Research article

Autoantibody Profiles and Clinical Correlations in Systemic Sclerosis: A Cross-Sectional Study

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Article Info

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Keywords

Systemic sclerosis, Antinuclear antibodies, Anti-Scl-70, Interstitial lung disease

Abstract

Background: Systemic sclerosis (SSc) is a rare autoimmune disease marked by immune dysregulation, vascular injury, and fibrosis. Antinuclear antibodies (ANA) are pivotal for diagnosis and prognosis, yet Indian data remain scarce. This study assessed ANA patterns and autoantibody profiles in SSc and their clinical relevance.

Methods: A cross-sectional study was conducted in the Department of Dermatology, AIIMS Patna, from September 2022 to September 2023. Eighty-five patients fulfilling ACR/EULAR criteria for SSc were enrolled. Clinical features were documented using a structured proforma, and patients were classified into diffuse cutaneous (dcSSc) and limited cutaneous (lcSSc) subsets. ANA screening was performed by indirect immunofluorescence (IIF) on HEp2 cells, followed by autoantibody profiling with line immunoassay (LIA). Statistical analyses were performed using JAMOVI v2.3.28, with $p<0.05$ considered significant.

Results: Of 85 patients, 70 (82.4%) were female (ratio of 5:1), with a mean age of 34.7 ± 10.7 years. dcSSc accounted for 55.3%. Common manifestations included arthralgia (69.4%), respiratory symptoms (68.2%), and interstitial lung disease (ILD) (64.1%). Homogeneous ANA was the predominant IIF pattern (41.2%), followed by centromere (10.6%) and speckled (8.2%). LIA revealed anti-Scl70 as the most frequent autoantibody (58.3%), significantly associated with ILD and Raynaud's phenomenon. Anti-U1 snRNP and anti-PMScl antibodies correlated with musculoskeletal involvement.

Conclusion: SSc in this cohort showed female predominance with frequent arthralgia and respiratory symptoms. Anti-Scl-70 antibodies were most common, strongly linked to diffuse disease and ILD. ANA profiling provides disease characterization in Indian SSc.

Introduction

Systemic sclerosis (SSc) is a heterogeneous, uncommon autoimmune connective tissue disease characterized by a triad of immune dysregulation, widespread microvascular injury, and progressive fibrosis affecting the skin and internal organs [1,2]. The estimated prevalence in India is approximately 12 per 100,000 population, with a striking female predominance [1-3] and a reported female-male ratio ranging from 3:1 to 8:1 [3,4]. The pathogenesis of systemic sclerosis is complex and involves endothelial dysfunction, chronic inflammation, and aberrant fibroblast activation, leading to excessive extracellular matrix deposition. Clinically, SSc affects not only the skin but also several internal organs, including the lungs, heart, kidneys, gastrointestinal tract, and musculoskeletal system, resulting in diverse clinical manifestations and variable disease outcomes [2,5,6]. Based on the extent of cutaneous involvement, SSc is classified into two major subsets: (lcSSc) and dcSSc [4,7]. lcSSc is characterized by fibrosis confined to the acral regions, including the face and distal extremities, whereas dcSSc involves the trunk and proximal limbs and is frequently associated with earlier and more severe internal organ involvement [1,7]. Autoantibodies are a hallmark of SSc and play a pivotal role in diagnosis, prognosis, and disease stratification. ANA are the most frequently detected [2,8]. IIF on HEp2 cells is considered the gold standard for screening [8] because of its high sensitivity and ability to identify distinct staining patterns. Enzyme-linked immunosorbent assay (ELISA), although more specific, allows detection and quantification of individual autoantibodies; however, in recent years, LIA has emerged as a reliable and efficient technique for ANA profiling. This method enables simultaneous detection of multiple nuclear and cytoplasmic antigens on a single nitrocellulose strip, offering faster processing time with diagnostic performance comparable to ELISA [9]. Given the established associations between specific autoantibodies and distinct clinical phenotypes, a comprehensive autoantibody profiling provides valuable insights into disease stratification, prognosis, and clinical management of patients with systemic sclerosis [2,10]. Among the autoantibodies, anti-topoisomerase I (Anti Scl70), anti-centromere, and anti-PMScl are particularly relevant, as they correlate with distinct clinical phenotypes and organ involvement. For instance, Anti Scl70 is strongly associated with ILD [5,6], while anti-centromere antibodies are more frequent in lcSSc and linked to vascular manifestations [4]. Despite extensive research in Western populations, data from Indian cohorts remain limited. Regional variations in clinical presentation and autoantibody distribution highlight the importance of population-specific studies [10]. This study was therefore undertaken to evaluate the clinical features, ANA patterns, and autoantibody profiles of SSc patients from Bihar and to explore their associations with disease subtypes and organ involvement.

Materials and Methods

Study Design and Setting

This was an observational cross-sectional study conducted in the Department of Dermatology and the Department of Biochemistryat All India Institute of Medical Sciences (AIIMS), Patna, Bihar, India, between September 2022 and September 2023. All patients presenting to the outpatient and inpatient services with a diagnosis of SSc during the study period were approached for inclusion.

Study Population

A total of 85 adult patients meeting the American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) classification criteria for SSc were enrolled [7,11]. The following groups were excluded during patient selection:

Individuals with overlap connective tissue diseases Individuals with other autoimmune rheumatic disorders lacking features of systemic sclerosis

Clinical Evaluation

Demographic details, disease duration, and clinical features were recorded using a structured proforma. Organ involvement was assessed through clinical examination and relevant investigations, including high-resolution computed tomography (HRCT) of the thorax for ILD [6], urine analysis for proteinuria, and gastrointestinal symptom evaluation.

Clinical Classification

Patients were further categorized into dcSSc and lcSSc subtypes based on the extent of skin involvement. dcSSc was defined by the rapid progression of skin thickening from fingers to trunk, often accompanied by severe constitutional symptoms, pulmonary fibrosis, and cardiac or renal involvement. lcSSc was characterized by long-standing Raynaud's phenomenon, limited skin thickening confined to the face and distal extremities, and milder systemic features. Patients without skin thickening were also included in the lcSSc group.

Autoantibody Testing

Serum samples of all the selected patients underwent ANA screening by IIF, subsequent ANA profiling by using LIA. For IIF, serum samples were tested using the EUROIMMUN biochip technique (Germany), which employs HEp2 cells and monkey liver tissue sections. Samples were diluted 1:100 in phosphate-buffered saline and incubated with fluorescein-labelled anti-human globulin. Fluorescence was visualized under an inverted microscope at 10× and 40× magnification, with intensity compared against positive and negative controls [12].
For autoantibody profiling, LIA was performed using commercially available (human diagnostics, made in India, in collaboration with Germany) strips coated with nuclear and cytoplasmic antigens. Patient sera were incubated with the strips, and bound antibodies were detected using horseradish peroxidase (HRP)labeled antihuman IgG. The presence of specific autoantibodies (including anti-Scl70, anti-centromere (CENPB), anti-PMScl, anti-Ku, and anti-U1snRNP) was visualized as brown bands following the addition of substrate and stop solutions, as per accordance with the manufacturer's protocol [13].

Statistical Analysis

Data were entered into Microsoft Excel and analyzed using JAMOVI version 2.3.28. Continuous variables were expressed as mean ± standard deviation (SD). Between group comparisons for continuous variables were performed using independent samples 't' test. Categorical variables were compared using Chisquare test. A p-value <0.05 was considered statistically significant.

Results

Demographic and Clinical Characteristics

A total of 85 systemic sclerosis (SSc) patients were enrolled. The mean age at evaluation was 34.7 ± 10.7 years, with a meandisease duration of 43.7 ± 35.4 months. There was a marked female predominance (82.4%; femaletomale ratio 5:1). Diffuse cutaneous systemic sclerosis was observed in 47 patients (55.3%), while 38 patients (44.7%) had limited cutaneous systemic sclerosis.

Clinical Features

The most frequent clinical manifestations included joint pain (69.4%), respiratory symptoms (68.2%), and ILD confirmed on HRCT thorax (64.1%). Skin thickening was observed in 55.3% of patients, gastrointestinal symptoms in 42.4%, Raynaud's phenomenon in 38.8%, and proteinuria in 27.1% (Table 1).

Table 1: Clinical features of Systemic Sclerosis patients (n=85).

Clinical Feature	Frequency (n)	Percentage (%)
Joint pain	59	69.4
Respiratory symptoms	58	68.2
Interstitial lung disease	55	64.1
Skin thickening	47	55.3
Gastrointestinal symptoms	36	42.4
Raynaud's phenomenon	33	38.8
Proteinuria	23	27.1

Values are presented as numbers (percentage). HRCT= high-resolution computed tomography.

ANA Patterns by IIF

The distribution of ANA patterns detected by IIF according to disease subtype and gender is presented in Table 2. ANA screening revealed that the homogeneous pattern was the most common (41.2%), followed by centromere (10.6%), speckled(8.2%), and nucleolar (7.0%) patterns. Mixed ANA patterns were observed in 15.2% of patients, while 17.6% tested negative by IIF. The homogenous pattern was distributed across both dcSSc and lcSSc subtypes and was more frequent in females.

Table 2: ANA patterns by IIF and distribution by disease subtype and gender.

ANA patterns by IIF	Total (%)	Types of SSc	Types of SSc	Gender	Gender
ANA patterns by IIF	Total (%)	Diffuse	Limited	Female	Male
Homogeneous	35 (41.1%)	24	11	29	6
Nucleolar	6 (7.0%)	4	2	6	0
Speckled	6 (8.2%)	4	2	5	1
Centromere	9 (10.6%)	3	6	7	2
Mixed Homogeneous -Nucleolar	5 (4.7%)	2	3	4	1
Mixed Homogeneous -Speckled	5 (5.9%)	1	4	3	2
Mixed Homogeneous -Nucleolar-Nuclear Membrane	2 (2.3%)	1	1	2	0
Mixed Speckled-Cyt oplasmic	2 (2.3%)	1	1	1	1

ANA patterns by IIF	Total (%)	Types of SSc	Types of SSc	Gender	Gender
Negative	15 (17.6%)	7	8	13	2

Values are presented as a number (percentage). ANA= antinuclear antibody; IIF= indirect immunofluorescence; dcSSc= diffuse cutaneous systemic sclerosis; lcSSc= limited cutaneous systemic sclerosis

Organ Involvement and ANA Associations

The association between ANA patterns detected by IIF and organ system involvement is summarized in Table 3. Homogenous ANA was strongly associated with pulmonary disease (47.4%), while centromere antibodies correlated with renal and hepaticinvolvement. Negative ANA cases also demonstrated organ involvement, particularly pulmonary manifestations (Table 3). These associations reinforce the prognostic role of ANA patterns in systemic sclerosis.

Table 3: Association between ANA patterns and organ involvement.

ANA Pattern	Pulmonary n (%)	Musculoskeletal n (%)	Renal n (%)	Hepatic n (%)	CNS n (%)
Homogeneous	27(47.4)	4(30.8)	4(50.0)	0(0)	1(100)
Nucleolar	6(10.5)	1(7.7)	0(0)	0(0)	0(0)
Speckled	5(8.8)	1(7.7)	1(16.7)	0(0)	0(0)
Centromere	5(8.8)	0(0)	1(16.7)	1(50.0)	0(0)
Mixed homogeneous-nucleolar	3(5.3)	1(7.7)	0(0)	0(0)	0(0)
Mixed homogenous-speckled	2(3.5)	1(7.7)	0(0)	0(0)	0(0)
Mixed Homogeneous -Nucleolar-Nuclear membrane	1(1.7)	1(7.7)	1(16.7)	1(50.0)	0(0)
Mixed speckled and cytoplasmic	2(3.5)	0(0)	0(0)	0(0)	0(0)
ANA Negative	6(10.5)	4(30.8)	0(0)	0(0)	0(0)
Total	57(100)	13(100)	6(100)	2(100)	1(100)

Values are presented as percentages. IIF=indirect immunofluorescence; CNS= central nervous system.

Autoantibody Profiles by LIA

The distribution of autoantibody profiles according to disease subtype and gender is shown in Table 4. Autoantibody profiling using LIA yielded positive results in 84 patients, while one patient tested negative despite having clinical features consistent with systemic sclerosis.

Anti-Scl70 was the most frequent autoantibody, detected in 58.3% of patients. It was more common in dcSSc (61.7%) compared to lcSSc (54.1%) and was predominantly observed in females. Other autoantibodies included anti-PMScl (8.3%), anti-CENPB (6.0%), anti-Ku (3.6%), and anti-U1snRNP (3.6%). Mixed antibody profiles were identified in 20.2% of patients (Table 4).

Table 4: Distribution of autoantibody profiles according to disease subtype and gender.

Autoantibody	Total (%)	dcSSc (%)	lcSSc (%)	Female (%)	Male (%)
Anti-Scl-70	49 (58.3)	29 (61.7)	20 (54.1)	40 (58.0)	9 (60.0)
Anti-PM-scl	7 (8.3)	6 (12.8)	1 (2.7)	6 (8.7)	1 (6.7)
Anti-CENP-B	5 (6.0)	2 (4.3)	3 (8.1)	4 (5.8)	1 (6.7)
Anti-Ku	3 (3.6)	2 (4.3)	1 (2.7)	3 (4.3)	0
Anti-U1-snRNP	3 (3.6)	2 (4.3)	1 (2.7)	2 (2.9)	1 (6.7)
Mixed	17 (20.2)	6 (12.8)	11 (29.8)	14 (20.2)	3 (20)

Autoantibody	Total (%)	dcSSc (%)	lcSSc (%)	Female (%)	Male (%)
Total	(n=84) 100%	(n=47) 100%	(n=37) 100%	(n=69) 100%	(n=15) 100%

Values are presented as a number (percentage). LIA=line immunoassay; dcSSc=diffuse cutaneous systemic sclerosis; lcSSc=limited cutaneous systemic sclerosis.

Autoantibodies and Organ System Involvement

The association between autoantibodies detected by LIA and organ system involvement is summarized in Table 5. Anti-Scl-70 antibodies were most frequently associated with pulmonaryinvolvement, followed by musculoskeletal and renal involvement. Other autoantibodies, including anti-U1-snRNP, anti-PM-Scl, and anti-CENP-B, were observed less frequently and across multiple organ systems.

Table 5: Association of Autoantibodies with Organ Involvement.

Organ System	Anti-Scl-70 n (%)	Anti-U1-snRNP n (%)	Anti-PM-Scl n (%)	Anti-CENP-B n (%)	Other/Mixed n (%)
Pulmonary (n = 57)	35 (61.4)	5 (8.8)	2 (3.5)	2 (3.5)	13 (22.8)
MKS (n = 13)	6 (46.2)	1 (7.7)	1 (7.7)	0	5 (38.4)
GIT (n = 2)	1 (50.0)	0	0	0	1 (50.0)
Renal (n = 6)	3 (50.0)	1 (16.7)	0	1 (16.7)	1 (16.7)
CVS (n = 2)	1 (50.0)	0	0	0	1 (50.0)
Hepatic (n = 2)	0	0	1 (50.0)	0	1 (50.0)
CNS (n = 1)	0	1 (100)	0	0	0

Values are presented as a number (percentage). MKS=Musculoskeletal, GIT= Gastrointestinal system, CVS= cardiovascular system, CNS= Central nervous system

Association Between Autoantibodies and Specific Clinical Features

The association between specific autoantibodies and selected clinical features is presented in Table 6. A statistically significant association was observed between anti-Scl-70 antibodies andRaynaud's phenomenon (p = 0.002) as well as interstitial lung disease (p = 0.001). Anti-U1snRNP and anti-PM-scl were inversely linked to joint pain (p=0.024) and Raynaud's phenomenon (p=0.005).

Table 6: Clinical associations of specific autoantibodies in Systemic Sclerosis *(n=85).*

Antibody	Character	Negative (N/n)	Positive (F/f)	p
Anti-Scl-70	Raynaud's phenomenon	3/24	30/61	0.002
	ILD	9/24	46/61	0.001
	Joint pain	15/24	44/61	0.386
Anti-U1-snRNP	Raynaud's phenomenon	30/73	3/12	0.005
	ILD	48/73	7/12	0.618
	Joint pain	54/73	5/12	0.024
Anti-PM-scl	Raynaud's phenomenon	33/74	0/11	0.005
	ILD	50/74	5/11	0.152
	Joint Pain	51/74	8/11	0.798

Values are numbers. The table demonstrates the distribution of selected clinical features among patients positive and negative for Scl70, U1snRNP, and PMScl auto-antibodies. ILD= Interstitial lung disease. Values are presented as numbers. p-values < 0.05 were considered statistically significant.
N = antibodynegative patients with the clinical feature; n = total antibodynegative patients; F = antibodypositive patients with the clinical feature; f = total antibodypositive patients

Discussion

Systemic sclerosis is a heterogeneous autoimmune disorder with variable clinical manifestations and immunological profiles. In the present study, a marked female predominance and mean age of onset (34.7 years) were observed, with females accounting for 82.4% of the study population and a female-to-male ratio of 5:1. This finding is consistent with previous Indian studies and it reflects the well-recognised female preponderance of SSc reported globally [1]. The mean age of patients in our cohort was comparable to that reported by Sharma et al., further supporting demographic similarities within Indian populations [1]. dcSSc was more frequently observed than the limited cutaneous subtype in the present study. This distribution is broadly comparable to the findings of Pradhan et al. in a western Indian cohort, although slight differences in proportions may reflect regional variations, referral patterns, and differences in sample size [2]. The higher prevalence of dcSSc in our cohort may also explain the increased frequency of internal organ involvement observed.

The most common clinical manifestations in our study were joint pain, respiratory symptoms, and skin thickening. The high prevalence of joint pain is consistent with previous reports [14-16], although variability in reported musculoskeletal involvement across studies has been attributed to differences in diagnostic criteria and symptom definitions [5,10,11,17]. Respiratory involvement was common in our cohort, with ILD detected in a substantial proportion of patients. Although the frequency of pulmonary involvement in our study was higher than that reported in some large longitudinal cohorts, this discrepancy may be explained by differences in study duration, sample size, and the use of high-resolution computed tomography for detection [5,6,10].

ANA testing by IIF demonstrated that the homogeneous pattern was the most frequently observed ANA pattern in our cohort. This finding is in agreement with prior studies that have highlighted the predominance of homogeneous and nucleolar patterns in systemic sclerosis [2]. The presence of ANA negativity in a subset of patients highlights the clinical heterogeneity of SSc and highlights the importance of comprehensive immunological evaluation. Autoantibody profiling by line immunoassay revealed anti-Scl-70 as the most prevalent autoantibody, particularly among patients with diffuse cutaneous disease and females. This finding aligns with previous studies demonstrating a strong association between anti-Scl-70 antibodies and dcSSc, as well as severe internal organ involvement, especially pulmonary involvement and Raynaud's phenomenon [5]. The observed association between anti-Scl-70 antibodies and interstitial lung disease in our study further reinforces the prognostic significance of this autoantibody [4,5]. Given the established role of autoantibodies in the diagnosis and prognostic stratification of SSc, comprehensive immunological profiling was undertaken. Indirect immunofluorescence (IIF) on HEp-2 cells remains the reference method and gold standard for antinuclear antibody (ANA) screening in systemic autoimmunerheumatic diseases, including SSc [8,17]. Large cohort studies have reported ANA positivity in approximately 85–95% of SSc patients [18,19], rendering true ANA-negative disease relatively uncommon. In the present study, however, 17.6% of patients were ANA-negative by IIF. This comparatively higher rate may be attributed to methodological and immunological factors. Certain SSc-associated autoantibodies may produce weak, low-titre, or atypical fluorescence patterns that are difficult to interpret, particularly at higher screening dilutions, thereby increasing the likelihood of under-detection [20]. Additionally, variability in antigen expression on HEp-2 substrates and technical aspects of assay performance may further influence sensitivity. Patients with "Seronegative" SSc (negative on IIF) may possess rare or novel antibodies, such as anti-U11/U12 RNP, which can be detected by specialized immunoblot assays [21]. Pulmonary involvement emerged as the most frequently affected organ system in the present cohort, with anti-Scl-70 antibodies showing the strongest association. Similar variations in autoantibody distribution and clinical correlations have been reported in international cohorts from New Zealand, Brazil, and Poland, where distinct antibody profiles were observed across different populations [22–24]. These findings highlight the influence of geographic and ethnic factors on autoantibody expression while reinforcing the prognostic relevance of comprehensive serological profiling. This observation is consistent with earlier reports identifying ILD as a leading cause of morbidity and mortality in SSc [6,14]. Musculoskeletal involvement remained clinically significant due to the high prevalence of joint pain, a finding also supported by previous literature [11,15,16]. Significant inverse associations were also observed between anti-U1-snRNP and Raynaud's phenomenon and joint pain; this indicates that anti- U1snRNP may define a distinct subgroup with musculoskeletal involvement but less vascular disease [25]. Similarly, PMScl antibodies, classically linked to polymyositis–systemic sclerosis overlap, were inversely associated with Raynaud's phenomenon in our cohort, in line with EUSTAR. which demonstrated [25,26] that anti-PMScl antibodies are associated with overlap syndromes, particularly myositis and arthritis. Raynaud's phenomenon and digital ulcers were less frequent in anti-PMScl patients compared to other antibody subsets. ILD was present but showed more favourable functional outcomes than in anti-Scl70-positive patients. Overall, these findings emphasize the value of ANA profiling in systemic sclerosis, not only for diagnosis but also for risk stratification and prognostic assessment. Early identification of high-risk patients, particularly those with Anti-Scl70 antibodies, may facilitate timely monitoring and intervention, thereby improving clinical outcomes. These results contribute to the growing body of evidence supporting the integration of immunological markers into the routine evaluation and management of SSc [10].

Conclusion

This study demonstrates a clear female predominance in SSc and highlights the heterogeneous clinical and immunological profile of the disease. Joint pain and pulmonary involvement were the most common clinical manifestations, with ILD representing thepredominant internal organ involvement. Anti-Scl-70 emerged as the most frequent autoantibody and showed significant associations with dcSSc, ILD, and Raynaud's phenomenon, while anti-U1-snRNP and anti-PM-Scl antibodies were associated with

specific clinical features, with musculoskeletal involvement but less vascular disease. These findings underscore the clinical relevance of comprehensive ANA profiling in SSc and support its role in disease stratification, prognostication, and informed clinical management.

Authors contribution

S.K. and D.D. contributed to the conception and design of the study and were involved in acquisition, analysis, and interpretation of data. **L.P.K., M.M., B.K., and A.B.** drafted the manuscript and revised it critically for important intellectual content. **S.P.** provided clinically relevant samples for the assay. **B.N.N.** contributed to data analysis and interpretation.

All authors have approved the final draft for publication purposes and are accountable for the accuracy and integrity of the work. The corresponding author Dr. Sushil Kumar is responsible for ensuring that the descriptions are accurate and agreed by all author.

Data Availability Statement

The data supporting the findings of this study are not publicly available due to ethical and privacy restrictions but are available from the corresponding author upon reasonable request.

Acknowledgments

The authors would like to acknowledge the Department of Dermatology, AIIMS Patna, for providing clinical support and laboratory facilities required for the conduct of this study. The authors also thank all the patients who consented to participate in this research.

Artificial Intelligence (AI) Use Statement

Artificial intelligence tools (Chat GPT, Copilot) were used for language editing and improving the clarity of the manuscript. The authors take full responsibility for the content, interpretation, and conclusions of the study.

Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Ethical Approval

The study was approved by the Institutional Ethics Committee of AIIMS Patna (AIIMS/Pat/IEC/PGTh/Jan22/50). Written informed consent was obtained from all participants before enrolment. This study was in compliance with the ethical principles for medical research involving human subjects, in accordance with the Declaration of Helsinki.

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