Antinuclear Antibodies: A demographic profile from a Tertiary Healthcare Centre of Delhi

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Abstract

Background: Anti-nuclear autoantibodies (ANA), have explicitly been linked to immune complex-mediated autoimmune diseases.Detection and quantitation of autoantibodies are therefore crucial in the diagnosis and treatment.

Objectives: This study aims to evaluate the demographic profile of patients with ANA positivity in a tertiary health care Centre in Delhi,India.

Patients and methods:A total of 938 samples of suspected patients were analyzed over a period of three years from February 2020 to February 2023 for the presence of antinuclear antibodies. Serum ANA levels were measured by ELISA. The data were collected retrospectively and analyzed using SPSS v 23 (IBM Corp.) software.

Results: A total of 104 (11.09%) samples were positive for ANA. Females constituted the majority 84(80.77%) of the total positive samples. Females in the age group of 21 to 40 years showed the highest ANA positivity. Maximum autoimmune disease suspected samples were received from the Medicine Department, followed by Dermatology, Ophthalmology, and Orthopedics departments.58.6% (61 out of 104) of the samples received were from the Indoor patient Department (IPD) as compared to 41.34% (43out of 104) from Outdoor patient department (OPD) (P =0.4592).

Conclusion: Regular follow up of patients with positive ANA might be beneficial for early detection of the related autoimmune conditions. However, more long term follow up studies are needed to support this hypothesis.

Keywords: Antinuclear antibodies; SLE; Autoimmune disorder.

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Introduction

Autoimmune diseases (ADs) are chronic pathological conditions precipitated by the loss of immunological tolerance to self-antigens, which can cause either systemic or organ-specific damage. When immunologic tolerance breaks down, an immune reaction against self-molecules occurs, which results in autoimmune diseases **(Smith and Germolec, 1999)**. Autoimmune diseases have become substantially more prevalent over the past few years owing to factors other than genetic predisposition (**Kumar et al., 2021).**In India,the prevalence of these autoimmune diseases is 10%,(**Premkumar et al.,2014)** compared to a global prevalence of 5.3% (**Cooper et al.,2009**).

It is unclear what triggers the immune response to self-molecules, but studies have suggested links to infections, genetics, and environmental factors (**Smith and Germolec,1999**).The preponderance of connective tissue diseases typically involves an immune humoral response in the presence ofautoantibodies against intracellular antigens. This phenomenon has been linked tomany diseases, including polymyositis, dermatomyositis, mixed connective tissue disease, systemic lupus erythematosus (SLE), and systemic sclerosis (**Fernandez et al., 2003**).

Autoimmune diseases are becoming increasingly common from both clinical and laboratory perspectives. Detection and quantitation of autoantibodies are therefore crucial in the diagnosis and treatment of these diseases (**Meroni and Schur, 2010**). SLE is a systemic autoimmune disorder with a highly heterogenous clinical presentation, primarily affecting women of child bearing age. But this disease can strike at any age, with the symptoms emerging as early as infancy or later in life(**Feng et al., 2014**).

SLE was initially identified in1941 as one of the connective tissue diseases (**Kumar et al., 2009**). SLE can clinically present in a variety of unpredictable ways, making the diagnosis difficult to make and delaying the initiation of treatment (**Heinlen et al., 2007**).

The anti-nuclear autoantibodies (ANA),which have explicitly been linked to immune complex-mediated pathogenesis and end-organ damage, were among the earliest evidence of immune abnormalities found in SLE patients (**Silverman et al., 2008**). ANA testing is relatively non-specific, and false-positive results cause pointless medical evaluations. Even though the absence of ANA virtually excludes the chance of lupus, this fact does not negate the possibility of the disease (**Wandstrat et al., 2006**). About 25% of people have detectable ANAs and 2.5% of people havelevels that are substantially elevated. The enduring presence of autoreactivity in the human population raises the possibility that ANA play a significant role in the normal immune response(**Li et al., 2008**).

Detection and quantitation of autoantibodies are therefore crucial in the diagnosis and treatment of these diseases (**Meroni and Schur, 2010**).This study aims to assess the demographic profile of the patients with ANA positivity in a tertiary healthcare Centre in Delhi.

Patients and Methods

In this study, aanalysis of 938 samples received at the serology division of the Microbiology Department,University College Medical Sciences & GTB Hospital from suspected autoimmune disorder patients was done overa period of three years from February 2020 to February 2023 to look for the presence of antinuclear antibodies.The blood sample received in a vacutainer which was allowed to clot by leaving it undisturbed

atroom temperature. After 15-30 minutes, the sample was centrifuged at 2,000 x g for 10 minutes. The resulting serum was stored at -20^{0}C for testing.

Test Procedure

ANA Screen, an ELISA-based method, for the qualitative detection ofANA autoantibodies in human serum was performed as per the manufacturer's instructions (CalbiotechInc. (CBI), California).The Ab (antibody) index was calculated bydividing the OD value of each sample by the cut-offvalue. The antibody index was interpreted as"No detectable ANA" if it was less than 0.9, a value between 0.9-1.1was borderline , and a value of>1.1 was "Detectable ANA".

Statistical Analysis

The data were entered into an MS Excel spreadsheet program. The software SPSS v 23 (IBM Corp.) was usedfor data analysis. Descriptive statistics were elaborated in the form of means/standard deviations and medians for continuous variables. Frequencies and percentages were used for categorical variables.The Chi-square test was used to compare groups in categorical data. P-value <0.05 was taken as statistically significant.

Results

A total of 938 samples of the suspected patients were analyzed for the presence of ANA antibodies over a duration of 3 years between 2019-2022. A total of 104 (11.09%) samples were positive for ANA (**Fig.1**).

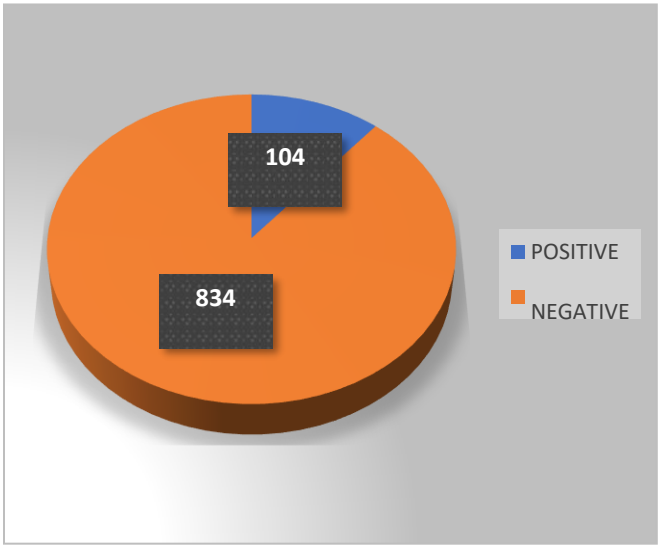

Fig.1. Overall percentageof ANApositivity among all samples (n=938)

(**Fig.2**) depicts the gender-wise percentage distribution in ANA-positive patients.Females constituted the majority 84 (80.77%) of the total positive samples, whereas the males comprised the remaining.

The age-group-wise distribution of ANA positivity among males and females is depicted in (**Fig.3**) .Females in the age group of 21to 40 years showed the highest ANA positivity,while those older than 60 showed the least positivity without any inclination toward either gender. The age of the patients ranged from 2 to 94 years, with a mean of 33.9±15.65 years and median of 33 years.

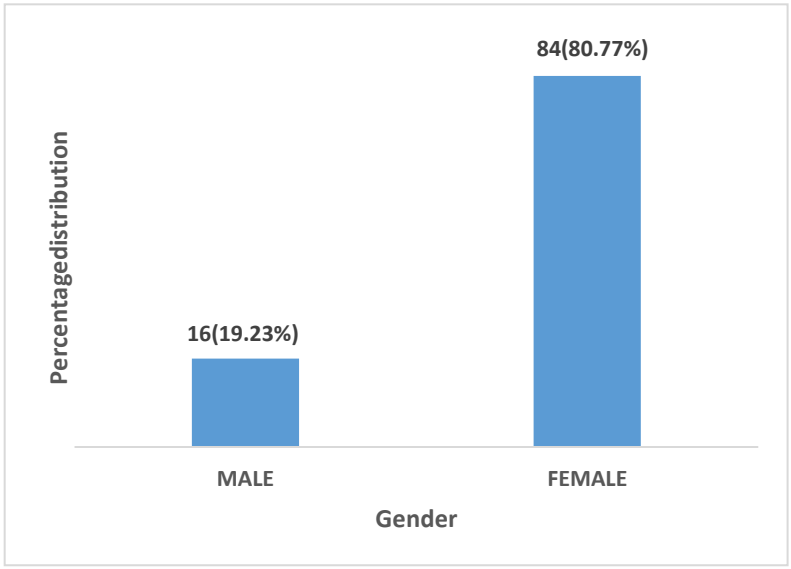

Fig.2. Gender-wise percentage distribution in ANA-positive patients (n=104)

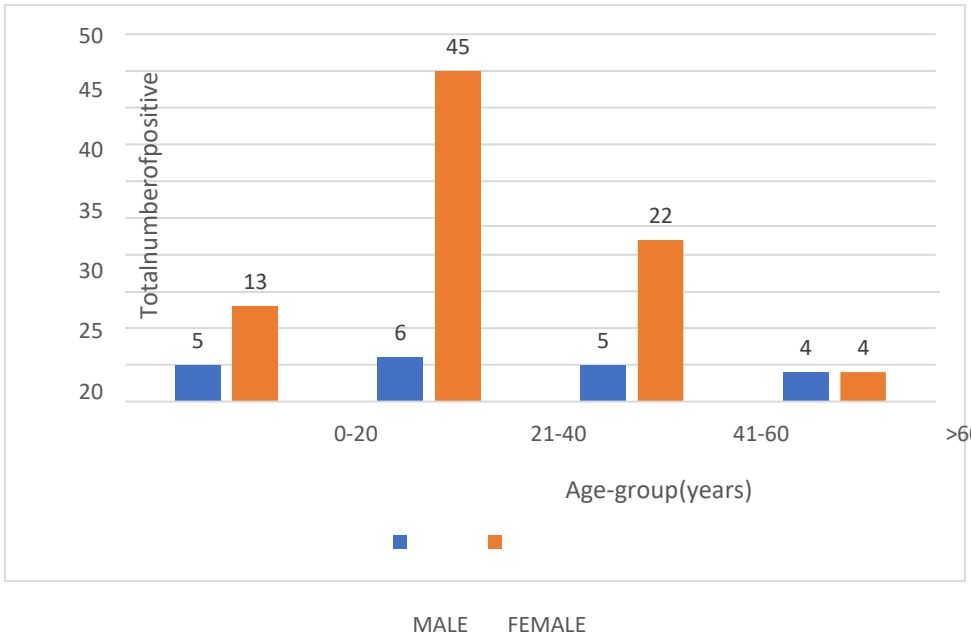

Fig.3. Age-groupwisedistributionofANApositivityamongmaleandfemalepatients (n=104)

In (**Table.1**), the overall distribution and positivity of the samples across various clinical departments are shown. A total of 720 samples were from the Medicine department, 55 each from Dermatology and Ophthalmology departments, 43 from Orthopedics, 31 from Gynaecology, 23 from Paediatrics, and11 were from the Surgery departments.

Table1. Overall distribution and positivity of the samples across the various clinical departments(n=938)

Department	Total samples	Total positive samples (%)
Medicine	720	84(80.77%)
Surgery	11	0 (0%)
Dermatology	55	8 (7.69%)
Ophthalmology	55	1 (0.96%)
Gynaecology	31	4 (3.85%)
Pediatrics	23	2 (1.92%)
Orthopedics	43	5 (4.81%)
Total	938	104

Table.2) depicts the distribution of samples across the IPD and OPD of the various clinical disciplines. The majority of the samples were from the Medicine ward, followed by Dermatology, Ophthalmology, and Orthopedics departments.Maximum ANA positivity was seen in samples from Orthopedics OPD (18.60%).

Table 2. Distribution of samples across the IPD and OPD of the various clinical disciplines(n=938)

Department	Location	Number of samples	Positive samples
Medicine	**OPD**	261	33(4.58%)
	IPD	459	51(7.08%)
Surgery	**OPD**	6	0 (0%)
	IPD	5	0 (0%)
Dermatology	**OPD**	23	2 (3.63%)
	IPD	32	6(10.9%)
Ophthalmology	**OPD**	12	0 (0%)
	IPD	43	1 (1.81%)
Gynaecology	**OPD**	18	0 (0%)
	IPD	13	0 (0%)
Paediatrics	**OPD**	7	0 (0%)
	IPD	16	2 (8.69%)
Orthopaedics	**OPD**	26	8(18.60%)
	IPD	17	1 (2.32%)

Discussion

Systemic auto immune diseases including SLE can impact any organ and exhibit a wide range of clinical and serological characteristics (**Sisó et al, 2008**). Diagnosis, both by clinical as well as laboratory methods in the early phase is thus very important to prevent damage to various organ systems. However, with currently available diagnostic tests it is very challenging to make early and accurate identification of lupus because of low sensitivity and specificity(**Li et al., 2011**).

The prevalence of benign autoimmune disorders is not well-documented because routine autoantibody levels are not frequently assessed in the general community. As previously stated, antinuclear antibodies are present in some autoimmune diseases as well as in other non-autoimmune conditions (**Fernandez et al., 2003**). ANA testing is thus, recommended for individuals who have disease manifestations in two or more organ systems (**Wandstrat et al., 2006**).

Our study reported an overall positivity of 11.09% for ANA.Since more than 99% of SLE patients have significant levels of this autoantibody found at some point during the course of the disease, ANA positivity is essentially necessary to establish a diagnosis of lupus. However, due to the low prevalence of SLE and also because approximately 2.5% of the general population has significantly elevated ANA levels (**Wandstrat et al., 2006;Li et al.,2011**), positive ANA values should be cautiously interpreted in healthy subjects without clinical signs. On the other side, antibodies may appear in the serum many years before the diagnosis of autoimmune disease. Moreover, the presence of ANA in the human population suggests that antinuclear antibodies might be an important component of the normal immune response. Thus,it is also true that subjects who are in preclinical SLE stages are represented in the ANA-positive healthy population. Bearing in mind that ANAs can be present in healthy subjects as well as those with non-rheumatic diseases, the monitoring of their amount and further rheumatologic diagnosis should be mentioned only in cases with features suggestive of autoimmune diseases.The presence of ANA without signs of connective tissue disorders does not require periodic rheumatic check-ups (**Li et al., 2011**). A recent study reported an ANA positivity rate of 7.09%, similar to our study findings (**Semchuk et al., 2007**). In contrast to our findings, a study found a higher ANA prevalence rate of 22.6% (**Fernandez et al.,2003**).

Our study reported considerably higher ANA values in females as compared to males and was statistically significant($P < 0.0001$).The higher prevalence of ANA antibodies in females is associated with increased adipose tissue content in women which is capable of producing pro-inflammatory cytokines and estrogen which can further cause different autoimmune disorders including SLE. Pregnancy and childbirth make an intense changes in female hormonal levels resulting in immune modulation and may resultin the subsequent development of SLE (**Ganguli et al.,2019**).Our result is consistent with the findings of another study done to study the risk factors of ANA positivity in healthy persons (**Li et al., 2011**). Similar results were found in another study on the Canadian rural population (**Semchuk et al., 2007**). In the majority of the studies,ANA positivity was found to be higher in female patients, suggesting that female patients may be at increased risk for autoimmune diseases.However, the association between strong ANA positivity and the female gender is still not fully understood. Women in the agegroup of 21 to 40years had the

maximum ANA positivity in our study suggesting that younger females might have an immune dysregulation that makes them more susceptible to SLE-like conditions. Similar results have been found in other studies (**Wandstrat et al., 2006; Li et al., 2011;Feng et al., 2014**).

According to a study on the associations of clinical features and prognosis with age in patients with SLE, the female-to-male gender ratio decreases with age. This trend likely reflects the association between SLE and estrogen status because older female patients produce less estrogen(**Feng et al., 2014**).

SLE affects multiple systems, but skin and joints are the most commonly affected (**Mckinley et al., 1995**). In our study, maximum SLE suspected samples were received from the Medicine department, followed by Dermatology, Ophthalmology, and Orthopedics departments. A study reported arthritis to be the most common clinical manifestation of SLE (**Ahmed et al., 2017**).Constitutional symptoms in the form of fever, fatigability, and weight loss are also seen in SLE (**Mckinley et al., 1995; Ahmed et al., 2017**).Lupus nephritis, neurological disorders, and hematological abnormalities are common manifestations of this disease (**Cervera et al., 2003; Mok et al.,2014; Ahmed et al.,2017**).

In our study, 58.6% (61 out of 104) of the samples received were from the indoor admitted patients departments as compared to 41.34% (43 out of 104) from the out-patient department though it had no statistical significance.

This study has certain limitations. Firstly, our study was conducted on a small population, a study involving more subjects would yield more conclusive results. Our study is based on laboratory confirmed cases representing only the tip of the iceberg in the overall pattern of disease. Thirdly,ANA testing by ELISA is useful only as an initial screening approach, but is not disease specific. Hence, Immunofluorescence assay (IFA) test having high disease specificity would be more confirmatory. Further studies involving IFA testing for autoimmune diseases in future would,thus, be more conclusive and will give us a more detailed picture.

Conclusion

Despite its poor specificity, the elevated ANA titer remains one of the diagnostic indicators for autoimmune diseases even though the elevated titer can also be due to drugs, some infectious diseases,and malignancies.The identification of this disease in its early stages could be made possible by a careful interpretation of the ANA results and by taking into consideration the clinical and demographic profile of the patients.Regular follow up of patients with positive ANA might be beneficial for early detection of the related autoimmune conditions. However, more long-term follow-up studies are needed to support this hypothesis.

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References

- **Ahmed N, Shigidi M, Al AN, Abdelrahman H, Taha E. (2017).** Clinical features and antinuclear antibodies profile among adults with systemic lupus erythematosus and lupus nephritis:across-sectional study. Pan Afr Med J, 27:114.
- **Cervera R, Khamashta MA, Font J, Sebastiani GD, Gil A, Lavilla P,et al.(2003).**Morbidity and

Mortality in Systemic Lupus Erythematosus During a 10-Year Period: A Comparison of Early and Late Manifestations in a Cohort of 1,000 Patients. Medicine(Baltimore), 82(5):299–3.

- **Cooper GS, Bynum MLK, Somers EC. (2009).** Recent insights in the epidemiology of autoimmune diseases: Improved prevalence estimates and understanding of clustering of diseases.J Autoimmun,33(3–4):197–207.
- **Feng X, Zou Y, Pan W, Wang X, Wu M, Zhang M, et al. (2014).** Associations of clinical features and prognosis with age at disease onset in patients with systemic lupus erythematosus.Lupus,23(3):327–34.
- **Fernandez SAV, Lobo AZC, Najjar Z, de Oliveira P, Fukumori LMI, Périgo AM, et al.(2003).** Prevalence of antinuclear autoantibodies in the serum of normal blood donors. Rev.Hosp. Clín. Fac. Med,58(6):315-319.
- **Ganguli P, Chakrabarty BK, Chopra M, Ray S. (2019).** Antinuclear Antibodies Status and Related Risk Factors in Non pregnant Adult Indian Women. Indian J Med Biochem,23(2):250–3.
- **Heinlen LD, McClain MT, Merrill J, Akbarali YW, Edgerton CC, HarleyJ B,et al. (2007).** Clinical criteria for systemic lupus erythematosus precede diagnosis, and associated autoantibodies are present before clinical symptoms. Arthritis Rheum, 56(7):2344–51.
- **Kumar U, Kanjilal M, Ramakrishnan L, Thangavelu M .(2021).** Prevalence of pre-clinical autoimmunity in the normal adult population residing in a metropolitan city of India: A cross-sectionalstudy. EurJ Rheumatol, 5;8(2):79–83.
- **Kumar Y, Bhatia A, Minz R (2009).** Antinuclear antibodies and their detection methods in diagnosis of connective tissue diseases:a journey revisited. Diagn Pathol, 4(1):1.
- **Li QZ, Karp DR, Quan J, Branch VK, Zhou J, Lian Y, et al. (2011).** Risk factors for ANApositivityin healthy persons. Arthritis Res Ther, 13(2):R38.
- **Mckinley PS, Ouellette SC, Winkel GH. (1995).** The contributions of disease activity, sleep patterns, and depression to fatigue in systemic lupus erythematosus. Arthritis Rheum, 38(6):826–34.
- **Meroni PL, Schur PH .(2010).** ANA screening: an old test with newr ecommendations. Ann Rheum Dis, 69(8):1420–2.
- **Mok CC, Yap DY, Navarra SV, Liu Z hong, Zhao M hui, Lu L, et al.(2014).** Overview of lupus nephritis management guidelines and perspective from Asia: Overview of lupus nephritis management guidelines and perspective from Asia. Nephrology, 19(1):11–20.
- **Prem Kumar B, Srinivasa Murthy M , Rajagopal K , Nagaprabu VN,Sree Madhuri P. (2014).** Epidemiology of Autoimmune Disorders with Special Reference to Rheumatoid Arthritis from a Tertiary Care Center. Indian J Pharm Pract,30;7(3):50–60.
- **Semchuk KM, Rosenberg AM,**

McDuffie HH, Cessna AJ, Pahwa P, Irvine DG. (2007). Antinuclear Antibodies and Bromoxynil Exposure in a Rural Sample. J Toxicol Environ HealthA,70(7):638–57.

- **Silverman GJ, Srikrishnan R, Germar K, Goodyear CS, Andrews KA, Ginzler EM, et al.(2008).** Genetic imprinting of autoantibody repertoires in systemic lupus erythematosus patients.ClinExp Immunol, 153(1):102–16.
- **Sisó A, Ramos-Casals M, Bové A, Brito-Zerón P, Soria N, Muñoz S, et al. (2008).** Previous antimalarial therapy in patients diagnosed with lupus nephritis: Influence on outcomes and survival. Lupus,17(4):281–8.
- **Smith DA, Germolec DR.(1999).** Introduction to immunology and autoimmunity.Environ Health Perspect, 107(5):661-665.
- **Wandstrat A, Carrjohnson F, Branch V, Gray H, Fairhurst A, Reimold A, et al. (2006).**Autoantibody profiling to identify individuals at risk for systemic lupus erythematosus.J Autoimmun,27(3):153–60.