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Antinuclear antibodies by IIF-ANA method in systemic rheumatic diseases

Sistemik romatizmal hastalıklarda IIF-ANA metoduyla antinükleer antikorlar

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Abstract

Purpose: Antinuclear antibodies have been used for years for diagnosis and follow-up of systemic rheumatic diseases. Indirect immunofluorescence assay (IIF) is the gold standard to reveal the presence of ANA (ANA). This study intends to investigate by the IF-ANA method the relation of ANA staining patterns, titration and antibodies types with systemic rheumatic diseases.

Materials and methods: The study included 215 patients, who were being followed up in our clinic with the diagnosis of systemic rheumatic disease between September 2015 and December 2015, who had an ANA-positive test and underwent subgroup analysis. ANA was tested using IIF method and ANA profile was tested by using a commercial kit with immunoblotting. ANA staining patterns, titration results, and results of antibodies types were compared with types of systemic rheumatic disease.

Results: In the study, the most frequent ANA staining pattern was granular (34.4%) and homogeneous (33.5%) while the most frequent result in the subgroup analysis was R0-52 (28.8%), SS-A (24.1%), and ds-DNA (19.06%) positive. The most frequent ANA titration was 1/100, 1/320, 1/1000 and above, respectively.

Conclusion: Although specific antibody positivity is higher in SLE and SSc, the existence of specific antibodies in different percentages in all the rheumatic diseases indicates the importance of using ANA with the clinic findings in the diagnosis and follow-up of systemic rheumatic disease.

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Keywords: Anti-nuclear antibodies,indirect immunofluorescence assay,systemic rheumatic diseases.

Özet

Amaç: Antinükleer antikor, sistemik romatizmal hastalıkların tanı ve tedavisinde yıllardır kullanılmaktadır. İndirekt immünfloresan (IIF) yöntem antinükleer antikor (ANA) varlığını tespit etmede altın standart yöntemdir. Bu çalışmanın amacı sistemik romatizmal hastalıklarda ANA boyanma tipleri, titrasyon ve antikor tiplerinin ilişkisini araştırmaktır.

Gereç ve yöntem:Çalışmaya Eylül-Aralık 2015 tarihleri arasında kliniğimize başvuran, ANA sonucu pozitif olup subgrup analizi yapılmış olan, yeni sistemik romatizmal hastalık tanısı konulmuş toplam 215 hasta dahil edildi. ANA, IIF yöntemiyle, ANA subgrupları immünblot yöntemi ile ticari kitler kullanılarak çalışıldı. ANA boyanma tipleri, titrasyon ve antikor tipleri, sistemik romatizmal hastalık tipleri ile karşılaştırıldı.

Bulgular:Çalışmamızda en sık ANA boyanma tipi granüler (%34.3) ve homojen (%33.5) özellikte olup, antikor analizinde en sık R0-52 (%28.8), SS-A (%24.1) ve ds-DNA (%19.06) pozitifliği saptandı. ANA titrasyonu, sıklık sırasına göre 1/100, 1/320, 1/1000 ve üstü şeklinde idi.

Sonuç: Spesifik antikor pozitifliklerinin en sık sistemik lupus eritematozus ve sistemik skleroderma'da görülmesine rağmen, değişen oranlarda tüm sistemik romatizmal hastalıklarda saptanması, tanı ve izlemde ANA'nın klinikle birlikte değerlendirilmesi gerektiğinin önemini göstermektedir.

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Anahtar sözcükler: Antinükleer antikor,indirekt immünfloresan,sistemik romatizmal hastalık.

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Introduction

Antinuclear antibodies (ANA) which are synthesized against the nucleus, nucleolus, cytoplasm, and cell surface antigens are used for diagnosis and follow-up of systemic rheumatic diseases. Target antigens are mainly structured as proteins, protein-macromolecular complex, protein-nucleic acid complex, and nucleic acid. However, ANA often develops against deoxyribonucleic acid (DNA)-protein and ribonucleic acid (RNA)-protein complexes [1,2].

In addition to systemic rheumatic diseases, ANA may also be detected in non-rheumatic diseases such as thyroid diseases, infectious diseases, malignant diseases and even in healthy individuals. ANA may be detected in low titers at 13-15% in healthy individuals, reaching 40-45% according to international reports. These antibodies can be seen more frequently in women and increase with age [3-5,6].

Although there are various laboratory tests for the detection of ANA, indirect immunofluorescence (IIF) is the most frequently used test in the diagnosis of systemic rheumatic diseases. It has high sensitivity and specificity. As positivity in low titers may also be observed in healthy individuals, most of laboratories have been defined the positivity significant at 1:160 titration in the diagnosis of systemic rheumatic disease [7,8].

This study intends to study retrospectively the relation of ANA staining patterns, titration results, and results of ANA subgroup analysis in patients followed up for diagnosis of systemic rheumatic diseases.

Materials and Methods

The study included the patients, followed up at the Immunology-Rheumatology polyclinic between September 2015 and December 2015 for diagnosis of systemic rheumatic disease, who had an ANA-positive test and underwent ANA profiling. The diagnosis of rheumatic diseases is based on ICD codes. Patients using anti-TNF drug were not included in the study. Patient files were examined. Age, gender, type of rheumatic disease, ANA staining pattern, titration, and subgroups were recorded. For this retrospective study, approval was obtained from the Ethics Committee for Non-invasive Clinical Studies at the Adnan Menderes University School of Medicine (No: 2016/839).

Antinuclear antibody test was performed in the laboratory of the Department of Medical Microbiology at the Adnan Menderes University School of Medicine.

IIF-ANA:

Patient blood samples were centrifuged at 3000 rpm for 10 minutes to separate their sera, which were then kept at -20°C until the performance of tests. ANA, antimitochondrial antibody (AMA), antismooth muscle antibody (ASMA) tests were performed in the same well by the indirect fluorescence antibody method using the kit (Euroimmun, Germany) incorporating Hep-20/10, liver (monkey) tissue, kidney tissue, and stomach tissue, in accordance with the manufacturer recommendations. Slides were examined under the fluorescent microscope (Nicon, EFD-3) at an objective lens magnification of x 40. Samples detected positive at 1:100 titration for ANA were diluted at 1:320 and 1:1000 to be studied again in the same way.

ANA Profile test:

Serum was studied by immunoblotting for Smith (Sm), Ribonucleoprotein/Smith (RNP/Sm), Sjögren's Syndrome-A (SS-A), Ro-52, Sjögren's Syndrome-B (SS-B), Scl-70 (topoisomerase 1), PM-Scl (Polymyositis-systemic sclerosis), Jo-1 (histidyl tRNA synthetase), Centromere B, double-stranded DNA (dsDNA), nucleosomes, histones, ribosomal protein (Ribosomal-P), AMA-M2 (Antimitochondrial antibody-M2) antibodies (IgG) using ANA profile 3 Euroline (Euroimmun-Germany) kit and Euroblotmaster incubation system at 1/100 dilution, in accordance with the manufacturer's recommendations. Antibodies detected on strips were evaluated semi-quantitatively (limit value, +, ++, +++, and ++++) using Euroline Scan program.

Statistical evaluation: SPSS 13.0 software was used for statistical evaluation of data. Gathered data was presented as mean ± standard derivation and percentage.

Results

The study enrolled a total of 215 patients, including 193 women and 22 men, with the age range of 19 to 78 years and average

age of 49.05±13.92 years. The diagnosis of patients enrolled in the study was as follows: 51 patients with systemic lupus erythematosus (SLE), 45 with rheumatoid arthritis (RA), 36 with undifferentiated connective tissue diseases (UCTD), 33 with Sjögren's Syndrome (SjS), 23 with systemic sclerosis (SSc), 8 with RA+SjS, 8 with ankylosing spondylitis (AS), 5 with RA+SjS+primary biliary cirrhosis (PBC), 5 with RA+SLE, and 1 with CREST Syndrome.

In the study the most frequent ANA staining pattern was granular (34.4%), homogeneous (33.5%), homogeneous-granular (12.6%) (Table 1).

Nuclear staining (homogeneous, granular, centromer, nucleolar, homogeneous-granular, granular-nuclear dot, nuclear membrane, peripheral) was present in 211 patients (98.1%), cytoplasmic staining in 4 patients (1.9%).

The most frequent ANA staining pattern by the type of rheumatic disease was as follows: homogeneous in SLE, RA, and UCTD, granular in SjS, RA, UCTD and SLE, homogeneous-granular in SLE, SSc and SjS (Table1).

In the subgroup analysis by the ANA profile test, the most frequently detected positivity was as follows: Ro-52 at 28.8%, SS-A at 24.1%, ds-DNA at 19.06%, SS-B at 13.4%, and RNP/Sm at 13.02% (Table 2).

In the comparison of ANA subgroups by the type of rheumatic diseases, the most frequently detected positivities were as follows: ds-DNA, SS-A, nucleosome, and RNP/Sm, Ro-52 in SLE; Ro-52 and ds-DNA in RA; Scl-70, PM-Scl, Centromere B, RNP/Sm and SS-A in SSc; Ro-52, SS-A, and SS-B in SjS; Ro-52, RNP/Sm, and PM-Scl in UCTD; ds-DNA, Scl-70, and PM-Scl in AS; Ro-52 and SS-A in RA+SjS; AMA M-2 and Ro-52 in RA+SjS+PBC; Ribosomal-P and nucleosome in RA+SLE; and Centromere B in CREST (Table 2).

The most frequent ANA titration was 1/100, followed by 1/320, 1/1000 and above. The most frequent ANA titration by the type of rheumatic disease was as follows: 1/1000 and above in SLE and RA+SLE; 1/1000 and above in one patient with CREST syndrome; 1/320 in RA+SjS+PBC; 1/100 in AS, RA, UCTD and RA+SjS (Table 3).

Discussion

IF-ANA test is the gold standard method for the ANA assay used in the diagnosis and follow-up of systemic rheumatic diseases. IF-ANA test produces results by the ANA staining pattern and titration. ANA staining patterns generally indicate antibodies synthesized against nuclear structures. Antibodies synthesized against cytoplasmic structures are not reported by some laboratories [1]. In this study, there was nuclear staining pattern in 211 patients (98.1%), cytoplasmic staining pattern in 4 patients (1.9%). Cytoplasmic staining pattern was present in RA and UCTD.

In this study, the most frequent ANA staining pattern was granular (34.4%) and homogeneous (33.5%). ANA staining patterns were similar to the results from other studies conducted in Turkey. In a study by Çelikbilek et al. [9], the most frequent staining pattern was homogeneous (23%) and granular (22%), as in another study by Yumuk et al. [10], which also observed homogeneous and granular pattern as the most frequent ones. Homogeneous staining pattern was most frequently present in SLE , RA and UCTD (34.7%, 26.4%, and 16.7%, respectively) while granular staining pattern was most frequently present in SjS, RA, UCTD and SLE (23%, 20.3%, 18.9% and 17.6%, respectively). In addition, homogeneous staining pattern and granular staining pattern were present at varying rates in almost all of the rheumatic diseases included in the study.

Patients with AS were also ANA positive, though this was not a connective tissue disease. In studies, ANA positivity was detected at 17.1% to 27.1 % in patients with AS [11,12]. In our previous study [13], none of the patients with AS had ANA positivity. As, from among the patients who presented at the clinic during this study, those who were ANA-positive and underwent the subgroup analysis were included in the study, the number of ANA-positive patients is unknown within the total number of patients with AS. But we know that ANA may also be positive in healthy population.

While the role of ANA is not known in most of rheumatic diseases, its role in the pathogenesis of SLE is obvious. In SLE, the prototype of autoimmune diseases characterized with multisystemic involvement, ANA, anti-dsDNA

and anti-Sm antibodies are included in the diagnosis criteria of the disease [14]. In patients with SLE, the frequency of anti-dsDNA and anti-Sm was reported by Soto et al. [15] to be 62% and 35% and by Scholz et al. [16] to be 58% and 20.1%. In this study, the frequency is 49% and 9.8% in SLE and 20% and 20% in SLE+RA. Our results were similar to this studies results. In addition, anti-Sm antibodies is also positive in RA+SjS+PBC at 20%. In SLE, anti-dsDNA was the most frequently detected autoantibody in this study. Also anti-RNP/Sm was most frequently positive in SLE (27.4%).

Some antibody types are closely related with rheumatic diseases, including anti-dsDNA and SLE or anti-Scl-70 and SSc, while other antibody types such as Ro/La may be intensely produced in many rheumatic diseases [14]. In this study, Scl-70 was positive in 9.76% of patients while the positivity rate was 69.5% in SSc, dsDNA was positive in 19.06% of patients while the positivity rate was 49% in SLE. In this study, among all rheumatic diseases, the most frequently detected antibody was Ro-52 (28.8%) and SS-A (24.1%). Ro-52 and SS-A were most frequently positive in SjS (78.7% and 63.6%) and RA+SjS (100% and 62.5%) and were also found to be positive at varying rates in some of other rheumatic disease. Ro-52 was most frequently present in seconder SjS. The literature reports the frequency of anti-Ro/SS-A in primary SjS at 40%-90% and in the secondary SjS at 10%-15% [17]. In accordance with the literature, this study detects similar results in primary SjS while results are higher than those of the literature for secondary SjS. SS-B was most frequently positive in SjS (48.4%), RA+SLE (20%), and RA+SjS+PBC (20%). The literature reports positivity of SS-B at 20-50% for primary SjS while the positivity of this is in accordance with the literature. Although its specificity is not as high as that of anti-dsDNA and anti-Sm, antiribosomal-P (10%) and anti-PCNA (2%) are also SLE-specific antibodies [1,18]. This study detected antiribosomal-P at 40% in RA+SLE and 9.8% in SLE, anti-PCNA at 5.88%in SLE. Detected at 95% in drug-related lupus, anti-histone antibodies may also be detected in asymptomatic individuals [19,20]. This study detected anti-histone antibodies at 4.18% and found positivity in SLE, SSc, SjS, UCTD and RA.

Antinucleosome antibodies, also known as anti-chromatin antibodies, were detected in patients with SLE at 50-90% [17] while this study detected them at 40% in RA+SLE and 29.4% in SLE. Antiribosomal P, anti-Sm and antinucleosome antibodies were detected in RA+SLE higher than SLE.

With sensitivity of 60% in CREST Syndrome and 32% in SSc anti-centromere antibodies were positive in 1 patient with CREST Syndrome and were detected at a lower rate (17.3%) than that of the literature in patients with SSc [7].

PM-Scl antibodies that are frequently positive in SSc and PM were most frequent in SSc at 17.3% but could not be evaluated for PM, which was not included in the working group [7]. Jo-1 antibodies that observed at 20% in PM/DM (dermatomyositis) and also detected positive in the antisynthetase antibody syndrome were positive in 2 patients, including 1 patient with RA and 1 patient with UCTD [21].

ANA may also be positive in healthy population at low titration. They may be positive in healthy population at 13.3% in 1/80 dilution, at 7% in 1/160 dilution, at 6% in 1/320 dilution, at 4.4% in 1/640 dilution, at 2.1% in 1/1280 dilution [22]. ANA positivity is generally associated with rheumatic disease clinic at high titration. In a study by Karakeçe et al. [23], the most frequent titration was 1/100 and the majority of patients were rheumatologic patients. In our study, the most frequent titration was 1/100, followed by 1/320 and 1/1000. The most frequent titration was 1/100 in patients with AS, RA.

There are some limitations to our study. First, a relatively small number of patients with some rheumatic diseases. Therefore, it is not exactly possible to compare ANA staining patterns or other results according to rheumatic diseases types. Second, there was no patient with PM/DM in this study. Third, we were unable to interpret the frequency of ANA positivity in this rheumatic diseases as this study included the patients who had an ANA-positive test and underwent ANA profiling.

In conclusion, this study evaluated the ANA staining patterns and specific autoantibody results in patients followed up at our clinic with the diagnosis of systemic rheumatic disease. In this study, some of specific autoantibody results were lower than those in the literature. This

was because our study included patients with multiple rheumatic diseases. Although ANA are highly specific for the diagnosis and follow-up of some systemic rheumatic diseases, including SLE and SSc, they may also be positive in many other systemic rheumatic diseases at certain percentages. Therefore, ANA results should be evaluated at the clinic before deciding on the diagnosis and treatment.

Conflict of interest: The authors declared no conflict of interest.

Table 1. ANA staınıng pattems by the type of rheumatic disease

Patterns N (%)	SLE (n=51)	RA (n=45)	SSc (n=23)	SjS (n=51)	UCTD (n=33)	AS (n=36)	RA+ SjS (n=8)	RA+ SjS+PBC (n=5)	RA+SLE (n=5)	CREST (n=1)
Homogeneous 72 (33.5)	25(34.7)	19(26.4)	3(4.2)	5(6.9)	12(16.7)	2(2.8)	2(2.8)	1(1.4)	3(4.2)	0
Granular 74 (34.4)	13(17.6)	15(20.3)	4(5.4)	17(23)	14(18.19)	2(2.7)	5(6.8)	3(4.1)	1(1.4)	0
Homogeneous-granular 27 (12.6)	8(29.6)	3(11.1)	7(25.9)	5(18.5)	3(11.1)	0	0	0	1(3.7)	0
Centromer 9 (4.2)	0	1(11.1)	4(44.4)	1(11.1)	1(11.1)	0	1(11.1)	0	0	1(11.1)
Nucleolar 21 (9.7)	1(4.8)	5(23.8)	5(23.8)	5(23.8)	3(14.3)	2(9.5)	0	0	0	0
Granular-nucleolar dot 5 (2.3)	2(40)	0	0	0	0	2(40)	0	1(20)	0	0
Cytoplasmic 4 (1.9)	0	1(25)	0	0	3(75)	0	0	0	0	0
Nuclear membrane 1 (0.5)	0	1(100)	0	0	0	0	0	0	0	0
Peripheral 2 (0.9)	2(100)	0	0	0	0	0	0	0	0	0

SLE: Systemic lupus erythematosus, RA: Rheumatoid arthritis, UCTD: Undifferentiated connective tissue diseases, SjS: Sjogren's syndrome, SSc: Systemic sclerosis, AS: Ankylosing spondylitis, PBC: Primary biliary cirrhosis, CREST: Calcinosis, Raynaud phenomenon, esophageal dysmotility, sclerodactyly and telangiectasia

Table 2. ANA subgroup results by the type of rheumatic disease; (n,%)

Subgroup N,(%)	SLE (n%)	RA (n%)	SSc (n%)	SjS (n%)	UCTD (n%)	AS (n%)	RA+ SjS (n%)	RA+ SjS+PBC (n%)	RA+SLE (n%)	CREST (n%)
SSA 52 (24.1)	17(33.4)	2(4.4)	4(17.3)	21(63.6)	2(5.55)	0	5(62.5)	1(20)	0	0
SSB 29 (13.4)	8(15.6)	2(2.2)	1(4.34)	16(48.49)	0	0	1(12.5)	1(20)	1(20)	0
RO-52 62 (28.8)	13(25.4)	5(11.1)	1(4.34)	26(78.7)	7(19.4)	0	8(100)	2(40)	0	0
Ds-DNA 41 (19.06)	25(49)	5(11.1)	2(8.6)	4(12.1)	3(8.3)	1(12.5)	0	0	1(20)	0
RNP-Sm 28 (13.02)	14(27.4)	3(6.6)	4(17.3)	1(3.03)	5(13.8)	0	0	1(20)	0	0
Sm 11 (5.11)	5(9.8)	1(2.2)	0	3(9.09)	0	0	0	1(20)	1(20)	0
Nucleosome 19 (8.83)	15(29.4)	1(2.2)	0	1(3.03)	0	0	0	0	2(40)	0
Ribosomal-P 10 (4.65)	5(9.8)	1(2.2)	0	1(3.03)	1(2.7)	0	0	0	2(40)	0
PCNA 5 (2.3)	3(5.88)	1(2.2)	0	0	1(2.7)	0	0	0	0	0
Histone 9 (4.18)	5(9.8)	1(2.2)	1(4.34)	1(3.03)	1(2.7)	0	0	0	0	0
Scl-70 21 (9.76)	1(1.96)	0	16(69.5)	1(3.03)	1(2.7)	1(12.5)	1(12.5)	0	0	0
Centromere B 14 (6.51)	3(5.88)	1(2.2)	4(17.3)	0	3(8.3)	0	1(12.5)	1(20)	0	1(100)
PM-Scl 14 (6.51)	2(3.92)	1(2.2)	4(17.3)	2(6.06)	4(11.1)	1(12.5)	0	0	0	0
AMA-M2 9 (4.18)	2(3.92)	2(4.4)	0	1(3.03)	0	0	0	4(80)	0	0
Jo-1 2 (0.93)	0	1(2.2)	0	0	1(2.7)	0	0	0	0	0

SLE: Systemic lupus erythematosus, RA: Rheumatoid arthritis, UCTD: Undifferentiated connective tissue diseases, SjS: Sjogren's syndrome, SSc:Systemic sclerosis, AS: Ankylosing spondylitis, PBC: Primary biliary cirrhosis, CREST: Calcinosis, Raynaud phenomenon, esophageal dysmotility, sclerodactyly and telangiectasia

Table 3. ANA tıtratıon by the type of rheumatic disease

	1/100 n (%)	1/320 n (%)	≥1/1000 n (%)	Total N
SLE	19 (37.2)	12 (23.5)	20 (39.2)	51
RA	33 (73.3)	8 (17.7)	4 (8.8)	45
SSc	8 (34.7)	8 (34.7)	7 (30.4)	23
SjS	13 (39.3)	11 (33.3)	9 (27.2)	33
UCTD	22 (61.1)	10 (27.7)	4 (11.1)	36
AS	7 (87.5)	1 (12.5)	0	8
RA+ SjS	5 (62.5)	3 (37.5)	0	8
RA+ SjS+PBC	1 (20)	3 (60)	1 (20)	5
RA++SLE (n %)	0	1 (20)	4 (80)	5
CREST (n %)	0	0	1 (100)	1
Total	108(50.2)	57 (26.5)	50 (23.2)	215

SLE:Systemic lupus erythematosus, RA: Rheumatoid arthritis, UCTD: Undifferentiated connective tissue diseases, SjS: Sjogren's syndrome, SSc: Systemic sclerosis, AS: Ankylosing spondylitis, PBC: Primary biliary cirrhosis,CREST: Calcinosis, Raynaud phenomenon, esophageal dysmotility, sclerodactyly and telangiectasia

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