

**ANTI-NUCLEAR ANTIBODIES IN DAILY CLINICAL PRACTICE: PREVALENCE
IN A TERTIARY CARE HOSPITAL.**

**DR. MAYURI KIRTIBHAI PATEL, DR. SHILPI SAHU, DR. ARPITA SINGH, DR.
SUPRIYA DUTTA, DR. NIRALI UPADHYAY**

Junior Resident.,MGM Institute of Health Science, Kamothe,Navi Mumbai, Maharashtra,
410206. ,Mayuri05031996@gmail.com

Professor and head of the Department of Pathology.,Drshilpisahu1974@gmail.com

Senior Resident.,arpisngh@gmail.com

Consultant Pathologist at Jupiter Hospital, Mumbai.

Junior Resident, MGM Institute of Health Science, Kamothe,

Navi Mumbai, Maharashtra, 410206.

(CORRESPONDING AUTHOR): DR. ARPITA SINGH

ABSTRACT

Introduction: The presence of anti-nuclear autoantibodies (ANA) is closely associated with immune complex-mediated autoimmune conditions. Accurate detection and measurement of these autoantibodies play a pivotal role in both diagnosing and managing such diseases. The indirect immunofluorescence method has emerged as the gold standard for detecting ANA, facilitated by advancements in serological testing for autoimmune disorders. ANA screening, while non-specific, offers a reliable, rapid, and comprehensive assessment, revealing diverse patterns indicative of various autoimmune disorders. **Materials and Methods:** Between January 2023 and June 2023, a total of 179 serum samples were subjected to ANA screening using the indirect immunofluorescence (IIF) method on Hep-2000 cells. **Observations and Results:** Among the 179 serum samples analyzed, 40 (22.35%) tested positive for anti-nuclear antibodies (ANA). Among the positive samples, speckled pattern was the most prevalent, observed in 20 cases (50%), followed by homogeneous pattern in 8 cases (20%), nucleolar pattern in 6 cases (15%), mixed pattern in 3 cases (7.5%), cytoplasmic pattern in 2 cases (5%) and Centromere in 1 case(2.5%). Notably, higher positivity rates were observed in the age groups of 20-40 years (35%) and 40-60 years (32.5%), with females showing a predominance. **Conclusion:** The speckled pattern emerged as the most prevalent among positive cases, closely followed by homogenous patterns. This underscores the importance of conducting observational studies to comprehensively grasp the epidemiology of autoimmune disorders and ascertain the predictive value of ANA indirect immunofluorescence (IIF) testing in clinical settings. Furthermore, exploring the feasibility of integrating ANA IIF as a screening tool for autoimmune disorders warrants consideration.

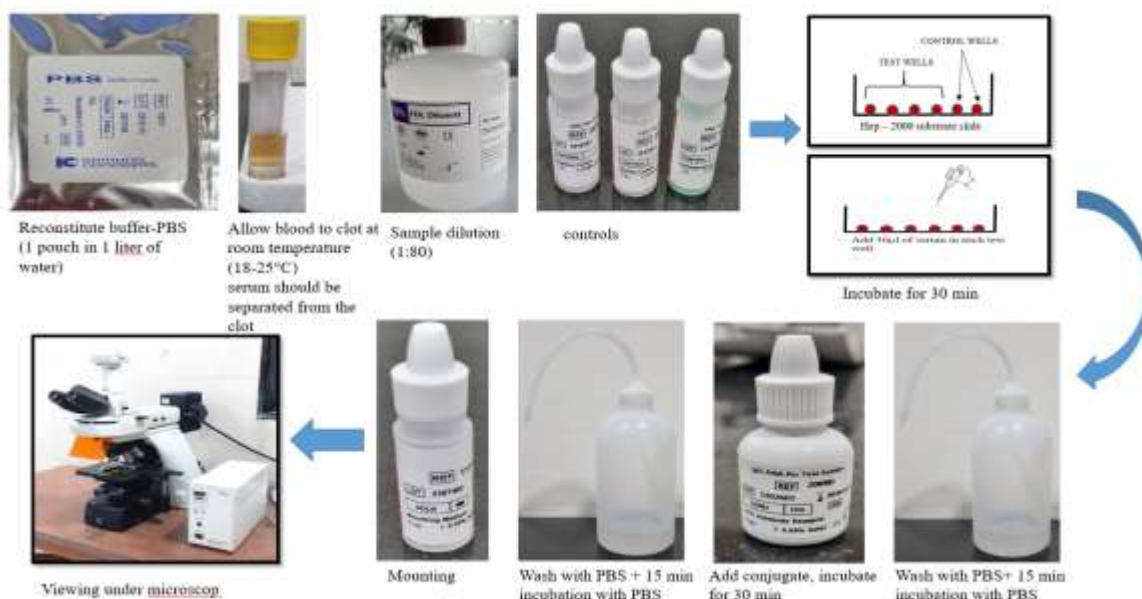
Key Words: Antinuclear antibody (ANA), Autoimmune, Hep- 2000, Indirect Immunofluorescence.

INTRODUCTION: Autoimmune diseases manifest through the immune system's misguided attack on the body's cells and tissues, resulting in tissue and organ damage driven by autoreactive elements like T lymphocytes and autoantibodies. Among these, autoantibodies are pivotal in diagnosis. Anti-nuclear antibodies (ANAs) constitute a group of antibodies recognizing macromolecules such as DNA, RNA, and proteins, with cytoplasmic components also targeted (1,2). These antibodies serve as crucial markers for diagnosing connective tissue diseases (CTDs) and autoimmune hepatitis, although their presence can extend to other autoimmune conditions, cancers, infections, and even healthy individuals (3,4). Precise detection and quantification of autoantibodies are vital for accurate diagnosis and treatment. Indirect immunofluorescence (IIF) using HEp-2 cells derived from human epithelial larynx cancer has emerged as the preferred method for ANA detection due to its simplicity, reliability, and cost-effectiveness. Nonetheless, interpreting IIF results can be time-consuming and prone to variability based on the microscopist's expertise (5). The introduction of serological testing for autoimmune disorders has further established ANA detection by IIF as the gold standard, providing a non-specific yet dependable and rapid screening method capable of identifying various patterns associated with autoimmune disorders.

AIM: This study aims to determine the prevalence of ANA patterns and assess their clinical significance, in patients presenting at a tertiary care hospital in Navi Mumbai, Maharashtra.

INCLUSION CRITERIA: This study includes all patients presenting at MGM Hospital with clinical suspicion of autoimmune disease, encompassing individuals with complaints of joint pain, generalized body pain, symptoms resembling systemic lupus erythematosus (SLE), paralysis, drug reactions, acute and chronic kidney disease, tuberculosis, as well as those with vague symptoms.

MATERIALS AND METHODS: A prospective study was conducted over six months from January 2023 to June 2023 at the Immunology section of the Department of Pathology, MGM Medical College, Navi Mumbai. A total of 179 cases were included in the study. ANA screening was performed using a commercial indirect immunofluorescence (IIF) test kit utilizing human epithelial tumor cell lines, specifically Hep-2 cells, as the substrate. Serum samples of patients were diluted with sample diluting buffered solution in a 1:80 ratio and incubated with the antigen substrate. The formation of a stable antigen-antibody complex occurred if ANAs were present. Non-specifically bound antibodies were removed through washing. Subsequently, the substrate was incubated with an anti-human antibody conjugated to fluorescein. In positive samples, a stable three-part complex formed, comprising fluorescent antibodies bound to human antinuclear antibodies, which in turn were bound to the nuclear antigen. This complex was visualized using a fluorescent microscope. Positive samples exhibited apple-green fluorescence in the cell nuclei, indicative of specific patterns corresponding to the distribution of nuclear antigens within the cells. Negative samples did not display a discernible pattern of nuclear fluorescence.



OBSERVATIONS AND RESULTS: The current study adopts a laboratory-based descriptive observational approach and was conducted within the Immunology section of the Department of Pathology, MGM Medical College, Navi Mumbai. A total of 179 serum samples received by the Pathology department at MGM Medical College, Navi Mumbai, were included in the study.

Table 1: Distribution of Positive and Negative Sample.

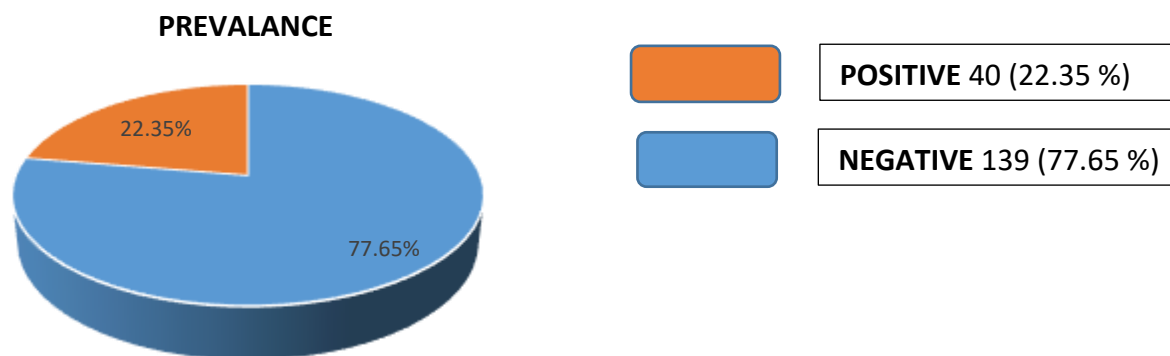


Table 1 compares the demographics of people with autoimmune disorders to healthy individuals. Out of 179 samples, 40 positive (22.35%) and 139 negative (77.65%)

Table 2: Comparison of ANA Prevalence between Healthy Individuals and Symptomatic Patients

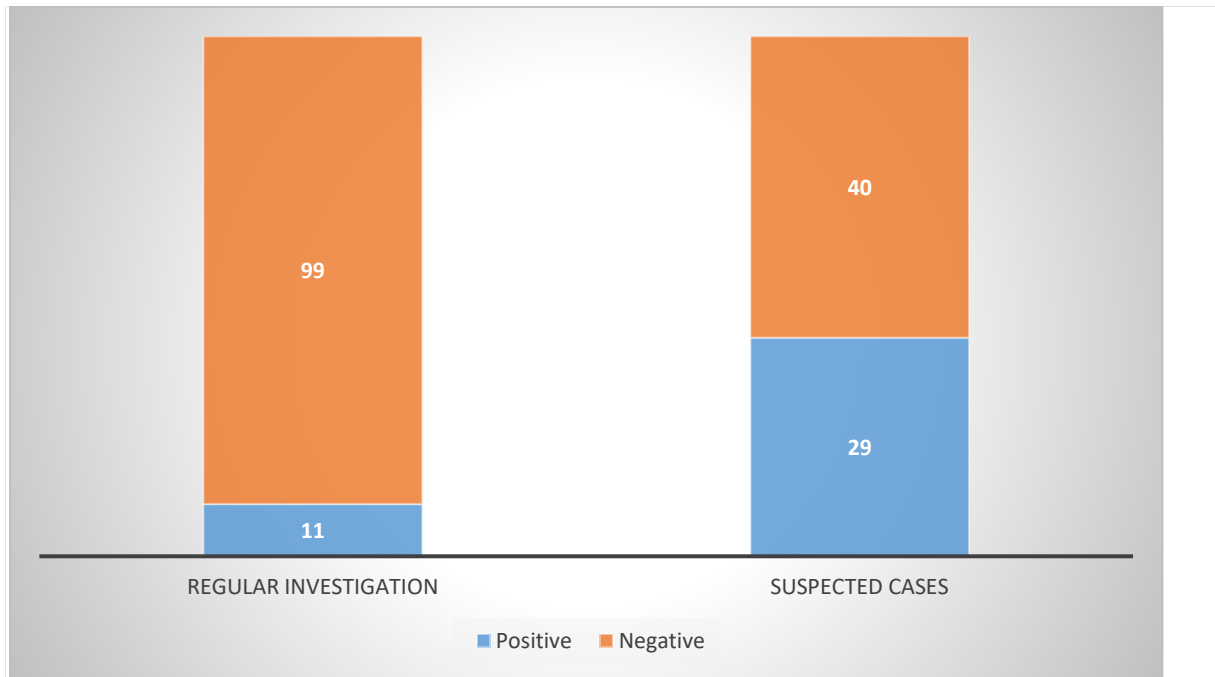


Table 2 shows among a total of 69 suspected cases, ANA positivity was observed in 29 instances, while among 110 healthy individuals, 11 cases tested positive for ANA.

Table 3: Gender-wise distribution of cases

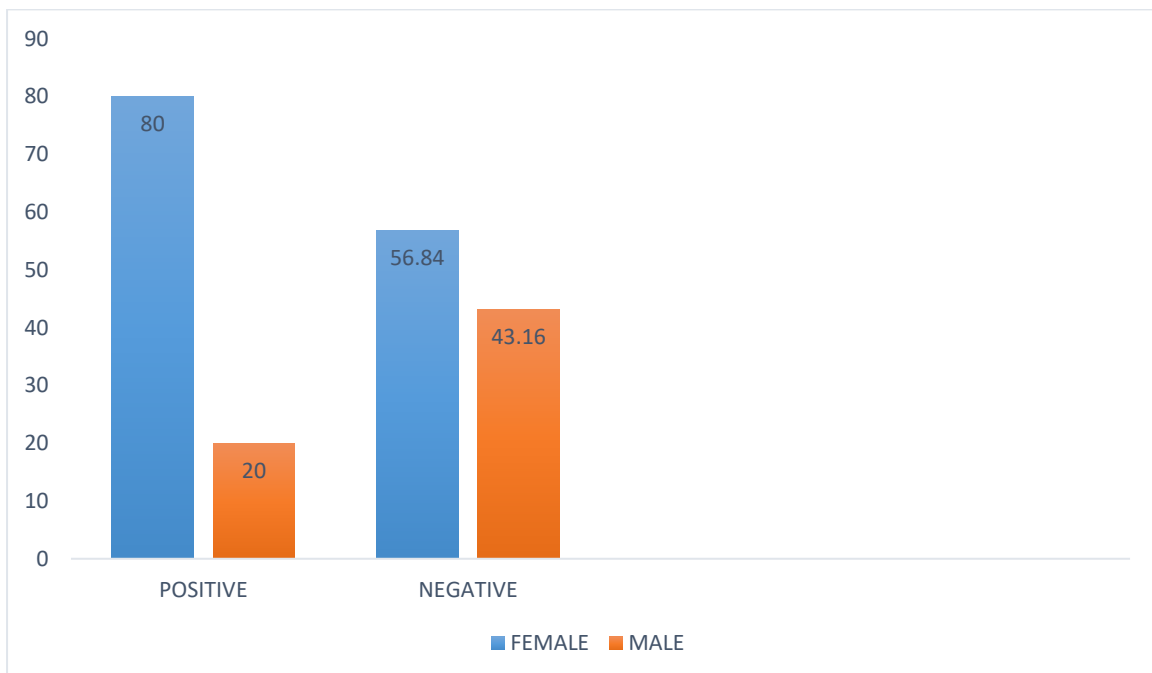


Table 3 shows the ANA-positive male patient ratio of 8 (20%) to positive female patients of 32 (80%).

Table 4: Age group distribution

AGE	POSITIVE	NEGATIVE	TOTAL
1-20 YEAR	12 (30%)	21	33
21-40 YEAR	14 (35%)	52	66
41-60 YEAR	13 (32.5%)	48	61
61-80 YEAR	01 (2.5%)	17	18
> 80 YEAR	00 (0%)	01	01
TOTAL	40	139	179

Table 4 shows the age distribution of those enrolled in the study. The most afflicted age group was 21-40 years (14%), followed by 41-60 years (13%) and 1-20 years (12%). 61-80 (2.5%) years.

Table 5: Prevalence of different ANA patterns:

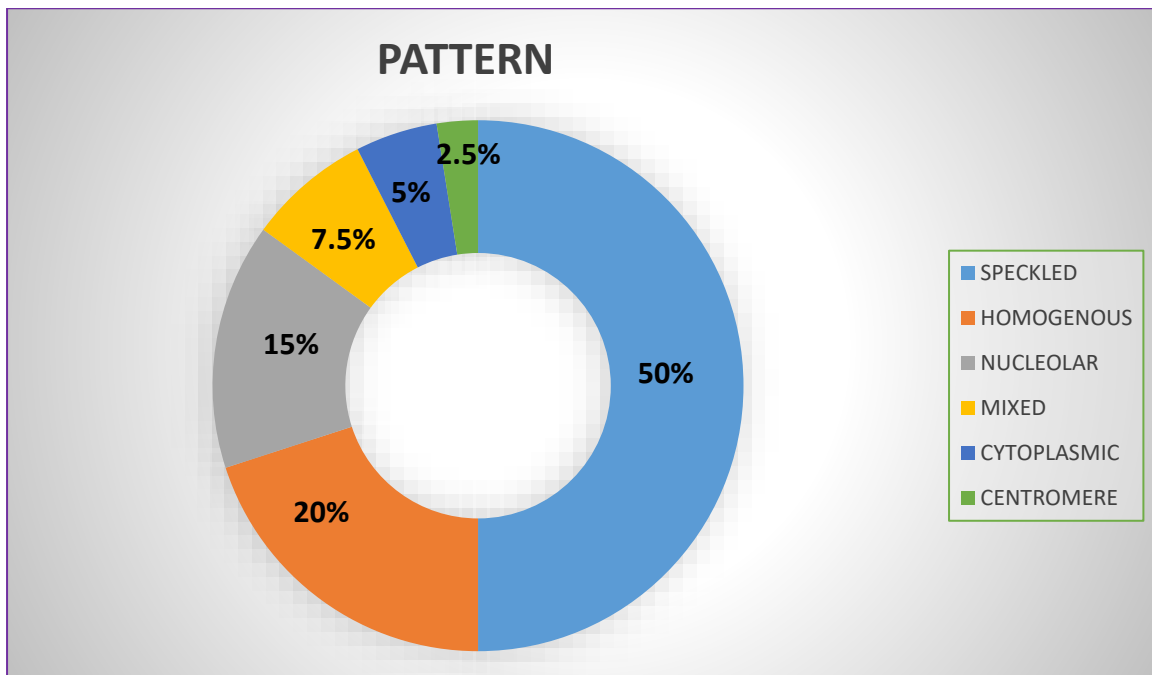


Table 5 shows that speckled is the most common pattern, followed by a homogeneous pattern.

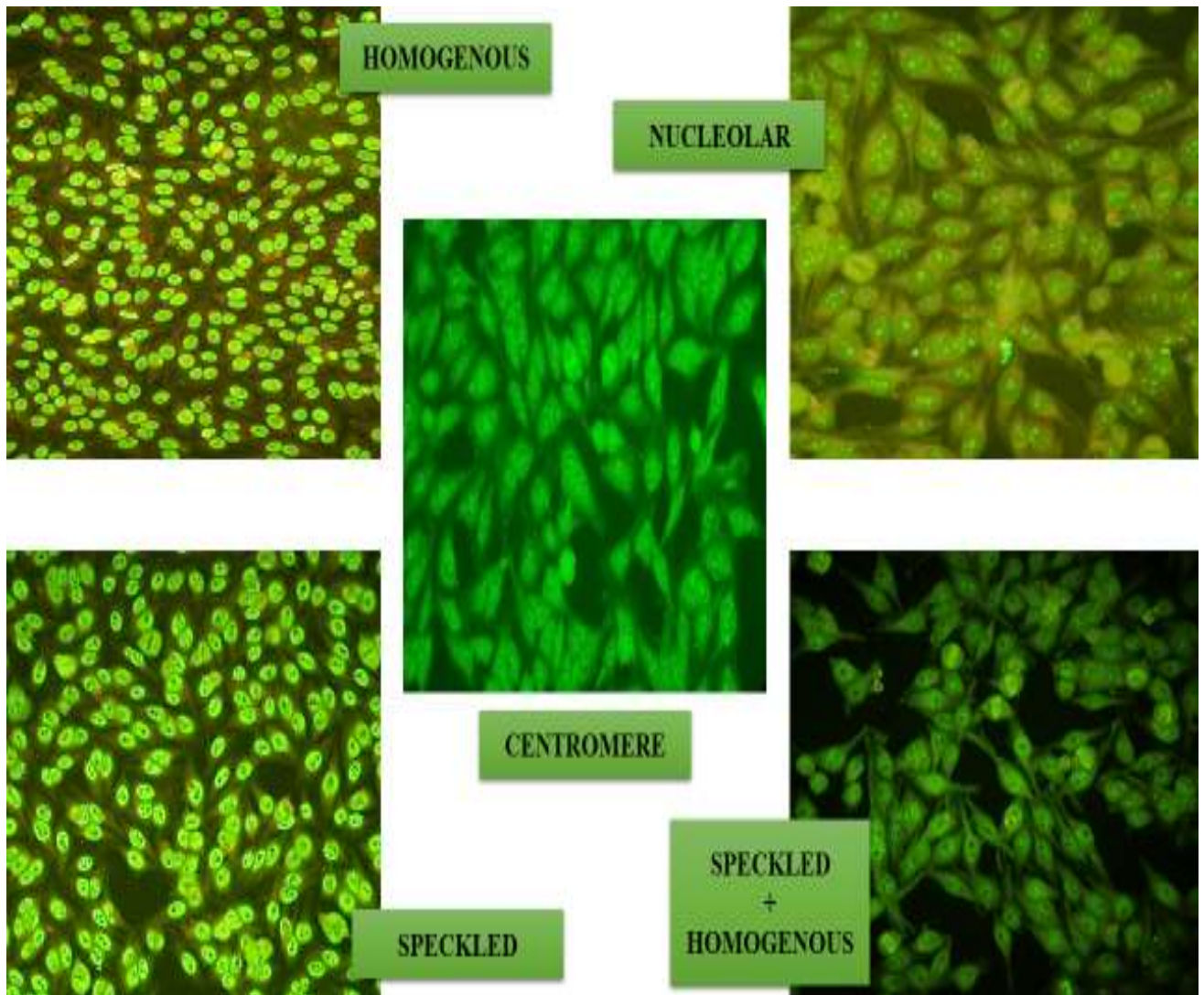
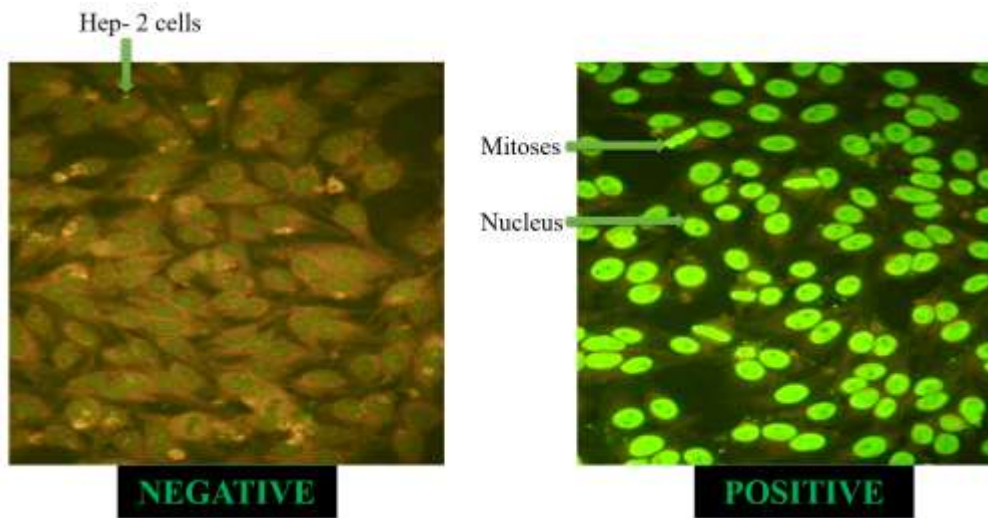
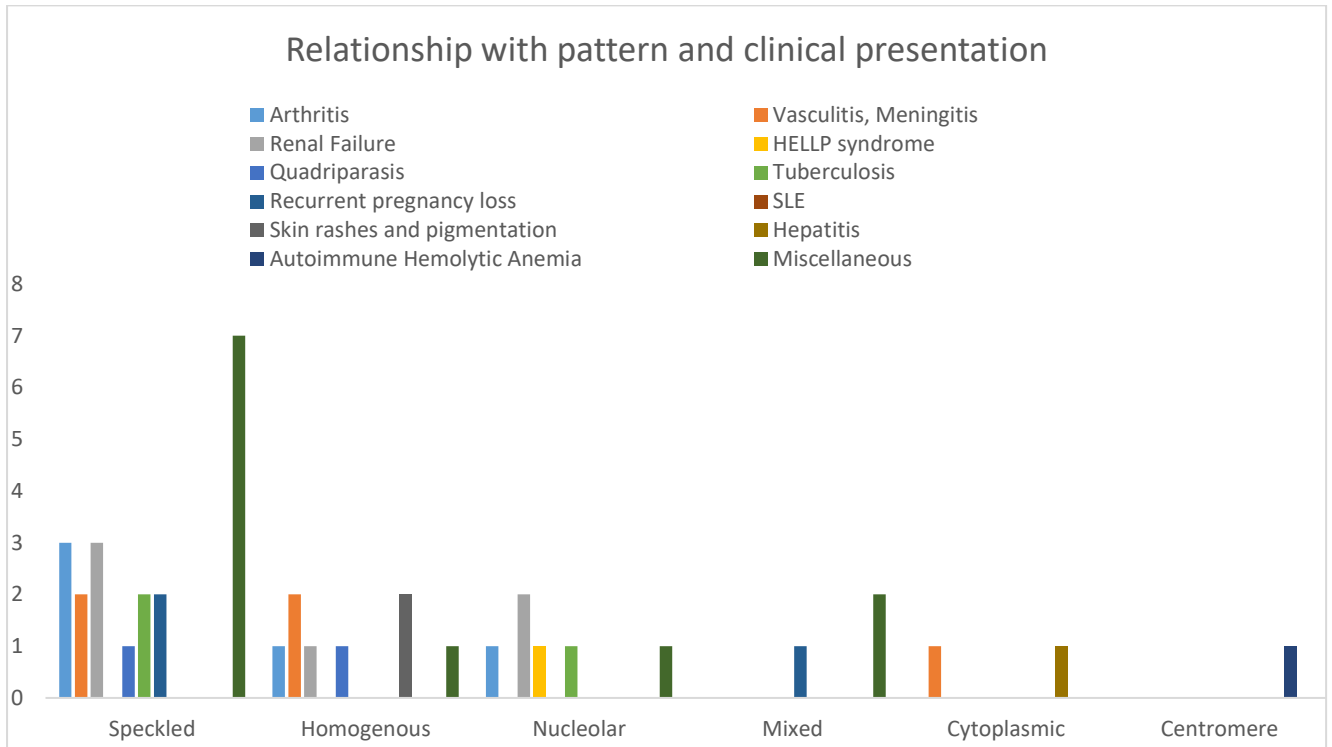


Table 6: Based on symptoms

DISCUSSION: The discussion underscores the significant role of autoimmune serology within India's clinical domain, emerging as a commonly requested diagnostic tool (6). Within this array of tests, the anti-nuclear antibody (ANA) test stands out, often sought for its diagnostic support potential. The utilization of human epithelial cell lines (Hep-2) as a substrate for ANA testing has notably augmented sensitivity (7). It is crucial to approach the interpretation of ANA positivity comprehensively, taking into account pattern, titer, antigen specificity, and clinical context (8). Despite the inherent subjectivity, certain ANA patterns demonstrate specificity, proving immensely beneficial in clinical diagnosis. As ANA serves as a screening test, confirmation is typically pursued through Line Immunoassay for further validation.

The current investigation centered on a patient-based analysis, encompassing the ANA test results of 179 individuals. The evaluation involved assessing ANA positivity utilizing Immunofluorescence (IIF) on Hep-2 cell lines. Among the sampled population, 40 patients tested positive for ANA, constituting 22.35% of the total, while 139 patients tested negative, comprising 77.65%. Notably, among male patients, 8 out of 40 (20%) tested positive, whereas among females, 32 out of 40 (80%) tested positive. Regarding age distribution, individuals aged 21-40 years demonstrated the highest frequency of ANA positivity at 14%, followed by the 41-60 age group at 13%, and the 1-20 age group at 12%. Those aged 61-80 years exhibited the lowest ANA positivity rate at 2.5%. The speckled pattern emerged as the most prevalent ANA pattern, with arthritis being the predominant associated condition.

Conversely, individuals manifesting symptoms akin to systemic lupus erythematosus (SLE) or drug reactions predominantly displayed a homogeneous pattern. In the present study among a total of 69 suspected cases, ANA positivity was observed in 29 instances, while among 110 healthy individuals, 11 cases tested positive for ANA. This observation highlights that ANA positivity occurs among healthy individuals as well, indicating that it alone may not serve as a confirmatory test. Further correlation with line immunoassay is necessary for confirmation.

Other studies conducted in India, such as the one by Minz et al. in Chandigarh, revealed a comparable frequency of ANA positivity to the findings of this study (9). Akmatov et al. in Germany and Satoh et al. in the U.K. reported ANA positivity rates of 33% and 13.8%, respectively (10,11), while Sremec Nada et al. in China and Weyand Banhuk et al. in the USA demonstrated notably lower prevalence rates (12,13). The well-established female predominance in autoimmunity remains a subject of inquiry, with estrogen posited as a potential modulator of autoimmunity, as indicated by Parks et al. (14)

Autoimmune disorders are particularly prevalent in the 20 to 40 age group, contrasting with Satoh et al.'s findings, which noted the highest prevalence in the 40 to 60 age bracket (11).

The correlation between autoimmune diseases and specific antinuclear antibody (ANA) patterns underscores the complex interaction between immune dysregulation and clinical manifestations. Certain ANA patterns demonstrate unique associations with distinct autoimmune conditions, offering crucial diagnostic insights. For example, the speckled ANA pattern is frequently linked to systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA), while the homogeneous pattern often corresponds with conditions such as drug-induced lupus and scleroderma. Moreover, the nucleolar pattern is commonly observed in patients with systemic sclerosis and systemic scleroderma. The centromere pattern is associated with the CREST form of scleroderma, and the cytoplasmic pattern is linked to autoimmune hepatitis and primary biliary cirrhosis. These associations underscore the diagnostic value of ANA patterns in distinguishing specific autoimmune diseases, aiding clinical decision-making, and guiding treatment strategies. However, it's crucial to emphasize that while ANA patterns provide valuable diagnostic clues, their interpretation should be combined with clinical presentation, laboratory findings, and other supplementary tests to ensure precise diagnosis and appropriate management of autoimmune disorders.

Table 7: Comparing studies done in India and worldwide

AUTHOR	PLACE OF STUDY	STUDY DESIGN	SAMPLE SIZE	ANA PREVALENCE	AGE	FEMALE	MOST COMMON ANA PATTERN
PRESENT STUDY	Maharashtra, India	Patient-based	179	22.35%	20-40	28.83 %	SPECKLED
Gupta P et al	Raipur, India	Patient-based	536	33%	37+18	74%	Nuclear speckled
Weyand Banhuk F et al	Brazil	Patient-based	172	4.1%	-	-	Nuclear Homogenous
Akmatorv MK et al	Germany	Population-based	1199	33 %	49(38– 60)	56.2%	Nuclear speckled
Minz RW et al	Chandigarh, India	Patient-based	650	18.9%	42 (20– 50)	75.9%	Nuclear speckled
Sebastian Wet al	Bangalore, India	Patient-based	5066	38.2%	-	-	Nuclear Homogenous
Satoh M et al	USA	Population-based	4754	13.8%	50–59	9.6%	Nuclear Homogenous

CONCLUSION: Antinuclear antibodies (ANA) serve as indicative markers of systemic autoimmune responses, rendering them valuable as both screening tools and aids in the diagnosis of systemic autoimmune diseases. Among ANA patterns, speckled emerged as the most prevalent, often accompanied by homogenous patterns and exhibiting a notable female predominance. Despite its non-specific nature, ANA screening proves reliable, expedient, and cost-effective. Therefore, observational studies are imperative not only for elucidating the epidemiology of autoimmune disorders but also for assessing the predictive value of ANA Immunofluorescence (IIF) in clinical settings. Moreover, consideration should be given to the potential incorporation of IIF as a screening tool for autoimmune diseases (15).

REFERENCES:

1. Pisetsky DS. Antinuclear antibody testing-misunderstood or misbegotten? Nat Rev Rheumatol 2017;13:495-502.

2. Tebo AE. Recent approaches to optimize laboratory assessment of antinuclear antibodies. *Clin Vaccine Immunol* 2017;24:e00270-17.
3. Pasquali J, Goetz J. Que faire en présence d'anticorps antinucléaires chez l'adulte ? In: *Lupus érythémateux*. 2013. p. 103.
4. Peng SL, Craft JE. Anti-nuclear Antibodies. In: *Kelley and Firestein's Textbook of Rheumatology (Tenth Edition)*. 2017. p. 817
- 5 P. Kern, M. Kron, and K. Hiesche Measurement of Antinuclear Antibodies: Assessment of Different Test Systems. *Clin Diagn lab immunol* 2000 Jan; 7(1): 72-78.
6. Peene I, Meheus L, Veys EM, De Keyser F. Detection and identification of antinuclear antibodies (ANA) in a large and consecutive cohort of serum samples referred for ANA testing. *Ann Rheum Dis*. 2001;60(12):1131–1136. doi: 10.1136/ard.60.12.1131.
7. V. Choudhary, A. Sharma and V. Sharma Determination and Prevalence of Antinuclear Antibody (ANA) Patterns in Autoimmune Disorders in a Tertiary Care Hospital, Jaipur 2022 January-March 7(1):29
8. Pasquali J, Goetz J. Que faire en présence d'anticorps antinucléaires chez l'adulte ? In: *Lupus érythémateux*. 2013. p. 103.
9. Minz R W, Kumar Y, Anand S et al. Antinuclear antibody positive autoimmune disorders in North India: an appraisal. *Rheumatol Int*. 2012;32 (09):2883–2888.
10. Akmatov M K, Röber N, Ahrens W et al. Anti-nuclear autoantibodies in the general German population: prevalence and lack of association with selected cardiovascular and metabolic disorders-findings of a multicenter population-based study. *Arthritis Res Ther*. 2017;19(01):127.
11. Satoh M, Chan EK, Ho LA, et al. Prevalence and sociodemographic correlates of antinuclear antibodies in the United States. *Arthritis Rheum*. 2012;64(7):2319- 2327. doi:10.1002/art.34380.
12. Nada T, Kozmar A, Sremec J, Anić B, Batinić D Properties of Uncommon Indirect Immunofluorescence Staining Patterns Determined during Antinuclear Antibody Detection on HEp-2 Cells. *J Clin Med*. 2021 Sep; 10(17): 386-392.
13. Weyand Banhuk F, Corrêa Pahim B, Jorge Sandro A, and Menolli Andrade R Relationships among Antibodies against Extractable Nuclear Antigens, Antinuclear Antibodies, and Autoimmune Diseases in a Brazilian Public Hospital. *Autoimmune Dis*. 2018; 985-990.
14. Beeson P B. Age and sex associations of 40 autoimmune diseases. *Am J Med*. 1994; 96(05):457–462.

15. V. Choudhary, A. Sharma and V. Sharma Determination and Prevalence of Antinuclear Antibody (ANA) Patterns in Autoimmune Disorders in a Tertiary Care Hospital, Jaipur 2022 January-March 7(1):31