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Microbiology Section

Antinuclear Antibodies Profiling in Patients with Autoimmune Diseases: A Cross-sectional Study from Tertiary Care Centre in Southern India

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ABSTRACT

Introduction: Over past few decades, autoimmune disorders have been increasing in India but adequate research on it hasn't been done yet. Antinuclear antibodies (ANA) serve as one of the most important hallmarks of autoimmunity. Recent studies and research are focusing on demonstrating the correlation between the wide range of ANA and various autoimmune disorders.

Aim: To profile ANA and correlate their patterns with clinical manifestations in patients with autoimmune diseases at a Tertiary Care Centre in South India.

Materials and Methods: The present cross-sectional study was carried out for 12 months (from February 2023 to January 2024) at SRM Medical College Hospital and Research Centre, Kattankulathur, Chennai, Tamil Nadu, India. Patients presenting with rheumatic symptoms and referred for ANA testing using the Indirect Immunofluorescence (IIF) method were included in the study. The study population comprised both male and female participants, with age groups ranging from paediatrics to geriatrics. A total of 526 blood samples (3-5 mL) were collected from patients with rheumatic complaints and processed to obtain serum. IIF was performed using the Mosaic HEp-20-10/Liver (Monkey) BIOCHIP kit to detect ANA patterns. Positive samples (n=67) were further evaluated using EUROLINE ANA Profile 3 Plus DFS70 IgG immunoblot for specific autoantibodies, and the results were correlated with the clinical diagnosis of autoimmune disorders. The collected data were analysed using IBM Statistical Package for Social Sciences (SPSS) Statistics software. Descriptive statistics and inferential tests such as the Chi-square test was applied. A p-value of <0.05 was considered statistically significant.

Results: A total of 526 patients were investigated for ANA testing. A total of 67 samples positive for IIF-ANA were further tested using the immunoblot method. Out of the 67 samples processed/tested for presence of the human antibodies specific to 16 nuclear, mitochondrial and cytoplasmic antigens, 55 (82%) samples showed positivity. Out of the 67 samples, 49 (73.13%) were female and a significant number of the patients were in the age groups 31-40 (16, 23.8%) and 41-50 years (16, 23.8%). Speckled pattern was most frequently observed 21 (31.3%). The majorly identified autoantibodies were against U1RNP 18 (26.8%) and nucleosomes 18 (26.8%).

Conclusion: A higher- ANA pattern positivity could be correlated with a high prevalence of autoimmunity in this geographical location, promoting its use as a means for detecting suspected autoimmune illnesses.

Keywords: Autoimmunity, Immunoblot, Indirect immunofluorescence assay, Speckled pattern

INTRODUCTION

An autoimmune disease occurs as a result of structural or functional damage caused due to the action of the immune system against its self-antigens [1]. Immunological tolerance offers a mechanism wherein, the immune system becomes incapable of attacking or acting against its own self-antigens. Any compromise in these mechanisms leads to autoimmune diseases. Different autoimmune diseases involve localised or systemic manifestations.

The exact reason causing autoimmune diseases still remains unknown but a lot of theories point towards an immune system that becomes excessively active and attacks the body following an injury or infection. Certain risk factors like age [2,3], gender [4,5], genetics, hormones, environmental factors, mutations etc., could be possibly increasing the chance of an individual to develop an autoimmune disease. As the knowledge of autoimmune disorders expands, research on prevention is becoming more important. Environmental factors appear to play a large impact in the development of autoimmune illnesses, even though the amount of hereditary influence is often seen [6].

The ANA testing is done by using IIF assay [7]. It is considered as the Gold standard for ANA testing. The fluorescence is characterised by certain patterns. According to ICAP, International Consensus on ANA patterns, there are 24 nuclear, cytoplasmic and mitotic patterns [8]. IIF patterns are correlated with particular ANA subtypes, and ANA testing benefits greatly from pattern recognition. A homogenous fluorescence pattern usually indicates that the antibodies are focused on nucleosomes, histones, or dsDNA. Antibodies to membrane proteins may be seen as a membranous pattern. Speckled fluorescence patterns are correlated with antibodies to other nuclear antigens. Anti Smith antibodies exhibit a speckled pattern of fluorescence. A finely speckled pattern is formed by anti-SSA/Ro and anti-SSB/La. Discrete speckles in cells passing through interphase are antibodies directed against the centromeres. Antibodies against Deoxyribonucleic Acid (DNA) topoisomerase are linked to nucleolar speckles (Scl-70). The cytoplasmic speckled pattern indicates the presence of aminoacyl-tRNA synthetase (Jo-1) antibodies [9].

Although IIF-ANA continues to be the most common first testing method, additional techniques such as immunoassays and Enzyme-Linked Immunosorbent Assays (ELISA) provide confirmatory testing for particular ANAs. In many autoimmune disorders, the presence of autoantibodies against cell nuclei (ANA) is a crucial diagnostic

marker. The different cell nuclear components (biochemical compounds found in the cell nucleus) are the target of antibodies against nuclear antigens. These include ribonucleoproteins, nucleic acids, and proteins found in cell nuclei. They are a common discovery in a variety of illnesses, especially rheumatic types. Diagnosis of specific rheumatic diseases and their distinction from other autoimmune diseases depend on differential antibody diagnostics against nuclear antigens. Hence, it is crucial to detect these antibodies in the sera [9]. Line immunoassays/immunoblot methods are widely used for further testing the specificity of the antigens. The immunoblot technique provides a strip coated with different antigens that are then incubated with the patient's sera to get a result which is interpreted to check whether the sera show positivity to a particular antibody.

The current investigation uses a test kit which offers a qualitative in-vitro method for detecting human autoantibodies that belong to the IgG class against sixteen distinct antigens in blood or plasma, which includes histones, SS-B, PM-Scl, Ro-52, nRNP, Nucleosomes, SS-A, DFS 70, AMA-M2, PCNA, Sm, dsDNA, Ribosomal P-protein, CENP B, Scl-70 and Jo-1 [10].

Autoimmune diseases often present with vague symptoms, making early diagnosis challenging [11]. ANAs are key markers for these conditions, but data on their patterns and prevalence in South India is limited. This study aimed to fill that gap by analysing ANA patterns and autoantibody profiles in patients at a Tertiary Care Centre, aiding in better diagnosis and management.

The primary objective of the study includes identifying ANA patterns using IIF Assay. The secondary objective of the study included determining the antibodies to extractable nuclear antigens through line immunoassay and immunoblot methods, specifically utilising the EUROLINE ANA profile 3 plus DFS70 (IgG).

MATERIALS AND METHODS

The present cross-sectional study was performed for a span of 12 months (February 2023 to January 2024) at Department of Microbiology, SRM Medical College Hospital and Research Centre, Kattankulathur, Tamil Nadu, India. Samples for study were collected from patients after obtaining written informed consent, in accordance with the guidelines approved by the Institutional Ethics Committee of SRM Medical College Hospital and Research Centre. Institutional Ethics Approval was obtained for this study (Ethics clearance number: SRMIEC-ST0123-286).

Sample size calculation: The sample size was calculated based on a study done by Gupta P et al., [12]. In the study conducted at All India Institute of Medical Sciences (AIIMS), Raipur, India, analysed a total of 644 patient reports. Out of these, 44% patients were sent for antibody reactivity testing and majority of them, 66% showed a positive reaction to one or more of the specific antibodies. These values were used in the current study for sample size calculation.

Depending on the statistician's calculations, the sample size was obtained which was n=67.

Sample size calculation:

The sample size was calculated using the formula:

$n=4pq$

d^2

$p=66$, $q=100-p=44$, $d=0.2 \times 66 =13.2$

$n=4\times66\times44 /(13.2)^2$

$n=11,616/174.24$

$n=66.6$

$n\sim67$

Inclusion criteria: Patients who were prescribed ANA testing by IIF by different specialty departments such as patients clinically diagnosed with Rheumatoid arthritis, Systemic Lupus Erythematosus (SLE), Sjogren's syndrome, Polymyositis, Dermatomyositis, Mixed Connective Tissue Disease (MCTD).

Exclusion criteria: Patients who were not prescribed ANA testing were excluded from the study.

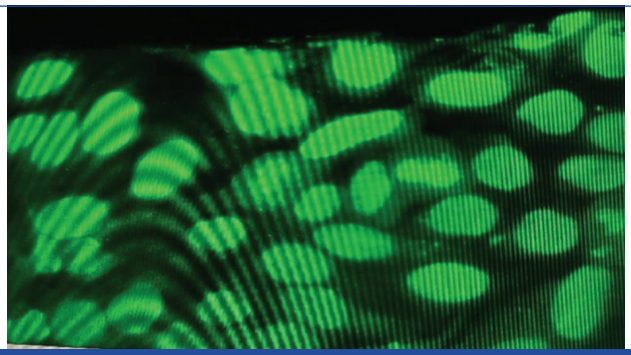
Study Procedure

The blood samples were received from various departments from patients of all ages with various rheumatic complaints. A 3-5 mL of blood was collected in a red capped vacutainer (without anticoagulants, serum tube). The blood samples were subjected to centrifugation at 3000 rpm for 15-20 minutes to obtain the serum. The serum samples were stored at a temperature of 40°C until tested.

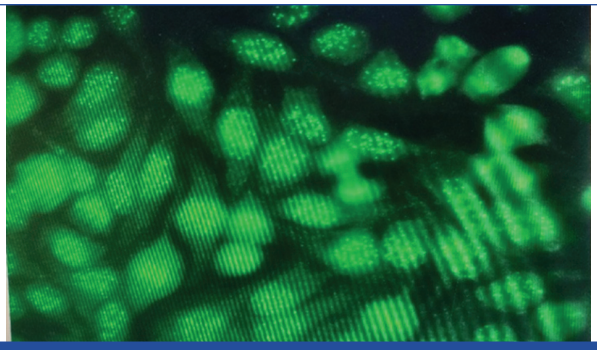
IIF test was performed using the kit Mosaic HEp-20-10/Liver (Monkey). The kit was provided by Everlife-CPC Diagnostics Pvt., Ltd., Gopalapuram, Chennai, 600086, Tamil Nadu, India. The test kit offered a qualitative or semi-quantitative in-vitro determination of human antibodies of the immunoglobulin class IgG against the cell nuclei in patients' serum samples to aid in the diagnosis of many autoimmune diseases, especially of the rheumatic form.

PBS-Tween solution was used to dilute the samples. The samples were diluted to 1:100 dilution. The reagent tray was taken. 30µL of the diluted sample (1:100) was taken using a micropipette and it was added to the reaction field without any bubbles. Following the same procedure, all the samples to be investigated were transferred to the reagent tray on to each of the reaction fields. The reaction fields on the reagent tray were designed in such a way that their geometry exactly fits the amount of the droplets that were placed on them, hence there was a chance for the commencement of individual reaction simultaneously. The BIOCHIP slides were coated with a substrate combination which constitutes Hep-20-10 & primate liver (monkey), for precise distinguishing and identification of the ANA. The BIOCHIP slide was then taken out and it was placed very carefully on the reagent tray, making sure that the individual samples remain separate and do not come in contact with each other. This was incubated for a period of 30 minutes at room temperature. After incubation, the slide was washed using the PBS-Tween solution. This was followed by incubation with the conjugate for 30 minutes, followed by washing. Then the slide was placed on to the cover glass with the BIOCHIPs facing downwards. A fluorescence microscope was used to evaluate the results [Table/Fig-1-6]. The microscope used was Nikon fluorescence microscope which uses blue filter- 450 nm and light source- mercury lamp.

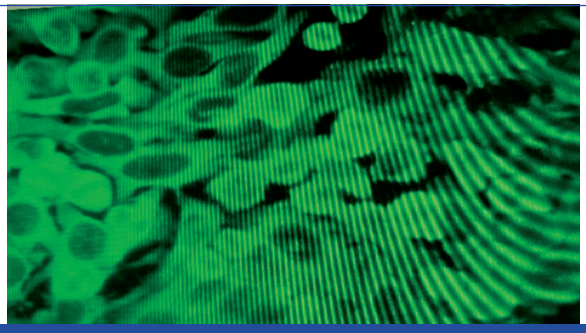
The samples tested positive for IIF, were then subjected to EUROLINE ANA profile three plus DFS 70 IgG. The kit was provided by Everlife-CPC Diagnostics Pvt., Ltd., Gopalapuram, Chennai, 600086, Tamil Nadu, India. This test offered a qualitative, in-vitro method for detecting autoantibodies (human) that belong to class IgG against sixteen distinct antigens in blood or plasma, which includes histones, SS-B, PM-Scl, Ro-52, nRNP, Nucleosomes, SS-A, DFS 70, AMA-M2, PCNA, Sm, dsDNA, Ribosomal P-protein, CENP B, Scl-70 and Jo-1.



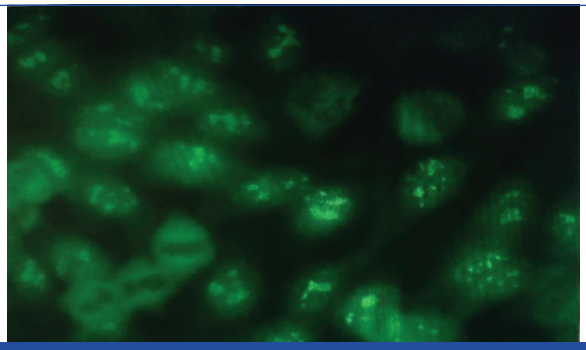
[Table/Fig-1]: Homogenous pattern.



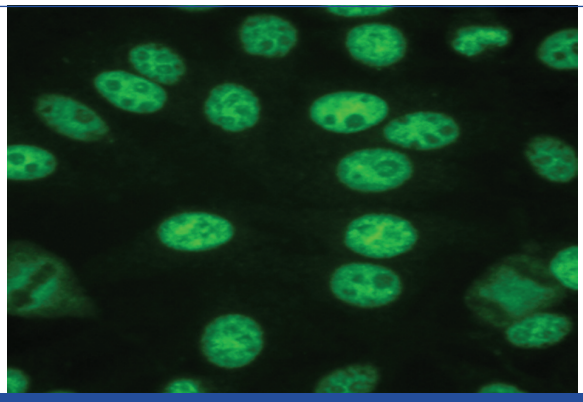
[Table/Fig-2]: Centromere pattern.



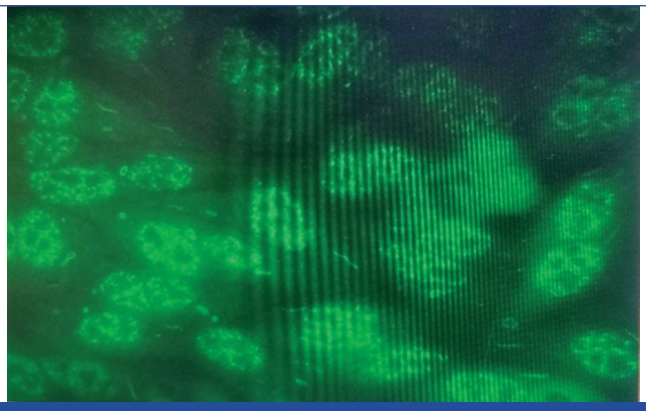
[Table/Fig-3]: Cytoplasmic fluorescence.



[Table/Fig-4]: Nucleolar pattern.



[Table/Fig-5]: Fine speckled pattern.



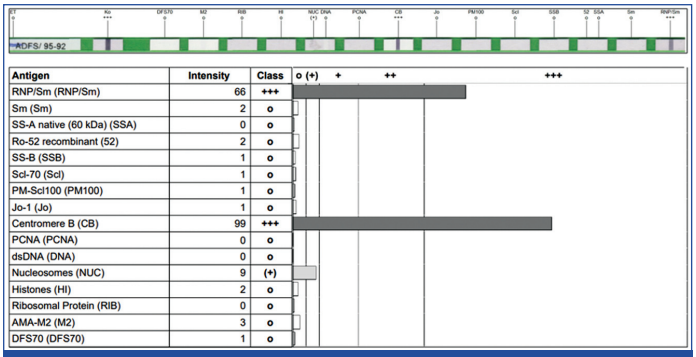
[Table/Fig-6]: Coarse speckled pattern.

The test kit and the components of the test kit were taken out of the refrigerator (2-80°C) and were allowed to reach room temperature (18-250°C). Once all the components of the kit were brought to room temperature, the desired number of strips for the test was taken out of the package. Each strip was placed in an empty channel of the incubation tray. Once the strips were taken out, the surface of the strips was checked properly for any damage. If the surface of the strip showed any kind of damage, it was not used for testing. The surface of the strip had chances to get affected by the condensation when the test kit was taken from refrigeration. Hence, utmost care was taken while handling the strips and the strips were taken out of the package only after all the contents were brought to room temperature (18-250°C). After taking out the required number of strips from the package, it was tightly closed and refrigerated (2-80°C) again to maintain the stability. The strips were inserted into the incubation tray's empty channel. The strip's number ought to be displayed. On a shaker (Gel rocker, at Department of Microbiology, SRM MCH &RC), the incubation tray was set. For incubation, 1.5 mL of sample buffer was added in to channel with strip. Then incubation was done for five minutes at room temperature on gel rocker. The incubation period was tracked starting from when the gel rocker was turned on, and it was turned off five minutes later.

The liquid in the incubation tray was aspirated off after the incubation time of five minutes. Then a micropipette (supplied by Thermo scientific Finnpipette F3 Variable Volume Pipettor, Vol-100-1000 µL) was taken with a clean pipette tip. A 1.5 mL of the diluted serum sample was filled in the channel of the incubation tray. Then incubation was done again at room temperature (18-250°C) for a period of 30 minutes on the gel rocker. This was followed by washing the strips. The liquid in the channel of the incubation tray was aspirated off. Then 1.5 mL of working strength wash buffer was added to the channel and the shaker was turned on for five minutes. After five minutes, shaking was stopped and the wash buffer in the channel was aspirated off. The wash buffer of volume 1.5 mL was again added to the channel, shaken for five minutes, the same procedure was repeated for the third time. This completed an intermediate washing step (3 x 5 minutes). Once the washing procedure was done, 1.5 mL of diluted enzyme conjugate was pipetted into channel. This was incubated on the rocking shaker for a period of 30 minutes. Following incubation, the liquid from the channel was aspirated and washing was performed following the washing procedure mentioned above. Subsequently, 1.5 mL of the substrate solution was pipetted into the incubation tray's channel. The liquid was withdrawn out of the channel after this was incubated on a rocking shaker for ten minutes. Washing procedure was repeated. The washing procedure of this step differs from the previous steps. 1.5 mL of distilled water was used for washing. Distilled water (1.5 mL) was pipetted in to the channel and shaker was turned on for one minute after which the shaker was stopped and the distilled water was aspirated off, the same procedure was repeated two more times. Once this procedure was done, the strip was taken out of the channel of the incubation tray and it was placed on the evaluation protocol, it was allowed to air dry for five to ten minutes and then it was evaluated. EUROLine Scan software (EUROLineScan YG0006-0101 Release version:3, Software version: 3.4, 3.4.37 build 2274, EUROIMMUN Medizinische Labordiagnostika AG Seekamp 31, 23560 Lubeck, Germany) was used to evaluate the strips wherein the intensity of each positive antibody was indicated by a band [Table/Fig-7].

STATISTICAL ANALYSIS

Statistical analysis was performed using IBM SPSS software and Chi-square test was utilised to find out the association between clinical diagnosis, Immunofluorescence and immunoblot.



Antigen	Intensity	Class
RNP/Sm (RNP/Sm)	66	+++
Sm (Sm)	2	o
SS-A native (60 kDa) (SSA)	0	o
Ro-52 recombinant (52)	2	o
SS-B (SSB)	1	o
Scl-70 (Scl)	1	o
PM-Scl100 (PM100)	1	o
Jo-1 (Jo)	1	o
Centromere B (CB)	99	+++
PCNA (PCNA)	0	o
dsDNA (DNA)	0	o
Nucleosomes (NUC)	9	(+)
Histones (HI)	2	o
Ribosomal Protein (RIB)	0	o
AMA-M2 (M2)	3	o
DFS70 (DFS70)	1	o

[Table/Fig-7]: Showing positive results against the antigens RNP/Sm and CENP B. RNP: Ribonucleoprotein; Sm: Smith; Scl: Scleroderma; CENP B: Centromere protein B; PCNA: Proliferating Cell Nuclear Antigen; dsDNA: double stranded Deoxyribonucleic acid; AMA M2: Anti mitochondrial M2 antibody; DFS 70: Dense fine speckled 70

RESULTS

The samples were received from different departments from patients with rheumatic complaints. The samples were then tested for the presence of ANA using IIF. All the samples that showed positive results in IIF assay were then subjected to ANA profiling using EUROLINE ANA Profile Three Plus DFS70 (IgG) which contains a panel of 16 antibodies. The 67 samples that tested positive for IIF-ANA were further analysed using the immunoblot method [Table/Fig-8].

[Table/Fig-8]: Representing the immunoblot results of the EUROIMMUN ANA Profile 3 Plus DFS70 IgG test. The blot in [Table/Fig-8] includes five patient samples (numbered 1 to 5), each tested against a panel of autoantigens including DFS70, M2, Ribosomal P, Histones, Nucleosomes, dsDNA, PCNA, CB, Jo-1, PM100, Scl-70, SSA, Ro-52, SSB, Sm, and RNP/Sm. Autoantibody reactivity is indicated using symbols denoting intensity (e.g., +, ++, +++) or "o" for negative reactions.

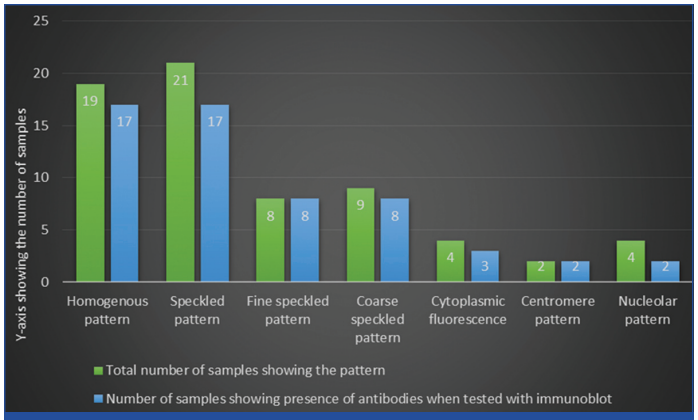
Among the 67 samples tested for EUROIMMUN ANA Profile 3 Plus DFS70 IgG, 55 (82%) samples were positive for antibodies specific to extractable nuclear antigens. Of the 67 samples, 49 (73.13%) were from female patients, with the majority belonging to the age groups 31-40 years 16 (23.8%) and 41-50 years 16 (23.8%) The most common pattern observed was speckled 21 (31.3%) [Table/Fig-9].

The predominant autoantibodies identified were against U1RNP 18 (26.8%) and nucleosomes 18 (26.8%) [Table/Fig-10].

Pearson's Chi-square test was used for the statistical analysis, wherein the correlation of clinical diagnosis with IIF pattern was made and the p-value came out to be insignificant (p=0.144). A value below 0.05 is considered significant. Another correlation of IIF pattern with the antibody detected with the immunoblot was done, which showed p-value, p=0.006, which falls under the significant category. ANA detected was co-related with the clinical diagnosis to obtain a significant value of p<0.01 which accounts for 1% level of significance [Table/Fig-11-14].

DISCUSSION

Immunological serology is a vital diagnostic method for disorders of the connective tissue. The 67 samples positive for IIF were subjected to testing by immunoblot. In present investigation, the most abundantly seen antibodies were RNP/Sm (U1RNP/Sm) and antibodies against Nucleosomes, which were 18 (26.8%) each, followed by antibodies against Sm, dsDNA and Ro-52 (anti-Sm,



[Table/Fig-9]: Showing the details of immunoassay of the samples with different immunofluorescence patterns.

Antibody	Number of patients showing positivity for the antibody	Percentage
RNP/Sm (U1RNP/Sm)	18	26.8%
Sm	13	19.4%
SS-A	8	11.9%
Ro-52	13	19.4%
SS-B	3	4.4%
Scl-70	7	10.4%
PM-Scl 100	1	1.4%
Jo-1	1	1.4%
Centromere B	5	7.4%
PCNA	0	0
dsDNA	13	19.4%
Nucleosomes	18	26.8%
Histones	6	8.9%
Ribosomal P. Protein	4	5.9%
AMA M2	2	2.9%
DFS 70	2	2.9%

[Table/Fig-10]: Showing the number of patients positive for specific ANA and percentage using the Line immunoassay/immunoblot EUROLINE ANA Profile 3 Plus DFS70 (IgG).
U1 RNP: U1 Ribonucleoprotein; Sm: Smith; Scl: Scleroderma; CENP B: Centromere protein B; PCNA: Proliferating Cell Nuclear Antigen; dsDNA: double stranded Deoxyribonucleic acid; AMA M2: Anti mitochondrial M2 antibody; DFS 70: Dense fine speckled 70

Chi-square tests	Value	df	p-value
Pearson Chi-square	715.951[a]	624	0.006
Likelihood ratio	242.980	624	1.000
No. of valid cases	67		

[Table/Fig-11]: Chi-square test demonstrating a statistically significant association between patient age and hospital ward distribution.

Chi-square tests	Value	df	p-value
Pearson Chi-square	41.340[a]	16	<0.001
Likelihood ratio	48.722	16	0.000
No. of valid cases	67		

[Table/Fig-12]: Chi-square test demonstrating the significant association between patient gender and hospital ward distribution.

Chi-square tests	Value	df	p-value
Pearson Chi-square	128.813[a]	91	0.006
Likelihood ratio	106.277	91	0.131
No. of valid cases	67		

[Table/Fig-13]: Chi-square analysis demonstrating the significant association between IIF patterns and specific extractable nuclear antigen autoantibodies detected by EUROLINE ANA Profile 3 Plus DFS70 (IgG).

Chi-square tests	Value	df	p-value
Pearson Chi-square	105.400a	91	0.144
Likelihood ratio	88.174	91	0.564
No. of valid cases	67		

[Table/Fig-14]: Chi-square test demonstrating the association between Anti-nuclear Antibody (ANA) immunofluorescence patterns and clinical diagnosis.

anti-dsDNA, anti-Ro-52), which were 13 (19.4%) each. Another similar study conducted in Central India by Gupta P et al., also showed similar results wherein the most commonly found antibody was U1 RNP/Sm (17.49%) followed by SS-A antibody [12].

In a study conducted by Peene I et al., SS-A and SS-B antibodies were most abundantly detected, differing from the current study [11]. Another study conducted by Guo Y-P et al., in China also included line immunoassay and 44.2% were positive for the autoantibodies. The antibodies that had the highest frequency were anti-Ro 52 which was 19%, anti-M2 was 17.8%, and anti-SSA was 14.3% which has a decent deviation from the current study [13].

There is growing consensus that unknown gene-environment interactions constitute the root cause of autoimmune diseases and autoimmunity, despite the fact the precise mechanisms behind their development remain unclear [14]. Numerous genetic risk factors for autoimmune disorders have been identified owing to technological improvements made possible by the human genome project and its related investigations. Nevertheless, nothing is understood about the even greater impact of environmental variables [15].

In the current investigation, a total number of 526 samples were tested by IIF (Immunofluorescence Assay) for a span of one year (February 2023 to January 2023). All the samples that were taken for IIF were blood samples, which were centrifuged to obtain the serum and the serum thus obtained was used for testing. A total of 67 samples positive for IIF were taken and tested by immunoblot (EUROLINE ANA Profile 3 Plus DFS 70 IgG). The results obtained from the IIF and immunoblot technique were assessed based on different criteria. It was observed that the patients belonging to the age group 31-50 years, showed the highest positivity (47.6%) for immunofluorescence. There was a very clear gender bias that can be seen in the study wherein 49 out of 67 patients were female which accounts for 73% and 18 were male (27%). A study conducted in the general population of China by Guo Y-P et al., from July 2011 to September 2013, also showed the gender bias in autoimmune disorders wherein the prevalence of ANA was higher in samples from female patients than in males, similar to the current study [13]. The study by Guo Y-P et al., included 20,970 samples, among which 10,550 were male and 10,420 were female. Of the 20,970 sera tested using IIF, 1,243 samples were positive for ANA-IIF and out of these positive samples, 903 were female and 340 were male [13]. In the current study, this gender bias can also be supported by segregating the patients on the basis of the ward/location. This ward/location-wise distribution reported 25 (37.3%) patients out of 67, from the female medical ward.

After performing IIF assay using EUROIMMUN Mosaic HEp-20-10/liver, it was observed that speckled pattern was seen most often (21 (31.3%) out of 67) followed by homogenous pattern {19 (28.3%) out of 67}. A similar prospective, retrospective study was conducted for a period of 27 months, by Wendy Sebastian et al, wherein 5066 samples were processed out of which 319 samples were tested using both immunofluorescence assay and line immunoassay method. Out of these 319 samples, 122 samples which accounts for 38.2% were positive for immunofluorescence. These 122 samples were then tested using the line immunoassay and of 122, 101 samples were positive which is 82.8%. The most abundantly seen IIF pattern was homogenous 46 (45.5%) followed by speckled pattern 36 (35.6%) [16]. But in the current study, at SRM hospital setting, speckled pattern 21 (31.3%) was most abundantly seen followed by Homogenous pattern 19 (28.3%).

In another study conducted in Bolu which is located in northwestern Turkey by Mengeloglu Z et al., the distribution of ANA patterns based on the symptoms and findings, the ANA positivity rate, and the patterns' correlation with specific diseases were assessed. In line with the current investigation, one of the most often reported patterns was speckled but along with the speckled pattern, another most abundantly reported pattern in the study was nucleolar pattern, which was seen in a rather lesser number in the current study [17].

In a routine laboratory study, 924 patients with suspected systemic rheumatic disease had their sera tested for ANAs using HEp-2 cell assays at Charité Universitätsmedizin, Berlin. This was done by Egerer K et al., both an automation with pattern recognition and a thorough visual investigation by an experienced personnel to analyse ANA data. Blinding of the samples was done for assessment reasons. A total of 546 sera (59.1%) were found to be positive, 140 sera (15.1%) to be mildly positive, and 238 sera (25.8%) to be negative for ANAs according to the automated system [18].

The present study highlights the importance of ANA profiling as a crucial diagnostic tool in the early identification and management of autoimmune rheumatic diseases. Timely detection of specific autoantibodies can guide clinicians toward accurate diagnosis, appropriate treatment strategies, and better patient outcomes. The integration of both IIF and immunoblot techniques enhances diagnostic precision by identifying both ANA patterns and antigen-specific profiles.

In the future, larger multicentric studies with longitudinal follow-up could provide deeper insights into the prognostic value of ANA profiles. Additionally, integrating ANA profiling with clinical scoring systems and emerging biomarkers could refine diagnostic algorithms and contribute to the development of personalised medicine approaches in autoimmune disease management.

Limitation(s)

The limitations of the study were the decreased number of ANA positive samples analysed in the study, the lack of heterogeneity in the age groups of the study population and the limitation pertaining to the study population representing a single geographical area. A follow up multicentric study with a larger sample size involving patients from various parts of the country is imperative for confirming the results of the present study.

CONCLUSION(S)

A greater fluorescence intensity in the initial screening is associated with a higher rate of confirmation of antibodies on immunoassay. The integration of ANA testing using IIF and immunoblot enhances the diagnostic precision by correlating specific immunofluorescence patterns with the detection of particular autoantibodies. This combined approach is essential for the accurate diagnosis and management of autoimmune disorders.

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