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Age-wise Distribution of Antinuclear Antibody (ANA) Patterns by Indirect Immunofluorescence Assay (IFA): A Cross-Sectional Study at a Tertiary Care Hospital in Central India

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Abstract

Background: *Antinuclear antibody (ANA) testing using indirect immunofluorescence assay (IFA) plays a pivotal role in the early diagnosis and classification of autoimmune disorders. Age-related variations in ANA patterns have been observed, influencing clinical interpretations and subsequent management.*

Objective: *This study aimed to investigate the distribution of ANA patterns among various age groups in patients from a tertiary care center in Central India, utilizing the IFA technique on HEp-2 cells.*

Methods: *A cross-sectional observational study was conducted at SAIMS Medical College and PG Institute, Indore, from January 1, 2025, to May 15, 2025. ANA-positive serum samples (n=211) were analyzed. The fluorescent patterns were categorized according to the International Consensus on ANA Patterns (ICAP) and stratified by age groups: 0–20, 21–40, 41–60, and over 60 years.*

Results: *The most frequent pattern was nucleoplasm speckled (30.3%), followed by cytoplasmic homogenous (25.6%). The 21–60 years age groups demonstrated the highest pattern diversity. Rare patterns like few nuclear dots and cell nucleoli peripheral were seen in isolated cases.*

Conclusion: *There is a notable age-dependent variation in ANA patterns. Recognizing these patterns can improve diagnostic precision and guide further clinical investigation.*

Keywords: *Antinuclear Antibody (ANA), Indirect Immunofluorescence Assay (IFA), Autoimmune Diseases, Age-related Patterns, HEp-2 Cells*

Introduction

Autoimmune diseases are marked by various markers. Antinuclear antibodies (ANAs) are immunological markers commonly associated with a variety of autoimmune diseases, particularly systemic lupus erythematosus (SLE), systemic sclerosis, and Sjögren's syndrome. These autoantibodies target nuclear components and can be detected using the indirect immunofluorescence assay (IFA) on HEp-2 cells. The IFA method remains the gold standard for ANA detection due to its sensitivity and its ability to display distinct fluorescence patterns corresponding to specific autoantibodies.

Understanding ANA patterns and their implications is essential, especially in resource-limited settings where early and accurate diagnosis is critical. The International Consensus on ANA Patterns (ICAP) has helped streamline the interpretation of IFA results, classifying them into nuclear, nucleolar, and cytoplasmic staining categories. However, studies focusing on the age-wise distribution of ANA patterns remain sparse, particularly in the Indian subcontinent.

Immune system functionality changes with age, influencing autoantibody production. Pediatric patients may show transient positivity, while adults and elderly individuals tend to develop autoantibodies in association with disease. This study seeks to provide a detailed age-wise distribution of ANA patterns in Central India, aiming to bridge the knowledge gap and contribute to better clinical utilization of ANA IFA results.

Materials and Methods

Study Design: Cross-sectional, observational study
Location: Department of Microbiology, SAIMS Medical College and PG Institute, Indore, Madhya Pradesh, India
Duration: January 1, 2025-May 15, 2025
Sample Size: 211 ANA-positive serum samples
Testing Method: Indirect immunofluorescence on HEp-2 cells

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Inclusion Criteria: All ANA-positive results during the study period

Exclusion Criteria: ANA-negative samples
Pattern Classification: Based on ICAP guidelines

Results

Table 1: Overall Pattern Distribution

Pattern	Number	Percentage (%)
Nucleoplasm speckled	64	30.3
Nucleoli speckled	8	3.8
Nuclear homogenous	18	8.5
Cytoplasmic speckled	44	20.8
Cytoplasmic homogenous	54	25.6
Cytoplasmic filamentous	14	6.6
Mitotic cells positive	5	2.4
Few nuclear dots	1	0.5
Cell nucleoli peripheral	3	1.4
Total	211	100.0

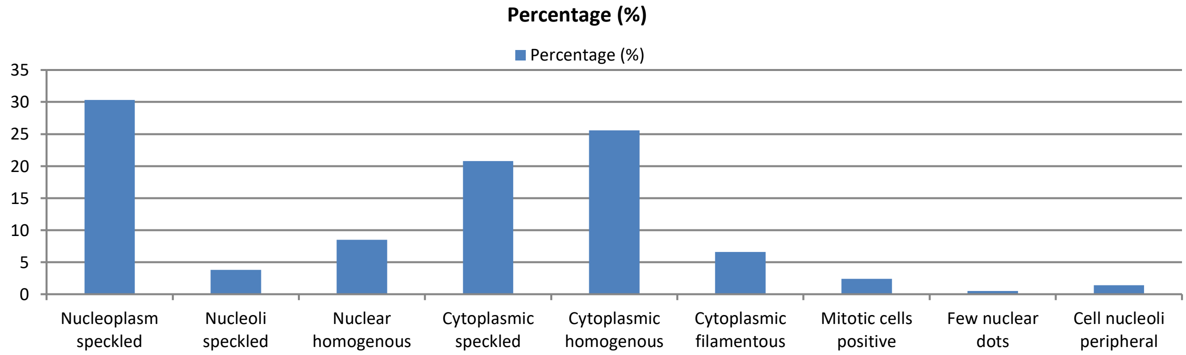

Figure 1: Overall Pattern Distribution

Table 2: Age-wise Distribution

Pattern	0–20 yrs	21–40 yrs	41–60 yrs	>60 yrs
Nucleoplasm speckled	1	24	21	18
Nucleoli speckled	1	2	3	2
Nuclear homogenous (Cell nuclei Homogenous)	0	7	8	3
Cytoplasmic speckled	2	18	12	12
Cytoplasmic homogenous	5	12	21	16
Cytoplasmic filamentous	0	2	7	5
Mitotic cells positive	0	3	2	0
Few nuclear dots	0	0	1	0
Cell nucleoli peripheral	0	1	2	0

Table 1 summarizes the frequency and percentage of different ANA patterns observed across all 211 positive samples. The data is categorized based on the International Consensus on ANA Patterns (ICAP). The most common pattern was nucleoplasm speckled (30.3%), followed by cytoplasmic homogenous (25.6%).

Figure 1 is a visual representation (likely a bar or pie chart) of the data shown in Table 1. It graphically illustrates the proportional distribution of each ANA pattern among the total 211 samples.

This table 2 breaks down the same ANA patterns by four age groups: 0–20 years, 21–40 years, 41–60 years, and >60 years. The 21–60 year age groups showed highest pattern diversity, especially for nucleoplasm speckled and cytoplasmic homogenous patterns.

This figure is a grouped bar chart that visually displays the distribution of different ANA patterns across the four age categories mentioned in Table 2. It provides an age-comparative view of pattern prevalence, highlighting differences in frequency and diversity of patterns with age.

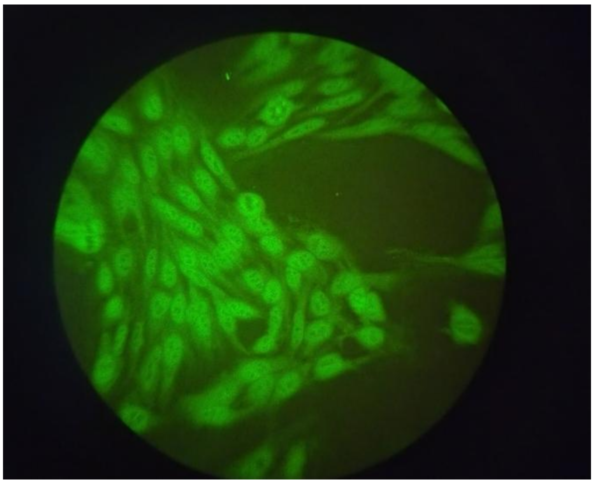

Figure 3: Nucleoplasm speckled pattern

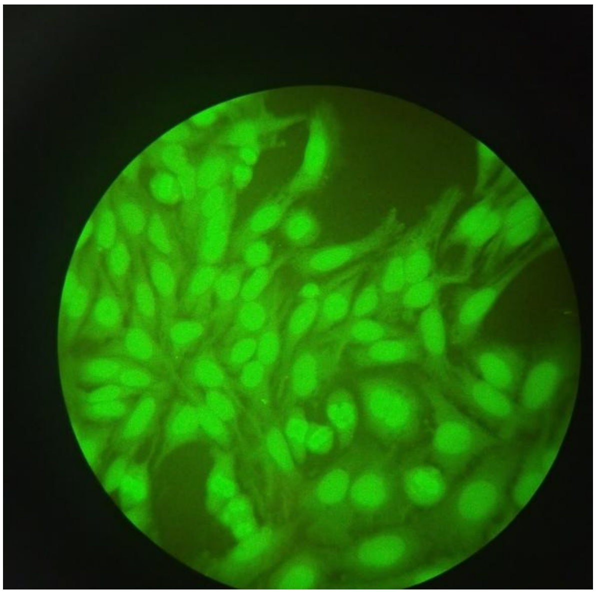

Figure 4: Cell nuclei Homogenous

Discussion

The findings of this study highlight a diverse and age-influenced pattern distribution of ANA in patients. The nucleoplasm speckled and cytoplasmic homogenous patterns were the most frequently observed, suggesting a potential correlation with diseases common in young to middle-aged adults.

The predominance of nucleoplasm speckled pattern in 21–40 and 41–60 age groups is consistent with its known association with mixed connective tissue disease and early SLE, conditions often presenting in early to mid-adulthood. Cytoplasmic homogenous patterns were observed across all age groups but were especially prominent in the 41–60 age bracket, aligning with its relation to autoimmune myopathies and anti-signal recognition particle antibodies.

Interestingly, the cytoplasmic filamentous pattern, though less frequent, was seen more in older patients, possibly indicating late-onset myositis or primary biliary cirrhosis. The low prevalence of rare patterns such as few nuclear dots and cell nucleoli peripheral aligns with global epidemiological data.

These observations underscore the need for age-specific reference data in ANA interpretation, which can help avoid over- or under-diagnosis in specific populations. Furthermore, the study supports the utility of pattern-based ANA reporting as a cost-effective preliminary tool in autoimmune disease screening, especially in tertiary care centers serving large populations.

Conclusion

ANA pattern interpretation by IFA demonstrates significant age-related variation. The nucleoplasm speckled and cytoplasmic homogenous patterns were predominant, particularly among adults aged 21–60 years. Recognizing these trends is crucial for accurate diagnosis and management of autoimmune diseases.

References

[1] Tan EM, Feltkamp TEW, Smolen JS, et al. Range of antinuclear antibodies in "healthy" individuals. *Autoimmun Rev*. 2012;11(12):A593-A598.

[2] Maity S, Patel KB, Ojha U; Pattern Distribution and Gender Correlation of Antinuclear Antibody by Indirect Immunofluorescence Assay: A Retrospective Study from Central India", IJSDR - International Journal of Scientific Development and Research (www.IJSDR.org), ISSN:2455-2631, Vol.10, Issue 6, page no.b89-b93, June-2025.

[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] Fritzler MJ. Advances and applications of multiplexed diagnostic technologies in autoimmune diseases. *Lupus*. 2015;24(4-5):400-405.

[5] Satoh M, Chan EKL, Ho LA, et al. Prevalence and sociodemographic correlates of antinuclear antibodies in the United States. *Arthritis Rheum*. 2012;64(7):2319-2327.

[6] Chan EKL, Damoiseaux J, Carballo OG, et al. Report of the second international consensus on ANA pattern (ICAP) workshop. *Autoimmun Rev*. 2016;15(5):416-420.

[7] Mahler M, Miyachi K. Cytoplasmic staining patterns on HEp-2 cells: what's old and what's new? *Auto Immun Highlights*. 2016;7(1):3.

[8] Meroni PL, Schur PH. ANA screening: an old test with new recommendations. *Ann Rheum Dis*. 2010;69(8):1420-1422.

[9] Dellavance A, Gabriel Jr A, Nuccitelli B, et al. The relevance of the cytoplasmic patterns observed in the anti-nuclear antibody indirect immunofluorescence test. *Clin Chem Lab Med*. 2020;58(3):401-407.

[10] Bizzaro N, Antico A, Platzgummer S, et al. Automated antinuclear immunofluorescence antibody screening: A comparative study of six computer-aided diagnostic systems. *Autoimmun Rev*. 2021;20(2):102740.

[11] Petri M, Orbai AM, Alarcón GS, et al. Derivation and validation of the Systemic Lupus International Collaborating Clinics classification criteria for systemic lupus erythematosus. *Arthritis Rheum*. 2012;64(8):2677-2686.