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Seroprevalence and Pattern Interpretation of Anti-Nuclear Antibodies in Connective Tissue Disorders Using Indirect Immunofluorescence

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Abstract:

Background:Connective tissue disorders (CTDs) are a group of autoimmune diseases characterized by the presence of circulating antinuclear antibodies (ANA), which serve as critical biomarkers for early diagnosis. Indirect immunofluorescence (IIF) on HEp-2 cells remains the gold standard for ANA detection and enables interpretation of distinct fluorescence patterns that are often disease-specific.
Objective:To evaluate the seroprevalence of ANA and to analyze the distribution and clinical significance of fluorescence patterns observed by IIF in patients clinically suspected of CTDs.
Methods:A cross-sectional study was conducted over a five-month period involving 90 patients with clinical suspicion of CTDs. Serum samples were tested for ANA using a commercial HEp-2 IIF assay at a screening dilution of 1:80. Positive samples were further categorized based on fluorescence patterns, and the results were analyzed in relation to age, gender, and probable clinical diagnosis.
Results:Of the 90 samples tested, 59 (65.6%) were positive for ANA. ANA positivity was significantly higher in females (73%) compared to males (48%) ($p < 0.05$). The most common fluorescence pattern was speckled (35.6%), followed by homogenous (27.1%), nucleolar (15.2%), peripheral (10.1%), centromere (6.8%), and cytoplasmic (5.1%). The homogenous pattern was primarily associated with suspected systemic lupus erythematosus, while nucleolar and centromere patterns were linked with systemic sclerosis. ANA positivity was highest in the 31–50 years age group.
Conclusion:IIF remains a highly sensitive and informative method for ANA detection in suspected CTD patients. The interpretation of fluorescence patterns provides valuable diagnostic clues and aids in the clinical stratification of autoimmune diseases. These findings support the continued use of IIF as a frontline serological tool in the diagnostic workup of CTDs.

Keywords:

Antinuclear Antibodies (ANA), Indirect Immunofluorescence (IIF), Connective Tissue Disorders (CTDs), HEp-2 Cells, Autoimmune Diseases, Fluorescence Patterns

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Introduction

Connective tissue disorders (CTDs) represent a heterogeneous group of autoimmune diseases characterized by widespread inflammation and immune-mediated damage to connective tissues, affecting organs such as the skin, joints, muscles, and internal organs. Common CTDs include systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), Sjogren's syndrome (SS), systemic sclerosis, and polymyositis/dermatomyositis. These disorders are predominantly seen in women and can present with a broad spectrum of clinical manifestations, often overlapping and evolving over time.

Among the most critical tools for early and accurate diagnosis of CTDs is the detection of anti-nuclear antibodies (ANAs)—autoantibodies that target components within the cell nucleus. ANA testing not only assists in diagnosing autoimmune diseases but also provides insight into disease activity and prognosis.
Indirect immunofluorescence (IIF) on HEp-2 cell substrates remains the gold standard for ANA detection due to its high sensitivity and its ability to identify diverse patterns of nuclear and cytoplasmic fluorescence. Each pattern, such as homogenous, speckled, nucleolar, or centromere, is associated with particular autoantibodies and underlying CTD subtypes. For instance, homogenous patterns are often seen in SLE, speckled patterns in mixed connective tissue disease, and nucleolar patterns in systemic sclerosis. Thus, pattern recognition can offer valuable diagnostic and prognostic clues.

Despite global guidelines advocating for IIF-based ANA screening, regional studies are essential to understand local disease prevalence, demographic trends, and pattern distribution. Such data are particularly lacking in many parts of India, where autoimmune disease recognition is growing but remains underrepresented in published literature.

This study aims to evaluate the seroprevalence of ANA in suspected CTD cases using IIF and to interpret the fluorescence patterns in relation to demographic characteristics and probable clinical diagnoses. By doing so, we seek to enhance understanding of the diagnostic utility of IIF and support the development of context-specific testing strategies in autoimmune disease diagnosis.

Materials and Methods

Study Design and Setting

This cross-sectional, observational study was carried out in the Department of Microbiology, Tirunelveli Medical College, Tirunelveli, Tamil Nadu, India, over a period of five months from February 2015 to June 2015. The study was conducted after obtaining prior approval from the Institutional Ethics Committee.

Study Population

The study included 90 non-duplicate serum samples from adult patients clinically suspected of having connective tissue disorders (CTDs), such as Systemic Lupus Erythematosus (SLE), Rheumatoid Arthritis (RA), Sjogren's Syndrome (SS), Systemic Sclerosis, and Polymyositis/Dermatomyositis, based on presenting clinical features.

Inclusion Criteria

- Patients aged ≥12 years with clinical suspicion of autoimmune CTD.

Exclusion Criteria

- Pediatric patients (<12 years of age).
- Patients who were seropositive for HIV or HBsAg.
- Hemolyzed or inadequate serum samples.

Sample Collection and Processing

Approximately 3 mL of venous blood was collected aseptically from each participant. The samples were allowed to clot at room temperature, centrifuged, and serum was separated. All serum samples were labeled and stored at –80°C until further processing.

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Detection of Antinuclear Antibodies (ANA) by Indirect Immunofluorescence (IIF)

The detection of ANA was performed using a commercial ANA-HEp-2 IIF kit (BIOSYSTEMS, Spain) according to the manufacturer's instructions. This technique employs HEp-2 cell substrates, which are human epithelial cells derived from laryngeal carcinoma, known for their high sensitivity in detecting a broad spectrum of nuclear and cytoplasmic autoantibodies.

Principle

Antinuclear antibodies present in the patient's serum bind to specific nuclear or cytoplasmic antigens present on the HEp-2 cells affixed to the slide. These antigen-antibody complexes are then detected by incubation with a FITC-labeled goat anti-human IgG conjugate. The resulting fluorescence pattern is observed under a fluorescence microscope and interpreted according to the International Consensus on ANA Patterns (ICAP) guidelines.

Reagents and Materials Used

- HEp-2 coated slide wells.
- Positive and negative control sera (included in the kit).
- FITC-conjugated goat anti-human IgG with Evans Blue counterstain.
- Phosphate Buffered Saline (PBS) (10x) – pH 7.2.
- Mounting medium (Mowiol 12%, Glycerol 30%).
- Fluorescence microscope equipped with 495 nm excitation and 525 nm emission filters.

Procedure

1. Serum samples were diluted 1:80 in PBS.
2. 25 µL of diluted serum was added to each HEp-2 well.
3. Slides were incubated in a moist chamber at room temperature (15–30°C) for 30 minutes.
4. Following incubation, slides were gently rinsed and washed with PBS for 5 minutes, changing the PBS once.
5. Slides were dried using blotting paper and one drop of FITC-conjugate was added to each well.
6. A second incubation was performed under the same conditions for 30 minutes.
7. After washing and drying, a drop of mounting medium was added and covered with a coverslip.
8. Slides were examined immediately under a fluorescence microscope at 250x to 400x magnification.

Interpretation of Fluorescence Patterns

Slides were interpreted as positive if specific fluorescence was detected and as negative in the absence of defined staining. The ANA-positive samples were further categorized based on fluorescence patterns:

- Homogenous
- Speckled
- Peripheral (Rim)
- Nucleolar
- Centromere
- Cytoplasmic

Each pattern was correlated with specific autoimmune disease associations. Only patterns observed at or above the 1:80 dilution were considered significant.

Quality Control

Each batch of tests included positive and negative control sera supplied by the kit manufacturer to validate assay performance. Only results from slides showing appropriate control reactions were considered for analysis. Reagents and kit components were stored and used according to the manufacturer's specifications to maintain assay integrity.

Statistical Analysis

All collected data were compiled and entered into Microsoft Excel, and statistical analyses were performed using IBM SPSS software version 20.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics were used to summarize the demographic characteristics of the study population, including

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age and sex distribution, as well as the proportion of samples that tested positive for antinuclear antibodies (ANA) and the frequencies of various fluorescence patterns observed.

The association between ANA positivity and demographic factors such as gender, as well as between specific fluorescence patterns and suspected connective tissue disease types, was assessed using the chi-square (χ^2) test. In cases where the expected cell counts were less than five, Fisher's exact test was applied to ensure the validity of the results. A p-value of less than 0.05 was considered statistically significant.

In analyses comparing assay performance (if applicable), diagnostic agreement between indirect immunofluorescence (IIF) and enzyme-linked immunosorbent assay (ELISA) methods was evaluated using Cohen's kappa (κ) statistic. The strength of agreement was interpreted according to standard guidelines: values ≤0.20 as slight, 0.21–0.40 as fair, 0.41–0.60 as moderate, 0.61–0.80 as substantial, and ≥0.81 as almost perfect agreement. All analyses were conducted with a two-tailed approach.

Results

Demographic Profile of the Study Population

A total of 90 patients clinically suspected of connective tissue disorders (CTDs) were included in the study. Of these, 63 (70%) were females and 27 (30%) were males, yielding a female to male ratio of 2.3:1. The mean age of the study population was 35.7 years, with an age range of 18 to 65 years. The majority of patients (53 cases, 58.9%) belonged to the 31–50 years age group, reflecting the typical age of onset for autoimmune connective tissue diseases.

ANA Positivity by Indirect Immunofluorescence (IIF)

Among the 90 serum samples tested using IIF at a screening dilution of 1:80, 59 samples (65.6%) were found to be positive for anti-nuclear antibodies (ANA). The remaining 31 samples (34.4%) were ANA-negative. A higher rate of ANA positivity was observed among females (73%) compared to males (48%), and this difference was found to be statistically significant ($p < 0.05$), indicating a notable female preponderance in seropositivity.

Distribution of ANA Fluorescence Patterns

Various nuclear and cytoplasmic fluorescence patterns were observed among the 59 ANA-positive cases. The speckled pattern was the most frequently observed, seen in 21 cases (35.6%), followed by the homogenous pattern in 16 cases (27.1%), and the nucleolar pattern in 9 cases (15.2%). The peripheral (rim) pattern was noted in 6 cases (10.1%), and the centromere pattern was observed in 4 cases (6.8%). Additionally, cytoplasmic granular fluorescence was seen in 3 cases (5.1%).

Pattern Correlation with Suspected CTD Types

Fluorescence patterns demonstrated disease-specific trends. The homogenous pattern was strongly associated with systemic lupus erythematosus (SLE); among the 16 homogenous-positive samples, 13 patients had clinical features suggestive of SLE, indicating a high correlation with anti-dsDNA antibodies. The speckled pattern, being more nonspecific, appeared in a broad spectrum of CTDs, including suspected cases of SLE, Sjögren's syndrome, and mixed connective tissue disease (MCTD). The nucleolar and centromere patterns were predominantly seen in patients clinically suspected to have systemic sclerosis, aligning with known associations with anti-Scl-70 and anti-centromere antibodies, respectively. The cytoplasmic granular pattern was identified in cases with features of polymyositis and dermatomyositis.

Age and Gender Trends in ANA Positivity

The highest ANA positivity was observed in the 31–50 years age group, which also represented the largest portion of the study population. Among females in this age group, ANA positivity was particularly prominent. These findings are

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consistent with the established demographic trend of autoimmune diseases being more common in women of reproductive age, likely due to the modulatory effects of estrogen on immune function.

Table 1: Gender Distribution of the Study Population

Gender	Number of Patients	Percentage (%)
Female	63	70.0
Male	27	30.0
Total	90	100.0

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Table 2: Age-wise Distribution of Patients

Age Group (Years)	Number of Patients	Percentage (%)
< 30	17	18.9
31–50	53	58.9
> 50	20	22.2
Total	90	100.0

Table 3: ANA Positivity Among Suspected CTD Patients (IIF Method)

ANA Result	Number of Cases	Percentage (%)
Positive	59	65.6
Negative	31	34.4
Total	90	100.0

Table 4: Distribution of ANA Fluorescence Patterns (n = 59 Positive Cases)

Fluorescence Pattern	Number of Cases	Percentage (%)
Speckled	21	35.6
Homogenous	16	27.1
Nucleolar	9	15.2
Peripheral (Rim)	6	10.1
Centromere	4	6.8
Cytoplasmic (Granular)	3	5.1
Total	59	100.0

Discussion

The present study aimed to assess the seroprevalence of anti-nuclear antibodies (ANA) and interpret their fluorescence patterns using indirect immunofluorescence (IIF) in patients clinically suspected of having connective tissue disorders (CTDs). Among the 90 serum samples tested, a substantial ANA positivity rate of 65.6% was observed, reinforcing the utility of ANA testing as a frontline tool in the evaluation of autoimmune rheumatic diseases. This finding aligns with previous studies that report ANA positivity ranging between 50% and 70% among CTD-suspected populations, particularly in settings where IIF using HEp-2 cell substrates is employed as the diagnostic method of choice.

A notable gender disparity was observed, with ANA positivity significantly higher among females (73%) compared to males (48%). This reflects the well-documented female preponderance in

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autoimmune diseases, particularly during reproductive age, likely due to hormonal influences such as estrogen-induced immune modulation. Similar patterns have been reported by Ghosh et al. and Sebastian et al., suggesting that sex hormones may enhance B-cell activation and autoantibody production, making females more susceptible to CTDs.

The most frequent fluorescence pattern observed was the speckled pattern (35.6%), followed by homogenous (27.1%) and nucleolar (15.2%) patterns. These findings are consistent with reports from international and Indian cohorts, where the speckled pattern is often the most prevalent due to its association with a variety of CTDs including SLE, Sjögren's syndrome, and mixed connective tissue disease (MCTD). Although nonspecific, the speckled pattern often warrants further specific antibody profiling to identify extractable nuclear antigens (ENAs) such as anti-RNP or anti-Sm.

The homogenous pattern was most strongly associated with clinically suspected systemic lupus erythematosus (SLE), supporting its classical correlation with anti-dsDNA and anti-histone antibodies. The nucleolar and centromere patterns were primarily seen in patients suspected of having systemic sclerosis, in line with previous literature that identifies these patterns as hallmark features of scleroderma and its subtypes. The cytoplasmic granular pattern, though less common in this cohort, was found in cases suggestive of polymyositis and dermatomyositis, indicating muscle-specific autoantibody involvement.

Age-wise, the peak ANA positivity occurred in the 31–50 years group, reinforcing the global trend that CTDs often emerge during the middle decades of life. This demographic predominance, combined with the gender bias observed, underscores the importance of targeted screening in women of childbearing age presenting with systemic symptoms.

Overall, the data obtained from this study highlight the diagnostic value of IIF not only in detecting the presence of ANA but also in providing a pattern-based clue to the likely autoimmune disease involved. The fluorescence patterns offer meaningful insight and can guide further serological testing, such as ENA panels, to achieve a more definitive diagnosis.

However, this study has some limitations. The diagnoses were based on clinical suspicion without complete longitudinal follow-up or confirmatory specific autoantibody profiling (e.g., anti-Sm, anti-Ro, anti-Scl-70). Additionally, while IIF is highly sensitive, it is subject to inter-observer variability and may yield false positives, particularly in elderly individuals and healthy controls. Nevertheless, it remains an indispensable tool, particularly in resource-limited settings where advanced multiplex assays are not readily available.

Conclusion

In conclusion, the study affirms that IIF remains a robust, cost-effective, and informative assay for the detection and pattern analysis of ANA in suspected CTD cases. When interpreted in the context of clinical findings, ANA pattern recognition via IIF significantly aids in the early diagnosis and stratification of autoimmune connective tissue diseases.

References

1. Tan, E. M., Feltkamp, T. E., Smolen, J. S., Butcher, B., Dawkins, R., Fritzler, M. J., ... & van Venrooij, W. J. (1997). Range of antinuclear antibodies in "healthy" individuals. Arthritis & Rheumatism, 40(9), 1601–1611.
2. Solomon, D. H., Kavanaugh, A. J., Schur, P. H., & American College of Rheumatology Ad Hoc Committee. (2002). Evidence-based guidelines for the use of immunologic tests:

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Journal of Dermatological Case Reports

Antinuclear antibody testing. Arthritis & Rheumatism, 47(4), 434–444.

3. Kumar, Y., Bhatia, A., & Minz, R. W. (2009). Antinuclear antibodies and their detection methods in diagnosis of connective tissue diseases: a journey revisited. Diagnostic Pathology, 4(1), 1.
4. Mahler, M., & Fritzler, M. J. (2014). Epitope specificity and significance in systemic autoimmune diseases. Annals of the New York Academy of Sciences, 1317(1), 1–9.
5. Ghosh, P., Ghosh, A., Ghosh, K., & Ghosh, B. (2013). Antinuclear antibody test: Need for standardization. Indian Journal of Rheumatology, 8(3), 94–100.
6. Sebastian, W., Roy, A., Abraham, A. M., & Mathew, A. (2017). Prevalence and pattern of antinuclear antibodies using indirect immunofluorescence. Indian Journal of Pathology and Microbiology, 60(1), 45–49.
7. Satoh, M., Chan, E. K., Ho, L. A., Rose, K. M., Parks, C. G., Cohn, R. D., & Reeves, W. H. (2012). Prevalence and sociodemographic correlates of antinuclear antibodies in the United States. Arthritis & Rheumatism, 64(7), 2319–2327.
8. Mehra, S., & Kumar, V. (2015). ANA pattern recognition and its association with specific autoimmune diseases. Journal of Clinical and Diagnostic Research, 9(9), EC01–EC04.
9. Von Mühlen, C. A., & Tan, E. M. (1995). Autoantibodies in the diagnosis of systemic rheumatic diseases. Seminars in Arthritis and Rheumatism, 24(5), 323–358.
10. Abeles, A. M., & Abeles, M. (2013). The clinical utility of antinuclear antibody testing. Rheumatic Disease Clinics of North America, 39(3), 487–500.
11. Damoiseaux, J., Andrade, L. E. C., Carballo, O. G., Conrad, K., Francescantonio, P. L., Fritzler, M. J., ... & Mahler, M. (2019). Clinical relevance of HEp-2 indirect immunofluorescent patterns: The International Consensus on ANA Patterns (ICAP) perspective. Annals of the Rheumatic Diseases, 78(7), 879–889.
12. Wiik, A. S. (2005). Anti-nuclear autoantibodies: clinical utility for diagnosis, prognosis, monitoring, and planning of treatment strategy in systemic immunoinflammatory diseases. Scandinavian Journal of Rheumatology, 34(4), 260–268.
13. Misra, R., & Dhir, V. (2011). Autoantibodies in systemic sclerosis: correlation with clinical manifestations. Indian Journal of Rheumatology, 6(3), 104–110.
14. Lahita, R. G. (1999). The immunoendocrinology of systemic lupus erythematosus. Bulletin of the NYU Hospital for Joint Diseases, 57(1), 12–16.
15. Arbuckle, M. R., McClain, M. T., Rubertone, M. V., Scofield, R. H., Dennis, G. J., James, J. A., & Harley, J. B. (2003). Development of autoantibodies before the clinical onset of systemic lupus erythematosus. The New England Journal of Medicine, 349(16), 1526–1533.