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The clinical significance of anti-DFS70 autoantibodies and its correlation with Vitamin D levels

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ABSTRACT

OBJECTIVE: This study aims to investigate the clinical significance of anti-dense fine speckled 70 (DFS70) autoantibodies and its association with systemic autoimmune rheumatic diseases (SARD) related autoimmune markers and Vitamin D levels.

METHODS: The study group consisted of 281 (mean age±SD: 45.31±15.89 years; 88.3% female) anti-DFS70 autoantibody-positive patients. All patients' sera in the study group were tested by ANA HEp-2 indirect immunofluorescent antibody (IIF) and immunoblotting (IB) methods (Euroimmun AG, Lübeck, Germany). Anti-DFS70 antibody-positive patients were divided into two subgroups. Anti-DFS70 antibody-positive patients with SARD were assigned as Group 1 (n=43), anti-DFS70 antibody-positive patients without SARD were assigned as Group 2 (n=238). A control group with anti-DFS70 negative patients with SARD were assigned as Group 3 (n=49, mean age±SD: 49.86±12.08; 79.6% female). The clinical characteristics, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), neutrophil/lymphocyte ratio, thrombocyte/lymphocyte ratio (TLR), rheumatoid factor (RF), anti-cyclic citrullinated peptide (anti-CCP), and 25-hydroxyvitamin D3 (25OHD3) levels were compared between three groups.

RESULTS: The majority (61.9%) of anti-DFS70 antibody positive patients had no specific diagnosis. Other systemic diseases were detected as allergic diseases (10.0%), hematological abnormalities (5.0%), thyroid diseases (3.6%), gastrointestinal system diseases (1.8%), malignancies (1.4%), and infections (1.1%). ESR, CRP levels, and TLR were lower in Group 2 than Groups 1 and 3 ($p<0.05$). RF and anti-CCP positivity rates were lower in Group 2 when compared with Groups 1 and 3 ($p<0.05$). 25OHD3 levels did not differ between three groups ($p=0.103$).

CONCLUSION: We observed that anti-DFS70 autoantibody may be associated with organ-specific autoimmune diseases, allergic diseases, and hematological disorders. Therefore, it is essential to evaluate these pathologies in patients positive for anti-DFS70 antibodies.

Keywords: Anti-dense fine speckled 70 antibody; immunoblotting method; indirect immunofluorescent antibody method; inflammation; Vitamin D.

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Autoantibodies targeting nuclear and cytoplasmic autoantigens are used as markers in the diagnosis and classification of systemic autoimmune rheumatic diseases (SARD), such as systemic lupus erythematosus (SLE), Sjogren's syndrome (SS), mixed connective tissue disease, and systemic sclerosis [1].

In recent years, studies have demonstrated a new autoantibody called anti-dense fine speckled 70 (DFS70) in healthy individuals, patients with interstitial cystitis, atopic dermatitis, alopecia, asthma, thyroid diseases, cataract, malignancy, and some inflammatory conditions [2–5]. The DFS pattern is characterized by the

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fine-granular fluorescence of the nuclei in the interphase and the metaphase chromatin [2]. The target antigen was called DFS70, since it causes autoantibody reactivity with a 70 kD protein in Western blot. Protein sequence analyses have shown that this antigen is identical to lens epithelium-derived growth factor (LEDGF), also known as DNA-binding transcription coactivator p75 [3–5]. DFS70/LEDGFp75 protein is commonly present in mammalian cells, and it is a multifunctional stress response protein that is related to cancer, inflammation, and other disorders [2, 6, 7].

The indirect immunofluorescent (IIF) staining method with Hep-2 cell substrate is the gold standard for detecting antinuclear antibodies (ANA) [1, 2]. DFS nuclear pattern is commonly detected by ANA HEp-2 IIF test in routine ANA screening. The ANA-HEp-2 IIF test is not always reliable for this pattern, and confirmation assays such as immunoblotting (IB) and enzyme-linked immunosorbent assay should be used to identify anti-DFS70 autoantibody and other concomitant SARD specific autoantibodies [2, 8]. In light of the existing literature, anti-DFS70 autoantibody presence helps to exclude the diagnosis of rheumatologic diseases in the absence of concomitant SARD specific autoantibodies [2, 8, 9]. Although studies show that the prevalence of anti-DFS70 autoantibodies are higher in healthy individuals and some inflammatory conditions, the mechanism underlying the appearance of anti-DFS70 antibody is not clear enough, and there is still lack of information about its clinical significance [3, 9].

Vitamin D has many effects on the immune system as well as its known role on calcium and bone homeostasis. Vitamin D can regulate innate and adaptive immune responses and has immunmodulatory functions. Vitamin D deficiency has been associated with increased susceptibility to autoimmune disorders [10]. Although the relationship between Vitamin D and SARD has been studied frequently, there are limited number of studies investigating the association between Vitamin D and anti-DFS70 antibody.

The present study primarily aimed to investigate the clinical significance of anti-DFS70 autoantibodies and its association with hematological, inflammatory, and other SARD related autoimmune markers. The second aim of this study is to determine the relationship between anti-DFS70 autoantibody presence and Vitamin D levels.

Highlight key points

- Anti-DFS70 autoantibody may be associated with organ-specific autoimmune diseases, allergic diseases, and hematological disorders.
- Anti-DFS70 antibody and SARD-related autoantibodies can also be detected together.
- SARD-related autoantibodies should be monitored together rather than confirming the DFS pattern alone.

MATERIALS AND METHODS

The study was conducted in accordance with the original Declaration of Helsinki. This study was approved by the non-interventional ethics committee of Balikesir University (date: 20.05.2020, approval number: 2020/78).

Patients

The study group consisted of anti-DFS70 autoantibody-positive patients admitted to Balikesir Ataturk City Hospital between 2019 and 2020 years. Anti-DFS70 antibody-positive patients are divided to subgroups as anti-DFS70 antibody-positive patients with SARD (Group 1), and anti-DFS70 antibody-positive patients without SARD (Group 2). Anti-DFS70 antibody-negative patients with SARD (control group) were assigned as Group 3.

Demographic characteristics (age, gender, etc.), signs and symptoms, comorbidities, presence of malignancy, presence of rheumatic disease, family history, and clinical data for the rheumatic disease were searched from the medical records. Recurrent outcomes of patients were not included in the study.

IIF-ANA Assay

All patients' sera in the study group were tested by ANA HEp-2 IIF and IB methods. Patients whose ANA-HEp-2 IIF DFS pattern confirmed with line immunoassay (LIA) (IB) were recruited.

The IIF-ANA screening test was performed using HEp20-10/liver biochip (Euroimmun AG, Lübeck, Germany) conjugated with a specific anti-human IgG (Euroimmun AG). Sera were considered positive for ANAs if IIF staining was observed at a serum dilution of 1:100 and patterns were evaluated as semi-quantitatively 1+ to 4+ according to the intensity of the positive control. IIF patterns were determined by the ICAP standards (www.ANApatterns.org) by the same laboratory specialist.

Extractable Nuclear Antigen (ENA) Analysis by LIA

Line immunoassay was performed using the Euroimmun Euroline ANA profile three-plus DFS70-IgG assay (Euroimmun AG, Lübeck, Germany) for ENA. Strip consisted of 16 autoantigens: RNP/Sm, Sm, SS-A, Ro-52, SS-B, Scl-70, PM-Scl, Jo-1, DFS-70, centromere protein B, proliferating cell nuclear antigen (Rib-P), dsDNA, nucleosomes, histones, ribosomal P-protein, and anti-mitochondrial antibody-M2 (AMA-M2). Semiquantitative results (negative, 1+, 2+, and 3+) were obtained using a scanner and EUROLineScan software (Euroimmun AG), automatically according to reaction intensity.

Other Assays

Rheumatoid factor (RF) was studied by nephelometric method, anti-cyclic citrullinated peptide (anti-CCP) was studied by chemiluminescent microparticle immunoassay method on Architect device (Abbott Diagnostics). AMA, anti-neutrophil cytoplasmic antibody, anti-smooth muscle antibody, anti-dsDNA, anti-endomysium, and anti-gliadin tests were studied by IIF method (Euroimmune AG). 25-hydroxyvitamin D3 (25OHD3) measurement was done using the chromatographic based method.

Infection and inflammatory markers such as HBsAg, anti-HCV, anti-HIV, and Brucella test results; neutrophil, lymphocyte, platelet values, neutrophil/lymphocyte ratio (NLR) and thrombocyte/lymphocyte ratio (TLR), erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP) were investigated retrospectively. Thyroid autoantibody (anti-TG, anti-TPO) results were searched from laboratory database of our hospital.

Statistical Analysis

The data obtained in the study were analyzed by SPSS 22.0 (SPSS INC, Chicago, IL, USA) program. Whether the groups showed normal distribution or not was tested by the Shapiro–Wilk Kolmogorov–Smirnov tests. As at least one of the groups did not conform to the normal distribution, Kruskal–Wallis test was used for comparing three or more groups. Categorical variables were given as a percentage and mean±standard deviation. Chi-square test was used to compare independent groups with categorical variables. $P<0.05$ was considered statistically significant.

RESULTS

The study group consisted of 281 patients (mean age±SD=45.31±15.89 years; 88.3% female) that was shown to have DFS pattern with ANA-HEp-2 IIF method and further validated to have anti-DFS70 antibody by IB method. 61% of the patients had no specific diagnosis. Other systemic diseases were detected as rheumatologic diseases (15.3%), allergic diseases (10.0%), hematological abnormalities (5.0%), thyroid diseases (3.6%), gastrointestinal system diseases (1.8%), malignancies (1.4%), and infections (1.1%) (Table 1). The demographic and clinical characteristics of all anti-DFS70 antibody-positive patients are given in Table 1.

Group 1 (anti-DFS70 antibody-positive patients with SARD) consisted of 43 of 281 (15.3%) patients. Group 2 (anti-DFS70 antibody-positive patients without SARD) consisted of 238 of 281 (84.6%) patients. Group 3 (n: 49) consisted of anti-DFS70 antibody-negative patients with SARD.

The mean age of Group 2 (43.55±15.57 years) was significantly lower than Group 1 (55.12±14.10 years), and Group 3 (49.86±12.08 years) ($p<0.001$). Gender of the patients was similar between the groups ($p=0.225$). Three of the groups differed in terms of clinical characteristics and laboratory results ($p<0.05$). Group 2 lower rates of arthralgia, myalgia, arthritis, sicca symptoms, and raynaud phenomenon compared to Groups 1 and 3 ($p<0.05$). ESR, CRP levels and TLR was lower in Group 2 than Groups 1 and 3 ($p<0.05$). NLR was similar among three groups ($p=0.138$). RF and anti-CCP positivity rates were lower in Group 2 when compared with Groups 1 and 3. 25OHD3 levels did not differ between three groups ($p=0.103$). The patients' characteristics, comorbid diseases, medications, and laboratory results for all the groups are given in Table 2.

Nine of 43 patients in Group 1 (anti-DFS70 antibody-positive patients with SARD) had comorbid diseases. The summary of patients' characteristics and autoantibody profile that have overlapping rheumatic and other systemic diseases in Group 1 are shown in Table 3.

DISCUSSION

DFS70/LEDGF/p75 is a growth/transcription factor localized in the cell nucleus [11]. Some factors, such as ultraviolet B light, hyperthermia, nutrient deprivation, and some chemotherapeutic agents, may increase

Table 1. Characteristics of anti-DFS-70 antibody positive patients

Group 1 [anti-DFS70 (+) SARD (+)]	%	Group 2 [anti-DFS70 (+) SARD (-)]	%
Rheumatoid arthritis	7.8	Patients without a specific diagnosis	61.9
Sjogren's syndrome	4.3	Allergic diseases	10.0
Undifferentiated connective tissue disease	1.4	Asthma	6.4
Systemic lupus erythematosus	1.1	A history of allergies	2.1
Scleroderma	0.7	Allergic rhinitis	1.1
		Idiopathic urticaria	0.4
		Hematological disorders	5.0
		Anemia	1.7
		Thrombocytopenia	1.1
		Coagulation disorder	0.7
		Secondary polycythemia	0.4
		Leukopenia	0.4
		FV Leiden mutation	0.4
		Idiopathic thrombocytopenic purpura	0.4
		Thyroid diseases	3.6
		Autoimmune thyroiditis [Anti-TPO/anti-TG(+)]	2.1
		Hypothyroidism	1.4
		Gastrointestinal system diseases	1.8
		Ulcerative colitis	0.7
		Elevation of liver enzymes	0.7
		Primary biliary cirrhosis [AMA-M2(+)]	0.4
		Malignancies	1.4
		Breast cancer	0.4
		Endometrial malignancy	0.4
		Basal cell carcinoma of the skin	0.4
		Giant cell bone tumor	0.4
		Infections	1.1
		Brucella	0.7
		HCV	0.4

Anti-DFS70: Anti-dense fine speckled 70; SARD: Systemic autoimmune rheumatic diseases; Anti-TPO: Anti-thyroid peroxidase; Anti-TG: Anti-thyroglobulin; AMA-M2: Anti-mitochondrial antibody-M2; HCV: Hepatitis C virus.

oxidative stress, leading to the activation of DFS70/LEDGFp75. DFS70/LEDGFp75 helps cellular protection against environmental stressors by activating the transcription of various protective genes [2, 12]. Since its first definition, many diseases and clinical conditions have been associated with anti-DFS70 antibodies [2, 3, 8]. Anti-DFS70 antibodies have previously been reported in healthy individuals, blood donors, various autoimmune disorders, cancer, and inflammatory conditions [2, 11, 13]. Although many studies have attempted to establish a clinical relationship with anti-DFS70 antibodies, a clear relationship has not been confirmed yet [8]. Once detected in different conditions, anti-DFS70 antibodies have been reported to remain generally stable for several years, but it is suggested that systematic studies are required to address this thoroughly [8, 14].

In our study, we analyzed the clinical and laboratory profiles of all anti-DFS70 antibody-positive patients who were admitted to our hospital. We have also investigated the comorbidities, medications, inflammatory markers, and other autoimmune antibodies to evaluate the patients from a comprehensive perspective. According to our results, the mean age of the patients with positive anti-DFS70 antibodies was 45.3 years, and 88.3%

TABLE 2. Patients' characteristics, comorbidities, and treatments

	Group 1 [anti- DFS70 (+) SARD (+)] (n=43)	Group 2 [anti-DFS70 (+) SARD (-)] (n=238)	Group 3 [anti-DFS70 (-) SARD (+)] (n=49)	p
Age (years; mean±SD)	55.12±14.10	43.55±15.57	49.86±12.08	<**0.001**
Gender (female: [%])	86	88.7	79.6	0.225
Clinical history (%)				
Smoking	9.3	12.2	28.6	**0.007**
Arthralgia	83.7	44.5	89.8	<**0.001**
Myalgia	58.1	37.0	61.2	**0.001**
Lumbago	2.3	7.6	16.3	**0.041**
Arthritis	58.1	-	61.2	<**0.001**
Xeroftalmia/Xerostomía	30.2	3.8	24.5	<**0.001**
Raynaud	18.6	0.4	20.4	<**0.001**
Comorbidities (%)				
HL, CAD	2.3	2.1	10.2	**0.015**
HT	7.0	3.8	4.1	0.632
DM	4.7	1.7	4.1	0.363
Depression, anxiety disorders	2.3	4.2	12.2	**0.045**
Epilepsy, migraine	-	0.4	-	0.824
Pregnancy	-	0.8	4.1	0.124
Laboratory values				
25OHD3 (ng/mL) (mean±SD)	20.84±11.27	21.88±13.33	15.99±8.35	0.103
RF (%)	40.5	2.5	55.1	<**0.001**
Anti-CCP (%)	35.0	0.6	44.9	<**0.001**
ESR (mmHg) (mean±SD)	29.25±16.93	22.39±10.91	33.43±23.38	**0.002**
CRP (ng/mL) (mean±SD)	0.59±0.58	0.42±0.38	0.78±0.92	**0.040**
NLR (mean±SD)	2.00±1.07	2.06±1.13	2.47±1.38	0.138
TLR (mean±SD)	151.88±58.96	127.62±68.16	149.40±65.33	<**0.001**
Medication				
Metabolic diseases (antihyperlipidemic, oral antidiabetics, anti-hypertensives beta-blocker, levothyroxine), n	6	24	6	
Psychiatric diseases (antidepressants), n	1	10	–	
Neurological diseases (anti-epileptics), n	–	1	2	
Allergic diseases (bronchodilators, antihistaminics), n	–	21	–	
Rheumatic diseases (DMARD, steroids, biologic agents, immuno modulatory/ supressant agents), n	37	–	43	

Anti-DFS70: Anti-dense fine speckled 70; SARD: SD: Standard deviation; Systemic autoimmune rheumatic diseases; RF: Rheumatoid factor; Anti-CCP: Anti cyclic citrilled peptide; ESR: Erythrocyte sedimentation rate; CRP: C-reactive protein; NLR: Neutrophil/lymphocyte ratio; TLR: Thrombocyte/ lymphocyte ratio; HL: Hyperlipidemia; CAD: Coronary artery disease; HT: Hypertension; DM: Diabetes mellitus; DMARD: Disease modifying anti-rheumatic drugs.

Table 3. Characteristics of patients with additional pathologies who diagnosed with rheumatic disease and positive anti-DFS70 antibodies

	Diseases/comorbidities	Autoantibodies	Age	Gender
1	SS and pancytopenia	Anti-DFS-70, SS-A, SS-B, Ro-52	36	Female
2	SS and allergy	Anti-DFS-70, SS-A	48	Female
3	SLE and anemia	Anti-DFS-70, Histone, Nucleosom, Sm, SS-A, Ro-52, anti-dsDNA	45	Male
4	SLE and kidney involvement	Anti-DFS-70, Histone, SS-A	43	Female
5	SLE and kidney involvement	Anti-DFS-70, Histone, Nucleosom, Sm, Rib-P	42	Female
6	RA and atopic dermatitis	Anti-DFS-70, RF, anti-CCP	50	Female
7	RA and AMA (+)	Anti-DFS-70, AMA-M2, RF	76	Female
8	UCTD, Raynaud, lung involvement	Anti-DFS-70, Sm/RNP, RF	66	Female
9	Scleroderma and interstitial lung disease	Anti-DFS-70, Scl-70	77	Female

SS: Sjogren's syndrome; RA: Rheumatoid arthritis; SLE: Systemic lupus erythematosus; UCTD: Undifferentiated connective tissue disease; RF: Rheumatoid factor; anti-CCP: Anti cyclic citrullinated peptide; Sm: Anti-smith antibody; Anti-DFS70: Anti-dense fine speckled 70; AMA: Anti-mitochondrial antibody; anti-dsDNA: Anti-double stranded DNA antibody.

were female. These results are in line with previous studies, which suggest that anti-DFS70 antibody appears at a younger age and more commonly in females [5, 9, 15, 16].

Anti-DFS70 antibody was reported between 2.0% and 21.6% in healthy individuals [3]. Analyzes of the data of 78399 individuals, including blood donors, healthy individuals, and patients who applied for routine ANA screening from 20 studies using different laboratory methods revealed that the positivity rate of the DFS pattern could reach up to 37% [11]. It has been suggested that isolated anti-DFS70 antibody can be used as an exclusion criterion for SARD in the absence of SARD-related autoantibodies [2, 8, 9]. At this point, an accurate diagnosis of the DFS pattern becomes crucial. An international study reported that the rates of accurately determining the DFS pattern are significantly lower than other classical ANA patterns and do not exceed 50% [17]. Correctly recognizing the DFS ANA IIF pattern and mixed IIF models composed of DFS as well as other ANA patterns presents a significant challenge. In conclusion, it seems indispensable that specific immunological tests are needed to confirm the presence of anti-DFS70 antibodies and to investigate the presence of other SARD-related autoantibodies before definitive results are reported to clinicians [8, 17]. The coexistence of autoantibodies may prevent ANA patterns to be recognized correctly. Instead of confirming the DFS pattern alone, the commonly recommended approach is using additional confirmation methods, including other autoantibodies [9, 11, 18]. As strength of our study, we have included patients with positive anti-DFS70 antibodies which were further confirmed with the IB method. SARD-related autoantibodies have also been investigated with this method.

According to our results, the majority (61.9%) of anti-DFS70 antibody-positive patients had no specific diagnosis. About 15.3% of the patients with positive anti-DFS70 antibodies were also positive for other SARD related autoantibodies and were diagnosed with rheumatic diseases. The diagnoses of these patients were RA (7.8%), SS (4.3%), UCTD (1.4%), SLE (1.1%), and scleroderma (0.7%). In the previous studies, other SARD related autoantibodies were found in approximately 11% of the patients that were also positive for anti-DFS70 autoantibody [2, 3, 9, 19, 20]. Studies reported anti-DFS70 antibodies as 2.6–11.1% in RA [19, 21], 4.3–28.6% in SS [3, 5, 15, 19], 1.8–5.7% in SLE [3, 15, 21], 0.6–5.7% in scleroderma [3, 5, 21], and 8.3–40% in UCTD patients [3, 18, 19]. The variability in anti-DFS70 antibody positivity rates among different studies may result from the differences in laboratory methods, whether a confirmation method is used, and the characteristics of the study Groups 3. Anti-DFS70 antibodies that are present in SARD are usually accompanied by other SARD-related autoantibodies [2]. In a study assessing the long-term outcomes of individuals with anti-DFS70 antibodies, none of the individuals developed SARD over an average follow-up of 4 years, who were tested negative for any other disease-specific autoantibodies [14].

In the previous studies with SLE patients, there were no clinical differences between patients with and without anti-DFS70 antibodies, suggesting that anti-DFS70 antibodies may not be associated with disease activity [2, 21]. Similarly, our study reported no clinical differences between the two SARD groups. In addition, classic SLE-associated autoantibodies have been reported with the anti-DFS70 antibody in all SLE patients except one [21]. We observed that some patients with anti-DFS70 antibodies have other autoantibodies associated with SARD, and some of these patients had accompanying allergic and hematological disorders. It is not yet clear whether these underlying pathologies trigger formation of anti-DFS70 antibody in patients with rheumatic diseases. Large sample sized studies with well-defined SARD patients could help understanding whether the presence of anti-DFS70 autoantibodies is incidental or associated with a particular clinical phenotype, comorbidity, or therapy.

The relationship of anti-DFS70 antibody with a specific clinical condition has not been demonstrated yet [8]. Our results showed that 10% of these patients had accompanying allergic diseases, and asthma (6.4%) was the most common condition. Asthma was reported as 4.0–16% in anti-DFS70 antibody positive patients in the previous studies [5, 21]. Hematological abnormalities were found in 5% (1.7% were anemia) thyroid diseases in 3.6%, and gastrointestinal system diseases in 1.8% of our patients. Among these, the presence of inflammatory and autoimmune diseases was remarkable. Autoimmune thyroiditis (2.1%) was the most common thyroid disease. In the patient group with gastrointestinal diseases; one patient had primary biliary cirrhosis with positive AMA-M2 autoantibody, and two patients had ulcerative colitis. Dellavance et al. [22] reported anti-DFS70 antibodies in various conditions, including organ-specific autoimmune diseases and inflammatory conditions. Their results showed that this autoantibody was common among people without SARD diagnosis, and 16% of those individuals had autoimmune thyroiditis. Few studies are investigating the association of anti-DFS70 antibodies with the presence of an organ-specific autoimmune disease. However, a higher frequency than SARD had shown only in patients with autoimmune thyroiditis (between 6.0 and 47.8%) [19, 21, 22].

In our study, infectious diseases such as HCV and Brucella infections were detected in 1.1% of the patients. It was previously reported that the anti-DFS70 antibody was detected in 7.4% of the patients with infectious conditions such as sinusitis, urinary tract infection, Adenovirus, HCV, and Toxoplasma gondii infections [22].

In many types of cancer, DFS70/LEDGFp75 is overexpressed. It acts as an oncoprotein promoting cancer cell proliferation and migration, angiogenesis, clonogenicity, stress survival, chemoresistance, and tumor growth [2, 23]. This protein has been associated with inflammatory, autoimmune conditions, and cancer. Although LEDGF/p75 is a stress survival oncoprotein, currently, little is known about its expression in tumors [24]. The anti-DFS70 antibody is most frequently reported in prostate cancer (17.2–22.3%), but it has also been detected in many different malignancies to a lesser extent (1.8%) [3]. In particular, it is more frequently detected in colon, thyroid, and breast cancers [24]. Given the emerging role of DFS70 / LEDGFp75 as an oncoprotein in various cancer types, relatively high frequencies of anti-DFS70 autoantibodies are expected in cancer patients [8]. In this study, malign diseases were determined in 1.4% of the patients. These malignancies included breast cancer, endometrial malignancy, basal cell carcinoma of the skin, and giant cell tumor of bone.

We observed lower 25OHD3 levels in the anti-DFS70 antibody-negative SARD group (Group 3) compared to the anti-DFS70 antibody-positive SARD group (Group 1). However, this difference was not statistically significant. Low levels of 25OHD3 have been associated with a higher ANA prevalence [10, 25, 26]. Moreover, an inverse correlation between 25OHD3 levels and the presence of autoantibodies in connective tissue diseases has also been reported. For example, 25OHD3 deficiency was associated with high serum anti-CCP levels in RA, and high RF levels in SS [27, 28]. The relationship between SARD and 25OHD3 has been studied extensively. However, the relationship between anti-DFS70 antibody positivity and 25OHD3 levels is investigated in a limited number of studies. Carbone et al. [29] reported higher 25OHD3 levels in anti-DFS70 antibody-positive individuals compared to healthy controls and SLE patients. This result was attributed to the natural protective properties of anti-DFS70 antibodies [29, 30]. 25OHD3 has numerous effects on the cells of the immune system. By suppressing B cell proliferation, differentiation, and secretion of immunoglobulins, it can cause decreased autoantibody production. 25OHD3 has recently been shown to have the ability to suppress autoimmunity-related Th17 production, thus pro-inflammatory IL-17 production, and to increase regulatory T cells, along with the other immunomodulatory effects [10]. Prospective cohort studies are needed in determining the relationship between 25OHD3 and anti-DFS70 antibodies.

The limitation of this study is its retrospective nature. However, a relatively larger sample size with the inclusion of patients whose DFS pattern was confirmed is the strength of the study. Furthermore, a detailed analysis of the clinical characteristics and laboratory results of the patients was performed. This study is currently one of the few studies assessing the association between anti-DFS70 antibody and 25OHD3 levels. Thus, the results can make valuable contributions to the existing knowledge.

In conclusion, the anti-DFS70 antibody has been widely used as a marker to exclude SARD diagnosis. Therefore, further analysis of anti-DFS70 autoantibody with confirmation assays becomes inevitable to perform the correct diagnosis. In this way, unnecessary further tests and treatments would be avoided. It has been observed that anti-DFS70 autoantibody may be associated with organ-specific autoimmune diseases, allergic diseases, and hematological disorders. Therefore, it is essential to evaluate these pathologies in patients positive for anti-DFS70 antibodies. Anti-DFS70 antibody and SARD-related autoantibodies can also be detected together. Consequently, we think that SARD-related autoantibodies should be monitored together rather than confirming the DFS pattern alone. We suggest that this approach would yield a more effective diagnosis and follow-up.

Ethics Committee Approval: The Balikesir University Clinical Research Ethics Committee granted approval for this study (date: 20.05.2020, number: 2020/78).

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