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ANA Profile in Population of West Bengal

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Abstract

Antinuclear antibodies (ANA) are significant biomarkers that are used to detect and classify autoimmune connective tissue disorders more efficiently. This paper provides a thorough overview of all the ANA profiling procedures, their testing advancements, clinical significance, and future directions. This study aimed to analyze the ANA profiles along with the prevalence of specific antibodies in patients presumed to have been diagnosed with autoimmune disorders in an Eastern Indian tertiary care hospital. The results were correlated with demographic data to conclude. ANA profiles of 48 patients from Peerless Hospitex Hospital, Kolkata were evaluated between January 24 to June 15, 2024. Out of them, 13 patients (27%) had positive ANA profiles. The most prevalent autoantibodies detected were SS-A and SS-B, which occurred in 7 patients (53.85%), followed by PM-Scl in 4 patients (30.77%). The study demonstrates how the ANA profile varies among different age groups, with the highest prevalence of 6 patients (46.15%) seen in the 60–75 age range. The clinical symptoms of diseases like autoimmune hepatitis, polymyositis, dermatomyositis, PSS (progressive systemic sclerosis), SLE (Systemic Lupus Erythematosus), and Sjogren's syndrome align with the ANA profile findings. This study offers insights into the distribution of autoantibodies targeting antigens present in ANA profiles, using clinical samples collected from a 550-bed tertiary care hospital in Eastern India. The findings underscore the need for comprehensive diagnostic approaches to autoimmune disorders.

Keywords: Antinuclear Antibody (ANA), Autoimmune Diseases, Demographic Correlation, ANA Profiling, Immunoblotting, Indirect Immunofluorescence, ELISA.

INTRODUCTION

ANA are antibodies that target a person's own cell nuclei components. They are important for the classification and diagnosis of connective tissue disorders (CTDs) caused by autoimmunity. ANA profile contains a wide variety of autoantibodies, each associated with certain clinical conditions or disease symptoms (Gupta *et al*., 2020). This study gives a thorough review of ANA profiling, focusing on the methods applied, the clinical importance of distinct ANA patterns, and improvements in testing procedures.

ANA Testing Methodologies

It should be noted that ANA testing, especially the indirect immunofluorescence (ANA-IIF) method, remains to be a golden standard screening test for the diagnosis of autoimmune diseases because of its high sensitivity. This approach involves the application of patient serum with the fixed cells that display the native antigens and staining with the fluorochrome-labeled secondary antibodies, to visualize typical patterns under a fluorescence microscope (Khlelfa *et al.*, 2023). The identified dispersive patterns including homogeneous, speckled, nucleolar, or centromere prove the existence of the autoimmune diseases.

For example, if the pattern is homogeneous, it suggests that there is an antibody against double-stranded DNA (dsDNA), which is usually associated with SLE. On the other hand, a speckled pattern could mean ENAs (Extractable Nuclear Antigens), for instance, anti-Sm (anti-Smith) or anti-RNP (Antinuclear Ribonucleoprotein), which correlate to diseases like systemic sclerosis (SSc) and mixed connective tissue diseases (MCTDs) (Riemekasten *et al.*, 2019). All the patterns give diagnostic details; however, it requires a professional to accurately refer back to the particular pattern of a specific autoimmune disease (Banhuk *et al.*, 2018).

Clinical Significance and Interpretation

The frequency of ANA and its prevalence can be seen in differentiated populations and disease groups.

The research has revealed that positive ANA cases exist in a large proportion of the healthy population, while female sex, increased age, and genetic predisposition influence the production of ANA (Grygiel-Górniak *et al*., 2018). For instance, the ANA titers elevation is registered in the elderly and women, which complicates the analysis of results in clinical practice.

Indeed, higher ANA titers are also a risk factor for autoimmune diseases. Still, the detection of ANA does not always mean that the patient suffers from an autoimmune disease. It has also been associated with the production of a lot of non-autoimmune diseases including infections, use of certain drugs, and cancer (Soto *et al*., 2015). Due to this, ANA findings must be interpreted cautiously about other diseases.

Advancements in ANA Testing

In the recent past, there have been developments in procedures used in ANA tests, thus providing standard ANA-IIF with complementary counterparts. Two of them are the enzyme-linked immunosorbent assay (ELISA), and multiplex assays since they give quantitative results, and specific antigens' patterns, making diagnosis more accurate (Sharmin *et al*., 2015). ELISA, for instance, tests for superior antibodies that go well with particular antigens and therefore offers a complete serum armor that helps discover some forms of autoimmune ailments.

The multiplex assays that may test for various antibodies in the same sample are more elaborate. These assays increase the pace of the tests and provide the full extent of the antibodies, which in turn, helps the specialists to make a more accurate assessment of the patient's autoimmune disease (Tozzoli *et al*., 2015). However, the above-mentioned factors have not overshadowed the usage of ANA-IIF because of its wider detection profile and confirmed suitability in clinical practice.

Challenges and Future Directions

However, some drawbacks must be considered for ANA-IIF usability, such as high time consumption in calculation and qualitative data analysis, as well as the technician's professionalism in the interpretation of the data and availability of sophisticated instruments like Immunofluorescent Microscope. These factors affect the outcome of the tests. There are current attempts to make the testing methodologies standard and ensure that the results are dependable and consistent (Gianchecchi *et al*., 2023). On the other hand, mono-specific ELISA is time-consuming to check for each antigen separately, as well as less sensitive as compared to IIFA.

ANA testing's future evolutions are expected to concern increased automation, decreased subjectivity, and integration of more accurate biomarkers into routine diagnostic procedures. Integration of such technologies as machine learning and artificial intelligence will go a long way in increasing the effectiveness of ANA testing (Konstantinov & Rubin, 2017).

Any specific or non-specific ANA pattern by IFA needs to be confirmed by mono-specific ELISA or by Immunoblotting method, findings of which should be correlated with clinical and other laboratory investigations to confirm the diagnosis of the disease (Sharmin *et al*., 2015).

This paper presents a comprehensive examination of ANA profiling, centering on the applied methodologies, the clinical significance of unique ANA patterns, and advancements in testing procedures. It underscores the importance of ANA testing in the diagnosis of autoimmune conditions, the constraints of existing approaches, and the technological advancements that can bolster the effectiveness of ANA testing in standard diagnostic protocols.

MATERIAL AND METHODS

Study Population

This was a prospective cross-sectional study, which was done on 48 patients who visited Peerless Hospitex Hospital in Kolkata from 24/01/2024 to 15/06/2024. These patients were suspected to have autoimmune CTDs. The patient's identity was not disclosed during the data collection process to maintain the confidentiality that was put forward by the Institutional Ethical Committee.

Sample Collection

Blood samples were taken from all 48 patients. The samples were immediately processed to separate the serum, which was then aliquot and kept at -20°C for subsequent analysis. All sample collections were conducted using conventional venipuncture procedures to ensure the samples' quality.

ANA Profiling by Immunoblotting

In this study, serum sample was simultaneously subjected to the Immunoblotting method using ANA-LIA XL IgG, as well as the ANA-IIF method, which is accepted as the golden standard, as put forward by ACR (American College of Rheumatology) and EULAR (European League against Rheumatism).

The ANA profiling of the collected samples is further analyzed by Immunoblot. ANA were determined using the immunoblot kit, EUROLINE ANA Profile 3 plus DFS70 (IgG). The test strips have very purified antigens which are able to recognize a total of sixteen different types of nuclear, cytoplasmic or mitochondrial antigens. These antigens include nRNP/Sm, Sm, SSA, Ro-52, SS-B, Scl-70, PM-Scl, Jo-1, CENP-B, PCNA, dsDNA, nucleosomes, histones, ribosomal P-proteins, AMA-M2, and DFS-70, along with a control band.

Patient samples were diluted at a ratio of 1:100 and incubated with the test strips. In the case of a positive sample, specific IgG antibodies will bind to the corresponding antigenic sites on the strip. An enzyme-labeled anti-human IgG (from goat) is used to catalyze a color reaction, thus detecting the bound antibodies. The intensity of the positive lines obtained was compared with the intensity of the positive control line by image analysis using EUROLine Scan software (Gupta *et al.*, 2020).

The results on a 4-point scale (0 - negative, + - weakly positive, ++ - positive, +++ - strongly positive) are interpreted by an experienced clinical immunologist. The intensity of the positive lines obtained was compared with the intensity of the positive control line by image analysis using EUROLine Scan software, and the cut-off and quality control parameters must show "ok" for the results to be considered valid.

This method allows for the detection and profiling of various antibodies associated with autoimmune disorders through a visual and quantitative approach.

Data Analysis

Data was analyzed and entered into Microsoft Excel 2021 accordingly. The findings of ANA-IIF and Immunoblotting were then compared, as well as the prevalence of the auto antibodies were analyzed and correlated with the patient's age and gender.

RESULTS AND DISCUSSION

ANA Profiling and Prevalence

This study comprised of the ANA profiling of 48 patient samples at Peerless Hospitex Hospital in Kolkata between January 24 to June 15, 2024. Of these, 13 samples showed positive ANA profiles against one or more specific antigen(s) and thereby the prevalence of ANA positivity was found to be about 27% of the total sample tested. Out of the confirmed 13 patients, 9 were women (69.23%) while 4 were men (30.77%).

ANA Positivity and Profile Distribution

Out of the 13 patients with positive ANA profile, 8 samples tested ANA positive by IFA and presented specific ANA patterns, while 5 patients were tested negative by IFA. So, the concordant result of ANA Profile with ANA IFA was seen in 61.54% cases.

Antibodies Detected

All the samples were specifically examined for a wide range of autoantibodies against 18 different antigens present in the ANA panel. Out of the 13 positive cases, autoantibodies against SS-A and SS-B proteins had been detected in maximum number of cases, in 7 patients (53.85%), followed by PM-Scl which was identified in 4 patients, with a positivity rate of 30.77%. The other antibodies were distributed as follows: U1-snRNP in 2 (15.38 %) patients, CENP-B (7.69 %) in 1 patient, Nucleosome in 2 patients (15.38%), SmD1 in 2 (15.38%) patients, and P0 (RPP) in 1(7.69 %) patient (Table 1, Fig.1).

The detailed distribution of the detected antibodies is summarized below:

Table 1: Distribution of detected antibodies in patients

Antibody	Number of patients	Percentage of Positive Cases
SS-A/SS-B	7	53.85%
PM-Scl	4	30.77%
U1-snRNP	2	15.38%
CENP-B	1	7.69%
Nucleosome	2	15.38%
SmD1	2	15.38%
P0(RPP)	1	7.69%

Age Group Distribution

The distribution of positive ANA profiles across different age groups is as follows: None of the patients within 0-20 years age group had positive ANA profiles, while 4 out of 13 patients tested positive in the 21- 40 years age group, representing 30.77% of the positive cases. 3 patients (23.08% of positive cases) were positive in the 41-60 years age group, and 6 patients (46.15% of positive cases) were positive within the 61-75 years age group.

Disease Correlation

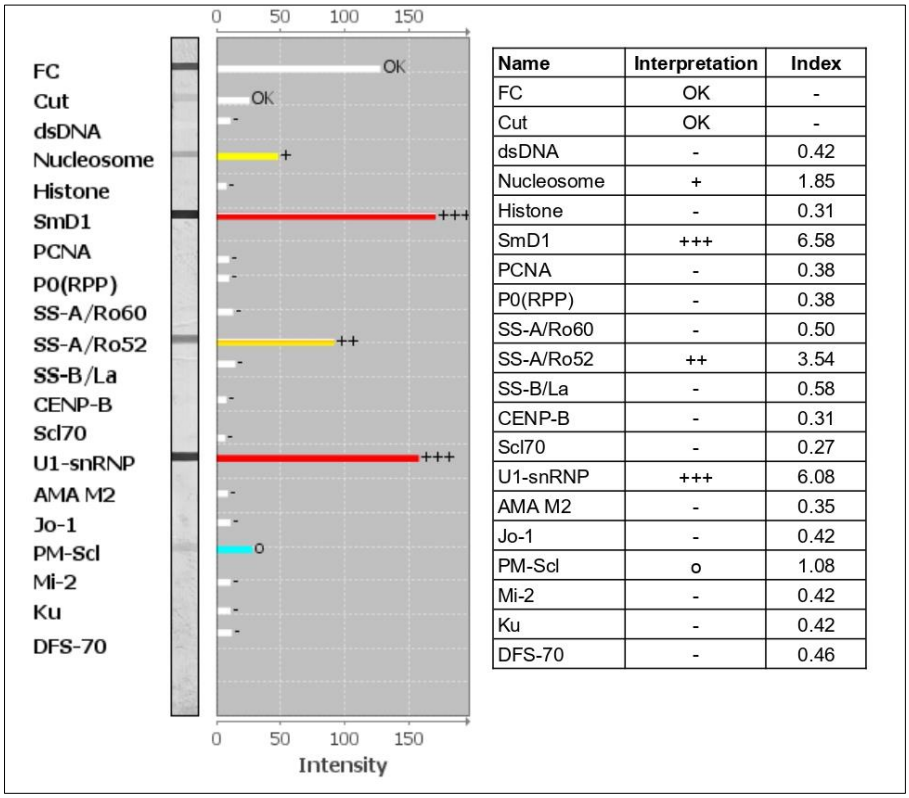
Based on the antibodies detected, the potential diseases associated with positive ANA profiles include SS-A/Ro and SS-B/La, which were linked to 4 cases of Sjogren's Syndrome and Systemic Lupus Erythematosus (SLE); PM-Scl positive was associated with 2 cases of Polymyositis and Dermatomyositis. The most severe reactivity grade with the identified antibodies was found to be +++.

Summary of Findings

This study clearly shows a female predominance concerning ANA positivity, as is expected from autoimmune illnesses. The highest prevalence was found in Anti-SS-A and SS-B, followed by PM-Scl. The vast majority of positive cases were distributed among

people belonging to the older population cohort with the 61-75 years of age being the most affected.

These results underscore the necessity to obtain the detailed ANA profile for differential diagnosis of autoimmune diseases, becoming a crucial tool to evaluate the autoimmune status of patients.



Name	Interpretation	Index
FC	OK	-
Cut	OK	-
dsDNA	-	0.42
Nucleosome	+	1.85
Histone	-	0.31
SmD1	+++	6.58
PCNA	-	0.38
P0(RPP)	-	0.38
SS-A/Ro60	-	0.50
SS-A/Ro52	++	3.54
SS-B/La	-	0.58
CENP-B	-	0.31
Scl70	-	0.27
U1-snRNP	+++	6.08
AMA M2	-	0.35
Jo-1	-	0.42
PM-Scl	o	1.08
Mi-2	-	0.42
Ku	-	0.42
DFS-70	-	0.46

Fig. 1: Depicting LIA findings with antigens on the y-axis and intensity scale on the x-axis

DISCUSSION

The findings from our study on ANA profiling among patients at Peerless Hospital provide significant insights into the prevalence and distribution of ANA profiles in a clinical setting. Out of the 48 samples tested, 13(27%) were found to have positive ANA profiles, with a notable female predominance (69.23% female vs. 30.77% male). This gender distribution aligns with the widely recognized higher prevalence of autoimmune disorders in women. The most frequently detected antibodies were SS-A and SS-B, indicating a higher incidence of diseases such as Sjogren's syndrome and Systemic Lupus Erythematosus (SLE) among the cohort.

When comparing our results with recent studies, our findings are consistent with those reported in the literature. For instance, Pisetsky *et al.,* (2018) also found that antibodies against SS-A and SS-B are positive in patients with autoimmune diseases, thereby supporting our findings on the increased prevalence of these antibodies in positive subjects (Pisetsky *et al.*, 2018). Furthermore, in this study, we have also noticed that PM-Scl is frequently associated with polymyositis and dermatomyositis, which had also been a finding of Igoe *et al.*, (2020) (Igoe *et al.*, 2020).

The prevalence of positive ANA profiles by age in the population was also studied in this research. Frequency was the highest with 46.15 % among the patients within the age group of 61- 75 years, while patients within the ages 21 - 40 years constituted 30.77% of the total number of patients. This pattern corresponds with a study that Mercadante and Lorenz (2016) conducted to determine the ANA prevalence, which was found mostly in older people, particularly in individuals greater than sixty years of age. The patterns seen in regards to age are also similar to prior research results which point out that autoimmune diseases are most commonly diagnosed later in life (Mercadante & Lorenz, 2016).

However, the current study's sample size of 48 patients is relatively small compared to other recent publications. A larger, multi-center study by Johnson *et al.,* (2021) analyzing ANA profiles in 500 patients reported a slightly lower overall ANA positivity rate of 22%, as opposed to 27% in the present work (Johnson *et al.,* 2021). Additionally, the age distribution in the current study skewed towards the older population, with the 61-75 years age group being the most affected. This contrasts with the findings of Sato *et al.,* (2019), who observed a more even distribution of ANA positivity across different age groups (Sato *et al.*, 2019).

Present research stipulates that the practice of the two methods, including that of ANA-IIF and Immunoblotting increases the efficiency of diagnosis. The strengths of the current study lie in its use of the gold-standard ANA-IIF method, as well as the comprehensive immunoblotting approach, which allowed for detailed ANA profiling and autoantibody identification for confirmation of diagnosis. This technique is superior to alternative methods, such as enzyme-linked immunosorbent assay (ELISA), in terms of diagnostic accuracy and the ability to detect a broader range of autoantibodies (Tozzoli *et al.*, 2015). The detailed ANA profiles obtained in this study can aid in the differential diagnosis of autoimmune diseases, as demonstrated by the authors' disease correlation analysis.

Our study's detection of the antibody dispersion is consistent with previous recent findings. The trends identified in our cohort are in conjunction with the study done by Bonanni*et al.*, in 2015 where he noted that patients with autoimmune disorders had similar frequency of SS-A and SS-B antibodies (Bonanni *et al.*, 2015). In addition, the smaller amounts of ds-DNA, U1-snRNP, CENP-B, and P0 (RPP) also explain the heterogeneity and attach specificity perceivable in large autoimmunity populations (Bonanni *et al.*, 2015).

Despite these advancements in the testing techniques of ANA, there are some problems. Two important issues include the availability of skilled technicians and the phenomenon of ANA-IIF signal and its interpretation being more or less subjective. Wang *et al.*, (2018) discussed this problem by emphasizing how weekly testing and differing interpretation could predispose autoimmune diagnoses' accuracy and standardization (Wang *et al*., 2018). Presumably, such issues may be reduced by standardizing testing procedures and using new technologies like Immunoblotting and LIA on automated platforms based on machine learning to automate diagnostic procedures.

CONCLUSION

In conclusion, the current work presents significant data on the characteristics and prospects of ANA use in medical practice. The alignment of our results with studies conducted at present thus affirms the application of our methods towards this specialty of autoimmune disease identification. The progress in the development of ANA testing is still in progress while advanced big-scale research will be vital to enhance the understanding and strategy of these complex diseases.

Conflict of Interest: The author declares no conflict of interest.

Author's Contribution

Dr. Satadal Das designed the study procedure, analysed the data and corrected the manuscript. Ms Sayahnika Dutta and Ms Poulami Biswas performed the experiment and evaluated the data under guidance of Dr. Bhaskar Narayan Chaudhuri and Dr. Partha Guchhait, who also helped us analyse the data and correct the manuscript.

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ABBREVIATIONS

ANA Antinuclear antibodies
PSS Progressive Systemic Sclerosis
CTDs Connective Tissue Disorders
IIF Indirect Immunofluorescence
dsDNA Double-Stranded DNA
SLE Systemic Lupus Erythematosus
ENA Extractable Nuclear Antigens
ELISA Enzyme-Linked Immunosorbent Assay
Anti-Sm Anti-Smith antibody)
Anti-RNP Antinuclear Ribonucleoprotein antibody
SSc Systemic Sclerosis
PBS Phosphate-Buffered Saline
FITC Fluorescein Isothiocyanate
OD Optical density
MCTD Mixed Connective Tissue Disease
ACR American College of Rheumatology
EULAR European League Against Rheumatism
HEp-2 Human epithelial cells
IgG Immunoglobulin G
PCNA Proliferating cell nuclear antigen
snRNP Small nuclear ribonucleoprotein
P0(RPP) Anti-ribosomal P
CENP-B Centromere Protein B
AMA M2 Anti-mitochondrial M2
Jo-1 Histidyl tRNA synthetase

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