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COMPARISON OF DETECTION OF ANTI-NUCLEAR ANTIBODIES BY INDIRECT IMMUNOFLUORESCENCE ASSAY ON HEP-2 CELLS VS. LINE IMMUNO-ASSAY

Microbiology

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ABSTRACT

Introduction: The gold standard set for detection of Anti-nuclear Antibodies (ANA) is Indirect Immunofluorescence Assay (IFA). This study targets the comparison between Line Immunoassay and Indirect Immunofluorescence Assay (IFA). **Material Methods:** 748 samples were reported by ANA-IFA and ANA -LIA in the duration of November 2021 to July 2022. **Results:** 157(59.92%) positive by ANA-IFA, 105(40.07%) positive by ANA-LIA **Conclusion:** ANA-IFA are much more sensitive and specific than ANA profiles

KEYWORDS

Antinuclear antibody (ANA), Indirect immunofluorescence (IIF), Line immune assays (LIA)

INTRODUCTION

Antinuclear antibodies (ANA) are a specific type of autoantibody that binds and destroys structures inside the nucleus of the cell. There are now antibodies against various compartments of the cell which are referred to as ANA. [1,2]

Autoantibodies can be detected in the serum of affected individuals to diagnose these diseases. Anti-nuclear antibodies are referred to as such, but they actually react with various other cellular structures as well, including the cell surface, nucleoli and cytoplasm, as well as nuclei. [3] The ANA test is widely used as a screening test for connective tissue disorders, even though its sensitivity varies and it often results in false positive results for various conditions. [3]

Anti-nuclear antibodies are commonly detected by immuno fluorescence (IF) assays and line immunoassays (LIA). IFA (immunofluorescence assay) detects auto antibodies by looking for specific staining patterns that are associated with specific clinical conditions. [4,5,6] Antigen specific LIA assays allow us to detect the presence of specific auto antibodies (ANA profiles) that target specific antigens. [1,2,3]

An immunofluorescence assay is the gold standard in ANA interpretation, but it is expensive, requires skills, and requires proper handling of specimens. IFA detection of ANA does not always yield a specific clinical diagnosis; it is usually necessary to perform an antibody assay in order to determine the clinical syndrome. Further diagnostic testing is needed if borderline ANA-IF staining (such as 1+ positive and weak positive results) creates confusion. In the case of MCTD, for example, the presence of an antibody anti-U1RNP can be used to diagnose the disease, but in other autoimmune diseases, the ANA IFA pattern and antibody profile overlap. Both immunofluorescence and ANA profiles can be used for the detection of ANA, but this increases the cost and time interval for diagnosis, since each laboratory has its own turnaround times. In some cases, an ANA profile may not detect the antibodies when the specific antigen in the kit is not present, in which case the diagnosis and subsequent treatment of the disease may be delayed. The purpose of this study was to compare ANA results obtained from 748 patients with autoimmune diseases who underwent both immunofluorescence (IF) and immunoprofile (ANA profile) ANA testing simultaneously. A correlation was finally found between these results and the disease that had been diagnosed. [1,2,3]

In this study, ANAs-IFA patterns are correlated with ANA profile subtyping, ANA profiles subtyping, and diseases diagnosed that are related to ANA profiles subtyping.

MATERIALS AND METHODS

A laboratory-based observational study was conducted in the Department of Molecular Biology, Mahatma Gandhi Medical College& Hospital, Jaipur (Rajasthan) from Nov 2021 to Aug 2022. A total of 748 samples were used in this study. As per standard recommendations, indirect immunofluorescent techniques were used to determine ANA using HEp-2 Cell Line Substrate; a titre of 1:180 was considered positive for IF. According to the kit manual, ANA profiles were performed using IMTEC-ANA-LIA MAXX to detect antibodies against ds DNA, nucleosomes, histones, Sm D1, PCNA, RPP/Po, SSA/Ro60Kd, SSA/Ro 52 kD, SSB/La, CENP - B, Scl 70, U1sn RNP, AMA M2, Jo -1, PM-Scl, Mi -2 and Ku.

Profile methods were compared with standard IF tests for ANA detection. The results of these two diagnostic assays (IFA and ANA profile) were also correlated with the diagnosed disease. A homogeneous pattern is found in patients diagnosed with SLE by the IF method; by double-stranded DNA (dsDNA); by histone antibodies in the ANA immunoprofile method, indicating co-relationship. [7] As shown in Table 1, common ANA-IFA patterns and antibodies are associated with clinical significance.

OBSERVATIONS AND RESULTS

The present study was a laboratory based observational study conducted in the Department of Molecular Biology, MGMC&H, Jaipur (Rajasthan) from Nov 2021 to Aug 2022. A total of 748 samples was included in the study. Demographic data (such as age, sex, in-patient, out-patient status) of the patients were recorded.

Table 1: ANA -IFA patterns and antibodies. [3]

Location	Pattern	Target antigen	Clinical association
Nucleus	Homogeneous	dsDNA	SLE, Drug induced SLE, other Rheumatic diseases
		Histones	
		Nucleosomes	
	Speckled	Sm	SLE, Sjogren's syndrome, mixed connective tissue disease, rheumatic disease, Scleroderma
		U1-snRNP	
		SSA/Ro	
		SSB/La	
		Cyclin1 (PCNA)	
		CENP A, B, C	

	Centromere	Sp-100, MND, NSp-I	CREST form of scleroderma, primary biliary cirrhosis
	Nuclear membrane	Nuclear lamins, Fibrillarin	Lupoid hepatitis, SLE, RA, primary biliary cirrhosis
Nucleolus	Nucleolar	Pm-Scl	Scleroderma, Scleroderma/ Myositis, Sjogren's syndrome, connective tissue disease
		RNA polymerase	
		NOR90	
		Th-Tho	
		Mitochondria	
Cytoplasm	--	Actin	Autoimmune hepatitis, myositis, primary biliary cirrhosis, SLE
		Vimentin	
		Golgi apparatus	
		Jo-1	
		Ribosomes	
		SSA	
SSA	--	--	Sjogren's syndrome, SLE, Neonatal Lupus
Mitotic cells	Centriole		Unknown
	Mid-Body		Unknown
	Spindle fibers		Rheumatic disease

Table 2: Distribution of Positive and Negative Samples

Total Sample	Positive Sample	Negative Sample
748	262(35.02%)	486(64.97%)

Table 3: Distribution of ANA-IFA and ANA-LIA Positive samples

Total Positive sample	ANA -IFA Positive	ANA-LIA Positive
262	157(59.92%)	105(40.07%)

Table 4: Distribution of common Immunofluorescence patterns

Location	Pattern	Positive Number
Nucleus	Speckled	129 (49.23%)
	Homogeneous	60 (22.90%)
	Centromere	7 (2.67%)
	Nuclear membrane	2 (0.76%)
Nucleolus	Nucleolar	12 (4.58%)
Cytoplasmic	-	48 (18.32%)
SSA	-	4(1.52%)

Table 5: Distribution of specific antibodies in different ANA patterns

Target antigens	Positive number
SSA/RO	30(28.57%)
Histones	11(10.47%)
Nucleosomes	11(10.47%)
Ds DNA	10(9.52%)
SSB/La	10(9.52%)
U1-Sm RNP	8(7.61%)
PmSCL-70	7(6.66%)
Sm D1	6(5.71%)
PCNA (cycline)	3(2.85%)
RPP	3(2.85%)
KU	2(1.90%)
AMA	1(0.95%)
CENP	1(0.95%)
KD	1(0.95%)
JO-1	1(0.95%)

DISCUSSION

The detection of antinuclear antibodies (ANA) for autoimmune diseases is one of the few investigations in Medicine that play such an important role in diagnosis. Studies have shown that the majority of ANA positive results are false positives when referred to a tertiary care centre following unnecessary testing. [8]

In this study, 748 patients with autoimmune diseases were analysed for correlation between IFA results and ANA profiles.

The results of ANA tests detected by IFA vs. LIA in patients suspected of having autoimmune diseases have been compared in many studies. [9,10,11] But few studies have examined the results in patients who have been diagnosed with autoimmune diseases. [12,13,14] In the present study, both tests (IFA and ANA profile) were utilized in order to establish a diagnosis and were then compared with IFA patterns for determining the diagnosis; the disease diagnosed was correlated with both the test results.

In autoimmune diseases, a preponderance of female patients is often observed; in the current study, 68.3% of participants were female, similar to other studies. [15,16,17,18]

In 1 in 80 dilution, 157 patients (59.92%) were found to have positive ANA IFA, as were 105 (40.05%) by ANA profile. A similar finding was found by Latha M et al, [15] who reported that ANA IFA was positive in 71.3% of 279 samples; 159 samples (56.9%) were also positive for ANA IF and Line Immuno Assays. An East India study found that 394 samples tested both for ANA IFA and ANA profile had 138 positive IFA results (35.02%) in 1 in 40 dilutions and 114 (82.6%) were also positive line immune assay results. [16] In that study, were mostly speckled patterns 129(49.23%) followed by homogeneous 60 (22.90%), cytoplasmic 48 (18.32%), nucleolar patterns 12 (4.58%), Centromere 7 (2.67%), and SSA 4 (1.52%) respectively.

There are 40 (15%) cases in which an ANA IFA is positive, but an ANA profile is negative. Due to the fact that ANA profiles often exclude some rare auto antibodies via the lack of specific antigenic substrates that can be detected by IF staining, this may account for the failure to detect some rare auto antibodies by ANA profiles.

We reviewed these 40 (15%) cases for their ANA pattern (28 cases were speckled, 7 were nucleolar, 3 were homogeneous cytoplasmic). Baronaite et al. analysed ANA results from 400 patients using IFA and line immunoassay (LIA) techniques, and the LIA method was found to be less reliable for analysing nuclear and speckled reactivity patterns. [10]

In this study, ANA profiles target the following antigens: SSA/RO 30, Histones 11, Nucleosomes 11, SSB/La 10, DS DNA 10, U1Sn RNP 8, Pm SCL 7, Sm D1 -6, PCNA (Cyclin)3, RPP 3, Ku 2, AMA, CENP, KD JO-1 -1.

A total of 12 samples (11.42%) were found to be positive for ANA- LIA profiles in this study, But negative for ANA - IFA. DsDNA antibodies were observed in six samples, SSA/Ro 52, antibodies in four samples, PCNA antibodies and U1-SnRMP antibodies in one sample. ANA-IFA cannot detect SSA and dsDNA antibodies, resulting in negative immunofluorescence. An immunofluorescence negative result might be caused by the absence of autoantigens or a low number of autoantigens during the preparation process. [19] Sebastian et al, [20] noted that 13.5% which were found positive by ANA-LIA and negative by ANA-IFA. [20]

If clinical features are strongly suggestive in a given clinical setting, it is important to perform further immune profile investigations even if IFA is negative. Thus, it is important to realize that certain auto antibodies may not stain by IFA method, while others present in the patient's serum may not be detected by ANA profile if it does not contain that particular antigenic substrate. Therefore, ANA-IFA is considered to be the gold standard screening test and LIA profile to be the confirmatory test. [21]

According to various studies, ANA-IFA are much more sensitive and specific than ANA profiles. [12,22,23] In the present study, we found 58% of the patients' ANA IFA patterns were co-related to the disease; 40% of patients had ANA profiles co-related to the disease. According to Priyadarshini et al [9], we found similar findings in the present study.

CONCLUSIONS

In contrast to immunofluorescence assay, ANA profile correlations with diseases are less. Additionally, ANA-IFA are much more sensitive and specific than ANA-LIA. Comparing immunofluorescence with ANA profile, immunofluorescence remains the preferred method for detecting autoimmune disorders.

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