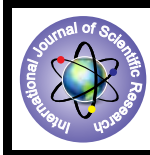


A Comparative Study of the ELISA and IF Methods for Detection of Antinuclear Antibodies Characteristic of Connective Tissue Diseases



Medical Science

KEYWORDS : Autoimmune diseases, Antinuclear Antibody, Immunofluorescence, ELISA

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ABSTRACT

Anti-nuclear antibody (ANA) testing is widely used as a screening test in autoimmune diseases. The most commonly used method for ANA testing is ANA immunofluorescence assay (ANA-IFA). This study compared the diagnostic performance of Enzyme linked immunosorbent assay (ELISA) with IFA for the detection of ANA. 321 samples were from patients with clinical suspicion of autoimmune diseases. Of the 321 test samples, IFA was positive in 53 (16.5%), negative in 268 (83.5%) cases. ELISA was positive in 47 (14.6 %), negative in 274 (85.4%) cases. 44 (13.7%) samples were positive by both methods. 266 (82.9%) samples tested negative by both. 9 (2.8%) samples were IFA positive but ELISA negative. 3 samples (1%) were ELISA positive but IFA negative. Comparing with the gold standard IFA, the sensitivity of ELISA was 83% and the specificity was 98.8%. ANA by ELISA is less sensitive than IFA using the HEp 2 substrate.

INTRODUCTION

The antinuclear antibody (ANA) test is a standard screening assay for detecting multiple antibodies that may be produced by a patient with an autoimmune or ANA associated rheumatic disease (AARD) such as Systemic Lupus Erythematosus (SLE), scleroderma, CREST syndrome (Calcinosis, Raynaud's phenomenon, Esophageal motility abnormalities, Sclerodactyly and Telangiectasia), Sjogren's syndrome, Mixed connective tissue disease (MCTD), Polymyositis, and Dermatomyositis.[1] Their detection with high sensitivity and specificity is therefore of utmost importance.

Although there are several methodologies available to screen ANA, the American College of Rheumatology (ACR) issued a statement declaring HEp-2 indirect immunofluorescence (IIF) as the preferred method for ANA screening.[2,3] The IFA test is currently considered the "gold standard" for testing for ANAs in clinical practice the IFA test permits the detection of antibodies to more than 30 different nuclear and cytoplasmic antigens comprising more than 50 auto antibodies.[4] There are 5 to 6 indirect immunofluorescence (IIF) nuclear patterns that are commonly reported by most laboratories, namely, homogeneous, speckled, nucleolar, centromere, peripheral/rimmed, and proliferating cell nuclear antigen (PCNA).

Disadvantages of immunofluorescence testing include the complexity of the procedure and requirement of a fluorescent microscope, which may not be available in many laboratories.[5] The ANA-IFA is a subjective assay requiring skilled personnel and is a manual assay with a significant amount of hands-on time.[1] Therefore, an ANA-enzyme immunoassay (ANA-EIA) is an attractive alternative to ANA-IFA. The advantages of ELISA testing include the speed and simplicity of the assay also mass produced coating antigen preparations may be more consistent from lot to lot than immunofluorescence cell substrates.[5] Some ELISA assays approach immunofluorescence in their sensitivity and specificity for the identification of antinuclear antibodies.[4] No technical expertise is required to interpret

the ELISA readings. Disadvantages of ELISA testing include reduced antigen diversity leading to decreased sensitivity. The present study was done with the objective of detection of ANA by immunofluorescence assay using HEp-2 cell substrate and Enzyme immunoassay in patients with suspected autoimmune diseases. This study also compared the diagnostic performance of the ELISA method for ANA screening compared with HEp-2 ANA using IFA testing.

MATERIALS AND METHODS

This was a Cross sectional comparative study. The institutional ethical committee clearance was obtained prior to conducting the study. The blood samples were collected after obtaining informed consents from the patients. A total of 321 samples were collected from patients with suspected autoimmune diseases during the period of 2014-15. Serum was separated by centrifugation and the serum samples were stored in the deep freezer at -20°C till further testing.

ANA Immunofluorescence Assay was performed using the Bio systems Immunofluorescence Kit. The procedure was carried out according to the

kit manufacturer's instructions. The serum samples were diluted 1/80. 1 drop each of the

Control and Test sera were placed on each slide well, ensuring to cover it completely. Serum anti nuclear antibodies bind to the corresponding antigens present in the HEp-2 cells coated on the slides. After an incubation of 30 minutes, the slide was drained and rinsed with phosphate buffered saline. The resulting antigen antibody complexes are detected by means of a fluorescein labeled anti human globulin. After a further incubation for 30 minutes, the slide was rinsed, mounting medium was added and then it was examined under the fluorescent microscope. The different patterns of fluorescence observed were Homogenous, Peripheral, Speckled, Nucleolar and centromere.

ANA ELISA was performed according to the Kit (Calbio-tech) Manufacturer's instructions. Briefly, diluted serum

samples are added to wells coated with purified nuclear antigens. ANA specific antibody, if present, binds to the antigen. All the unbound material is washed away and the enzyme conjugate is added to the antigen antibody complex. After washing off the excess enzyme conjugate, the plate is incubated to allow the hydrolysis of the substrate by the enzyme. The intensity of the colour generated is proportional to the amount of the IgG specific antibody in the sample.

RESULTS

321 samples were tested by both immunofluorescence (IFA) and ELISA. Out of 321 samples, 84 were males and 237 were females. Of the 321 test samples from patients with suspected autoimmune diseases, IFA was positive in 53 (16.5%), negative in 268 (83.5 %) cases. ELISA was positive in 47 (14.6 %), negative in 274 (85.4%) cases. 44 (13.7%) samples were positive by both methods. 266 (82.9%) samples tested negative by both. 9 (2.8%) samples were IFA positive but ELISA negative. 3 samples (1%) were ELISA positive but IFA negative. Table 1 shows the percentage positivity of ANA by ELISA and IFA. Table 2 shows the comparison between ANA detection by ELISA and IFA. Out of the 84 males, 2 (2.3%) were positive by IFA but out of the 237 females enrolled in the study, 54 (22.8%) were positive by IFA.

The majority of the patients who tested positive for antinuclear antibodies belonged to

the age group of 21 to 30 years followed by 31 to 40 years. The fluorescence patterns observed in the IFA positive samples were Homogenous in 22 samples, Speckled in 21 samples, Nucleolar in 7 samples, Cytoplasmic in 6 samples each. (Figure - 1)

Sensitivity and specificity of ELISA in comparison to IFA was calculated.

Comparing ELISA with the gold standard IFA, the sensitivity of ELISA was found to be 83% and specificity of ELISA was 98.8%. The sensitivity of IFA was 93.6% and the specificity of IFA was 96.7%. As shown in Table 2, compared to ANA-IFA, ANA-ELISA had low sensitivity (83% versus 93.6%), higher specificity (98.8% versus 96.7%). The sensitivity of IFA was found to be greater than that of ELISA. IFA could detect Anti nuclear antibodies in samples that were negative by ELISA. Moreover samples that showed low titre value by ELISA were positive by IFA.

DISCUSSION

In this study, ANA positives were more among the females (22.8%) than the males (2.3%). This correlates with the findings of Hayashi et al who reported that of the 111 patients with SLE, 104 were women and were men, and the median age was 35 years.[6] In the present study, most of the ANA positives belonged to the age group of 21 to 30 years followed by 31 to 40 years.

ELISA for ANA was positive in 47 (14.6%) out of 321 patients suspected to have autoimmune diseases and IFA was positive in 53 (16.5%) out of 321 patients. IFA showed more positives than the ELISA in the test group. This may be because ELISA was not able to detect all the types of antinuclear antibodies in the patient samples. Alternatively, it could be because ELISA could not detect low positives.

In the current study, comparing ELISA with the gold standard IFA, the sensitivity of ELISA was found to be 83% and specificity of ELISA was 98.8%. The sensitivity of IFA was

93.6% and the specificity of IFA was 96.7%. The findings of the present study correlated with Gneiwiek's study in which ANA-ELISA demonstrated equivalent sensitivity and somewhat higher specificity compared to ANA-IFA.[1] In contrast to the present study, Susan Copple et al showed that IFA test had only 80% sensitivity for 30 confirmed SLE serum samples, while the Bio-Rad, Phadia, Aesku, and Inova ANA ELISAs demonstrated excellent screening sensitivities of 96.6%, 96.6%, 90%, and 96.6%, respectively. The lack of agreement between test methods may reflect differences in the array of antigens present in the various assays.[4]

The different Fluorescent patterns observed were homogenous in 39.3%, speckled in 37.5%, nucleolar in 12.5% and cytoplasmic in 10.7% of the IFA positive samples. Sebastian et al have observed homogenous pattern in 45.5%, speckled pattern in 35.6% of the ANA positive samples.[7] Sunitha et al reported that a cytoplasmic granular pattern in 37 % of the samples and a homogenous pattern in 23 % of the samples.[8]

Conclusion

Antinuclear antibodies (ANA) are the hallmark of autoantibody production in autoimmune disease. Their detection with high sensitivity and specificity is therefore of utmost importance. Various detection methods are in use. The lack of agreement between test methods may reflect differences in the array of antigens present in the various assays. Therefore, it can be concluded from this study that ANA by ELISA is less sensitive than IFA using the HEP 2 substrate. So it is recommended that ANA detection by IFA to be followed by the labs in screening for autoimmune diseases even though ANA detection by immunofluorescence is more expensive.

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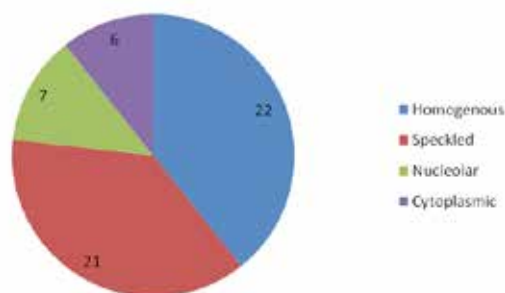
Table 1
Results for ANA by IFA and ELISA

Methods	Result	No of samples	Percentage
IFA	Positive	53	16.5%
	Negative	268	83.5%
ELISA	Positive	47	14.6%
	Negative	274	85.4%

Table 2
Comparative analysis of ANA detection by IFA and ELISA

IFA	ELISA		Total
	ANA +	ANA -	
ANA +	44	9	53
ANA -	3	265	268
Total	47	274	321

Figure-1 Patterns of Immunofluorescence



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