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Evaluation of immunoblotting test results in patients with positive antinuclear antibodies

Antinükleer antikorların pozitif saptandığı hastalarda immunoblotting test sonuçlarının değerlendirilmesi

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ABSTRACT

Objective: Autoimmune diseases occur as a result of the immune response to self antigens and tissues of the organism and the detection of autoantibodies is very important in the diagnosis of these disorders. Antinuclear antibodies (ANA) autoantibodies generated against to nuclear/ cytoplasmic components of the cell are important diagnostic criteria for connective tissue diseases. Currently, a number of methods are available for the detection of ANA. The gold standard method for the detection of ANA is Indirect Immunofluorescence Antibody (IIFA) assay using Hep2 (human laryngeal epidermoid carcinoma). When positive results are observed, Extractable Nuclear Antigen(ENA) tests follow as a means of confirming the diagnosis. Identification of the specific extractable nuclear antigens is warranted because this may further differentiate between the distinct types of autoimmune connective tissue diseases. In this study, were analyzed retrospectively that ENA test results in patients with positive ANA IIFA test.

Methods: Antinuclear antibodies were tested for a total of 3000 patients admitted to various clinics of Ondokuz Mayıs University Medical Faculty. Each serum sample was studied at 1: 100 dilution in accordance with the

ÖZET

Amaç: Otoimmün hastalıklar, organizmanın kendi doku ve hücrelerine karşı immün yanıt gelişmesi sonucu oluşmaktadır ve bu hastalıkların tanısında otoantikorlar büyük önem taşımaktadır. Anti nükleer antikor (ANA) adı verilen, hücre nükleusu ve/veya sitoplazmasındaki nükleer yapılara karşı gelişen otoantikorlar, bağ doku hastalıklarında önemli bir tanı kriteridir. Günümüzde, ANA pozitifliğinin saptanması amacıyla geliştirilmiş bir çok yöntem bulunmaktadır. ANA tespiti için altın standart yöntem, Hep2 (insan laryngeal epidermoid karsinoma) hücreleri kullanılarak gerçekleştirilen Indirekt Immünofloresan Antikor (IIFA) testidir. Pozitif sonuçlar gözlendiğinde, tanıyı doğrulamanın bir yolu olarak, ekstrakte edilebilir nükleer antijen (ENA) testleri takip eder. Spesifik ekstrakte edilebilir nükleer antijenlerin tanımlanması, otoimmün bağ dokusu hastalıklarının farklı tipleri arasındaki ayrımı sağlayabilir. Bu çalışmada, ANA IIFA testi pozitif olan hastalarda ENA test sonuçlarını retrospektif olarak inceledik.

Yöntem: Ondokuz Mayıs Üniversitesi Tıp Fakültesi'nin çeşitli kliniklerine başvuran toplam 3000 hasta için Anti nükleer antikorlar test edildi. Her serum numunesi 1: 100 dilüsyonda

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manufacturer's recommendations (Euroimmun AG, Lübeck, Germany) and staining pattern with fluorescence intensity was evaluated with immunofluorescence microscope. ENA was investigated by immunoblotting method (Euroimmun AG, Lübeck, Germany) in 640 ANA positive sera.

Results: Distribution of the patients included in the study, 2192 (73.07%) were female and 808 (26.93%) were male. 640 samples detected as ANA positive. When we look at the distribution of ANA patterns in positive samples; granular 173 (27.03%), granular and cytoplasmic granular 98 (15.31%), homogenous and granular 67 (10.47%) were the most common. When the ENA profiles of the ANA-positive samples were examined, 557 (83.7%) were found to be positive and 83 (12.97%) were negative. According to our study, SSA (26.88%), SSB (17.81%), Sm / RNP (17.66%) were the first three places in the ENA positivite.

Conclusion: Our ANA positivity rate was found to be compatible with the literature. The pattern distribution is similar to the data of our region. After the first screening with IIFA, looking for different antigens with the immunoblot test; not only it is a cost effective approach;but also facilitate the diagnosis of autoimmune diseases.

Key Words: ANA (AntinuclearAntibody), Indirect Immunofluorescence Antibody (IIFA), Extractable Nuclear Antigen (ENA)

üreticinin (Euroimmun AG, Lübeck, Germany) önerileri doğrultusunda çalışılmış, immunfloresan mikroskopta boyanma paterni ve floresans şiddeti değerlendirilmiştir. ANA pozitif olarak tespit edilen 640 numunede immunoblot yöntemi (Euroimmun AG, Lübeck, Germany) ile ENA test edilmiştir.

Bulgular: Çalışmaya alınan hastaların 2192'si (%73,07) kadın, 808'i (%26,93) erkekti. 640 numune ANA pozitif olarak tespit edilmiştir. Pozitif örneklerde ANA paternlerinin dağılımına baktığımızda;173 granüler (%27,03), 98 granüler+sitoplazmik granüler (%15,31), 67 homogen+granüler(%10,47) en sık saptanmıştır. ANA pozitif örneklerin ENA profilleri incelendiğinde 557'si (%83,7) pozitif, 83'ü (%12,97) negatif bulundu. ENA pozitifliklerinde ilk üç sırayı SSA (%26,88), SSB (%17,81), Sm/RNP (%17,66) aldı.

Sonuç: ANA pozitiflik oranımız literatür ile uyumlu bulunmuştur. Patern dağılımı da bölgemiz verilerine benzerdir. IIFA ile ilk tarama ardından immunoblot test ile farklı antijenlere bakılması; maliyet etkin bir yaklaşım olmakla birlikte; otoimmün hastalıkların tanısını kolaylaştıracaktır.

Anahtar Kelimeler: ANA (Antinükleer antikor), İndirekt İmmunfloresan antikor (IIFA), Ekstrakte Edilebilir Nükleer Antijen (ENA)

INTRODUCTION

Antinuclear antibodies (ANA) are originally referred to autoantibodies that produced against nuclear antigens and some other antigens that present in the cell cytoplasm or membrane (1). The presence of ANA is used as a screening test for the diagnosis of autoimmune diseases especially for rheumatologic disorders. Approximately 25% of the community has ANA positivity but the prevalence of significantly elevated levels is about 2.5% which indicates an autoimmune disease. The gold standard for the

detection of ANA is indirect immune fluorescence assay (IIFA) that has a lot of advantages like the detection of patterns which indicate certain diseases (2,3,4). However, it is labour-intensive, and technical interpretation of the results can be subjective (5).

Some proteins found in the nucleus of the cell can be extracted using saline, and are called Extractable Nuclear Antigens (ENAs). Among ENAs, Smith (Sm) antigen is a nonhiston acidic ribonucleoprotein with low molecular weight; SS-A is a protein playing role in the process of mRNA, SS-B is a phosphoprotein playing role as a cofactor for RNA polymerase III; Scl-70 antigen is defined as DNA topoisomerase I, and Jo-1 is the histidyl-tRNA synthetase enzyme (6). Analysis of the reactivity to ENAs may be of help to distinguish between the different types of autoimmune connective tissue diseases (7). It is known that, presence of antibodies against the Smith (Sm) antigen are specific for SLE (8), and the presence of anti-Sjogren's Syndrome (SS)A and/or-SSB antibodies are a sign for Sjogren's Syndrome (9, 10). In addition to having diagnostic potential, detection of anti-ENA antibodies is prognostically significant, too. Presence of anti-SSA in the circulation of the pregnant woman might cause neonatal lupus erythematosus and/or congenital heart block in the newborn (11,12), and presence anti- of Topo-I antibodies anticipates a more serious course of disease in systemic sclerosis (SSc) (13). In this study, it is aimed to evaluate extractable nuclear antigen test results in patients who were determined positive for anti-nuclear antibody, retrospectively.

MATERIAL and METHOD

Ondokuz Mayıs University Clinic Researchs Ethic Committee approval (Date: 01.10.2021 and Number: B.30.2.ODM.0.20.08/579) was obtained for this study.

ANA and anti ENA test results were retrospectively evaluated from clinical samples sent to Medical Microbiology Laboratory between January 2016-December 2018.

Antinuclear antibodies were tested for a total of 3000 patients admitted to various clinics of Ondokuz -Mayıs University Medical Faculty. Each serum sample was dilueted as 1:100, and the presence of ANA and pattern was evaluated with ANA-IFA. The commercial IIFA kit (Euroimmun AG, Lübeck, Germany), which contains HEP-2 and monkey liver cells together as a tissue for ANA IIFA testing, was used. In accordance with the manufacturer's recommendations, patient serum were studied with 1/100 dilution titers. Prepared preparations were evaluated at a fluorescence microscope (Euroimmun AG, Lübeck, Germany) at a magnification of 400x. The results were reported qualitatively (+, ++, +++, ++++) according to the fluorescence intensity of the slides and their patterns.

The samples that were detected as ANA positive subsequently tested for ENA. Samples were stored at - 20°C till the study. A total of 640 ANA-positive samples were tested for anti-ENA using. Anti-ENA profile immunoblot method (Euroimmun AG, Lübeck, Germany) was studied.

RESULTS

Of the patients included in the study, 2192 (73.07%) were female and 808 (26.93%) were male.

The distribution of the clinics as follows; rheumatology 1285 (42.83%), physical therapy 345 (11.5%) and hematology 213 (7.10%)(Table 1). Total of 640 (21.33%) serum samples were found to be positive for ANA IIFA. The distribution of ANA patterns in positive samples; speckled n=173 (27.03%), speckled and cytoplasmic granular n=98 (15.31%), homogen and speckled n=67 (10.47%) were the most common (Figure 1and Figure 2). It was detected that 557 (83.7%) ENA positive and 83 (12.97%) ENA negative in ANA positive patients. The ENA profiles of the ANA-positive samples were; SSA (26.88%), SSB (17.81%), Sm / RNP (17.66%) were the firstthree places in the ENA positivity (Table 2).

Table 1. Distribution of the clinics

Clinic	Number(%)
Pediatric Nephrology	31 (1,03)
Child Health and Diseases	49 (1,63)
Internalmedicine	82 (2,73)
Dermatology	123 (4,10)
InfectiousDiseases	30 (1,00)
Physical Medicine and Rehabilitation	345(11,50)
Gastroenterology	59(1,97)
Chest Diseases	180(6,00)
Eye diseases	161(5,37)
Hematology	213(7,10)
Nephrology	113(3,77)
Neurology	187(6,23)
Rheumatology	1285(42,83)
Pediatrics Other[1]	36(1,2)
Other*[2]	106(3,53)

Pediatrics Other*[1]: PediatricAllergy, PediatricSurgery, PediatricEndocrinology, PediatricInfection, PediatricGastroenterology, PediatricHematology, PediatricImmunology, PediatricCardiology, PediatricNeurology, Newborn.

Other*[2]: Emergency, Anesthesiology, Neurosurgery, Endochrinology, General Surgery, Thoracic Surgery, Gynecology and Obstetrics, Cardiovascular Surgery, Cardiology, Otorhinolaryngology, Oncology, Orthopedics andTraumatology, Psychiatry, Urology, Intensive Care Unit.

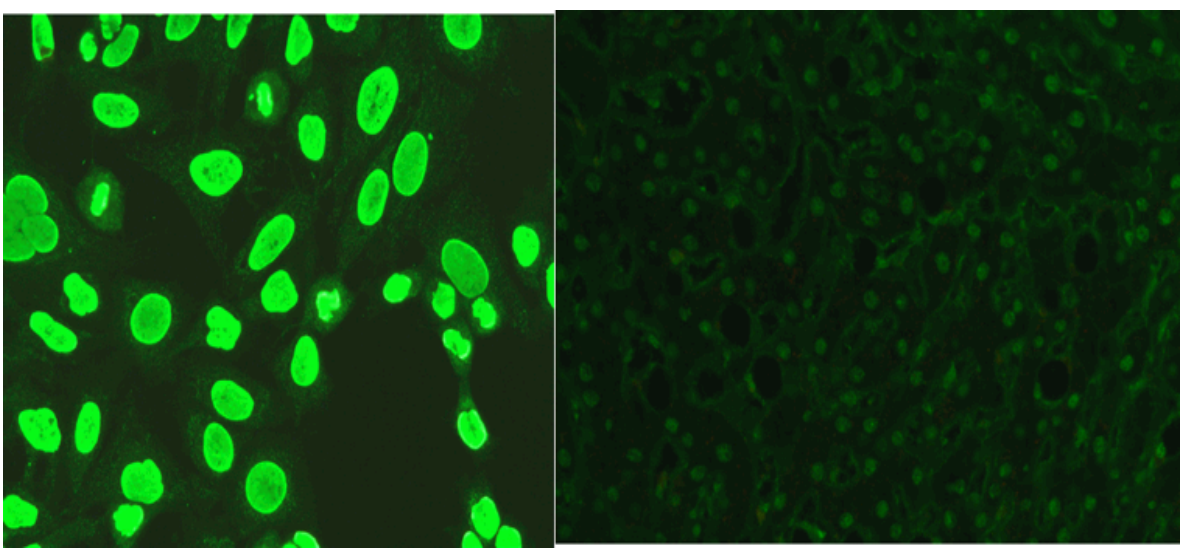

Figure 1. Homogen pattern in Hep-2 (x400) and liver tissue

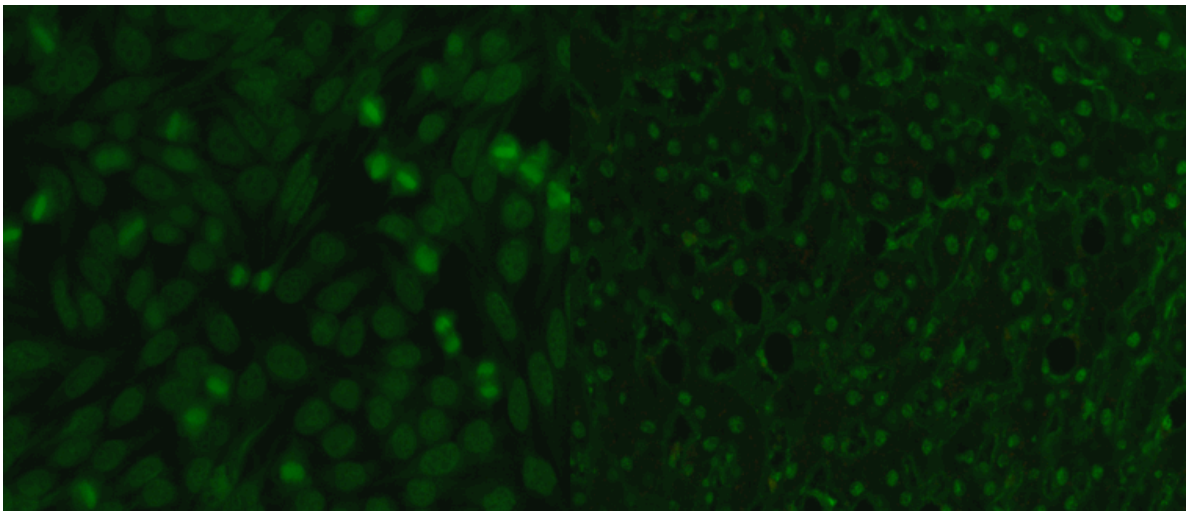

Figure 2. Homogen + speckled pattern in Hep-2 (x40) and liver tissue

Table 2. Comparison of ANA patterns and ENA profiles of the patients

		ENA							
		Anti Scl-70	Anti JO-1	Anti-ss-A	Anti-ss-B	Anti Sm/RNP	Anti-Sm	Total	%
ANA Patterns	Homogen+Speckled	10	10	10	12	16	9	67	10,47%
	Cytoplasmic granular + Nuclear membrane	3	1	2	5	1	2	14	2,19%
	Speckled	13	25	50	29	32	24	173	27,03%
	Speckled + Cytoplasmic granular + Homogen	5	0	1	2	4	3	15	2,34%
	Speckled + Dense fine speckled	2	5	5	4	1	2	19	2,97%
	Homogen	3	1	4	0	4	2	14	2,19%
	Speckled + Cytoplasmic granular	3	8	39	17	17	14	98	15,31%
	Nucleolar	2	1	3	1	3	1	11	1,72%
	Speckled + Nucleolar	0	2	18	9	1	3	33	5,16%
	Cytoplasmic granular	0	8	3	4	2	3	20	3,13%
	Other	18	8	22	16	19	10	93	14,53%
	Negative	9	13	15	15	13	18	83	12,96%
	Total	68	82	172	114	113	91	640	100,00%
	%	10,63%	12,81%	26,88%	17,81%	17,65%	14,22%	100,00%	

DISCUSSION and CONCLUSION

The autoantibodies which are seen in autoimmune diseases are against to nuclear and cytoplasmic components of the cells (13). Early detection of autoantibodies has always played an important role in predicting and diagnosing autoimmune disorders, especially for the patients suspected with overlapped syndromes and complex conditions. Anti-nuclear antibodies (ANA), as detected by indirect immunofluorescence, are hallmarks of autoimmune connective tissue diseases. Identification of the specificity for extractable nuclear antigens (ENA) is warranted because this may further differentiate between the distinct types of autoimmune connective tissue diseases (7).

The presence of Antinuclear antibody is more common in women. Sex hormones (especially estrogens) play a significant role in the development of autoimmune diseases and predispose the female sex to more frequent occurrence of these diseases (14). This results with the knowledge of the autoimmune diseases are more frequent in women(13,15,16,17). And in this study, the majority of the patients with suspected autoimmune disease were women (73.07%).

In our study, most of the samples were sent from the rheumatology department (42.83%) to our laboratory with the suspicion of autoimmune disease. This result is similar with the study of Karakeçe et al. (18).Likewise; in the study of Çelikbilek et al., they found the most common positivity in rheumatology department (17). Since autoantibodies has played a consolidate role in diagnosis of systemic autoimmune disorder, it is expectable that positivity rates are higher in the samples of the patient admits to of rheumatology department.

Anti-nuclear antibodies (ANA) represent important diagnostic markers in various autoimmune rheumatic conditions (e.g., systemic lupus erythematosus (SLE), Sjögren's syndrome, systemic sclerosis, dermato/poly myositis, mixed connective tissue diseases, and rheumatoid arthritis), with an increasingly recognized relevance to disease prediction and prognosis (13). In our study, ANA positivity rate was found to be 21.33%. ANA positivity rates were reported to be between 8-35% in Turkey (13,19).

The American College of Rheumatology (ACR) and international committees recommend HEp-2 IIF as the Standard screening method for ANA detection (4,5,21). Up to 30 different ANA staining patterns have been described today including both nuclear as well as cytoplasmic staining patterns. The most common patterns include the homogeneous, speckled, nucleolar, and centromere pattern(7). This study, when the positivity of patients with ANA IIFA was examined, the highest observed ANA pattern was found to be speckled. In the study from Trabzon province, Kaklıkkaya et al. found that the most common pattern was speckled pattern similar like our study (21). This result is similar to the literature (16,17,20,23).

Identification of the specificity for extractable nuclear antigens (ENA) is provided because this may further differentiate between the distinct types of autoimmune connective tissue diseases. At the same time some of ANA patterns are very rare and most technicians in routine laboratories are not trained to distinguish them all. (7). In our study, ENA positivity was found in 83.7% of ANA IIFA positive samples. Yumuk et al. found 79.5% ANA positivity in their study (23). According to our study, SSA (26.88%), SSB (17.81%), Sm / RNP (17.66%) were the first three places in the ENA positivity in ANA-positive samples. According to the results of ENA test that Afşar et al. performed in ANA positive patients in 2007, they detected at least one antigen in 171 of 215 (79.5%) patients and antibodies against the SS-A/Ro-52 antigen were the most common antibodies(3). Also, Yumuk et al. detected antigen positivity with ENA in 126 (60.5%) of 208 ANA positive patients and antibodies against SS-A antigen were the most. (23)

In our study, no antigen was detected in ENA test in 12.97% of ANA positive patients. Antibodies against

seven antigens are sought in our test strip. Therefore, there may be antibody positivity to antigens other than the seven antigens we studied. The presence of other antigens should be considered in the presence of clinical suspicion in patients who have been positively detected by the IIFA test and whose ENA test is negative.

In conclusion, ANA IIFA should be used as a screening test for suspected autoimmune disease. ENA testing should be performed for detection and verification of the relevant antigen. ENA tests are both diagnostically and prognostically significant because they support the detection of specific antigens especially in the diagnosis of rheumatological diseases such as SLE, Sjogren's Syndrome, Systemic sclerosis.

ETHICS COMITTEE APPROVAL

* The study was approved by Ondokuz Mayıs University Clinic Researchs Ethic Committee (Date: 01.10.2021 and Number: B.30.2.ODM.0.20.08/579).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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