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RESEARCH ARTICLE

Evaluation of Anti-Nuclear antibody test results in clinical practice

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ABSTRACT

Objective: Aim of this study is to evaluate anti-nuclear antibody (ANA) test results obtained between 2009 and 2011.

Methods: Of a totally 5068 cases tested for ANA by indirect immunofluorescence method (IIFA), randomly chosen 982 ANA-positive cases were reviewed in terms of gender, level and pattern of fluorescence, anti-dsDNA (anti-double stranded DNA) and anti-extractable nuclear antigen (ENA) profile. Anti-dsDNA levels and anti-ENA profiles were determined by enzyme linked immune assay (ELISA) and immune-blotting (IB), respectively.

Results: Sex distribution of ANA positive patients was determined as 756 (77%) females and 226 (23%) males. Fifty per cent of the cases were from rheumatology department, 20% from gastroenterology and 30% from other units. Fluorescence levels were considered borderline or weak positive in 62.6% of the samples. The most frequent patterns were homogeneous (23%), speckled (22%), homogeneous-speckled (15.5%) and nucleolar (13.5%). Anti-dsDNA were studied in 759 ANA positive patients and 66 (8.7%) samples were found positive, being 44 of them (68.8%) with homogeneous pattern and the rest with speckled, nucleolar, nuclear dots, centromeric or midbody patterns. Totally 131 (31.6%) of 414 samples studied for anti-ENA profile were found positive. The first four frequent profiles were SSA (34.4%), SSA-SSB (16.8%), Scl70 (16%) and Sm/RNP (9.2%).

Conclusion: Our results are similar with the current related literature. It is known that autoantibodies can be detectable before clinical symptoms being apparent, especially in SLE. Therefore, borderline or weak fluorescence levels should also be reported and the patients having them should be followed-up carefully. *J Microbiol Infect Dis 2015;5(2): 63-68*

Key words: Antinuclear antibody, indirect immune-fluorescence assay, extractable nuclear antigen antibodies

Anti-nükleer antikor test sonuçlarının klinik uygulamada değerlendirilmesi

ÖZET

Amaç: Bu çalışmanın amacı 2009-2011 yıllarında laboratuvarımızda yapılan ANA (antinükleer antikor) tetkik sonuçlarının retrospektif olarak değerlendirilmesidir.

Yöntemler: Bu dönemde laboratuvarımızda toplam 5068 serum örneğinde indirekt immünofloresans antikor yöntemiyle (IIFA) ANA varlığı araştırıldı. Rastgele örnekleme ile seçilen ANA-pozitif 982 olgu cinsiyet dağılımı, ışıma düzeyleri ve paternleri, dsDNA sonuçları ve ekstrakte edilebilir nükleer antijen (ENA) profilleri açısından incelendi. ANA ölçümü için doku olarak HEP-2 ve maymun karaciğeri hücrelerini birlikte içeren ticari IIFA kiti kullanıldı. Hasta serumlarının 1/100 sulandırım titresi ile çalışıldı. Sonuç verilirken ışıma titresi ve derecesi ile birlikte homojen, benek, sentromer gibi paternler de rapor edildi. Anti-dsDNA düzeylerinin tayininde Enzim İmmün Assay (ELISA) yöntemi kullanıldı. 20 IU/mL üzerindeki değerler pozitif kabul edildi. Anti-ENA profili immünoblot yöntemi ile bakıldı.

Bulgular: ANA sonucu pozitif bulunan hastaların 756'sı (%77) kadın, 226'sı (%23) erkekti. Örneklerin %50'si Romatoloji, %20'si Gastroenteroloji, %30'u diğer birimlerdendi. %62.6 örnekte sınırda veya zayıf ışıma pozitifliği gözlendi. En sık 4 ışıma paterni sırasıyla homojen (%23), benek (%22), homojen-benek (%15.5) ve nükleolar (%13.5) olarak saptandı. ANA pozitif 759 hastada ELISA ile anti-dsDNA çalışılmış; 66'sı (%8.7) pozitif, 693'ü (%91.3) negatif bulundu. Anti-dsDNA pozitif 44 örnekte (%68.8) homojen ışıma paterni; kalan örneklerde ise benek, nükleolar, nükleer dots, sentromer ve midbody paternleri gözlenmiştir. ANA pozitif 414 örnekte ise immünoblot yöntemi ile anti-ENA profili çalışıldı. Bunların 131'i (%31.6) pozitif, 283'ü (%68.4) negatif bulundu. ENA pozitifliklerinde ilk dört sırayı SSA (%34.4), SSA-SSB (%16.8), Scl70(%16), Sm/RNP (%9.2) aldı.

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Sonuç: Sonuçlarımız ilgili literatür sonuçlarıyla benzerdir. Özellikle SLE'de, klinik semptomlardan önce otoantikorların pozitifleşebildiği bilinmektedir. Bu olasılık göz önünde bulundurularak serum örneklerinde zayıf/sınırda ışıma gözlenen hastaların yakın takibe alınmasını ve bu ışıma düzeylerinin sonuç raporunda belirtilmesi gerektiğini düşünmekteyiz.

Anahtar kelimeler: Antinükleer antikor, indirekt immün-flörasan tetkiki, ayrıştırılabilir nükleer antijen antikorlar

INTRODUCTION

Anti-nuclear antibody (ANA) is a common name for the antibodies against the contents of the cell nucleus. The detection of ANA is used as 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 patterns which indicate certain diseases.[1-3] There are three types of patterns; nuclear (homogeneous, granular, nuclear lamine, centromeric, nuclear dots, proliferating cell nuclear antigen), cytoplasmic (granular, filaments like actin, vimentin, cytokeratin, lysosomal-like, Golgi apparatus) and mitotic (spindle, midbody, centrosomoses). As the antibodies not only against to the nuclear parts but also cytoplasmic and mitotic elements, the terminology of ANA was discussed for a change to appropriate term like anticellular antibodies.[4,5] After a positive result, a further examination is done with anti-double stranded DNA (anti-dsDNa) and anti-extractable nuclear antigen (anti-ENA) profile which contain specific antigens like SSA/Ro, SSB/La, Sm, Scl-70 for clarifying the diagnosis.[1-6]

The aim of this study is to look for features of ANA results including gender and department of the patients, ratio of positiveness, fluorescence titre levels and patterns, anti-dsDNA and anti-ENA profiles during a three year period. For this reason, the results of ANA tests obtained between 2009-2011, were evaluated retrospectively.

METHODS

5068 serum samples were tested for ANA by IIFA between 2009 and 2011. Randomly chosen 982 ANA-positive cases were reviewed in terms of sex, level and pattern of fluorescence, anti-dsDNA and ENA profile.

Detection of ANA levels

IIFA which uses a combination of HEp-20-10 cells and monkey liver tissue as substrates (Euroimmune Medizinische Labordiagnostica AG, Germany) was used for screening ANA. Test was performed with 1/100 dilution of the serum samples. The results were reported as negative or positive with the fluorescence levels and patterns like homogeneous, granular, nucleolar, etc.

Detection of anti-dsDNA levels

ELISA (Organtec Diagnostika GmbH, Germany) was performed for testing anti-ds DNA. Levels above 20 IU/mL were considered positive according to the test protocol.

Detection of anti-ENA profiles

Immunblotting was done by using Euroline Anti ENA profile plus 1 IgG assay (Euroimmun Medizinische Labordiagnostica AG, Germany).

All tests were performed by using the instructions in the kit inserts.

RESULTS

Gender distribution of ANA positive patients was determined as 756 (77%) females and 226 (23%) males.

Table 1 shows the distribution of ANA positive patients to the medical departments. The majority of the ANA positive patients were from rheumatology (50%), gastroenterology (20%) and physical medicine and rehabilitation (5%) departments. Other ANA positive patients were from pulmonary diseases, dermatology, neurology, internal diseases, hematology, nephrology, infectious diseases, endocrinology and other departments.

The fluorescence levels and the patterns of ANA positive samples were shown in Table 2 and 3 respectively. The most frequent four patterns were homogeneous (23%), granular (22%), homogeneous-granular (15.5%) and nucleolar (13.5%) in our study. The majority of ANA positive samples (62.6%) had only borderline or weak fluorescence level in our study. The indicative fluorescence levels were found as; 1+ (24.5%), 2+ (7%), 3+ (3.3%) and 4+ (2.3%).

Table 1. The distribution of ANA positive samples according to the departments

Department	Number	%
Rheumatology	491	50.0
Gastroenterology	196	20.0
Physical Medicine and Rehabilitation	49	5.0
Pulmonary Diseases	40	4.1
Dermatology	40	4.1
Neurology	39	4.0
Internal Diseases	29	3.0
Hematology	29	3.0
Nephrology	9	0.9
Infectious Diseases	20	2.0
Endocrinology	20	2.0
Other	20	2.0
Total	982	100

Table 2. The distribution of fluorescence levels of ANA positive samples

Fluorescence levels	Number	%
Borderline	352	36.0
Weak	259	26.6
1+	238	24.5
2+	68	7.0
3+	31	3.3
4+	21	2.3
Mix	3	0.3
Total	982	100

Table 3. The distribution of patterns of ANA positive samples

Patterns	Number	%
Homogeneous	226	23.0
Granular	212	22.0
Homogeneous/granular	152	15.5
Nucleolar	133	13.5
Midbody	37	3.7
Cytoplasmic granular	36	3.6
Homogeneous/nucleolar	32	3.2
Nuclear dots	23	2.3
Granular/nucleolar	22	2.2
Centromere	18	1.8
Nuclear lamine	12	1.2
Other	80	8.0
Total	982	100

Anti-dsDNA was ordered from 759 out of 982 (77.3 %) ANA positive patients; 66 (8.7%) of them were positive. Homogenous pattern was determined in 44 (68.8%) anti-dsDNA positive samples. Granular, nucleolar, nuclear dots, centromeric and midbody patterns were determined from the rest of the positives.

Anti-ENA profile was ordered from 414 (42%) of the ANA positive patients and 131 (31.6%) of them were found positive. The distribution of the extractable anti-nuclear antigens was shown in Table 4. According to our study, the most frequent four antigens were SSA (34.4%), SSA-SSB (16.8%), Scl70 (16%) and Sm/RNP (9.2%) respectively.

Table 4. The distribution of ENA results

ENA	Number	%
SSA	45	34.4
SSA/SSB	22	16.8
Scl 70	21	16.0
Sm/RNP	12	9.2
SSB	9	6.8
Jo1	9	6.8
Histon	7	5.4
SSA/scl70	2	1.5
sm	2	1.5
SSB/sm	1	0.8
SSA/sm/RNP	1	0.8
Total	131	100

DISCUSSION

The autoantibodies which are seen in autoimmune diseases are against to nuclear and cytoplasmic components of the cells. The target antigens are ribonucleoproteins for anti-Sm, anti-RNP, anti-SSA/Ro, anti-SSB/La; DNA topoisomerase Type 1 for anti-Scl70; centromere for anti-centromere transfer; histidyl-tRNA synthetase for Jo-1 and double-stranded DNA for anti-dsDNA.[1]

Autoantibody detection tests have been used for the diagnosis and to monitor the therapy of the autoimmune diseases for almost 50 years. Today, autoantibody detection is done mostly by ready to use, economic and standardized commercial tests.[2] Indirect immune-fluorescence antibody (IIFA), immunodiffusion (ID), immunoblot (IB), ELISA and the new laser antigen measurement technologies are the main methods for autoimmunity.[3-6] IIFA is still the golden standard method for evaluation of ANA

which is the major test to find an autoimmune disease.[7]

ANA positivity rate found in our female patients (77%) is consistent results with the knowledge of the autoimmune diseases are more frequent in women.[8,9] This predominance was researched by Leo and et al. According to their study, the hormone profile, fetal microchimerism and some strategic genes which are on the sex chromosomes are playing role on this relationship.[10]

As expected, the most of the positive ANA results were from rheumatology department (50%) in our study. This result is similar with the recent study of Karakeçe et al. which was done in an university hospital in Turkey.[11] ANA is a very valuable test for the diagnosis of SLE (93% sensitivity) and scleroderma (85% sensitivity). It is also important for diagnosing Sjögren's syndrome, secondary Raynaud, polymyositis/dermatomyositis and rheumatoid arthritis.[12]

Surprisingly, the second frequent ANA positivity rate was in patients of the gastroenterology department (20%) in our study. The presence of ANA is found in a lot of chronic hepatobiliary diseases like viral hepatitis, drug induced hepatic disease, primary biliary cirrhosis, primary sclerosing cholangitis, non-alcoholic steatohepatitis, and alcoholic hepatitis. The main reason of ANA positivity is not the stimulation by an immunogen but the destruction of the hepatic cells because of the inflammation and necrosis. ANA is a very important diagnostic criterion especially in type 1-autoimmune hepatitis, along with anti-smooth muscle cell antibody. The most frequent patterns are homogeneous and granular in autoimmune hepatitis and nuclear lamine is the second one.[13-15]

Of all ANA positive samples, 62.6% had only borderline or weak fluorescence level in this study. As the clinical association of borderline/weak fluorescence levels are a subject of discussion, some researchers did not reported because they accepted as negative. Low titers of autoantibodies are seen in healthy people, relatives of the autoimmune patients and patients who have chronic inflammatory disease or cancer without having an autoimmune base. These kinds of antibodies are usually in IgM type of low affinity and polyreactive.[16] Most of the ANA positive people don't developed an autoimmune disease and this is consisted with low prevalence of rheumatologic disorders (5-7%) despite the rate of ANA presence.[9] ANA positivity can be detected 20-30% at 1/40, 10% at 1/80 and 5% at 1/160 dilution in healthy people.[17] Li et al. suggest that the persistence of the positive ANA may be a part of the component of the normal immune response.[9] In the study of Mariz et al., ANA positive healthy people were followed up for four years of period and none of them developed any symptoms. Along the period, 72.5% of the ANA positivity persisted on the same level, while 27.5% of them dropped below 1/80 and were reported as negative. It has been emphasized that this follow up was done only from the healthy people who had ANA-Hep-2 patterns that were not specific for acute rheumatic diseases. The writers suggested that the low ANA positivity of the patterns like homogeneous and centromere which are related only to autoimmune diseases must be followed intensely.[18] The common recommendation is to report the fluorescence levels above 1+ as positive. The increase of ANA by age was proved by several studies.[16,19] In our laboratory, IIFA is performed at 1/100 dilution and all patterns even with the borderline fluorescence level are reported and the judgment of the importance of the positivity is left to the clinicians. This type of reporting might give a chance to the SLE patients whose ANA is positive considerably before the clinical symptoms which is not a rare probability and these patients must be followed carefully.[20] Of all ANA positive patients with borderline/weak fluorescence level, 43.3% were from rheumatology department in this study.

The most frequent four patterns were homogeneous (23%), granular (22%), homogeneous-granular (15.5%) and nucleolar (13.5%) in this study. This was similar with the results of other studies from Turkey. Güdücüoğlu et al. reported 152 homogeneous, 96 nucleolar, 82 granular pattern out of 367 ANA positive patients.[8] The most and dominantly seen pattern was found as homogeneous (51.2%) and this was followed by fine granular (6%), homogeneous/fine granular (6%) and homogeneous/nucleolar (6%) by Yılmaz et al. [1]. Likewise, Yumuk et al. reported homogeneous as the most frequent pattern and the second one was granular.[21] Also Karakeçe et al. found the most frequent patterns as nuclear (56.2; fine and coarse granular, homogeneous and nuclear membrane), nucleolar (16.2%), mitotic (14%) and cytoplasmic (13.6%).[11]

A new pattern is identified showing a nuclear distribution of dense fine speckles (DFS) also known as lens epithelium-derived growth factor p75 (LEDGF/p75) that recognized as a 70-kd protein was not distinguished from homogeneous-granular pattern at the time of the study. Some of the homogeneous-granular patterns will be change into

granular and chromosomal granular type of anti-DFS. This is an autoantibody which can be seen in some of the dermatologic disorders like atopic dermatitis, psoriatic conditions, asthma, interstitial cystitis and rheumatologic diseases like Sjögren's syndrome but its clinical importance is questionable as this pattern also present in 10% of normal population.[22,23]

Anti-dsDNA was positive in 8.7% of ANA positive patients in the study. The major pattern of anti-dsDNA positive samples was homogeneous (68.8%). The most seen pattern from SLE disease is homogeneous (60-70%) which shows the autoantibody presence against dsDNA and our results were parallel to the literature.[1,21,24] On the other hand, it must be considered that homogeneous pattern may also point to the autoantibodies against histon and nucleosomes. The presence of anti-dsDNA has prognostic value as the titer of anti-dsDNA is an important criterion of disease activity and also shows a correlation with lupus nephritis.[24-26]

First step of the algorithm of ANA and specific antibody testing in the diagnosis of rheumatic diseases is to screen ANA. The second step is testing for anti-ENA profile from positive samples. Anti-ENA profile test is an immunoblotting assay that uses only known antigens.[27-29] Therefore, ANA with IIFA is more sensitive than anti-ENA profile test. According to our study, the most frequent four antigens were SSA (34.4%), SSA-SSB (16.8%), Scl70 (16%) and Sm/RNP (9.2%) respectively. Anti-Sm antibodies are mostly found in SLE patients but they can only detected in 25-30% of them. Similarly, Scl70 is 100% specific for the diagnosis of systemic sclerosis. If SSA or/and SSB are detected, the result will direct us not only to diagnose Sjögren's syndrome but also to sub acute cutaneous SLE and neonatal lupus syndrome.[30] Some studies reported that some ENAs especially anti-SSA/Ro and anti-SSB/La antibodies can be missed on IIFA, although others demonstrated borderline fluorescence might contain these antibodies.[31] It should not be forgotten that anti-ENA profile may be negative depending on the positive ANA pattern.

Our three years' experience of testing autoantibodies was shared in this study. Reliable test results are very important for the health of the patients with autoimmune disorders. For being a dependable laboratory, having enough knowledge and experience about the chosen methods of autoantibody tests is mandatory. It should be remembered that clinic status of the patients are very important for considering the results of autoimmune tests especially ANA.[32]

A good relationship with the clinicians is also an indispensable component of confidential analysis and reporting.

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