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Original Research Article

Clinical Profile of Patients with Positive Antinuclear Antibodies in Tertiary Care Centre

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Abstract:

Introduction: Antinuclear antibodies (ANA) are a group of antibodies that bind to components of the nucleus. ANA is the telltale sign of systemic autoimmune disease and thus can be used as a screening tool for autoimmune disease.

Methodology: A prospective observational study was conducted at a tertiary care hospital from January 2019 to December 2019. This study aimed to evaluate the clinical presentation of patients with positive antinuclear antibodies. A hundred symptomatic patients with ANA-positive were selected for the study after institutional ethics committee clearance.

Result: Out of 100 patients, 83% were female and 17% were male. The most common clinical manifestations in this study were fever (72%), Anemia (63%), Joint pain (53%), Skin Rash (32%), Oral ulcer (24%), Thrombocytopenia and Renal involvement (23%).

Conclusion: Autoimmune disease should be suspected in patients presenting with fever, anaemia and joint pain, particularly in female patients of age groups 20-40 years. In this study female to male ratio was 8:1. For the confirmation and categorizing of disease the Extractable nuclear antibody ENA test should be advised for better patient care. Among the specific diagnoses after the ENA test, the most common connective tissue disorder was SLE (53.9%).

Keywords: Autoimmune disease, Antinuclear Antibody (ANA), Extractable nuclear antibody (ENA), Systemic lupus erythematosus (SLE), Overlap syndrome (OS), Mixed connective tissue disease (MCTD), Systemic sclerosis (SSc), Sjogren's syndrome (SjS), undifferentiated connective tissue disease (UCTD).

Introduction

Autoimmune serology like antinuclear antibodies (ANA) is one of the most frequently requested tests in India as a screening and supporting diagnostic tool for autoimmune disease [1].

Antinuclear antibodies are antibodies that react against primarily self-antigens in the nucleus [2]. Those antibodies specifically directed at "normal" proteins in the nucleus are referred to as antinuclear antibodies. While most individuals possess autoantibodies in minimal quantities, elevated levels of these autoantibodies suggest the presence of an autoimmune disease. Most people who have positive ANA do not suffer from an autoimmune disease, and most of them are also unlikely to develop one. This is related to the fact that the prevalence of all autoimmune disorders is 5-7% [3]. The detection of antinuclear antibodies is regarded as a significant complement to the diagnosis and categorization of ANA-associated rheumatic diseases, including conditions like Systemic Lupus Erythematosus (SLE), Systemic Sclerosis, Mixed Connective Tissue Disease, Sjögren's Syndrome, and others [4]. The female gender is a risk factor for ANA positivity, and positivity in young women is of greater concern [5].

The impact of ANA-associated rheumatic diseases and their complications on quality of life, as well as their implications for morbidity and mortality, have been underscored in various trials. The detection of ANA involves the application of serum to a human cell line (Hep-2 cells), followed by examination through immunofluorescence microscopy. A positive result is typically indicated by an antibody titer exceeding 1:80 using this method. It's important to

note that a positive ANA test signifies the presence of autoantibodies. Roughly 25% of patients with connective tissue diseases exhibit an overlap syndrome, showcasing characteristics of various conditions such as SLE, SSc, Polymyositis, or Dermatomyositis alongside Rheumatoid Arthritis and Sjögren's Syndrome, either concurrently or successively as the disease progresses.

Severity varies from a mild disease with rash and fever or arthritis to a serious illness with renal failure and central nervous system involvement [6].

According to Christie et al's study, individuals exhibiting "dense fine speckled (DFS) patterns" and possessing "isolated anti-DFS70 autoantibodies" may not necessitate an immediate referral to a specialist. Conversely, those with the classic "homogeneous" ANA pattern coupled with ds-DNA antibodies should undergo prompt assessment for an ANA-associated rheumatic disease (AARD). In this context, achieving an early and accurate diagnosis is a critical objective [7].

Healthcare providers request tests for anti-ENA antibodies to confirm a diagnosis of Connective Tissue Diseases (CTD) in individuals displaying indicative clinical features. Additionally, these tests may be employed to rule out CTDs in patients with limited or ambiguous clinical findings. Furthermore, they are utilized to sub-classify patients already diagnosed with CTDs into specific groups and, on occasion, to monitor disease activity [8].

The purpose of our study is to determine the clinical patterns of diseases in positive and symptomatic ANA patients at tertiary care centres.

Materials and Methods

One hundred Symptomatic patients with ANA-positive reports were selected for study at the Indira Gandhi Institute of Medical Sciences (IGIMS), Patna, Bihar, between January 2019 and December 2019. It was a prospective observational hospital-based study and the patients who participated in this study underwent a clinical evaluation and investigation according to the need. The study was approved by the Institutional Ethics Committee of IGIMS, Patna (approval number 666/IEC/IGIMS/2018, dated 09 December 2018). Data tracking was performed using Microsoft Excel.

Inclusion criteria:

1. Aged 20 years or older.
2. Symptomatic ANA-positive patients.
3. Patients willing to give informed consent for the study were selected.

Exclusion criteria:

1. Pregnant patients.
2. Unwilling or unable to comply with protocol.

Detailed history, general examination, and systemic examination were done for enrolled patients. Laboratory investigations (complete blood count, Kidney function test, Liver function test, Random Blood sugar level, Thyroid profile, HIV, ENA profile) were done. Rheumatoid factor and anti-CCP were done in patients presenting with symmetrical polyarthritis.

Creatine phosphokinase (CPK) in patients complaining of myalgia. ANA tests were done in the IGIMS lab by applying serum to the Hep2 cell line and then immunofluorescence microscopy was done. An antibody titre of >1:80 is usually considered positive with this method. Radiological investigations (chest x-ray, knee joint x-ray in patients complaining of Knee pain) were done. ENA test was done using the immunoblot method; the machine used was Euroblot. Antibodies tested were U1-RNP, ds-DNA, Anti-Sm, SS-A, RO-52, SS-B/La, Scl-70, PM-scl, Jo-1, Nucleosomes, Histones, Rib-P protein and AMA-M2. “Mucosal biopsy” in suspected cases of Sjögren’s syndrome was done [9].

Results

Among the total 100 ANA-positive patients, male patients were 17 and female patients were 83 respectively (Figure 1).

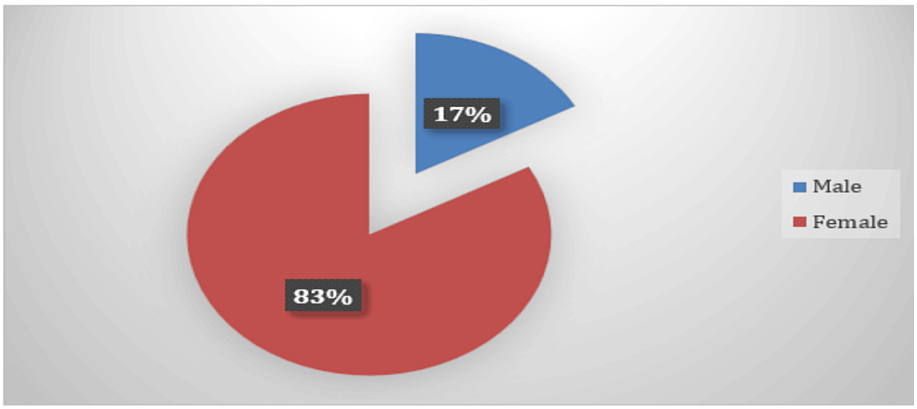

Figure 1: ANA-positive male and female patients

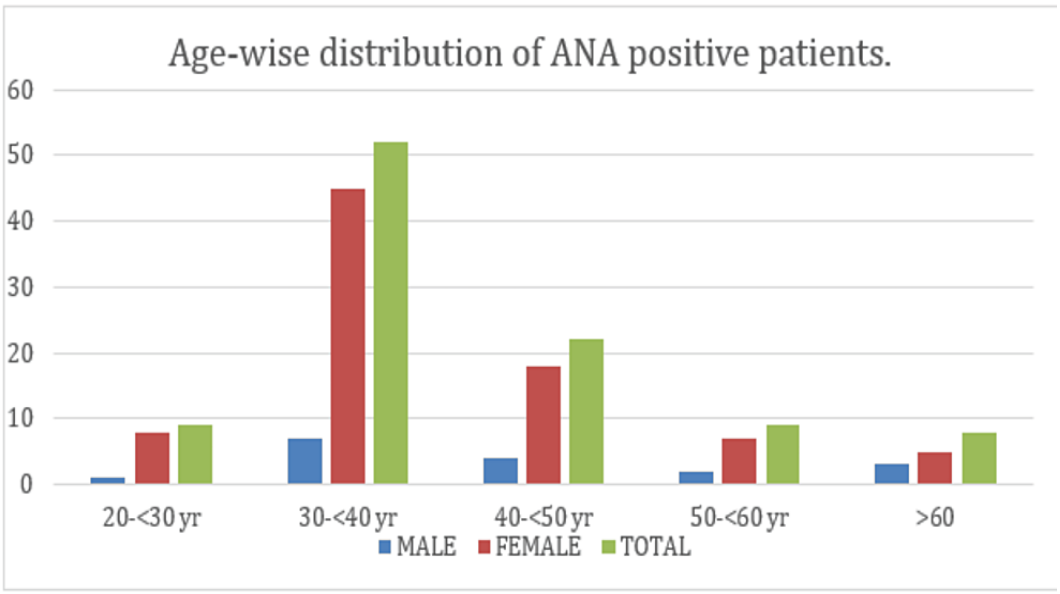

Figure 2: Age-wise group distributions of ANA-positive patients

Most patients are in age groups 30-40 years, and the next common age group is 40-50 years group (Table 1).

Table 1: Clinical manifestation of patients

Clinical Manifestations	Male	Female	No. Of Patients
Fever	9 (12.5%)	63 (87.5)	72
Skin Rash	0(0%)	32 (100)	32
Joint Pain	6(11.3%)	47 (88.6)	53
Renal Involment	5(21.7%)	18 (78.2)	23
Pleural Effusion	1(16.6%)	5 (83.3)	6
Anemia	5(7.9%)	58 (92)	63
Thrombocytopenia	3(13%)	20 (86.9)	23
Skin Thickening	0(0%)	6 (100)	6
Oral Ulcers	3(12.5%)	21 (87.5)	24
Raynaud's Phenomena	2(15.3%)	13 (86.6)	15
Psychosis	1(10%)	9 (90)	10

Table 2: Association between common symptoms and specific diseases

Clinical Manifestations	SLE	OS	MCTD	SSc	SJS	UCTD	No. Of Patients
Fever	44	15	8	0	0	5	72
Skin Rash	20	7	5	0	0	0	32
Joint Pain	30	12	7	0	1	3	53
Renal Involvement	14	5	3	0	0	0	23
Pleural Effusion	3	1	2	0	0	0	6
Anemia	42	11	8	0	0	2	63
Thrombocytopenia	14	4	4	1	0	0	23
Skin Thickening	0	3	0	3	0	0	6
Oral Ulcer	11	6	3	3	1	0	24
Raynaud's Phenomena	7	3	3	2	0	0	15
Psychosis	4	2	2	2	0	0	10

(SLE- Systemic lupus erythematosus, OS- Overlap syndrome, MCTD- Mixed connective tissue disease, SSc- Systemic sclerosis, SJS- Sjögren syndrome, UCTD- Undifferentiated connective tissue disease)

In this study, the most common clinical manifestations were fever (72%), (Table 1,2) Anemia (63%), Joint pain (53%), Skin Rash (32%), Oral ulcer (24%), Thrombocytopenia and Renal involvement (23%).

The symptomatic 100 patients who tested positive for ANA were subsequently advised to undergo extractable nuclear antigen (ENA) tests. Out of these, 89 patients received follow-up ENA test reports. Among them, 81 patients had positive ENA test results, while 8 patients showed negative ENA tests. Based on the ANA/ENA reports and clinical manifestations, we conducted specific diagnoses of

autoimmune diseases using different classification criteria.

Table 3: Specific diagnosis

Diagnosis	Male	%	Female	%	Number Of Cases
SLE	5	11.6%	43	89.5%	48
Overlap Syndrome	4	20%	16	80%	20
MCTD	2	18.1%	9	81.8%	11
Systemic Sclerosis	0	0%	3	100%	3
Sjogren's Syndrome	0	0%	2	100%	2
Undifferentiated CTD	1	20%	4	80%	5
Total	12	13.4%	77	86.5%	89

Systemic Lupus Erythematosus (SLE) was diagnosed according to the European League against Rheumatism (EULAR)/American College of Rheumatology (ACR) 2019 classification criteria [10].

Overlap syndromes were defined as conditions meeting the criteria of at least two connective tissue diseases occurring simultaneously or at different times in the same patient. Mixed Connective Tissue Disease is a systemic autoimmune disease characterized by overlapping features of at least two connective tissue diseases, which may include SLE, Systemic Sclerosis, Polymyositis, Dermatomyositis, and Rheumatoid Arthritis, along with the presence of anti-U_1-Ribonucleoprotein [11]. Sjögren's Syndrome diagnosis relied on the American College of Rheumatology classification criteria for Sjögren's Syndrome [12].

Among the specific diagnoses after the ENA test, the most common connective tissue disorder is found to be SLE (53.9%); Female to male ratio in our study is 8:1 for SLE. The second most common disorder was found to be overlap syndrome (22.4%), female to a male ratio is 4:1. The third most common disease is mixed Connective tissue disorder (12.3%), female to a male ratio is 4.5:1. Systemic sclerosis and Sjogren's syndrome are also confirmed in 3 and 2 patients respectively, and all the patients are females. 5 patients were not able to fulfil the criteria of any specific disease and because of symptomatic ANA positivity, were put into undifferentiated connective tissue disease (Table 3).

Discussion

In the present study,100 patients were enrolled. 83% were female, and 17% were male. Female gender could be a risk factor for an autoimmune disorder [13]. Now, studies have shown a relationship between female sex hormones and the occurrence of autoimmune disease. Sex hormones have a direct effect on immune cells of both innate and acquired immune systems [14].

The female-to-male ratio was 8:1 for SLE in our study whereas Ghosh et al. reported a female-to-male ratio of 14:1 [15]

Binoy et al reported a female-to-male ratio of 19:1 in SLE patients. Another Indian series by Malviya et al. had a female-to-male ratio of 8:1 in SLE [16]. Most patients belong to age groups of 30-40 years (52%) and the mean age was 36 years, while in the study done by Satoh M et al. common age group was 40 to 60 years of age [17]. The next common age groups were between 40 and 50 years in this study. In the present study, fever was the most common manifestation overall for ANA-positive patients, which was present in 72% of patients. The anaemia was present in 63%, while the joint pain (53%). Skin Rash (32%) and thickening of skin(6%) were found only in females in our study.

Among the specific diagnoses after the ENA test, the most common connective tissue disorder was SLE (53.9%); the female-to-male ratio in our study is 8:1 for SLE, which is similar to the study by Malviya et al. The second most common disorder found was overlap syndrome (22.4%). The third most common disease was mixed connective tissue disorder (12.3%). Systemic sclerosis and Sjogren's syndrome were also confirmed in 3 and 2 patients, respectively. Five patients were not able to fulfil the criteria of any specific disease and, given symptomatic ANA positivity, were put into undifferentiated CTDs.

Ranjana Walker-Minz et al in the study also reported female preponderance with an F: M ratio of 3:1 among ANA-positive patients. They also reported that SLE was the most common clinical diagnosis among ANA-positive CTDs, followed by scleroderma [18].

Limitation

This study was done in a small study group, so there is a need for a large multicentric study for a better understanding of the disease.

Conclusion

The consideration of autoimmune disease becomes crucial when patients exhibit symptoms such as fever and/or joint pain, especially among females aged 30-40.

In practical terms, the request for anti-ENA antibody testing is typically made after the confirmation of a positive ANA test.

Therefore, a two-stage testing approach is generally recommended, involving an initial ANA screening test followed by a confirmatory anti-ENA antibody test. The ENA test plays a significant role in supporting specific diagnoses and categorizing patients accordingly.

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