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Detection of Sensitivity and Specificity of Line Immuno Assay in Comparison with Indirect Immunofluorescence Assay for the Detection of Anti-Nuclear Antibodies in Diagnosis of Systemic Autoimmune Disorders

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A B S T R A C T

Systemic autoimmune diseases (SAD) are the diseases where multiple organs are involved in the presence of a large variety of auto antibodies directed against sub-cellular structures or molecules (E.g. nuclei, cytoplasm, mitochondria, DNA) and are characterized by presence of Antinuclear antibodies (ANA). Indirect immunofluorescence Assay (IIFA) on Hep-2 (human epithelial cell tumour line) is a classical technique for detection of ANA and is considered as "gold standard". Though positive fluorescence pattern on IIFA indicates the presence of ANA, however it does not allow precise identification of these antibodies. For this specialized techniques like ELISA, western blotting or line immunoassay (LIA) are employed 1) To detect sensitivity and specificity of Line immuno assay (LIA) in comparison with Indirect immunofluorescence assay(IIFA) for the detection of anti-nuclear antibodies in diagnosis of systemic autoimmune disorders. A cross-sectional study was conducted and a total of 150 clinically suspected cases of SAD of both sexes and above 18yrs of age from various departments were included in the study and blood samples collected were subjected to Indirect Immunofluorescence test on Hep-2 cells coated slides and Line immunoassay. 150 samples were analyzed for ANA by IIFA and LIA. Out of 150 samples, 54 samples were positive by IIFA. Line immunoassay was positive in 49 samples. Sensitivity and Specificity of LIA was found to be 72.2% and 89.58% respectively. Positive Coincidence Rate came out to be 79.59%. In contrast to other studies, our study gave an apt correlation of ANA detection by Line Immuno Assay and indirect immunofluorescence assay.

Introduction

Autoimmune disease is characterized by tissue injury due to breakdown of one or more of the basic mechanisms regulating immune tolerance leading to immunological reaction of the organism against its own tissues. Autoimmune diseases may occur as an isolated event (organ specific) or as systemic (non organ specific) autoimmune disease. Systemic autoimmune diseases are the diseases where multiple organs are involved in the presence of a large variety of auto antibodies directed against sub-cellular structures or molecules. (Eg:- nuclei, cytoplasm, mitochondria, DNA). Diseases in this group includes Systemic lupus erythematosis (SLE), Systemic

sclerosis/Scleroderma (SSc), undifferentiated connective tissue diseases or Mixed Connective tissue diseases (MCTD), Dermatomyositis /Polymyositis, Sjogren's syndrome (SS/SjS) (Jacinth Angel *et al.,* 2015). Systemic autoimmune disorders are characterized by presence of Antinuclear antibodies (ANA) in the blood of patients. ANA are a specific class of autoantibodies that have the capability of binding and destroying certain structures within the nucleus of the cells and are considered to be a serological hallmark of connective tissue diseases (Minz *et al.,* 2012).

The American College of Rheumatology (ACR) stated that ANA detection by IIFA on Hep-2 cells is considered as the gold standard (Damoiseaux and Cohen Tervaert, 2006) In a Clinically suspected cases of connective tissue disorders, ANA test is done, if positive further tests like Line immunoassay, ELISA, western blotting etc. are performed for the diagnosis of specific systemic autoimmune diseases. If negative no further autoantibody testing is performed (Alvarez *et al.,* 1999; Kavanaugh and Solomon, 2002).

The present study was carried out to compare Indirect Immunofluorescence test (GOLD STANDARD) with line immunoassay

Approval of the Institute's ethical committee was obtained to carry out the study

Materials and Methods

Settings

Study Place: Department of Microbiology, Gandhi Medical College, Secunderabad

Study design: Cross-sectional study

Study period: 12 months (JUNE 2016- JUNE 2017)

Inclusion criteria

Clinically suspected cases of Systemic autoimmune diseases of >18yrs of age and both sexes.

Exclusion criteria

Patients other than Systemic autoimmune diseases

Patients with Systemic autoimmune diseases with co-existing infectious diseases or carcinoma.

Sample size and duration of the study

150 Blood samples of suspected cases of systemic autoimmune diseases and over a period of 1 year

From June 2016 to June 2017, 150 blood samples from clinically suspected cases of Systemic autoimmune disease patients attending both outpatient and inpatient of Gandhi hospital, Secunderabad, referred to microbiology laboratory for Anti-Nuclear Antibodies (ANA) were included in study.

Under aseptic precautions 3ml of Blood samples collected, Serum separated out, alliquoted and stored in -20^0 C.

Repeated thawing is avoided. Samples and kit reagents are brought to room temperature 30 minutes before procedure

Antinuclear antibodies (ANA) detection by indirect immunofluorescence test is done using The Immuno Concepts HEp-2000® ANA Test System with transfected mitotic* human epithelioid cells (HEp-2 cells coated slides) and

All samples are further evaluated by line immunoassay (LIA)

Methods

Collected blood sample were brought to the laboratory and serum was separated using the standard protocol of the laboratory. IIFA was performed using The Immuno Concepts ANA Test System with mitotic human epithelioid cells (HEp-2) represents an advanced immunofluorescent system for detection of ANA. HEp-2 cells with mitotic figures have been shown to have greater sensitivity and yield sharper pattern recognition than classical mouse kidney substrate in detecting antibodies in progressive systemic sclerosis.

Positive and Negative controls were run with each test daily. Serum was diluted in 1:80 ratio (serum: diluent) (10 µl serum +790 µl diluent). 30 µl of the diluted serum was then put on each wells. This was then incubated at room temperature for 30 min. This step allowed the antibodies in the serum to react with the antigens coated on the wells.

The slide (wells) was then washed carefully and then dipped into the PBS for 10 min to remove the unbound antibodies. In the next step, FITC conjugate (Anti-human IgG conjugated to fluorescein isothiocyanate (FITC) was added to wells, to get bound to the antibodies and emit fluorescence. The FITC was again washed off carefully and dipped in PBS (in dark) for 10 min, to remove the unbound conjugate. The wells were then mounted using mounting medium. The visualization of the slide was then done under the fluorescence microscope at 40X. Based on the fluorescent intensity, samples were graded (+, ++, +++) and NEGATIVE.

Negative

A serum was considered negative for antinuclear antibodies if nuclear staining was less than or equal to the negative control well with no clearly discernible pattern. The cytoplasm may demonstrate weak staining, with brighter staining of the non-chromosomal region of mitotic cells, but with no clearly discernible nuclear pattern.

Positive

A serum was considered positive if the nucleus shows a clearly discernible pattern of staining in a majority of the interphase cells. The positive sample showed bright apple green fluorescence in the nuclei of the cells, with a clearly discernible pattern characteristic of the control serum that was used. The serum samples which were positive or negative by IFA method were further processed using Line Immuno Assay (LIA) using IMTEC-ANA-LIA MAXX kit. To perform LIA, nitro cellulose strips coated with 17 highly purified antigens as discrete lines (nRNP/Sm, SSA, Ro-52, SSB, Scl-70, PM-Scl, Jo-1, CENP-B, dsDNA, Nucleosomes, Histones, Ribosomal P-protein, AMA-M2) were used along with control band. The test procedure was as follows: Serum was diluted using DILUTION BUFFER in 1:110 and left on the horizontal shaker for 30 min. After this, 3 x washing was done with the WASH SOLUTION for 5 min each. This was then followed by adding CONJUGATE to the strip for 30 min. Again the washing step was repeated. To the washed strip, SUBSTRATE was added and left for 10 min. Afterwards, the reaction was stopped by adding STOP SOLUTION for 2 min. Then the strips were dried and evaluated by comparing with the intensity of Positive Control Line.

Results and Discussion

A Total of 150 samples of clinically suspected cases of Systemic autoimmune diseases from various Departments of Gandhi Hospital, Secunderabad, are included in the study. Antinuclear antibody (ANA) detection done by Indirect Immunofluorescence Assay (IIFA) and Line immunoassay (LIA) (Table 1 and 2).

Fig.1 Results with ANA-IIFA and line immunoassay tests in the study population

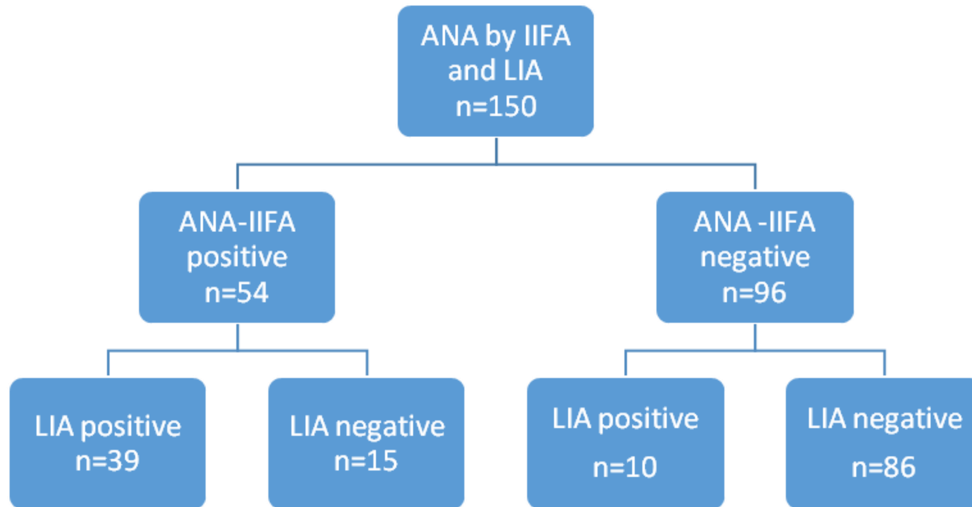

Table.1 Comparison between ANA detection by IIFA (Gold standard) and LIA

TEST		ANA DETECTION BY INDIRECT IMMUNOFLUORESECENT TEST(IIFA)		TOTAL
		POSITIVE	NEGATIVE	
BY LINE IMMUNO ASSAY(LIA)	POSITIVE	39 (True positive)a	10 (False positive) c	49 (a+c)
	NEGATIVE	15 (False negative)b	86 (True Negative)d	101 (b+d)
	TOTAL	54 (a+b)	96 (c+d)	150

Table.2 Sensitivity and specificity of line immunoassay in comparison with IIFA

Statistic	Formula	Value
Sensitivity	$\frac{a}{a+b}$	72.22%
Specificity	$\frac{d}{c+d}$	89.58 %
Disease prevalence	$\frac{a+b}{a+b+c+d}$	36.00%
Positive Coincidence value	$\frac{a}{a+c}$	79.59%
Negative Coincidence value	$\frac{d}{b+d}$	85.15 %

Total number of samples with ANA-IIFA and line immunoassay done: 150

Number of samples with ANA-IIFA positive with line immunoassay positive: 39

Number of samples with ANA-IIFA positive with line immunoassay negative: 15

Number of samples with ANA-IIFA negative with line immunoassay positive: 10

Number of samples with ANA-IIFA negative with line immunoassay negative: 86

In the current study, comparing LIA with the gold standard IIFA, the sensitivity of LIA was found to be 72.22% and specificity was 89.58%. Positive coincidence value was 79.59%. Prevalence of disorder found to be 36%.

In the present study, sensitivity and specificity of LIA was found to be 72.2% and 89.58% which is correlating with other studies like Sarojini Raman *et al.,* (2017), Sharmin *et al.,* (2014), Madhavi Latha and Anil Kumar (2014), Wendy Sebastine *et al.,* (2016) Prevalence of ANA was found to be 36% in present study which is also correlating with other studies of Sarojini Raman *et al.,* (2017), Shaily Garg *et al.,* (2017), Wendy Sebastine *et al.,* (2016), Akmatov *et al.,* (2017). Positive coincidence value was 79.59% correlating with Sarojini Raman *et al.,* (2017), Sharmin *et al.,* (2014), Wendy Sebastine *et al.,* (2016).

As the prevalence of Systemic autoimmune diseases is increasing there is a need for early and accurate diagnosis for prompt initiation of treatment to reduce disease morbidity and mortality, Hence detection of ANA by Indirect immunofluorescence test followed by profiling of Antinuclear antibodies by Line immunoassay will help in early and accurate diagnosis of systemic autoimmune diseases.

References

Alvarez, F., P. A. Berg, F. B. Bianchi *et al.,* (1999) "International autoimmune hepatitis group report: review of criteria for diagnosis of autoimmune hepatitis," Journal of Hepatology, vol. 31, no. 5, pp. 929–938, 1999.

Damoiseaux, J. G. M. C. and J.W. Cohen Tervaert, (2006) "From ANA to ENA: how to proceed?" Autoimmunity Reviews, vol. 5, no. 1, pp. 10–17, 2006

Jacinth Angel, Marin Thomas, Boppe Appalaraju (2015) cited 2017 Evaluation of ELISA and indirect Immunofluorescence in the diagnosis of autoimmune diseases and their interpretation in the clinical situation Department of Microbiology, PSG Institute of Medical Sciences and Research, Coimbatore, Tamil Nadu, India J Acad Clin Microbiol 17:7-11. DOI: 10.4103/0972-1282.158776

Kavanaugh, A. F., and D. H. Solomon, and The American College of Rheumatology ad hoc Committee on Immunologic Testing Guidelines, "Guidelines for immunologic laboratory testing in the rheumatic diseases: anti-DNA antibody tests," Arthritis Care and Research, vol. 47, no. 5, pp. 546–555, 2002

Madhavi Latha B, and Anil Kumar B (2014) Detection of antinuclear antibodies by indirect immunofluorescence method and its comparison with line immunoassay in a tertiary care hospital: A laboratory based observational study. J Med Sci Res 2(4):194-199 10.17727/JMSR.2014/2-034

Manas Akmatov *et al.,* Arthritis Research & Therapy (2017) 19:127 DOI 10.1186/s13075-017-1338-5

Minz RW, Kumar Y, An and S, *et al.,* (2012) Antinuclear antibody positive autoimmune disorders in North India: an appraisal. Rheumatol Int. 2012; 32:2883–2888.

Sarojini Raman, Amit Kumar Adhya, Prasant kumar Pradhan, Kanaklata Dash, Urmila Senapati (2017) Correlation of Indirect Immunofluorescence & Line Immunoassay Method in Detection of Autoimmune Diseases: an Observational Study at a Tertiary Care

Teaching Hospital Sch. J. App. Med. Sci., 2017; 5(7A):2520-252

Shaily Garg, Anshika Srivastava* and Santosh Prasad (2017): Correlation of Line Immuno Assay with Indirect Immunofluoresence Assay for the Detection of Anti-Nuclear Antibodies in Various Autoimmune Disorders; Journal of Autoimmune Disorders, Vol.3 No.3:37 2017

Sharmin S, Ahmed S, Abu Saleh A, Rahman F, Choudhury MR, Hassan MM (2014) Association of Immunofluorescence pattern of Antinuclear Antibody with Specific Autoantibodies in the Bangladeshi Population Bangladesh Med Res Counc Bull 2014; 40: 74-78

Wendy Sebastian, Atanu Roy, Usha Kini, Shalini Mullick (2016) Correlation of antinuclear antibody immunofluorescence patterns with immune profile using line immunoassay in the Indian scenario Indian Journal Of Pathology And Microbiology - 53(3), July-September 2010 DOI: 10.4103/0377-4929.68262

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