SK) dahil edildi. ELISA testi ile S100A8, S100A9, S100A12 ve S100A8/A9 (kalprotektin) protein seviyeleri tespit edildi. Parotis ve lakrimal bezlerin elastisite değerlendirilmesi 2D-SWE kullanılarak yapıldı.

Bulgular: Hasta ve sağlıklı kontrol grupları arasında yaş, cinsiyet ve vücut kitle indeksi açısından anlamlı fark yoktu. S100A9 ve S100A12 proteinlerinin ortalama düzeyleri amiloidoz (S100A9; 25,09±7,92 vs. 12,91±8,74, S100A12; 627,87±643,83 vs. 344,03±118,02, p=0,001) ve sarkoidoz (S100A9; 23,67±12,34 vs. 12,91±8,74, S100A12; 600,4±381,26 vs. 344,03±118,02 p=0,001) gruplarında sağlıklı kontrollere göre anlamlı olarak daha yüksek saptandı (p<0,05). Kalprotektin seviyeleri, amiloidoz grubunda IgG-RD ve kontrol grubuna göre önemli ölçüde daha yüksekti. Parotis ve lakrimal bezler için ortalama 2D-SWE skorları IgG4-RD grubunda kontrol, amiloidoz ve sarkoidoz gruplarına göre anlamlı derecede yüksekti (p<0,01). Elastikiyet kaybını yansıtan parotis bezlerindeki 2D- SWE skorları, IgG4-RD grubunda S100A9 düzeyi ile pozitif korelasyona sahipti (r=0,948, p=0,014).

Sonuç: Bu çalışmanın sonuçları, S100A9, S100A12 ve kalprotektinin umut verici biyobelirteçler olduğunu ve IgG4-RD, sarkoidoz ve amiloidozun ayırıcı tanısını kolaylaştırabileceğini düşündürmektedir. S100A9 ve parotis elastisitesi, IgG4-RD'li hastalarda hastalık aktivite durumunun değerlendirilmesi için yeni bir biyobelirteç ve araç olabilirler.

Anahtar Kelimeler: S100A8, S100A9, S100A12, kalprotektin, sonoelastografi

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Introduction

Amyloidosis, sarcoidosis, and IgG4-related disease (IgG4-RD) are systemic fibroinflammatory diseases, in which innate immunity and adaptive immunity play a prominent role. They may affect exocrine glands (such as salivary and lacrimal glands) causing dryness symptoms.[1] Diagnosis is based on a combination of clinical, laboratory, and radiographic findings with the exclusion of other disease entities.[2,3] Due to the heterogeneity in the location and size of organs which they involve, their clinical presentation is insidious, and the severity of the disease displays larger variation than other rheumatological diseases.

At the initial stage, pathologies associated with cellular infiltration are detected in the regions they involve, while in time they lead to fibrosis, resulting in irreversible organ defects.[4,5] Unfortunately, at early stages, diagnosis can not be made based upon clinical and laboratory findings, and diagnosis is established usually at later stages when organ damage presents with symptoms. Even though important advances have been made in the diagnosis of these diseases, there is an unmet need for specific early diagnosis, biomarkers for monitorization and prediction, and for diagnostic tools in routine clinical practice. Most diagnostic tests have limitations such as invasiveness, higher cost, or limited accessibility.[6,7]

The S100A protein family represents a large calcium-binding sub-family playing a regulative role in many cellular functions.[8] In physiological conditions, S100 proteins are present in low amounts in the body. However, they increase markedly under conditions of heat, trauma, infection, and in many inflammatory processes. S100 proteins, released by neutrophils and monocytes, activate innate immune pathways, mediated by cytokine-activated toll-like receptor for advanced glycation end (RAGE) products. RAGE-products, lead to an increase in the levels of tumor necrosis factor, interleukin-1β, and interleukin-6 cytokines.[9] Increased levels of inflammatory cytokines further stimulate neutrophils and macrophages, creating an inflammatory vicious circle. Hence, as activity and damage increase via positive feedback, serum S100 protein levels are further upregulated. Differential diagnosis of IgG4-RD includes amyloidosis and sarcoidosis, and diagnosis of these conditions can be challenging requiring novel biomarkers. To the best of our knowledge, there is no reported association between S100 proteins and sonoelastography of IgG4-RD.

Two-dimensional shear wave elastography (2D-SWE) is a newly emerged ultrasonography (US) imaging technique that can assess the elastic properties of tissues and help diagnose certain diseases by identifying local or diffuse alterations in the stiffness of tissues. We measure quantitative assessment of tissue elasticity in meters and kilopascals per second with this technique.[10,11] The utility of parotid and lacrimal sonoelastography has been shown both for supporting diagnosis and differentiating primary Sjögren's syndrome from tumoral lesions.[12]

Hence, in this study, we investigated the utility of measuring serum S100 proteins in a cohort of IgG4-RD patients. We wanted to study simple tests and tools, which may help in the determination of diagnosis and estimation of prognosis, such as S100A8, S100A9, S100A12, S100A8/A9 (calprotectin), and 2D-SWE in IgG4-RD, amyloidosis, sarcoidosis and if possible, to find a cut-off value for diseases.

Materials and Methods

This cross-sectional study included 27 amyloidosis, 17 sarcoidosis, and 13 IgG4-RD patients and 21 healthy controls (HC) which were followed in the rheumatology outpatient clinic. We included patients whether newly diagnosed or relapsing, who fulfilled the 2019 American College Rheumatology for IgG4-RD, the 1999 American Thoracic Society classification criteria for sarcoidosis, and the Tel Hashomer criteria for familial Mediterranean fever (FMF). The etiology of all amyloidosis patients was due to FMF. Individuals were examined and evaluated by an experienced rheumatologist. Healthy controls, who had no signs or symptoms participated to the study. Exclusion criteria were being a minor, lack of consent and having sialolithiasis, gland operation, parasympatholytic or antidepressant use, history of radiotherapy on the neck region, hepatitis B and C, osteoarthritis, cardiomyopathy, central nervous system diseases, malignancy, bacterial and viral infections, pregnancy, and Coronavirus disease-2019. Demographic data included age, gender, body mass index (BMI), and history of smoking. Laboratory tests included complete blood count (CBC), creatinine, uric acid, lipid profile, erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP).

Enzyme-linked Immunosorbent Assay (ELISA) for Serum S100 Proteins

The blood samples of all the participants were drawn into pro-coagulation tubes. The serum was collected immediately after centrifugation at 3000g for 15 minutes at 4 °C. Washing processes were done with a BIOTEK brand washing device (ELx50 Bioelisa Washer, Bio-Tec. Instruments, Inc.), and absorbance readings were carried out with a BIOTEK brand reader (ELx800 UV Universal Microplate Reader, Bio-Tec. Instruments, Inc.). Then the serum samples were stored at -80 °C until tested. Serum

S100A8, S100A9, S100A12, and calprotectin levels were analyzed using a commercial ELISA kit according to the manufacturer's instructions (Elabscience). All serum samples were diluted by 1:100. Results of S100A8, S100A9, S100A12, and calprotectin levels are expressed in nanograms per milliliter. Human S100A8, S100A9, S100A12, and calprotectin were studied with a commercially available ELISA kit (Boster Immunoleader En Rage PicoKineTMCA, USA). For S100A8 analytic measurement range was 0.63-40 ng/mL LoD: 0.38 ng/mL, for S100A9 it was 0.78-50 ng/mL, LoD: 0.47 ng/mL, S100A12 0.16-10 ng/mL LoD: 0.1 ng/mL and for calprotectin 1.56-100 ng/mL LoD: 0.94 ng/mL. absorbances for standards and samples were measured at 450 nm wavelength. Concentrations obtained from standard curves were expressed in ng/mL.

Ultrasound Examination

Ultrasonographic assessments (UA) of the lacrimal and salivary glands were conducted by two radiologists, using a LOGIQ S8 (GE Healthcare Systems, Waukesha, WI, USA) US device with a 9 MHz linear transducer. The radiologists did not have access to the medical records of the patients. Patients were NPO for 60 minutes prior to the examination. The examination continued with two-dimensional shear wave elastography. Elastography of the lacrimal and salivary glands were performed while both groups were in a supine location with the head turned to the across and the neck hyperextended. The participants were advised to breathe normally with their eyes closed. During the evaluation of the parotid glands with elastography, the patients were advised to breathe calmly and not to swallow. While the evaluation of the glands was made in both planes, their measurements were conducted only in the transverse plane (at the mandibular angle level for the parotid gland, at the middle of the gland level for the lacrimal gland). The 2D-SWE examinations were performed using a similar methodology described for liver evaluation.[13] The elasticity was defined with Young's modulus (elasticity modulus/estimated tissue stiffness), measured in kiloPascals (kPa). For conducting 2D-SWE evaluation, an adequate quantity of ultrasound gel was used, and no pressure was applied on the examined tissue. During the 2D-SWE evaluation, a sufficient proportion of ultrasound gel was used to show the tissue, and no pressure was applied to the any of groups. For the evaluation of the lacrimal gland, a 2-mm diameter region of interest (ROI) was placed in the middle of the lacrimal gland. For the parotid gland elastography evaluation, the probe was placed at a distance of 1.5 cm from the glandular capsule and a 3-mm ROI was placed in the superficial parenchyma in a homogeneous area. For both the lacrimal and parotid glands, three serial measurements were made in a similar procedure, at equal depth and localization. The average of the right and left gland elasticity measurements was used in the analyses (Figure 1A-D). Intraclass correlation levels between parotid and lacrimal gland elasticity values were evaluated for intra-rater and inter-rater reproducibility. (Supplementary Table 1).

The study was conducted after the ethical approval of the Ethics Committee of Gazi University complied with the Declaration of Helsinki (approval no: 2023.549). Informed consent was obtained from all patients and controls before the initiation of the study.

Statistical Analysis

For statistical analyses, NCSS (Number Cruncher Statistical System) 2007 (Kaysville, Utah, USA) program was used. In the evaluation of data, descriptive statistical methods [mean, standard deviation (SD), median, frequency, percentage, minimum, maximum] were utilized. Whether quantitative data were distributed normally was evaluated with the Shapiro-Wilk test and graphics. In the comparison of more than two groups between normally distributed quantitative parameters, One-Way ANOVA analysis, and was. When quantitative parameters were not normally distributed, in the comparisons between more than two groups, the Kruskal-Wallis tests and Dunn's test were used. Chi-square or Fisher's Exact tests were used to compare categorical variables. In the evaluation of the relationship between quantitative variables, Pearson's and Spearman's correlation tests were utilized for correlation analyses. In order to determine predictive values for parameters, diagnostic screening tests (sensitivity, specificity, PPV, NPV) and ROC analyses were used distinguishing IgG4-RD patients from healthy subjects. The optimal cut-off values for the sensitivity and specificity of S100A8, S100A9, S100A12 and S100A8/A9 in predicting IgG4-RD were calculated by using Youden's index. The p-value of <0.05 was considered statistically significant.

Results

Twenty-seven amyloidosis (12 female, 15 male; mean age 42.96±11.21 years), 17 sarcoidosis (14 female, 3 male; mean age 50.64±8.22 years), and 13 IgG4-RD (6 female, 7 male, mean age 51.58±10.74 years) patients presenting to the rheumatology outpatient clinic, and 21 HCs (12 female, 9 male; mean age 40.47±7.87 years) were included in the present study. There was no significant difference between patient and healthy control groups with respect to age, sex, and BMI. Patient groups were comparable in demographic characteristics, the prevalence of comorbidities,

involvements, and frequency of mucocutaneous symptoms (Table 1). Kidney (77.8%), lacrimal/salivary glands (37%), and gastrointestinal (GI) involvement (37%) were the most common region in amyloidosis, respectively, while lung (88.2%) and kidney (46.2%) involvement were most common in sarcoidosis and IgG4-RD, respectively.

While no significant difference was found between groups in terms of S100A8 protein values (p=0.221), S100A9 protein values were found to be significantly higher in amyloidosis (mean ± SD 25.09±7.92 ng/mL) and sarcoidosis (mean ± SD 23.67±12.34 ng/mL) groups than those in the control group (mean ± SD 12.91±8.74 ng/mL) (p=0.001). Likewise, S100A12 values were also found to be significantly higher in amyloidosis and sarcoidosis groups than in HCs [mean ± SD 627.87±643.83 ng/mL vs. 344.03±118.02 ng/mL, 600.4±381.26 ng/mL vs. 344.03±118.02 ng/mL, (p=0.001)] respectively. While calprotectin levels were found to be higher in the amyloidosis group than those in the IgG4-RD group (mean ± SD 810.76±772.58 ng/mL vs. 470.97±279.82 ng/mL, p=0.025) the difference did not reach statistical significance in sarcoidosis group (Table 2) (Figure 2A, 2B). In the statistical comparison, each disease was given a letter; a. control ; b. amyloidosis; c. IgG4-RD; d. sarcoidosis. (Table 2).

B-mode US evaluation revealed that all patients have increased echogenicity in parotid glands compared to HCs (p=0.001). Echogenicity did not differ significantly in lacrimal glands between groups. However, there was significant difference in the heterogeneous texture of lacrimal glands between groups (p=0.001). For the parotid glands, the mean shear wave elasticity mode values were significantly higher in the IgG4-RD patients compared with HCs, amyloidosis, and sarcoidosis groups (mean ± SD 16.27±3.48 vs. 9.89±2.60 kPa, 16.27±3.48 vs. 12.95±4.03 kPa, 16.27±3.48 vs. 12.84±3.25 kPa, all p=0.001), respectively (Table 3). For lacrimal glands, the mean shear wave elasticity mode values were significantly higher in the IgG4-RD patients compared with healthy subjects (mean ± SD 7.88±2.09 vs. 5.18±1.49 kPa, p=0.001), respectively.

In correlation analysis, 2D-SWE scores in parotid glands, reflecting loss of elasticity, had a positive correlation with S100A9 level in IgG4-RD (r=0.806; p=0.001) and amyloidosis (r=0.467; p=0.014) groups. In correlation analysis, in the amyloidosis group, a significant positive correlation was found between S100A12, (r=0.682; p=0.001) and calprotectin (r=0.847; p=0.001) values and S100A8. In both amyloidosis (r=0.644; p=0.001) and IgG4-RD (r=0.654; p=0.015) groups, a positive correlation was found between S100A12 and calprotectin values. In all groups, no significant relation was found between S100 values and traditional activity markers (ESR, CRP) (p>0.05) (Supplementary Table 2).

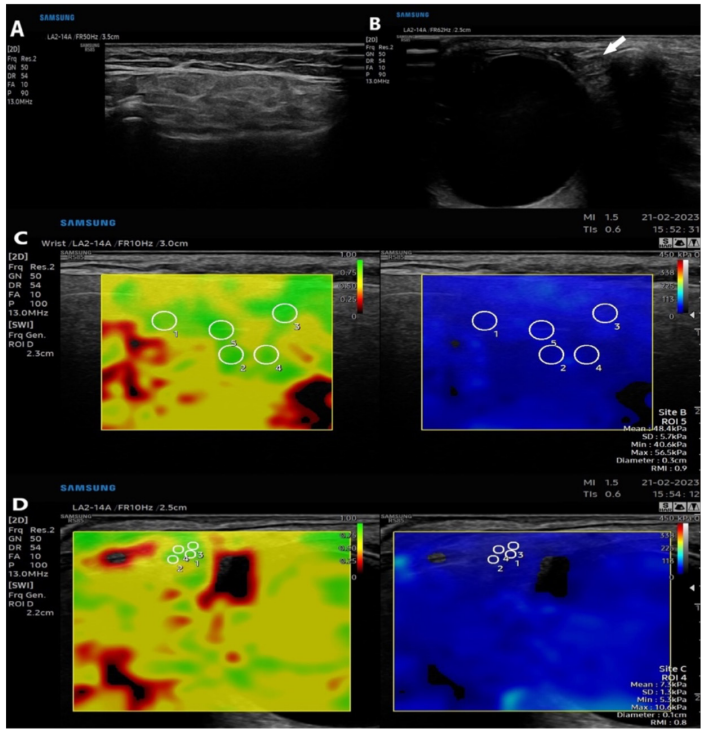

Figure 1. (A), Increased echogenicity and heterogeneous texture of parotid gland in the amyloidosis patient. (B) Heterogeneous texture of lacrimal gland in the IgG4-RD patient (white arrow), (C, D), Examples of the increased parotid and lacrimal elastography measurements performed in an IgG4-RD patient, respectively
IgG4-RD: IgG4-related disease

In amyloidosis patients, the cut-off value for S100A9 was determined as ≥12.7 ng/mL (sensitivity 96.3%, specificity 57.14%). Similarly, cut-off values for S100A12 and calprotectin were established to be ≥355.46 ng/mL (sensitivity 81.48%, specificity 71.43%) and ≥437.61 ng/mL (sensitivity 81.48%, specificity 47.62%), respectively (Table 4) (Figure 2C).

The mean shear wave elasticity cut-off scores that best discriminate IgG4-RD patients' parotid and lacrimal glands from HCs were 12.55 kPa and 5.5 kPa, respectively. A parotid gland 2D-SWE score of 12.55 kPa had a sensitivity and specificity of 92.3% and 80.95%, respectively. In the parotid elasticity ROC curve for IgG4-RD patients, the area under the curve was found to be 91.6% with a 5.5% standard error. The corresponding values were 86.3% and 6.2% respectively for lacrimal elasticity (Figure 2D).

Discussion

The present study is the first to evaluate the S100A8, S100A9, S100A12, and calprotectin of IgG4-RD, sarcoidosis, or amyloidosis patients as potential novel biomarkers. Our results suggest that S100A9, S100A12, and calprotectin proteins may be reliable, non-invasive biomarkers that can be used in the differential diagnosis of patients with suspected disease. Among investigated S100 proteins, S100A9 protein displayed the highest level of discriminatory power between amyloidosis cases and healthy controls. We demonstrated that S100A9 and S100A12 may have discriminatory power for not only amyloidosis but also sarcoidosis from healthy controls. And we evaluated the parotid/lacrimal gland involvement in these patients with 2D-SWE. Our results suggest that parotid/lacrimal US elastography could be a reliable, non-invasive auxiliary tool that can be used in the diagnosis of patients suspected of IgG4-RD. Furthermore, S100A9 biomarkers provided a prognostic value that was correlated well with the parotid elastography.

IgG4-RD, amyloidosis, and sarcoidosis are different multisystemic autoimmune diseases of unknown etiology in which immune dysregulation, genetic, and other unknown factors are in the pathogenesis.[1] Early diagnosis can be

Table 1. Demographic and clinical features of groups

	Amyloidosis (n=27)	Sarcoidosis (n=17)	IgG4-RD (n=13)	Control (n=21)	p
Age, years (mean ± SD)	43.11±11.19	46.94±5.89	48.85±8.93	43.81±6.93	0.100
Sex, female n (%)	12 (44.4)	14 (82.4)	6 (46.2)	12 (57.1)	0.073
BMI, kg/cm² (mean ± SD)	25.32±3.73	27.22±3.88	25.67±4.71	25.47±4.04	0.263
Smoking[1], n (%)	10 (37)	3 (17.6)	9 (69.2)	7 (33.3)	**0.041**
Disease duration, years	8 (2-20)	3 (1-17)	2 (1-6)	-	**0.001**
Involvements, n (%)					
-Lung	2 (7.4)	15 (88.2)	1 (7.7)	-	**0.001**
-Kidney	21 (77.8)	0 (0)	6 (46.2)	-	**0.001**
-Orbital	1 (3.7)	2 (11.8)	3 (23.1)	-	0.173
-Lacrimal/salivary glands	10 (37.0)	1 (5.9)	3 (23.1)	-	0.065
-GI	10 (37.0)	2 (15.4)	2 (11.8)	-	**0.003**
-Skin	4 (14.8)	5 (29.4)	0 (0)	-	0.093
-Lymph node	2 (7.4)	5 (29.4)	4 (30.8)	-	0.080
-Arthritis/arthralgia	20 (74.1)	6 (75.0)	2 (15.4)		**0.002**
Xerostomia, n (%)	8 (29.6)	9 (52.9)	4 (30.8)	-	0.178
Xerophthalmia, n (%)	6 (22.2)	9 (52.9)	3 (23.1)	-	**0.044**
Comorbidity, n (%)					
-Hypertension	17 (63.0)	5 (29.4)	4 (30.8)	2 (9.5)	
-Diabetes mellitus	2 (7.4)	4 (23.5)	0 (0)	0 (0)	**0.001**
-Coronary artery disease	3 (11.1)	1 (5.9)	4 (30.8)	0 (0)	
Treatment[2], n (%)					
-Corticosteroids[3]	1 (3.7)	14 (82.4)	11 (84.6)	-	**0.001**
-Colchicine	23 (85.2)	0 (0)	0 (0)	-	**0.001**
-Methotrexate	0 (0)	4 (23.5)	9 (69.2)	-	**0.001**
-Immunsupressants[4]	0 (0)	1 (5.9)	1 (7.7)	-	0.055
-Biologics[5]	20 (74.0)	0 (0)	2 (15.4)	-	**0.001**

GI: Gastrointestinal, [1]Active smoking, [2]Current treatment, BMI: Body mass index, [3]Current corticosteroid treatment, [5]Cyclophosphamide, azathioprine or mycophenolate, [5]Anakinra, canakinumab, rituximab, SD: Standard deviation, IgG4-RD: IgG4-related disease

Table 2. Evaluation of S100A8, S100A9, S100A12, calprotectin values, parotid/lacrimal gland shear wave elastography scores and laboratory measurements by groups

	Amyloidosis (n=27)	Sarcoidosis (n=17)	IgG4-RD (n=13)	Control (n=21)	p
S100A8, ng/mL	112.53±94.12	96.73±63.19	89.94±42.34	91.71±48.16	0.221
S100A9, ng/mL	25.09±7.92[a,b]	23.67±12.34[a,d]	21.12±13.82	12.91±8.74	**0.001**
S100A12, ng/mL	627.87±643.83[a,b]	600.4±381.26[a,d]	376.20±108.36	344.03±118.02	**0.001**
Calprotectin, ng/mL	810.76±772.58[b,c]	570.63±350.48	470.97±279.82	443.93±281[a,b]	**0.025**
Parotid elasticity, kPa	12.95±4.03[(a,b) (b,c)]	12.84±3.25[(c,d)]	16.27±3.48[a,c]	9.89±2.60	**0.001**
Lacrimal elasticity, kPa	6.55±1.71	6.18±2.15	7.88±2.09[(a,c)]	5.18±1.49	**0.001**
ESR (mm/h)	32.00±25.81[a,b]	17.69±14.55[c,d]	43.08±26.32[a,c]	14.05±12.7	**0.001**
CRP (mg/L)	12.78±23.67	11.93±8.98	20.62±38.79[a,c]	4.20±1.81	**0.047**
Platelet (x10³/uL)	239.67±63.6	306.53±101.4[b,d]	305.92±88.7[c,d]	244.9±41.4	**0.006**
Hemoglobin (g/dL)	12.73±2.07	13.33±1.45	12.79±1.85	13.72±1.84	0.197
Creatinine, mg/dL	2.20±2.41[a,b]	0.71±0.12[b,d]	0.83±0.26	0.70±0.13	**0.001**
Uric acid, mg/dL	6.13±1.69[a,b]	4.94±1.06	5.34±2.11	4.86±0.94	**0.021**
Total cholesterol, mg/dL	190.4±54.78	205.4±38.76	198.0±57.54	176.9±58.42	0.156
Triglyceride, mg/dL	196.6±127.9[a,b]	154.2±67.52	165.2±81.73	115.5±51.47	**0.019**
LDL, mg/dL	113.8±34.26	122.7±29.9	126.7±40.12	107.6±48.63	0.142
HDL, mg/dL	47.06±13.46	54.74±13.67	40.98±11.96	45.99±10.09	0.061

Values are presented as number (%), mean ± standard deviation or median [Q1-Q3]. CRP: C-reactive protein, ESR: Erythrocyte sedimentation rate, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, [a]Control, [b]Amyloidosis, [c]IgG4-RD, [d]Sarcoidosis, IgG4-RD: IgG4-related disease, kPa: KiloPascals

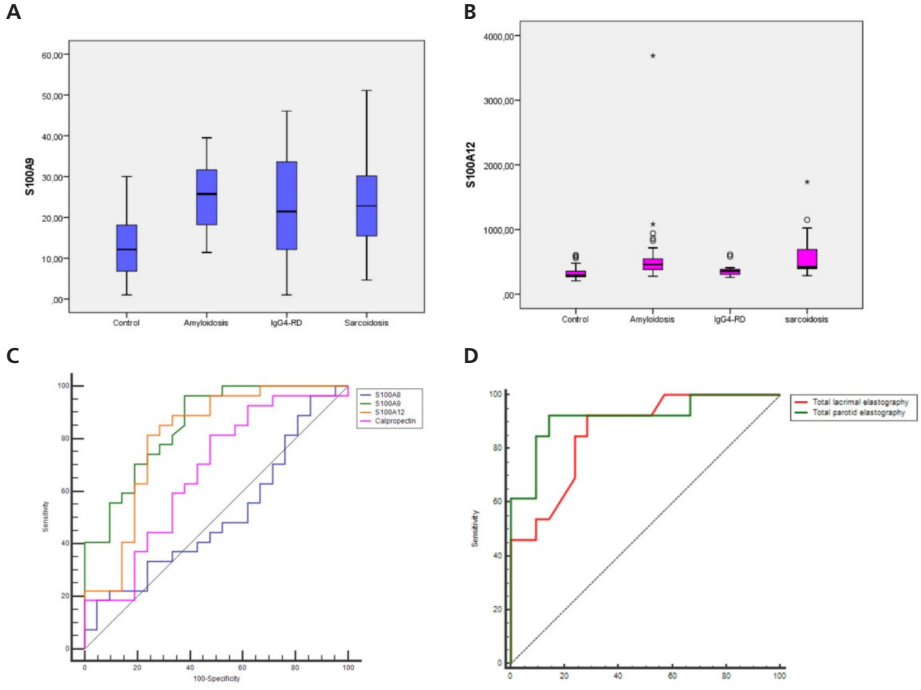

Figure 2. (A, B) Comparison of plasma S100A9 and S100A12 levels in amyloidosis, IgG4-RD, sarcoidosis patients and healthy groups, **(C)** Receiver operating characteristic curve analysis of S100A8, S100A9, S100A12 and calprotectin in amyloidosis. **(D)** Receiver operating characteristic curve analysis of parotid and lacrimal gland elasticity score in IgG4-RD. The area under the curve discriminates between IgG4-RD patients and healthy control subjects
IgG4-RD: IgG4-related disease

challenging. For definitive diagnosis gland and organ biopsies are required. However, both due to the invasiveness of the procedures and inadequate sampling may lead to a delay in diagnosis.[14] Biopsy can be challenging depending on the localization. Various studies have demonstrated that these diseases actually account for 25 to 50% of pseudoinflammatory diseases.[15] Unfortunately, we do not have adequate information on which patients will have a

Table 3. Comparison of elasticity (kPa) of parotid and lacrimal glands using two-dimentional shear wave elastography and B-mode ultrasonography

	Amyloidosis (n=27)	Sarcoidosis (n=17)	IgG4-RD (n=13)	Control (n=21)	p
Parotid gland elasticity (kPa)	12.95±4.03[b,c]	12.84±3.25[d,c]	16.27±3.48	9.89±2.60[a,c]	0.001
Lacrimal gland elasticity (kPa)	6.55±1.71	6.18±2.15	7.88±2.09	5.18±1.49[a,c]	0.001
P.G echogenicity, n (%)					
-Normal	14 (51.9)	9 (52.9)	6 (46.2)	21 (100)	0.001
-Increased	13 (48.1) [a,b]	8 (47.1) [a,d]	7 (53.8) [a,c]	0 (0)	
P.G texture, n (%)					
-Homogeneous	20 (74.1)	11 (64.7)	7 (53.8)	17 (81.0)	0.359
-Heterogeneous	7 (25.9)	6 (35.3)	6 (46.2)	4 (19.0)	
P.G posterior border, n (%)					
-Visible	18 (66.7)	10 (58.8)	7 (53.8)	15 (71.4)	0.724
-Invisible	9 (33.3)	7 (41.2)	6 (46.2)	6 (28.6)	
L.G echogenicity, n (%)					
-Normal	23 (85.2)	16 (94.1)	11 (84.6)	20 (95.2)	0.606
-Increased	4 (14.8)	1 (5.90)	2 (15.4)	1 (4.80)	
L.G texture, n (%)					
-Homogeneous	14 (51.9)	9 (52.9)	10 (76.9)	20 (95.2)	0.002
-Heterogeneous	13 (48.1)[a,b]	8 (47.1)[a,d]	3 (23.1)	1 (4.80)	

P.G: Parotid gland, L.G: Lacrimal gland, [a]Control, [b]Amyloidosis, [c]IgG4-RD, [d]Sarcoidosis, kPa: KiloPascals, IgG4-RD: IgG4-related disease

Table 4. ROC curve analysis of S100 proteins and elastography

	Cut-off value	Sensitivity	Specificity	AUC	Std. error	95% confidence interval	p
IgG4-RD							
Parotid elasticity	≥12.55	92.31	80.95	0.916	0.055	0.808-1000	0.001
Lacrimal elasticity	≥5.5	92.31	66.67	0.863	0.062	0.740-0.985	0.001
Amyloidosis							
S100A9	≥12.7	96.3	57.14	0.853	0.054	0.746-0.959	0.001
S100A12	≥355.46	81.48	71.43	0.804	0.068	0.671-0.938	0.001
Calprotectin	≥437.61	81.48	47.62	0.668	0.081	0.510-0.826	0.047

IgG4-RD: IgG4-related disease, AUC: Area under the curve, ROC: Receiver operating characteristic

more aggressive course of disease, and inadequate diagnosis and monitorization result in irreversible organ damage in clinical practice. Irreversible organ damage decreases the rate of response to current treatment as well as causing higher rate of morbidity and financial burden. Hence, it is important to establish the diagnosis, differential diagnosis, activity, and recurrence in these diseases at an early stage.

S100 proteins are small (10-12 kDA) cytosolic proteins which undergo conformational changes by binding calcium. They have basic functions in the body such as cell proliferation, cellular differentiation, energy metabolism, calcium homeostasis, recovery of cardiac tissue after cellular injury, and DNA repair. They regulate immunocomplex balance by playing a role both in pro-inflammatory pathways and anti-inflammatory pathways.[16] Previous studies have shown that S100 proteins were related to disease activity in several inflammatory diseases, such as juvenile rheumatoid arthritis, reactive arthritis, acute gout arthritis, psoriatic arthritis, and systemic lupus erythematosus.[17] In the present study, S100A9 and S10012 proteins were found to be significantly higher in amyloidosis and sarcoidosis patients than in healthy controls, whilst calprotectin was found to be higher only in amyloidosis patients than in the IgG4-RD group. In previous studies, it was reported that transthyretin amyloid (ATTR) amyloidosis patients with GI symptoms had high fecal calprotectin levels. In the present study, the highest calprotectin levels were found in amyloidosis patients with GI involvement. Therefore, if high serum calprotectin levels are detected in amyloidosis patients without GI involvement, these patients may undergo colonoscopy with biopsy in the early period, preventing GI pathologies from developing.

All new generation US devices are equipped with elastography features. Sonoelastography is a non-invasive

imaging method that can quantitatively evaluate tissue stiffness. 2D-SWE reduces operator-dependent limitations by providing a ROI box on top of the color-coded images and reveals a numerical value reflecting tissue stiffness. [18] Elastography has broad applications in liver and thyroid diseases for the assessment of tumor/nodular lesions and parenchymal fibrosis. In addition, its utility has been previously demonstrated in skin and tendon involvement of various rheumatic diseases.[19] In our study, parotid elastography scores were significantly higher in IgG4-RD than in the control group. For the cut-off value of 12.55 kPa, sensitivity, and specificity were found as 92.3% and 80.95%, respectively. In addition, S100A9 strongly correlated with lacrimal gland SWE values, reflecting the severity of the disease.

Study Limitations

A small sample size was a major limitation of our study. Another limitation was the absence of serial S100 proteins and sonoelastography measurements; therefore, we could not determine whether its levels changed with treatment or whether it may predict relapse.

Conclusion

S100A9, S100A12, and calprotectin might be promising biomarkers in IgG4-RD, amyloidosis, or sarcoidosis for diagnostic purposes. And this study suggests that the parotid gland lose elasticity in patients with IgG4-RD and amyloidosis. In addition, 2D-SWE, and especially the S100A9 biomarker, can be used as helpful tools that can facilitate prognosis in the early stages of the disease.

Ethics

Ethics Committee Approval: The study was conducted after the ethical approval of the Ethics Committee of Gazi University complied with the Declaration of Helsinki (approval no: 2023.549).

Informed Consent: Informed consent was obtained from all patients and controls before the initiation of the study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: H.K., Concept: H.K., A.K., A.E., H.Kü., M.A.Ö., Design: H.K., N.Y.D., M.N.C., S.A., H.P., A.K., A.E., H.Kü., M.A.Ö., Data Collection or Processing: H.K., N.Y.D., M.N.C., S.A., H.P., A.K., A.E., H.Kü., M.A.Ö., Analysis or Interpretation: H.K., N.Y.D., Literature Search: H.K., A.E., H.Kü., Writing: H.K.

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Supplementary Table 1. ICC scores for intra- observer and interobserver measurements in the sonoelastography findings

	Intraobserver (ICC)	p	Interobserver (ICC)	p
Parotid gland elasticity	0.983	**0.001****	0.864	**0.001****
Lacrimal gland elasticity	0.996	**0.001****	0.949	**0.001****
P.G echogenicity	0.989	**0.001****	0.991	**0.001****
P.G texture	0.987	**0.001****	0.864	**0.001****
P.G posterior border	0.944	**0.001****	0.880	**0.001****
L.G echogenicity	0.899	**0.001****	0.881	**0.001****
L.G texture	0.961	**0.001****	0.940	**0.001****

ICC: Intraclass correlation coefficient, P.G: Parotid gland, L.G: Lacrimal gland

Supplementary Table 2. Correlation of S100 proteins with parotid/lacrimal elastography scores, ESR and CRP in amyloidosis, sarcoidosis and IgG4-RD

	Amyloidosis (n=27)				Sarcoidosis (n=17)				IgG4-RD (n=13)			
	S100A9		S100A12		S100A9		S100A12		S100A9		S100A12	
	r	p	r	p	r	p	r	p	r	p	r	p
S100A8, ng/mL	-0.083	0.682	0.445	0.128	0.275	0.286	0.389	0.123	0.311	0.301	0.159	0.603
S100A9, ng/mL	1.000				1.000				1.000			
S100A12, ng/mL	-0.188	0.348	1.000		0.206	0.428	1.000				1.000	
S100A8/A9, ng/mL	-0.098	0.628	0,644	**0.001**	0.184	0.480	0.309	0.155	0.094	0.761	0.454	0.287
Parotid elasticity, kPa	0.948	**0.014**	0.970	0.08	0.213	0.411	0.213	0.411	0.806	**0.001**	0.374	0.209
Lacrimal elasticity, kPa	0.227	0.255	0.263	0.185	0.325	0.203	-0.028	0.914	-0.176	0.564	-0.465	0.109
ESR, mm/h	0.163	0.417	0.222	0.265	0.205	0.447	0.118	0.664	0.492	0.087	0.000	1.000
CRP, mg/dL	-0.029	0.887	0.063	0.759	0.080	0.761	0.321	0.209	0.366	0.219	0.088	0.775

CRP: C-reactive protein, ESR: Erythrocte sedimentation rate, IgG4-RD: IgG4-related disease, kPa: KiloPascals