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Original Article

Biochemistry Section

Comparative Analysis of Line Immunoassay with Immunofluorescence Assay for the Identification of Autoantibodies in Patients with Suspected Systemic Autoimmune Disorders: A Cross-sectional Study

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ABSTRACT

Introduction: Systemic Autoimmune Diseases (SAD) are the diseases, including Rheumatoid Arthritis (RA), Systemic Lupus Erythematosus (SLE), Sjogren's syndrome, polymyositis, dermatomyositis, etc. where multiple organs are involved in the presence of a large variety of autoantibodies directed against one's own immune system at various levels. They are one of the major causes of death and disability in all age groups and are characterised by the presence of Antinuclear Antibodies (ANA) in the blood of patients.

Aim: To comparatively evaluate the utility of Line Immunoassay (LIA) with Indirect Immunofluorescence Assay (IIFA) for the detection and identification of ANA in suspected SAD patients.

Materials and Methods: The present observational, cross-sectional study was conducted on the collected samples of 560 clinically suspected SAD patients attending the Outpatient and Inpatient Departments (Medicine, Paediatrics, Orthopaedics, Dermatology and General Surgery) at Shri Ram Murti Smarak Institute of Medical Sciences, Bareilly, Uttar Pradesh, India (tertiary care hospital), from March 2016 to March 2020. Samples were subjected to ANA testing by IIFA and LIA. All variables anti-dsDNA, anti-Smd1, SSA/Ro and U1 SnRNP, SSA/Ro, anti Proliferating Cell Nuclear Antigen (PCNA) and PO/RPP, SSB/La, anti CENP-B, Scl70, AMA-M2 and Mi-2 included in the study were presented in the form of percentages. Performance of LIA was reported in terms of sensitivity and specificity at 95% confidence interval. Data was analysed using Statistical Package for the Social Sciences (SPSS) version 22.0 (Chicago, IL, USA) for windows.

Results: Samples of both male (n=229) and female (n=331) patient of age group ranging from 0-90 years with mean age of 38.2 years were studied. Out of 560 samples, 197 (35.17%) patients were found to be ANA positive by IIFA. The LIA was positive in 175 (31.25%) patients. Of these 175 positive patients, 92 (52.57%) had a recognised autoimmune disease with the most common diagnosis was SLE, found in 38 (41.30%). In comparison to IIFA the sensitivity and specificity of LIA was found to be 82.13% and 98.62% respectively with 92.63% accuracy.

Conclusion: The combined analysis of IIFA and LIA can be very useful for rapid identification of ANA which strengthen the initiation of treatment at the earliest to reduce disease morbidity and mortality.

Keywords: Antinuclear antibodies, Autoimmunity, Rheumatic diseases, Sjogren's syndrome, Systemic lupus erythematosus

INTRODUCTION

Autoimmune diseases are a group of diseases where there is an underlying tissue injury due to breakdown mechanism regulating immune tolerance resulting in own tissue damage. Autoimmune diseases are further categorised into those affecting specific organ like Hashimoto's thyroiditis and Grave's disease and Systemic Autoimmune Diseases (SAD) like Systemic Lupus Erythematosus (SLE), Rheumatoid arthritis and Sjogren's syndrome [1]. Antinuclear Antibodies (ANA) are a group of autoantibodies produced by a person's immune system when it fails to distinguish between "self" and "nonself" which can lead to autoimmune diseases. Autoimmune diseases are one of the most important non communicable diseases affecting 8.5% of the population worldwide [2]. It affects people of all genders, races and ages but certain people have an increased risk of developing this condition [3].

Even as the numbers are increasing, SAD often remain undiagnosed and untreated particularly in the developing countries as they mostly present with vague symptoms and patient often presents late to the physician and for physician also there is always a diagnostic dilemma. Diagnosis of SAD is often challenging and depends upon factors like clinical history, physical examination, and serological testing for detection of specific autoantibodies [4]. The ANA test is one of the primary tests to diagnose a clinically suspected SAD but a significant number of patients with non rheumatic conditions, such as chronic hepatic diseases, neoplasia, as well as active infections like tuberculosis, malaria and even healthy individuals may be positive for ANA, especially at low levels [5]. The most commonly used technique (gold standard) for the detection of ANA is the Indirect Immunofluorescence Assay (IIFA) on HEp-2 cells [6,7]. Although, IIFA is an excellent screening test in expert hands its main drawback is that it does not allow the specific identification of these autoantibodies, hence the disease. So, additional testing is required, employing techniques such as immunoprecipitation in agar, western immunoblotting, Enzyme-linked Immunosorbent Assay (ELISA) [7-9]. These techniques allow the identification of single autoantibody in a single test again limiting the scope of diagnosis.

The human Line Immunoassays (LIAs) were developed as a confirmatory test for rapid autoantibody profiling with greater sensitivity and specificity [10-12]. The LIA is easy to perform and interpret and gives the results within two hours [11].

The aim of the present study was to comparatively evaluate the utility of Line Immunoassay (LIA) with Indirect Immunofluorescence Assay (IIFA) for the detection and identification of ANA in suspected SAD patients.

MATERIALS AND METHODS

The present observational, cross-sectional study was conducted on the collected samples of 560 clinically suspected SAD patients attending the Outpatient and Inpatient Departments (Medicine, Paediatrics, Orthopaedics, Dermatology and General Surgery) at Shri Ram Murti Smarak Institute of Medical Sciences, Bareilly, Uttar Pradesh, India (tertiary care hospital), from March 2016 to March 2020. Clinically suspected SAD patients were advised for IIFA and LIA for confirmation which is a routine investigation. Ethical approval was not needed for the current study as all samples were received for diagnosis and implied consent was there.

Inclusion criteria: All the samples received during above mentioned duration were included in the study.

Exclusion criteria: Patients having any infectious disease and trauma were excluded from the study.

Study Procedure

Total 560 patients were included in the study. A 2 mL of blood was collected in a BD Vacutainer® (Cat. No. 367812) from each patient and referred to the Central Research Laboratory of the Department of Biochemistry for ANA testing.

Serum extraction: After receiving blood sample, serum was separated using the standard protocol of the laboratory. Serum was stored at -20°C for further analysis. Repeated freeze/thawing was avoided. Samples and kit reagents were brought to room temperature 30 minutes before procedure [10].

Indirect immunofluorescence assay: The IIFA was done using the Immuno Concepts Hep-2000 ANA Test System (Cat No. 2140G-Ro) as per manufacturer's instructions. It is an advanced immunofluorescent system with transfected mitotic Human Epithelioid cells (HEp-2) for detection of ANA. The HEp-2 cells with mitotic figures have been shown to have increased sensitivity with more acute pattern than classical mouse kidney substrate in detecting antibodies in Progressive Systemic Sclerosis (PSS) [13].

Briefly, serum was diluted using diluent in 1:80 ratio. A 30 µL of the diluted serum was then put on each well on the slide and incubated at room temperature for 30 minutes. Then the slide (wells) was washed carefully with saline Phosphate Buffer Solution (PBS) to remove the unbound antibodies. Fluorescein Isothiocyanate (FITC) conjugate (anti-human IgG conjugated to FITC) was added to wells, to get bound to the antibodies and emit fluorescence. The FITC was again washed off carefully with PBS (in dark) for 10 minutes, to remove the unbound conjugate. In the last step, the wells were then mounted using mounting medium. Fluorescence microscope at 40X was used for visualisation of the slides. The samples were graded (+, ++, +++) and negative based on the fluorescent intensity [10]. Positive and negative controls were run with each test.

If nuclear staining was less than or equal to the negative control with no clear pattern a sample was considered negative for ANA. The cytoplasm may sometimes demonstrate weak staining, with brighter staining of the non chromosomal region of mitotic cells with no conclusive pattern.

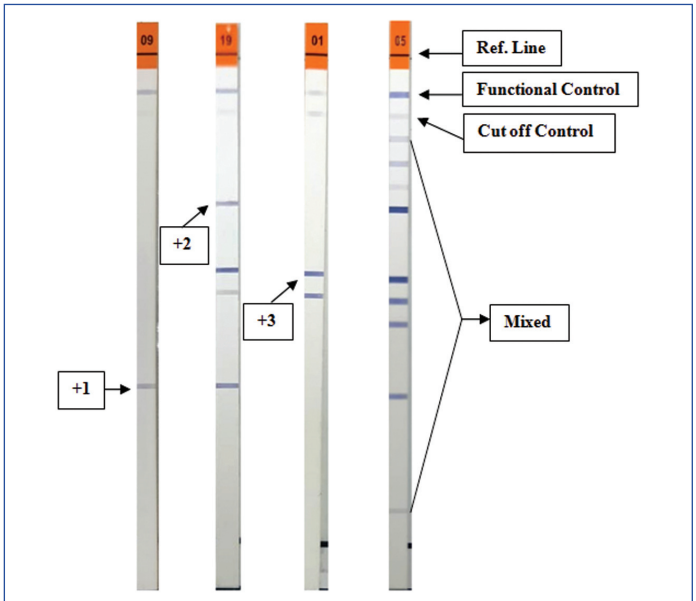
A serum was considered positive if a clear pattern of staining in a majority of the interphase cells in the nucleus was found. A bright apple green fluorescence in the nuclei of the cells is found in positive sample, with a clearly discriminating pattern characteristic of the control serum that was used [10].

Autoantibody detection with LIA: The serum samples were further processed with LIA using IMTEC-ANALIA MAXX kit (Cat. No. ITC92005). To perform LIA, nitro cellulose strips (orange colour coding) coated with 17 highly purified antigens as discrete lines were used along with two control bands (functional control and cut-off control) [Table/Fig-1]. The detection of autoantibody is based both on the presence of functional and cut-off control. The test results are valid if intensity of functional control is greater than cut-off control.

S. No.	Antigens	Identity	Disease association
1	dsDNA	Native	Systemic Lupus Erythematosus (SLE)
2	Nucleosome	Native	
3	Histone	Native	
4	SmD1	Peptide	
5	Proliferating Cell Nuclear Antigen (PCNA)	Recombinant	
6	PO (RPP)	Recombinant	
7	SS-A/Ro 60 kD	Native	Sjogren Syndrome/SLE
8	SS-A/Ro 52 kD	Recombinant	
9	SS-B/La	Recombinant	
10	CENP-B	Recombinant	CREST/Scleroderma
11	Scl70	Recombinant	
12	U1-snRNP	Recombinant	Mixed Connective Tissue Disease (MCTD)
13	AMA-M2	Native	Primary Biliary Cirrhosis (PBC)
14	Jo-1	Recombinant	Myositis
15	PM-Scl	Recombinant	
16	Mi-2	Recombinant	
17	Ku	Recombinant	

[Table/Fig-1]: List of antigens with their associated disease.

The test was performed according to the manufacturer's instructions. Briefly, serum was diluted using dilution buffer in 1:100 and left on the horizontal shaker for 30 minutes. After this, 3× washing was done with the wash solution for five minutes each. This was then followed by adding conjugate to the strip and left on the horizontal shaker for 30 minutes. Again, the washing step was repeated. To the washed strip, substrate was added and left for 10 minutes. Afterwards, the reaction was stopped by adding stop solution for five minutes. Then the strips were dried and evaluated. The test results were valid if functional and cut-off controls are visible and intensity of functional control was greater than cut-off control. The test result was negative, if no band was seen or seen with lower intensity in comparison to the cut-off control. The test was equivocal, if the intensity of the band was same as the cut-off control. The test result was positive, if a band exhibits a stronger intensity than cut-off control. Based on the color intensity, samples were graded (+, ++, +++) [Table/Fig-2] [10].



[Table/Fig-2]: Image showing +, ++, +++ intensities along with mixed pattern.

STATISTICAL ANALYSIS

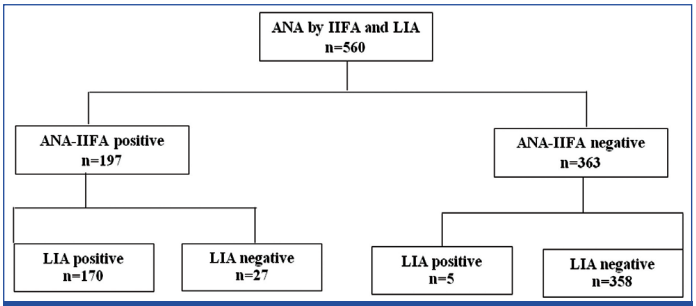
Performance of LIA was reported in terms of sensitivity and specificity at 95% confidence interval. All variables included in the study were presented in the form of percentage. Data were analysed using

Statistical Package for the Social Sciences (SPSS) version 22.0 (Chicago, IL, USA) for windows.

RESULTS

A total of 560 samples of, North Indian population of Rohilkhand region, which were clinically suspected of SAD were put to confirmation of disease. Samples of both male (n=229) and female (n=331) patient of age group ranging from 0-90 years with mean age of 38.2 years were tabulated in the different age groups to have better understanding of disease pattern.

Out of 560 samples, 197 (35.17%) were found to be IIFA positive and 363 (64.82%) were found to be IIFA negative. Using LIA, out of 560 samples 175 (31.25%) patients were found to be ANA positive, while remaining 385 (68.75%) were ANA negative [Table/Fig-3]. Comparing with IIFA (gold standard) the sensitivity and specificity of LIA was found to be 82.13% and 98.62% respectively [Table/Fig-4].



[Table/Fig-3]: Results with ANA- indirect immunofluorescent assay (gold standard) and line immunoassay tests in the study population.

Statistics variables	Value	95% CI
Sensitivity	82.13%	76.21%-87.09%
Specificity	98.62%	96.82%-99.55%
Positive likelihood ratio	59.62	24.91-142.71
Negative likelihood ratio	0.18	0.14-0.24
Positive predictive value	97.14%	93.42%-98.79%
Negative predictive value	90.63%	87.84%-92.84%
Disease prevalence	36.32%	32.36%-40.41%
Accuracy	92.63%	90.17%-94.64%

[Table/Fig-4]: Sensitivity and specificity of line immunoassay in comparison with indirect immunofluorescence assay (gold standard).

When samples were analysed in terms of gender, the female to male positive ratio was found to be 2.13:1 with total 119 positive females and 56 positive males [Table/Fig-5]. In terms of age groups, it was found that the positivity rate was maximum in the patients of 21-30 years age group followed closely by 31-40 years age group. Furthermore, maximum positive females were also found in 21-30 years age group [Table/Fig-5].

Variables	Total	With (+) LIA profile (n=175) (n,%)	With (-) LIA profile (n=385) (n,%)
Gender			
Female	331 (59.10%)	119 (68%)	212 (55.06%)
Male	229 (40.89%)	56 (32%)	173 (44.94%)
Age (years)			
0-10	26	6 (23.08%) (2 females, 4 males)	20 (76.92%)
11-20	65	16 (24.62%) (12 females, 4 males)	49 (75.38%)
21-30	124	48 (38.71%) (37 females, 11 males)	76 (61.29%)
31-40	99	32 (32.32%) (20 females, 12 males)	67 (67.68%)
41-50	109	35 (32.11%) (24 females, 11 males)	74 (67.89%)
51-60	75	20 (26.67%) (15 females, 5 males)	55 (73.33%)
61 and above	62	18 (29.03%) (9 females, 9 males)	44 (70.97%)
Total	560	175 (31.25%)	385 (68.75%)

[Table/Fig-5]: Age and gender wise distribution of the presence of auto antibodies (ANA).

Of these 175 positive ANA patients, 92 (52.57%) had a recognised autoimmune disorder while 83 (47.42%) did not. In 92 autoimmune disease patients, 66 (71.73%) were highly positive, 3 (3.26%) were moderate positive, 7 (7.60%) were low positive and 16 (17.39%) were mixed (having +3, +2 and +1 intensities) [Table/Fig-6]. The most common diagnosis was SLE, followed by MCTD, scleroderma, sjogren's syndrome, rheumatoid arthritis, dermatomyositis, Primary Biliary Cirrhosis (PBC), polymyositis, myositis and Guillain-Barré Syndrome (GBS) [Table/Fig-6].

S. No.	Diagnosis	No of patients (%)	ANA (+++)	ANA (++)	ANA (+)	Mixed
1	Systemic Lupus Erythematosus (SLE)	38 (41.30)	27	-	1	10
2	Scleroderma/Systemic sclerosis (SSc)	11 (11.95)	9		1	1
3	Mixed Connective Tissue Disorder (MCTD)	11 (11.95)	7	2	-	2
4	Sjogren's Syndrome (SjS)	10 (10.86)	9	-	-	1
5	Rheumatoid Arthritis (RA)	8 (8.69)	5	1	1	1
6	Dermatomyositis	6 (6.52)	2	-	3	1
7	Primary Biliary Cirrhosis (PBC)	4 (4.34)	4	-	-	-
8	Polymyositis	2 (2.17)	2			
9	Myositis	1 (1.08)	1	-	-	-
10	Guillain-Barré Syndrome (GBS)	1 (1.08)	-	-	1	-
Total		92	66	3	7	16

[Table/Fig-6]: Autoimmune disorders diagnosed among 92 patients with a positive ANA profile.

Among 83 non autoimmune disease patients, 34 (40.96%) were highly positive, 2 (2.40%) were moderate positive, 41 (49.39%) were low positive and 6 (7.22%) were mixed [Table/Fig-7]. Among these patients, pain, weakness and fever were the most common complaints [Table/Fig-7].

S. No.	Diagnosis	Number of patient (%)	ANA (+++)	ANA (++)	ANA (+)	Mixed
1	Pain	26 (31.32)	9	-	15	2
2	Weakness	25 (30.12)	9	1	13	2
3	Fever	12 (14.45)	8	-	3	1
4	COPD	6 (7.22)	2	1	3	-
5	Chronic kidney disease	4 (4.81)	3	-	1	-
6	Rashes	3 (3.61)	-	-	2	1
7	Gangrene	2 (2.40)	-	-	2	-
8	Anaemia	1 (1.20)	1	-	-	-
9	Breathlessness	1 (1.20)	-	-	1	-
10	Liver disorders	1 (1.20)	-	-	1	-
11	Mood disorder	1 (1.20)	1	-	-	-
12	Seizure	1 (1.20)	1	-	-	-
Total		83	34	2	41	6

[Table/Fig-7]: Diagnoses of non autoimmune disorders among 83 patients with a positive ANA profile.
COPD: Chronic obstructive pulmonary disease

Furthermore, of the 38 SLE patients with a positive ANA profile, 23 (60.52%) had positive anti-dsDNA, 22 (57.89%) had positive anti-Smd1, 14 (36.84%) had positive anti SSA/Ro 60 kD and U1 SnRNP, 13 (34.21%) had positive anti-histone and nucleosome, 12 (31.57%) had positive SSA/Ro 52 kD, 10 (26.31%) had positive anti Proliferating Cell Nuclear Antigen (PCNA) and PO/RPP, 7 (18.42%) had positive SSB/La, 1 (2.63%) had positive for anti CENP-B, Scl70, AMA-M2, and Mi-2. Occurrence of different autoantibodies in other autoimmune diseases was shown in [Table/Fig-8]. Furthermore, distribution of different autoantibodies in non autoimmune disease patients was shown in [Table/Fig-9].

DISCUSSION

Systemic autoimmune diseases are characterised by autoantibody patterns found in patient sera. The presence of ANA is critical for establishing the diagnosis of systemic autoimmune disorders and ANA are one of the most frequently ordered tests for this. Despite its high sensitivity and low specificity, screening for ANA is widely performed by IIF on HEp-2 cells as a first level screening test [6,7]. In IIFA positive fluorescence detects the presence of ANA but does not assist in confirmation of these autoantibodies and hence the disease. In recent years the ANA profiling has become widely used not only for evaluation but also in screening for autoimmune disorders despite

	Autoantibodies against SLE						Sjogren syndrome			CREST/ Scleroderma		MCTD	PBC	Myositis			
Disease	dsDNA	Nucleosome	Histone	SmD1	PCNA	PO (RPP)	SS-A/ Ro 60 kD	SS-A/ Ro 52 kD	SS-B/ La	CENP-B	Scl70	U1-snRNP	AMA-M2	Jo-1	PM-Scl	Mi-2	Ku
Systemic lupus erythematosus (38)	23 (+3) 2 (+1)	13 (+3) 1 (+2) 4 (+1)	13 (+3) 6 (+1)	22 (+3) 1 (+1)	10 (+3) 1 (+1)	10 (+3) 1 (+1)	14 (+3) 1 (+2) 2 (+1)	12 (+3) 1 (+2)	7 (+3) 1 (+1)	1 (+3) 1 (+1)	1 (+3) 1 (+1)	14 (+3) 2 (+1)	1 (+3) 1 (+1)	-	1 (+1)	1 (+3)	3 (+1)
CREST/ Scleroderma (11)	1 (+3) 1 (+1)	-	-	1 (+3)	-	1 (+3)	6 (+3)	2 (+3) 1 (+1)	1 (+3)	2 (+3)	2 (+3)	-	-	1 (+1)	-	1 (+1)	1 (+1)
Mixed connective tissue disorder (11)	-	1 (+1)	-	-	1 (+3)	-	1 (+1)	-	-	-	-	9 (+3) 2 (+2)	-	-	-	-	1 (+3)
Sjogren's syndrome (10)	-	-	-	-	-	1 (+3)	10 (+3)	10 (+3)	4 (+3) 1 (+1)	-	-	-	-	-	-	-	-
Rheumatoid arthritis (8)	1 (+3)	-	-	1 (+2)	-	-	5 (+3)	-	-	1 (+1)	1 (+3)	-	-	-	1 (+3)	-	1 (+1)
Dermatomyositis (6)	-	-	-	-	1 (+1)	-	2 (+3)	1 (+3)	-	-	-	1 (+1)	1 (+1)	-	1 (+1)	1 (+3) 1 (+1)	1 (+3)
Primary biliary cirrhosis (4)	-	-	-	-	-	-	-	-	-	-	-	-	4 (+3)	-	-	-	-
Polymyositis (2)	-	-	-	1 (+3)	-	-	-	-	-	-	-	2 (+3)	-	-	-	-	1 (+3)
Myositis (1)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1 (+3)	-	-
Guillain-Barré syndrome (1)	-	-	-	-	-	-	-	-	-	-	-	1 (+1)	-	-	-	-	-

[Table/Fig-8]: Distribution of autoantibodies in different autoimmune diseases.
PCNA: Proliferating cell nuclear antigen; CREST: Calcinosis, raynaud phenomenon, esophageal dysmotility, sclerodactyly, and telangiectasia

	Autoantibodies against SLE						Sjogren syndrome/SLE			CREST/ Scleroderma		MCTD	PBC	Myositis			
Disease	ds-DNA	Nucleosome	Histone	SmD1	PCNA	PO (RPP)	SS-A/ Ro 60 kD	SS-A/ Ro 52 kD	SS-B/ La	CENP-B	Scl70	U1-snRNP	AMA-M2	Jo-1	PM-Scl	Mi-2	Ku
Weakness (25)	1 (+1)	-	-	1 (+1)	5 (+1)	-	1 (+2) 1 (+1)	1 (+3)	3 (+3) 1 (+2) 2 (+1)	1 (+3) 1 (+1)	1 (+1)	4 (+1)	-	-	3 (+3) 2 (+1)	3 (+3) 1 (+1)	-
Fever (12)	1 (+1)	-	-	1 (+3)	-	-	7 (+3)	2 (+3) 1 (+2)	2 (+3)	-	1 (+3)	2 (+3) 2 (+1)	-	-	-	1 (+3)	-
Pain (26)	1 (+1)	-	-	2 (+3) 6 (+1) 1 (+2)	1 (+3)	1 (+1)	1 (+3) 1 (+1)	3 (+3)	1 (+3) 3 (+1)	-	1 (+1)	3 (+3) 1 (+1)	1 (+3)	-	2 (+1)	1 (+1)	1 (+3) 1 (+1)
Liver disorder (1)	-	-	-	-	-	-	-	-	-	-	-	-	1 (+1)	-	-	-	-
Mood disorder (1)	-	-	-	-	-	-	-	-	1 (+3)	-	-	-	-	-	-	-	-
Gangerine (2)	-	-	-	-	-	-	-	-	-	-	-	2 (+1)	-	-	-	-	-
Chronic obstructive pulmonary disease (6)	-	-	-	-	2 (+1) 1 (+2)	-	-	1 (+3)	1 (+3) 1 (+1)	-	-	-	-	-	-	-	-
Chronic kidney disease (4)	-	-	-	1 (+3)	-	-	2 (+3) 1 (+1)	1 (+3)	-	-	-	1 (+3)	-	-	-	-	-
Breathlessness (1)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1 (+1)	-	-
Anaemia (1)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1 (+3)	-	-
Redness/ rashes (3)	2 (+1)	-	-	1 (+3)	-	-	1 (+1)	-	-	-	-	-	-	-	-	-	-
Seizure (1)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1 (+3)	-	-

[Table/Fig-9]: Distribution of autoantibodies in different non autoimmune diseases.

its relatively high cost compared to the cost of the ANA test only. Because a characteristic profile of ANA is associated with different autoimmune diseases, identification of the fine specificity may provide valuable clues to the diagnosis. The LIA was developed as a confirmatory test for rapid autoantibody profiling [10,11,14,15]. The LIA is easy to perform and interpret with high sensitivity and specificity [10,11,14-16]. It detects a group of autoantibodies related to SAD like SLE, systemic sclerosis/scleroderma (SSc), undifferentiated connective tissue diseases or MCTD, dermatomyositis/polymyositis, Sjogren's syndrome (SS/SjS) [10,11,17].

A total of 560 samples of clinically suspected cases of SAD were included in the study. ANA detection was done by IIFA and LIA. Out of 560 samples, 197 (35.17%) were found to be IIFA positive. Using LIA, out of 560 samples, 175 (31.25%) patients were positive with ANA in their serum which is in concordance with other similar studies [10,11,14]. Among positive ANA patients, 92 (52.57%) were showing autoimmune diseases while 83 (47.43%) were not showing identifiable autoimmune conditions. The significance of a positive ANA test in clinically suspected autoimmune disease patients has been well established [18]. However, positive ANA test in non autoimmune disease patients is the result of various infections, drug therapies and haematological disorders [19].

Females (68%) were most commonly affected. The proportion of females getting affected with autoimmune diseases were high and the ratio of female: male was found to be 2.13:1. Gunnarsson R et al., (Norway) report a similar female predominance with a female: male ratio of 3:1 [20]. The age group between 21-30 years had the maximum number of patients (38.71%), which is in concordance with other similar studies by Angel J et al., and Greidinger EL [17,21]. Autoimmune diseases most often strike women of 20-50 years because of endocrinological changes due to puberty, pregnancy and menopause. These endocrinological changes occurring during various phases of women affect immune system due to untoward interaction between innate and adaptive immunity with fluctuating hormonal level in women with the release of pro and anti-inflammatory cytokines and hence making women more susceptible to autoimmune diseases [22,23].

The most common SAD in the present study was SLE with a prevalence rate of 41% that is similar to the study of Angel J et al., [17]. Among SLE autoantibodies, antibodies to dsDNA (60.52%) were frequently found followed by antibodies to SmD1 (57.89%), SS-A/Ro60 kD and U1Sn-RNP (36.84%), nucleosome and histone (34.21%), SS-A/Ro52 kD (31.57%), PCNA and PO (RPP) (26.31%) and SS-B/La (18.42%). Anti-dsDNA antibodies have been shown to correlate with disease activity and specific organ damage [24]. It was also included in the American College of Rheumatology (ACR) criteria, 1997 for the classification of SLE together with anti-Sm, antiphospholipid (anti-PL) antibodies, and ANA. Antibodies to SmD1 are considered a highly specific marker for SLE and are reported in 30-40% of these patients [25]. The present study data with SLE show a higher frequency of positivity to SmD1 (57.89%). Anti-RNP antibodies are noted in 40-50% of SLE patients but a very high titre of anti-RNP antibodies alone is highly characteristic of mixed connective tissue disease [25]. The present study results confirmed the previous finding of Didier K et al., by showing 100% positivity of anti-RNP antibodies in suspected mixed connective tissue disease patients [25]. Antibodies to SS-A/Ro were present in all suspected Sjogren's syndrome patients while earlier study showed that SS-A/Ro antibodies have been found in approximately 60-95% of patients with sjogren's syndrome and only 25-40% of SLE patients [25]. Patients with scleroderma tend to have antibodies to Scl-70, whereas anticentromere is noted with limited scleroderma (CREST syndrome) [25]. The present study data showed only 18% of anti Scl-70 and anti CENP-B antibodies, a highly specific marker for scleroderma were present in suspected scleroderma patients.

Limitation(s)

A very limited data on SAD diagnosis via IIFA and LIA is available in north Indian population particularly of the Rohilkhand region and the lesser frequency of test (560 samples in 4 years) again limits the research. Although, LIA is very good diagnostic test but it has the limitation to diagnose only 17 most common autoantibodies and we may be missing the other antibodies altogether.

CONCLUSION(S)

The sensitivity and specificity of LIA was found to be 82.13% and 98.62% respectively. The IIFA and LIA are useful diagnostic tools having good sensitivity and specificity and can be used for screening and prognostic purposes respectively. Burden of disease in female of child bearing age group (21-35 years) is significant and needs early diagnosis and treatment. Authors have useful diagnostic tests in the form of LIA and IIFA which should be available at primary and secondary care levels to help patient get the diagnosis and treatment at the earliest.

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