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Evaluation of Anti-DFS70 antibodies and DFS pattern in ANA positive individuals and ANA Associated Rheumatic Diseases

ANA Pozitif Bireylerde ve ANA İlişkili Romatizmal Hastalıklarda Anti-DFS70 Antikorlarının ve DFS Paterninin Değerlendirilmesi

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ABSTRACT

Aim: In this study we aimed to find the frequency of anti-DFS70 antibodies and DFS pattern in ANA positive individuals and ANA associated rheumatic diseases (AARDs).
Methods: In this study, 337 subjects who were evaluated in a rheumatology clinic with prediagnosis of rheumatic diseases with positive ANA test and had concurrent anti-extractable nuclear antigen (anti-ENA) antibodies results were retrospectively analyzed. Clinical diagnosis of patients and demographic characteristics were obtained from the patients' medical records.
Results: A total of 337 subjects (305 women, 32 men) were included in this study. The mean age was 49.8 ± 14.2 years. Of the 337 participants, 111 (32.9%) had an IIF-DFS pattern and 226 (67.1%) had a non-DFS pattern. Anti-DFS70 antibodies were positive in 20.1% of individuals. Sixty eight individuals had AARDs. An IIF-DFS pattern was observed in 22.1% and a non-DFS pattern was observed in 77.9% of individuals with AARDs ($p < 0.05$). Anti-DFS70 antibodies were positive in 13.2% of patients with AARDs. The frequency of AARDs was significantly lower in individuals with anti-DFS70 antibodies compared to individuals with other anti-ENAs antibodies ($p < 0.05$).
Conclusion: Anti-DFS70 antibodies may be present in patients with AARDs but AARDs are less prevalent in patients who had anti-DFS70 antibodies, compared with patients who had other anti-ENAs.

Key words: Antinuclear antibodies (ANA), Anti DFS70 antibody, DFS pattern

ÖZ

Amaç: Bu çalışmada, ANA pozitif bireylerde ve ANA ilişkili romatizmal hastalıklarda anti-DFS70 antikor ve DFS patern sıklığını bulmayı amaçladık.
Yöntem: Bu çalışmada romatoloji kliniğinde romatizmal hastalık ön tanısı ile değerlendirilen, ANA tetkiki pozitif olan ve eş zamanlı anti-ekstrakte edilebilir nükleer antijen (anti-ENA) antikor sonucu olan 337 kişi retrospektif olarak incelendi. Hastaların klinik tanıları ve demografik özellikleri hasta tıbbi kayıtlarından elde edildi.
Bulgular: Bu çalışmaya toplam 337 (305 kadın, 32 erkek) kişi dahil edildi. Yaş ortalaması 49.8±14.2 yıl idi. 337 katılımcının 111'i (%32.9) IIF-DFS paterne, 226'sı (%67.1) DFS dışı paterne sahipti. Bireylerin %20.1'inde anti-DFS70 antikoru pozitifti. 68 kişide ANA ilişkili romatizmal hastalık vardı. ANA ilişkili romatizmal hastalığa sahip kişilerin %22.1'inde IIF-DFS patern, %77.9'unda DFS dışı patern gözlendi ($p<0.05$). ANA ilişkili hastalığa sahip kişilerin %13.2'sinde anti-DFS70 antikoru pozitifti. Anti-DFS70 antikoruna sahip kişilerde diğer anti-ENA antikorlarına sahip kişilerle karşılaştırıldığında ANA ilişkili romatizmal hastalık sıklığı anlamlı ölçüde düşüktü ($p<0,05$).
Sonuç: Anti-DFS70 antikorlar ANA ilişkili romatizmal hastalıklarda bulunabilir ancak ANA ilişkili romatizmal hastalıklar anti-DFS70 antikorlarına sahip kişilerde diğer anti-ENA antikorlarına sahip kişilere kıyasla daha az yaygındır.

Anahtar kelimeler: Anti nükleer antikorlar (ANA), Anti DFS70 antikor, DFS patern

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INTRODUCTION

Antinuclear antibodies (ANA) are assay markers for diagnosis of ANA associated rheumatic diseases (AARDs) such as systemic lupus erythematosus (SLE), Sjögren's syndrome (SjS), mixed connective tissue disease (MCTD) and systemic sclerosis (SSc) [1]. The indirect immunofluorescence (IIF) assay is mostly used for the detection of ANA and proposed as a screening test by the American College Rheumatology (ACR) [2]. Anti-Dense Fine Speckled 70 (DFS70) antibodies were identified as related to specific IIF-ANA pattern. The DFS pattern is characterized by irregularly distributed, fine-granular fluorescence of the nuclei and has shown reactivity of the autoantibody with a 70 kD protein [3,4]. Anti-DFS70 antibodies are related with different status [5]. DFS70 pattern and anti-DFS70 antibodies have been stated more commonly in healthy individuals compared to systemic rheumatic diseases [6,7]. Anti-DFS70 antibodies have been defined as an eventual marker for the ruling out systemic autoimmune diseases (SARDs) [8].

In this retrospective study we aimed to determine the frequency of anti-DFS70 antibodies and IIF-DFS pattern in IIF-ANA positive individuals and patients with AARDs.

MATERIALS AND METHODS

In this retrospective study, 337 subjects with positive ANA test and who simultaneously had anti-ENAs antibodies results and who were evaluated with a prediagnosis of rheumatic diseases in the rheumatology outpatient clinic between 2018 and 2019, were consecutively included. ANA was detected using the indirect immunofluorescence (IIF) method (IIF Mosaic: Hep-20-10/Liver (Monkey) kit, Euroimmun, Germany). ANA at titer ≥ 1/160 was considered as positive. An ANA Profile 3 plus DFS70-IgG kit (Euroimmun, Germany) was used for the anti-ENAs test. Autoantibodies against nuclear ribonucleoprotein (nRNP), Smith (Sm), SS-A/Ro-52, SS-B, Scl-70, PM-Scl, Jo-1, centromere protein B (CENP-B), double-stranded DNA (dsDNA), nucleosome, histone, ribosomal P-protein and anti-mitochondrial antibodies (AMA) autoantigens were evaluated in the ANA profile. Demographic characteristics of the individuals, ANA pattern, anti-ENAs test results and diagnosis of AARDs were recorded from the electronic file. AARDs consisted of SLE, SS, undifferentiated connective tissue disease (UCTD), SSc, myositis, drug induced SLE and SLE/SSc overlap.

Statistics: Categorical data is shown as counts and percentages. Continuous data is shown as means and standard deviations (SD). The Pearson chi-square test was used to compare the qualitative results. The IBM-SPSS16 was used in all statistical analyses and the p value <0.05 was considered statistically significant. This study was performed according to the Declaration of Helsinki. Permission for the study was obtained from the Balıkesir University Faculty of Medicine Ethics Committee, decision number 190 in 04/12/2019.

RESULTS

In this study a total of 337 individuals (305 female, 32 male) with positive ANA and simultaneously anti-ENAs test results were included. The mean age was 49.87±14.29 years (female: 50.10±13.92 years, male: 47.75±17.49 years p>0.05). An IIF-DFS pattern was observed in 32.9% of 337 individuals.

Anti-DFS70 antibodies were positive in 68 (20.2%) of the 337 subjects (Table 1). Among these, 64 (94.1%) had isolated anti-DFS70 antibody (anti-DFS70 antibody positive, with other anti-ENAs being negative). Among anti-DFS70 antibody positive subjects, four had additional anti-ENAs specificity (1 anti-SS-A, 1 pm-scl, 1 anti-histone, 1 anti-SCL-70 antibody). Anti-DFS70 antibodies were present in 54 of the 111 (48.6%) subjects with IIF-DFS pattern (53 isolated anti DFS70 antibodies positive, 1 additional anti ENAs being positive). Fourteen of 226 (6.2%) subjects with other IIF-ANA patterns had anti-DFS70 antibodies. Among DFS pattern positive subjects, 58 individuals had >1/160 titer, 37 individuals had ≥1/320 titer, 14 individuals had ≥1/640 titer and 2 individuals had ≥1/1280 titer. Anti-DFS70 antibodies were positive in 85% of the subjects who had IIF-DFS titers ≥1/640 and 43.1% of the subjects who had >1/160 IIF DFS titer. The mean age was 46.93±14.17 in subjects who had anti-DFS70 antibodies and 50.62±14.25 in subjects who did not (p<0.05).

Sixty-eight of 337 (20.1%) individuals had AARDs

(19 SLE, 21 SS, 21 UCTD, 3 SSc, 1 myositis, 2 drug induced SLE, 1 SLE/SSc overlap). The DFS pattern was observed in 22.1% of (15/68) patients with AARDs. The frequency of AARDs was 13.2% (15/111) in individuals with DFS pattern and 23.5 % (53/226) in individuals with non DFS pattern ($p<0.05$). The anti-DFS70 antibodies were positive in 13.2% (n=9) of 68 patients with AARDs. The other anti-ENAs antibodies were positive in 42.6% (n=29) of 68 patients with AARDs. The frequency of AARDs was significantly lower in the individuals with anti DFS70 antibodies compared to those with other anti-ENAs specificity ($p<0.05$). Among 68 patients with AARDs, 6 patients had isolated anti-DFS70 antibody positivity and 3 patients had additional anti-ENAs specificity. Clinical diagnosis of anti-DFS70 positive subjects were 3 UCTD, 2 SLE and 4 SS. The distribution of AARDs, according to IIF-DFS and anti DFS70 antibody status, is shown in (Table 2).

Table 1. Frequency of IIF-DFS, anti-DFS70 antibody and AARD

	noun/percent	
	Total (337)	AARDs (68)
IIF-ANA		
-ANA positive	337	68
-DFS pattern positive	111 (32.9)	15 (22.1)
-Non DFS pattern	226 (67.1)	53 (77.9)
IB Anti ENA		
-Anti-DFS70 positive	68 (20.2)	9 (13.2)
-İsolated anti DFS70 positive	64 (18.9)	6 (8.8)
-Anti-DFS70 and additional ENAs positive	4 (1.2)	3 (4.4)
-İsolated anti ENAs positive	39 (11.6)	29 (42.2)
IIF-ANA and Anti ENA Combination		
-DFS pattern positive and anti DFS70 positive	54 (16.02)	7 (10.2)
-DFS pattern positive and isolated anti DFS70 positive	53 (15.72)	6 (8.8)
-DFS pattern positive and anti DFS70 additional anti ENAs positive	1 (0.29)	1 (1.4)
-DFS pattern positive and isolated anti ENAs positive	4 (1.18)	3 (4.4)
-DFS pattern negative and anti DFS 70 positive	13 (3.85)	3 (4.4)

The positive predictive value of IIF-DFS pattern to detect AARDs was 13.5% and the positive predictive value of anti-DFS70 antibodies to detect AARDs was 13.2% (Table 3).

Table 2. The frequency of anti-DFS70 antibody and IIF-DFS pattern in AARDs.

	IIF DFS70 pattern N (%)	Non IIF DFS pattern	Anti DFS70 antibody (+)	Anti DFS70 antibody (-)	Total (n)
Systemic Lupus Eritematozus (SLE)	6 (5.4%)	13 (5.8%)	2 (3%)	17 (6.3%)	19
Sjögren Syndrome (SS)	6 (5.4%)	15 (6.6%)	4 (6%)	17 (6.3%)	21
Undifferansiye konnective tissue disease	3 (2.7%)	18 (8%)	3 (4.5%)	18 (6.7%)	21
Systemic Sclerosis		3 (1.3%)		3 (1.1%)	3
Drug induced SLE		2 (0.9%)		2 (0.7%)	2
SLE/SS overlap		1 (0.4%)		1 (0.4%)	1
Myositis		1 (0.4%)		1 (0.4%)	1
Total	15	53	9	59	68

Table 3. The IIF-DFS pattern and anti DFS70 antibody in detection of AARDs

n=337	IIF DFS pattern positive	Anti DFS70 antibody positive
AARD negative (n=269)	96	59
AARD positive (n=68)	15	9
Total	111	68

Discussion: In this study, we aimed to investigate the frequency of IIF-DFS pattern and anti-DFS70 antibodies in ANA positive individuals and determine whether IIF-DFS pattern and anti-DFS70 antibodies were important to exclude the diagnosis of AARDs. The DFS pattern has been defined as the AC-2 pattern by an international ANA consensus algorithm. Although the intensely stained metaphase chromosome plate is an important feature of the AC-2 pattern, the entire speckled nuclear patterns with positive staining of the metaphase plate are not all AC-2. Mahler et al. developed the "pseudo-DFS" pattern description and stated that this pattern was responsible for most of the AC-2 patterns that did not show anti-DFS70 reactivity [9,10]. In our study we found IIF-DFS pattern in 32.9% (111) of the 337 individuals. Carter et al. reported a DFS pattern in 32.9% of 5339 ANA positive samples. and Dellevance et al. reported 37% DFS pattern among 13641 ANA

positive samples [5,7]. The presence of anti-DFS70 antibodies was reported from 60.2% to 91 % in subjects with IIF-DFS pattern [11,12]. In our study, anti-DFS70 antibodies were positive in 48.6% of subjects with IIF-DFS pattern and the percent of anti-DFS70 antibody positivity was lower at a low titer IIF-DFS pattern, compared to high titer IIF-DFS patterns. Similar to our study, Jeon et al. found that the anti-DFS70 antibody positivity was lower at low titer IIF- DFS pattern [13]. In our study, anti-DFS70 antibodies were not positive in all individuals who had an IIF-DFS pattern. We think that our results may be related to a pseudo DFS pattern and IIF-DFS positivity should be confirmed with the anti-DFS70 antibody test. Anti-DFS70 antibodies are rarely seen in systemic rheumatic diseases and it has been reported that they can be used to rule out SARDs [14]. Mahler et al. showed that anti-DFS70 antibodies were more common in healthy individuals (8.9%) than patients with SARDs (2.8%) [15]. Inversely in a recent study, Infantino et al. found no differences between AARDs and non-AARDs individuals though in their study, all of anti-DFS70 antibody positive AARDs patients had concomitant anti-ENAs specificity [1]. Muro et al. detected the anti-DFS70 antibodies in 4.4% of 500 subjects with different types of rheumatic diseases. They emphasized that patients with isolated anti-DFS70 antibodies were infrequently diagnosed with autoimmune rheumatic diseases [16].

On the other hand, Peker et al. found that the frequency of anti-DFS70 antibodies was statistically significantly higher in subjects with SARDs compared to donor serums [17]. Türkoglu et al. found that anti-DFS70 antibodies were positive in 91.7% of subjects with IIF-DFS pattern and they reported that all subjects had isolated positivity [18]. In our study, isolated positivity was found in 94.1% of anti-DFS70 antibody positive individuals and 13.2% of subjects with AARDs were positive for anti-DFS70 antibodies. Anti-DFS70 positivity was statistically significantly lower in individuals with AARDs.

Our report supports that anti-DFS70 antibodies may be present in patients with AARDs, but AARDs are less prevalent in patients who had anti-DFS70 antibodies. Our study differentiates itself from other studies related with anti-DFS70 antibodies because we examined the predictive value of DFS pattern and anti-DFS70 antibodies for the diagnosis of AARDs and we found that the positive predictive values of DFS pattern and anti-DFS70 antibodies were very low. This finding supports that the fact that anti-DFS70 antibody positivity is an important marker to exclude AARDs.

Limitations: This study was designed retrospectively. The study group consisted of individuals who were evaluated with a prediagnosis of rheumatic disease. Prospective monitoring of whether AARDs will develop in subjects with anti-DFS70 antibodies will contribute to the determination of the role of anti-DFS70 antibodies in rheumatic diseases.

Conclusion: Anti-DFS70 antibodies may be present in patients with AARDs, but AARDs are less prevalent in patients who had anti-DFS70 antibodies compared to patients who had other anti ENAs specificity.

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