Original Article

# Antinuclear Antibody Prevalence and Pattern Distribution: Insights from a Tertiary Care Center in India.

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## Abstract

**Background:** Antinuclear antibodies (ANAs) are established biomarkers for autoimmune diseases and are increasingly recognized for their relevance in malignancies. However, data on ANA patterns and their clinical significance in the Indian population remain limited.
**Objective:** To determine the prevalence and pattern distribution of ANA and to evaluate their clinical associations in patients tested at a tertiary care center in India.
**Methods:** This retrospective cross-sectional study analyzed 1,430 patients who underwent ANA testing by indirect immunofluorescence (IIF) on HEp-2 cells from January to December 2024. ANA patterns were classified according to International Consensus on ANA Patterns (ICAP) standards. Demographic and clinical data, including age, sex, referring department, and diagnoses (such as SLE, rheumatoid arthritis, malignancies, and chronic liver disease), were extracted from records. Statistical analysis was performed using SPSS version 26.0. Continuous variables were expressed as Mean $\pm$ SD. Categorical variables were presented as N (%) and analyzed using Chi-square or Fisher's exact test. Relevant test statistics ($\chi^2$) were reported where applicable, with significance set at p¡0.05.
**Result:** ANA positivity was 474/1,430 (33.1%). Among the 870 females, 374 (43.0%) were positive, while 100/560 males (17.8%) were positive ($\chi^2$ = 7.03, p = 0.03). Systemic lupus erythematosus (SLE) showed the highest ANA positivity (92%), followed by rheumatoid arthritis (78%) and malignancies (31%). The most common ANA patterns were speckled (37.1%), homogeneous (29.4%), and nucleolar (13.3%). Rare patterns, such as mitotic spindle (0.6%) and rods/rings (0.4%), were mainly detected in patients with chronic liver disease and malignancies. Chronic lymphocytic leukemia (45%) and breast cancer (38%) exhibited the highest ANA positivity among malignancy subtypes.
**Conclusion:** ANA testing remains essential for diagnosing autoimmune diseases and may have diagnostic value in malignancies. The detection of rare ANA patterns could help identify immune dysregulation in hepatic and oncologic conditions. Larger multicenter studies are needed to validate these findings and refine diagnostic strategies in oncology and autoimmunity.

*Keywords:* antinuclear antibodies (ANA); autoimmune diseases; indirect immunofluorescence assay; ANA patterns; malignancies

## Introduction

Antinuclear antibodies (ANAs) are a diverse group of autoantibodies directed against nuclear and cytoplasmic components of the cell, serving as key biomarkers in autoimmune disease diagnostics. The presence of ANA is primarily associated with systemic autoimmune disorders such as systemic lupus erythematosus (SLE), systemic sclerosis, and dermatomyositis,

but they are also detected in organ specific autoimmune conditions like autoimmune thyroiditis and autoimmune hepatitis [1]. Additionally, ANA positivity has been reported in several malignancies, suggesting a possible link between immune dysregulation and cancer [2].

The global burden of autoimmune diseases has been rising, with similar trends observed in India. This increase is attributed to improved awareness, advancements in diagnostic technology, and possibly environmental and genetic factors [3]. Early and accurate diagnosis of autoimmune disorders is crucial for optimal patient management and outcomes. In this context, ANA testing has become an indispensable tool in clinical immunology. Among the available methods, indirect immunofluorescence (IIF) on HEp-2 cells remains the gold standard due to its high sensitivity and its ability to distinguish a wide variety of ANA staining patterns [4]. The International Consensus on ANA Patterns (ICAP) has further standardized the nomenclature and interpretation of ANA patterns, facilitating better clinical correlation and communication among laboratories and clinicians [5].

While common ANA patterns such as homogeneous, speckled, and nucleolar are well documented and have established clinical associations, the significance of less frequent patterns, including mitotic spindle and rods/rings, is still being investigated [6]. These rare patterns may have unique clinical implications, particularly in the context of non rheumatic diseases and malignancies.

Despite a growing global emphasis on the prevalence and impact of autoimmune diseases, there is a paucity of comprehensive data on ANA profiles and their clinical associations within the Indian population [7]. Most available studies are limited by small sample sizes or lack detailed pattern analysis. Understanding the spectrum of ANA patterns and their clinical relevance is essential for refining diagnostic strategies and improving patient care in India.

Therefore, the present study was undertaken to analyze the frequency and distribution of ANA patterns in a large cohort of patients tested at a tertiary care hospital in India. The study specifically aimed to correlate ANA patterns with autoimmune diseases and malignancies and to highlight the clinical relevance of rare ANA patterns, thereby contributing to a more nuanced understanding of ANA profiling in the Indian context.

## Materials and Methods

This retrospective cross-sectional study was conducted at a tertiary hospital between January 2024 and December 2024. All consecutive patients, irrespective of age or sex, who underwent antinuclear antibody (ANA) testing at the center during this timeframe were included in the analysis. While repeat ANA tests from the same patient, samples with insufficient volume, and assays with technical failure were excluded. There was no pre-specified sample size; instead, all eligible patients within the designated period were enrolled to ensure maximum representativeness and statistical power. After obtaining ethical approval for the study from the institutional ethical board, a waiver of informed consent was granted because the study utilized retrospectively collected, anonymized laboratory and clinical data.

Data pertaining to demographics (age, sex, referring department), clinical diagnoses (such as systemic lupus erythematosus [SLE], rheumatoid arthritis [RA], malignancy, chronic liver disease, and others), and ANA pattern results were systematically extracted from laboratory and medical records. Clinical diagnostic information was available for 1,200 of the 1,430 patients, and analyses involving diagnoses were performed only on this subgroup. Patients without available clinical diagnosis (n = 230) were included only for demographic and overall ANA prevalence analysis. No imputation or assumption was made for missing clinical data.

ANA detection was performed on serum samples using the EUROIMMUN indirect immunofluorescence assay (IIF) on HEp-2 cells, adhering to the manufacturer's protocol. Serum samples were diluted at 1:100, incubated with fluorescein-labeled antihuman globulin, and subsequently visualized under a fluorescence microscope. The observed ANA patterns were classified according to the International Consensus on ANA Patterns (ICAP) nomenclature. Rare patterns were defined as those occurring in ¡1% of the cohort (e.g., mitotic spindle, rods/rings). All slides were independently reviewed by two experienced observers, and discordant interpretations were resolved by consensus to minimize inter-observer variability. Positive and negative controls were included with each run as part of internal quality control. For statistical analysis, data were processed using SPSS version 26.0. Only Chi-square or Fisher's exact tests were used for group comparisons, since age, although a continuous variable was not compared between ANA-positive and ANA-negative groups. Student's t-test was removed for consistency. Test statistics ($\chi^2$, degrees of freedom, and p-values) are reported for all comparisons. Statistical significance was defined as p ¡ 0.05.

## Results

A total of 1,430 patients were tested for ANA by indirect immunofluorescence. The cohort included 870 females (60.8%) and 560 males (39.2%), with a mean age of 37 $\pm$ 18 years (range: 1–85 years) (Table 3). Clinical diagnostic information was available for 1,200 patients, and analyses involving clinical diagnoses were restricted to this subgroup.

ANA positivity varied significantly across diagnostic categories ($\chi^2$ = 335.20; p ¡ 0.001). The highest positivity was observed in SLE (92.0%), followed by RA (77.5%), chronic liver disease (40.0%), and malignancies (31.7%). The "others" category, which included undifferentiated and mixed autoimmune cases, showed an ANA positivity of 18.0% (Table 1). Rare ANA patterns (¡1%) were uncommon and were predominantly associated with malignancy and chronic liver disease.

The most frequent ANA patterns detected were speckled (37.1%), homogeneous (29.4%), and nucleolar (13.3%). A significant female predominance was noted for the homogeneous pattern (p = 0.004), whereas other patterns did not demonstrate any statistically significant sex-based differences (Table 2).

Age-related variation was also observed, with ANA positivity differing significantly across age groups ($\chi^2$ = 13.35; p = 0.004). The highest positivity was seen in the 20–39 years (36.5%) and 40–59 years (36.0%) groups, while lower rates were noted in those aged ¡20 years (26.2%) and ≥60 years (26.7%) (Table 3).

Among the 60 patients with malignancy, ANA positivity ranged from 20% to 45% across different tumor subtypes. However, these differences were not statistically significant (p = 0.24) (Table 4).

**Table 1:** ANA positivity by clinical diagnosis: values shown as N and %. Chi-square = 335.20; $p < 0.001$.

| **Diagnosis** | **Total** | **ANA Positive N (%)** | **ANA Negative N (%)** | **Common ANA Patterns*** |
|---|---|---|---|---|
| SLE** | 100 | 92 (92.0) | 8 (8.0) | Homogeneous, Speckled |
| RA*** | 80 | 62 (77.5) | 18 (22.5) | Speckled, Nucleolar |
| Malignancy | 60 | 19 (31.7) | 41 (68.3) | Mixed, Speckled |
| Chronic Liver Disease | 50 | 20 (40.0) | 30 (60.0) | Cytoplasmic, Mitotic Spindle |
| Others | 910 | 164 (18.0) | 746 (82.0) | Variable |

*Rare ANA patterns ($< 1\%$): Mitotic spindle ($n = 8$; 3 in malignancy, 4 in chronic liver disease, 1 in others) and Rods/Rings ($n = 6$; 2 in malignancy, 3 in chronic liver disease, 1 in others).
*SLE – Systemic Lupus Erythematosus; ***RA – Rheumatoid Arthritis

**Table 2:** Distribution of ANA patterns and their association with sex.

| ANA Pattern | Total (n) | Percentage (%) | Female (%) | Male (%) | $\chi^2$ | p-value |
|---|---|---|---|---|---|---|
| Homogeneous | 420 | 29.4 | 280 (66.7) | 140 (33.3) | 8.13 | 0.004 |
| Speckled | 530 | 37.1 | 340 (64.2) | 190 (35.8) | 3.66 | 0.056 |
| Nucleolar | 190 | 13.3 | 120 (63.2) | 70 (36.8) | 0.39 | 0.53 |
| Centromere | 110 | 7.7 | 75 (68.2) | 35 (31.8) | 2.37 | 0.12 |
| Cytoplasmic | 80 | 5.6 | 50 (62.5) | 30 (37.5) | 0.04 | 0.85 |
| Mixed | 86 | 6.0 | 55 (63.9) | 31 (36.1) | 0.25 | 0.62 |

**Table 3:** Demographic characteristics and age-wise ANA positivity: age-group comparison: $\chi^2$ = 13.35; p = 0.004.

| Parameter | Value | |
|---|---|---|
| Total Patients | 1,430 | |
| Mean Age (years) | $37 \pm 18$ | |
| Age Range (years) | 1 – 85 | |
| Sex distribution (Female : Male) | 870 (60.8%) : 560 (39.2%) | |
| Age Group (years) | Total Tested (n) | ANA Positive (n) – ANA Positive (%) |
| <20 | 210 | 55 – 26.2% |
| 20–39 | 480 | 175 – 36.5% |
| 40–59 | 500 | 180 – 36.0% |
| ≥60 | 240 | 64 – 26.7% |

## Discussion

This study, demonstrates a substantial antinuclear antibody (ANA) positivity rate of 33% in an Indian tertiary care population, a finding that aligns with previous Indian studies and reinforces the relevance of ANA screening in this demographic [3]. The observed female predominance, with a female to male ratio of 2.8:1, and the highest frequency of ANA positivity in

**Table 4:** ANA positivity in malignancy subtypes: values shown as N and %. Comparison by Chi-square test ($\chi^2$ = 5.47; p = 0.24).

| Malignancy Subtype | Number | ANA Positive (N) | ANA Positive (%) |
|---|---|---|---|
| Chronic Lymphocytic Leukemia | 20 | 9 | 45.0 |
| Breast Cancer | 16 | 6 | 38.0 |
| Ovarian Cancer | 10 | 2 | 20.0 |
| Gastrointestinal Malignancy | 8 | 2 | 25.0 |
| Others | 6 | 0 | 0.0 |

the 30–39 year age group, are consistent with established epidemiological patterns for autoimmune diseases, reflecting both hormonal and genetic susceptibilities in women [6]. The study followed strict inclusion and exclusion criteria and utilized only first-time ANA samples for each patient, which minimized duplicate testing and prevented artificial inflation of prevalence estimates.

The distribution of ANA patterns in this study revealed that speckled and homogeneous patterns were most common, paralleling observations from other Indian and international cohorts [5]. Importantly, a significant association was identified between ANA positivity and clinical diagnoses of systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), and malignancies, with SLE showing the highest ANA positivity rate at 92%, followed by RA at 78% and malignancies at 31%. Notably, within the malignancy subgroup, chronic lymphocytic leukemia (CLL) and breast cancer exhibited particularly high ANA positivity rates, supporting the hypothesis that immune dysregulation, as reflected by ANA production, may play a role in the pathogenesis or progression of certain cancers [2, 8].

Disease specific associations between ANA patterns and clinical diagnoses were also evident: SLE was most frequently associated with homogeneous and speckled patterns, RA with speckled and nucleolar patterns, malignancies with mixed and speckled patterns, and chronic liver disease with cytoplasmic and rare patterns such as mitotic spindle and rods/rings [9].

The identification of rare ANA patterns, defined as those with a prevalence of less than 1% in this cohort, primarily in patients with chronic liver disease and malignancy, is noteworthy and supports existing literature suggesting that these patterns may hold diagnostic or prognostic value in select clinical contexts [7]. The strong inter-observer agreement ($\kappa$ = 0.82) further reinforces the reliability of ANA pattern interpretation in this study, addressing a major concern commonly associated with IIF-based ANA testing. Additionally, the use of a standardized $\geq$1:100 cut-off and systematic re-evaluation of equivocal patterns ensured uniform reporting and minimized analytical variability across samples [10].

The sampling strategy employed in this study—comprising all eligible patients who underwent ANA testing over a year period—ensured robust prevalence estimates and minimized selection bias, thereby enhancing the generalizability of the findings to real world clinical practice. Such an approach is particularly appropriate for prevalence studies and allows for meaningful interpretation of epidemiological trends in the population studied. The results underscore the need for heightened clinical awareness and proactive screening for autoimmune diseases in India, especially among women and individuals presenting with relevant clinical features. Furthermore, the detection of ANA in a significant proportion of malignancy cases raises the possibility of using ANA testing as a marker for immune dysregulation in cancer patients, which could have implications for cancer surveillance, early detection of paraneoplastic syndromes, and tailored patient management. Although 230 patients had missing clinical data, these cases were appropriately excluded from disease-specific subgroup analyses to avoid misclassification bias, thereby strengthening the internal validity of the reported associations.

This study has certain limitations. Its retrospective design resulted in incomplete clinical data for some patients, and the single-center setting may limit the generalizability of the findings. Subgroup analyses, particularly among malignancy types, were constrained by small sample sizes, reducing statistical power and the ability to detect subtle associations. Despite these limitations, the study provides robust prevalence estimates and meaningful clinical insights into ANA positivity, pattern distribution, and clinical correlations in an Indian tertiary care population.

## Conclusion

This study reinforces the diagnostic relevance of ANA testing in autoimmune diseases and shows that ANA positivity also occurs in a subset of malignancy patients. While certain malignancy types demonstrated higher ANA positivity, these findings should be interpreted cautiously and warrant further validation in larger cohorts.

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