International Journal of Medical and Pharmaceutical Research

Online ISSN-2958-3683 | Print ISSN-2958-3675
Frequency: Bi-Monthly
Available online on: https://ijmpr.in/

Original Article

Antinuclear Antibody Detection by Indirect Immunofluorescence Assay: A Hospital-Based Cross-Sectional Study

Dr Pallavi Magar[1], Dr Shilpi Sahu[2], Dr Arpita Singh[3]

[1]Junior Resident, Department of Pathology, MGM Medical College and Hospital,Kamothe, Navi Mumbai, Maharashtra, India – 410209
[2]Professor & HOD, Department of Pathology, MGM Medical College and Hospital,Kamothe, Navi Mumbai, Maharashtra, India – 410209
[3]Assistant Professor, Department of Pathology, MGM Medical College and Hospital,Kamothe, Navi Mumbai, Maharashtra, India – 410209

OPEN ACCESS

Corresponding Author:

Dr Shilpi Sahu
Professor & HOD, Department of Pathology, MGM Medical College and Hospital,Kamothe, Navi Mumbai, Maharashtra, India – 410209

Received: 25-06-2026
Accepted: 05-07-2026
Available online: 20-07-2026

ABSTRACT

Background: Antinuclear antibodies (ANAs) are important serological markers used in the diagnosis of autoimmune connective tissue disorders. The indirect immunofluorescence assay (IIFA) on HEp-2 cells is considered the gold standard for ANA detection because of its high sensitivity and its ability to identify characteristic immunofluorescence patterns that aid in clinical diagnosis.
Objectives: To determine the prevalence of ANA positivity, analyze the demographic characteristics and immunofluorescence pattern distribution, and correlate ANA results with clinical presentations in patients suspected of autoimmune disorders.
Materials and Methods: A hospital-based cross-sectional observational study was conducted at MGM Medical College and Hospital, Kamothe, Navi Mumbai, from October 2022 to November 2025. A total of 860 serum samples from patients clinically suspected of autoimmune diseases were included. ANA testing was performed using the ImmunoConcepts HEp-2000 Indirect Immunofluorescence Assay according to the manufacturer's protocol. Fluorescence patterns and intensity were evaluated using a Nikon ECLIPSE Ni-U fluorescence microscope. Demographic details, referring departments, clinical presentations, and ANA staining patterns were analyzed.
Results: Of the 860 patients, 228 (26.5%) were ANA positive, while 632 (73.5%) were ANA negative. Females constituted 76.8% (175/228) of ANA-positive patients, with a female-to-male ratio of approximately 3.3:1. The highest positivity was observed among individuals aged 15–49 years. The speckled pattern was the most common immunofluorescence pattern, accounting for 58.3% (133/228) of positive cases, followed by nucleolar (18.9%), homogeneous (14.9%), and cytoplasmic (8.8%) patterns. Fever with rash and joint pain were the most common clinical presentations, and the majority of referrals originated from the Department of Medicine.
Conclusion: ANA positivity was observed in approximately one-fourth of clinically suspected autoimmune disease patients, with a marked female predominance and highest prevalence in the reproductive age group. The speckled pattern was the predominant immunofluorescence pattern. HEp-2 cell-based IIFA remains the gold standard for ANA screening, and interpretation of ANA patterns in conjunction with clinical findings is essential for the early diagnosis and effective management of autoimmune disorders.

Keywords: *Antinuclear antibody, ANA, Indirect immunofluorescence assay, HEp-2 cells, Autoimmune diseases, Speckled pattern, Connective tissue disorders.*

INTRODUCTION

Antinuclear antibodies (ANAs) are a heterogeneous group of autoantibodies directed against various nuclear and cytoplasmic components of the cell. They are considered important serological markers in the diagnosis and classification of autoimmune connective tissue diseases (CTDs), including systemic lupus erythematosus (SLE), systemic sclerosis, Sjögren's syndrome, mixed connective tissue disease, polymyositis, and dermatomyositis. Although ANA positivity is not disease-specific, its detection, when interpreted in conjunction with clinical findings, plays a pivotal role in the early diagnosis and management of autoimmune disorders (1,2).

The indirect immunofluorescence assay (IIFA) performed on human epithelial type-2 (HEp-2) cells is regarded as the gold standard method for ANA detection because of its excellent sensitivity and ability to identify a wide range of autoantibodies. In addition to determining ANA positivity, IIFA provides valuable information regarding fluorescence patterns, which may indicate the presence of disease-specific autoantibodies and help guide further immunological investigations (3,4).

Several characteristic immunofluorescence patterns have been described, including homogeneous, speckled, nucleolar, centromere, cytoplasmic, and mixed patterns. Among these, the speckled pattern is the most frequently encountered and is associated with antibodies directed against extractable nuclear antigens. Homogeneous staining is commonly associated with anti-double-stranded DNA and histone antibodies seen in SLE, while nucleolar and centromere patterns are frequently associated with systemic sclerosis and limited cutaneous systemic sclerosis, respectively (5,6).

The prevalence of ANA positivity varies considerably depending on the study population, age, sex, ethnicity, and underlying clinical conditions. Females, particularly those of reproductive age, demonstrate a significantly higher prevalence of autoimmune diseases due to hormonal, genetic, and immunological influences. Consequently, ANA testing is most frequently requested in women presenting with symptoms suggestive of connective tissue disorders (7,8).

Although ANA testing is widely available, interpretation of results requires careful correlation with the patient's clinical presentation because low-titre ANA positivity may also occur in healthy individuals, elderly populations, chronic infections, malignancies, and certain drug-induced conditions. Therefore, understanding demographic characteristics and ANA staining patterns can improve diagnostic accuracy and reduce unnecessary investigations (9,10).

Limited data are available regarding ANA positivity and immunofluorescence pattern distribution in tertiary care centres in western India. Therefore, the present study was undertaken to determine the prevalence of ANA positivity among clinically suspected autoimmune disease patients, analyse demographic characteristics, evaluate immunofluorescence staining patterns, and correlate ANA findings with clinical presentations in a tertiary care hospital.

MATERIALS AND METHODS

Study Design and Setting

A hospital-based cross-sectional observational study was conducted in the Department of Pathology, MGM Medical College and Hospital, Kamothe, Navi Mumbai, over a period of three years from October 2022 to November 2025.

Study Population

The study included 860 consecutive serum samples received from patients clinically suspected of autoimmune disorders and referred for antinuclear antibody (ANA) testing from various clinical departments of the hospital. Patients of all age groups and both sexes were included.

Inclusion Criteria

- Patients with clinical suspicion of autoimmune diseases referred for ANA testing.
- Serum samples received during the study period with complete demographic and clinical information.

Exclusion Criteria

- Hemolysed, lipemic, or insufficient serum samples.
- Duplicate samples from the same patient.
- Samples lacking relevant clinical or demographic details.

Data Collection

Demographic variables including age and sex, referring department, clinical presentation, and provisional diagnosis were recorded from laboratory requisition forms and hospital records. Clinical indications were reviewed to assess their correlation with ANA test results.

Sample Collection and Processing
Approximately 3–5 mL of venous blood was collected under aseptic precautions. Blood samples were allowed to clot, and serum was separated by centrifugation. Serum samples were analyzed immediately or stored at 2–8°C until testing according to laboratory protocol.

ANA Detection by Indirect Immunofluorescence Assay (IIFA)
ANA testing was performed using the ImmunoConcepts ANA HEp-2000® Indirect Immunofluorescence Assay (Medsource Pvt. Ltd.), following the manufacturer's instructions. The assay employed HEp-2000 cell substrate slides containing seven reaction wells.

Briefly, diluted patient serum was incubated on HEp-2000 cell-coated slides. After washing, fluorescein isothiocyanate (FITC)-labelled anti-human IgG conjugate was added. Following a second washing step, slides were mounted and examined under a Nikon ECLIPSE Ni-U fluorescence microscope.

Interpretation of Results
Fluorescence intensity was graded semi-quantitatively as + to ++++. Positive samples were categorized according to the International Consensus on ANA Patterns (ICAP)-based immunofluorescence patterns, including:

- Speckled
- Homogeneous
- Nucleolar
- Centromere
- Cytoplasmic
- Mixed patterns

ANA positivity, fluorescence intensity, and staining patterns were documented for each patient.

Outcome Measures
The primary outcome was the prevalence of ANA positivity among clinically suspected autoimmune disease patients. Secondary outcomes included:

- Distribution of ANA patterns.
- Demographic characteristics of ANA-positive patients.
- Association of ANA positivity with clinical presentations and referring departments.

Statistical Analysis
Data were entered into Microsoft Excel and analyzed using SPSS version 25.0 (IBM Corp., Armonk, NY, USA). Continuous variables were expressed as mean ± standard deviation (SD), while categorical variables were presented as frequency and percentage. The association between categorical variables was evaluated using the Chi-square test or Fisher's exact test, wherever applicable. A p-value <0.05 was considered statistically significant.

Ethical Considerations
The study was conducted after obtaining approval from the Institutional Ethics Committee of MGM Medical College and Hospital, Kamothe, Navi Mumbai. Patient confidentiality was maintained throughout the study, and all data were anonymized prior to analysis. The study adhered to the ethical principles outlined in the Declaration of Helsinki.

RESULTS AND OBSERVATIONS
A total of 860 serum samples from clinically suspected autoimmune disease patients were tested for antinuclear antibodies (ANA) using the indirect immunofluorescence assay (IIFA). Of these, 228 patients (26.5%) were ANA positive, while 632 patients (73.5%) were ANA negative.

Table 1. Overall ANA Positivity among Study Participants

ANA Result	Number (n=860)	Percentage (%)
Positive	**228**	**26.5**
Negative	**632**	**73.5**
Total	**860**	**100.0**

Observation: Approximately one-fourth (26.5%) of clinically suspected autoimmune disorder patients were ANA positive.

Table 2. Gender Distribution among ANA-Positive Patients

Gender	Number (n=228)	Percentage (%)
Female	**175**	**76.8**
Male	**53**	**23.2**
Total	**228**	**100.0**

Observation: Females constituted more than three-fourths of ANA-positive patients, with a female-to-male ratio of approximately 3.3:1.

Table 3. Distribution of ANA Positivity According to Age Group

Age Group (Years)	Number	Percentage (%)
<15	15	6.6
15–49	132	57.9
50–64	51	22.4
≥65	30	13.1
Total	228	100.0

Observation: The highest ANA positivity was observed in the reproductive age group (15–49 years), accounting for nearly 58% of all positive cases.

Table 4. Distribution of ANA-Positive Cases According to Referring Department

Department	Number	Percentage (%)
Medicine	98	43.0
Dermatology	42	18.4
Rheumatology	35	15.4
Orthopaedics	18	7.9
Obstetrics & Gynaecology	15	6.6
Pediatrics	12	5.3
Others	8	3.5
Total	228	100.0

Observation: The Department of Medicine contributed the largest proportion of ANA-positive referrals.

Table 5. Distribution of ANA Immunofluorescence Patterns

ANA Pattern	Number (n=228)	Percentage (%)
Speckled	133	58.3
Nucleolar	43	18.9
Homogeneous	34	14.9
Cytoplasmic	20	8.8
Total	228	100.0

Observation: The speckled pattern was the predominant immunofluorescence pattern, followed by nucleolar and homogeneous patterns.

Table 6. Clinical Presentation among ANA-Positive Patients

Clinical Presentation	Number	Percentage (%)
Fever with rash	74	32.5
Joint pain	60	26.3
Fatigue	36	15.8
Generalized weakness	26	11.4
Lymphadenopathy	18	7.9
Hypertension	14	6.1
Total	228	100.0

Observation: Fever with rash was the most frequent presenting complaint, followed by joint pain and fatigue.

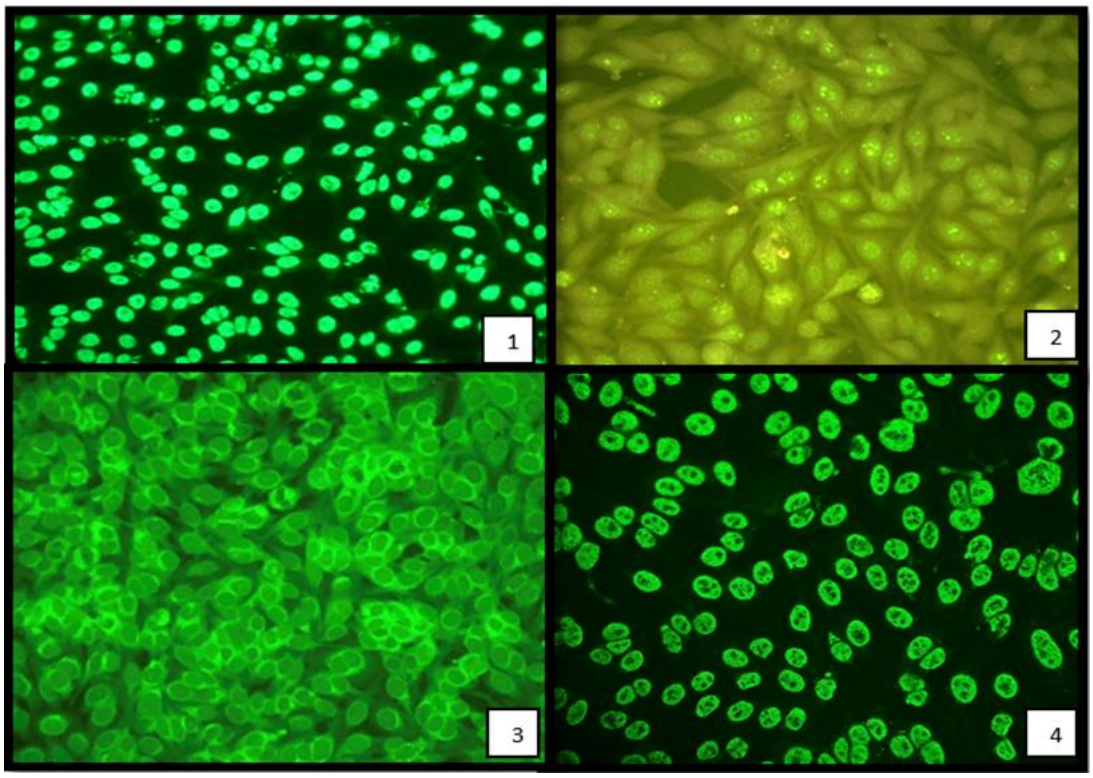

Figure 1 shows homogenous nuclear staining along with mitotic staining of +4 intensity, representing the homogenous ANA pattern. **Figure 2** depicts prominent fluorescent staining of nucleoli with 2+ intensity and absence of nuclear and mitotic staining, demonstrating the nucleolar ANA pattern. **Figure 3** shows intense cytoplasmic fluorescence staining of 3+ intensity with negative nuclear staining, representing the cytoplasmic ANA pattern. **Figure 4** demonstrates coarse speckled nuclear fluorescence of 3+ intensity with negative staining of nucleoli and mitotic cells, consistent with the speckled ANA pattern

DISCUSSION

The present cross-sectional study evaluated 860 patients clinically suspected of autoimmune disorders, among whom 228 patients (26.5%) were positive for ANA by indirect immunofluorescence assay. The observed positivity rate is comparable with several hospital-based studies from India that have reported ANA positivity ranging between 20% and 35% among clinically suspected autoimmune disease patients (1,11). The relatively high positivity observed reflects the selective referral of patients with strong clinical suspicion of autoimmune disorders.

A marked female predominance was observed in the present study, with 76.8% of ANA-positive patients being females, resulting in a female-to-male ratio of approximately 3.3:1. Similar observations have been reported by Gupta et al., who demonstrated significantly higher ANA positivity among females, particularly during the reproductive years (1). Autoimmune diseases are known to occur more frequently in women due to complex interactions among sex hormones, X-chromosome-linked immune regulatory genes, and environmental factors (7).

The highest frequency of ANA positivity was observed among individuals in the 15–49-year age group, corresponding to the reproductive age group. Similar age distribution has been described in previous Indian and international studies, where autoimmune connective tissue diseases predominantly affected young and middle-aged adults (8,12). Early identification of ANA positivity in this age group is clinically important because prompt diagnosis and treatment can prevent irreversible organ damage.

The Department of Medicine accounted for the highest number of ANA-positive referrals, reflecting the broad spectrum of systemic autoimmune diseases encountered in general medical practice. Patients presenting with prolonged fever, arthritis, skin manifestations, constitutional symptoms, and multisystem involvement are commonly evaluated initially by physicians before referral for immunological testing (11).

Among ANA-positive samples, the speckled pattern (58.3%) was the predominant immunofluorescence pattern, followed by nucleolar and homogeneous patterns. Similar findings have been reported by Gupta et al. and Rammohan et al., who identified the speckled pattern as the most frequent ANA staining pattern in patients with autoimmune disorders (1,11). The predominance of the speckled pattern may be attributed to antibodies against extractable nuclear antigens, which are

frequently encountered in systemic lupus erythematosus, Sjögren's syndrome, mixed connective tissue disease, and other autoimmune disorders.

The nucleolar pattern represented the second most common staining pattern in the present study and is commonly associated with systemic sclerosis and overlap syndromes (6). Homogeneous staining was also observed in a substantial proportion of patients and has been associated with antibodies against double-stranded DNA and histones, particularly in systemic lupus erythematosus (5).

The most common clinical presentation among ANA-positive patients was fever with rash, followed by joint pain, fatigue, generalized weakness, lymphadenopathy, and hypertension. These findings are consistent with the clinical manifestations commonly observed in autoimmune connective tissue diseases. Similar symptom profiles have been reported in previous studies evaluating ANA-positive patients in tertiary care hospitals (11,13).

The present study further reinforces the importance of HEp-2 cell-based indirect immunofluorescence assay as the preferred screening technique for ANA detection. Besides its high sensitivity, IIFA provides valuable information regarding fluorescence patterns that guide clinicians toward appropriate confirmatory antibody testing and facilitate accurate diagnosis (2,4). Although enzyme immunoassays are increasingly available, IIFA continues to be recommended by international expert consensus as the reference method for ANA screening (3).

The main limitation of the present study is that disease-specific autoantibody profiles, antibody titres, and long-term clinical follow-up were not evaluated. Future prospective studies incorporating ENA profiles, anti-dsDNA antibodies, complement levels, and clinical outcomes would provide a more comprehensive understanding of autoimmune disease patterns in the region.

Overall, the present findings demonstrate that ANA positivity is more common among females of reproductive age, with the speckled pattern being the predominant immunofluorescence pattern. Careful interpretation of ANA results in conjunction with clinical findings remains essential for the early diagnosis and appropriate management of autoimmune diseases.

CONCLUSION

The present study demonstrated an ANA positivity rate of 26.5% among patients clinically suspected of autoimmune disorders. A marked female predominance (76.8%) was observed, with the highest frequency occurring in the reproductive age group (15–49 years). The speckled pattern (58.3%) was the most common immunofluorescence pattern, followed by nucleolar and homogeneous patterns. Fever with rash and joint pain were the predominant clinical presentations among ANA-positive patients. These findings emphasize that indirect immunofluorescence assay (IIFA) on HEp-2 cells remains the gold standard screening method for ANA detection, providing valuable information through pattern recognition that aids in the diagnosis of autoimmune diseases. Correlation of ANA results with demographic characteristics and clinical features facilitates early diagnosis, appropriate clinical management, and timely immunological workup in patients with suspected autoimmune disorders.

REFERENCES

1. Gupta P, Agrawal P, Verma NR, Shah S, Patel S, Nanda R, Mohapatra E. Antinuclear antibody profiling in patients of a tertiary care centre in Central India. Indian J Clin Biochem. 2021;36(3):345–352.
2. Solomon DH, Kavanaugh AJ, Schur PH. Evidence-based guidelines for the use of antinuclear antibody testing. Arthritis Rheum. 2002;47(4):434–444.
3. Agmon-Levin N, Damoiseaux J, Kallenberg C, et al. International recommendations for the assessment of autoantibodies to cellular antigens referred to as anti-nuclear antibodies. Ann Rheum Dis. 2014;73(1):17–23.
4. Meroni PL, Schur PH. ANA screening: An old test with new recommendations. Ann Rheum Dis. 2010;69(8):1420–1422.
5. Chan EKL, Damoiseaux J, Carballo OG, et al. Report of the First International Consensus on Standardized Nomenclature of Antinuclear Antibody HEp-2 Cell Patterns (ICAP). Front Immunol. 2015;6:412.
6. Damoiseaux J, Andrade LEC, Fritzler MJ, et al. Clinical relevance of HEp-2 indirect immunofluorescent patterns. Ann Rheum Dis. 2019;78:879–889.
7. Fairweather D, Frisancho-Kiss S, Rose NR. Sex differences in autoimmune disease. Clin Rev Allergy Immunol. 2008;34(3):275–287.
8. Lahita RG. The role of sex hormones in systemic lupus erythematosus. Curr Opin Rheumatol. 1999;11(5):352–356.
9. Tan EM, Feltkamp TE, Smolen JS, et al. Range of antinuclear antibodies in healthy individuals. Arthritis Rheum. 1997;40(9):1601–1611.
10. Satoh M, Chan EKL, Sobel ES, et al. Clinical implication of autoantibodies in patients with systemic autoimmune diseases. Expert Rev Clin Immunol. 2007;3(5):721–738.

11. Rammohan MVNL, Begum S, Sudhaharan S, Talasila S, Teja VD. Analysis of antinuclear antibody test referral patterns in a tertiary care hospital over three years: A retrospective observational study.
12. Reshmy GS, et al. Antinuclear antibody profile in a tertiary care centre: Clinical correlation and pattern analysis. J Clin Diagn Res. 2021.
13. Fritzler MJ. Advances in the diagnosis and management of systemic autoimmune diseases using autoantibody testing. Autoimmun Rev. 2012;11(10):A652–A658.