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An Observational Study on Clinical and Biochemical Profile of Patients with Connective Tissue Diseases

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Abstract: ***Background: Connective tissue disorders (CTD) occur in 3–5% of the population. The advent of antibodies to extractable nuclear antigens (ENA) has become a reliable predictor to establish the diagnosis of CTD, sub classify patients into prognostic groups, and monitor disease activity. It is very vital to study the different presenting symptoms and signs of the above as well as the varied systemic involvement since early detection may slow down the progression of the disease. Aims and objectives: 1) To assess proportions of various connective tissue disorders. 2) Different presenting symptoms and signs of connective tissue disorders. 3) Different features of systemic involvement of connective tissue disorders. 4) Different biomarkers of connective tissue disorders. Material and method: A descriptive cross sectional study which included 50 patients based on inclusion and exclusion criteria was conducted in Sir T General Hospital, Bhavnagar, over a period from January 2021 to August 2021. Results and conclusions: The major manifestations in this study, were joint pain (50%), raised skin lesion (46%), itching (44%), discoloration of skin (40%), photosensitivity (34%) and Raynaud phenomenon (26%). SLE was the most common CTD accounting for (42%), followed by scleroderma (26%), MCTD (10%), rheumatoid arthritis (8%) and sjogren syndrome (6%). In this study, Gastrointestinal manifestations (48%) were the most common followed by musculoskeletal manifestation (44%), respiratory (38%), renal (26%), CVS (12%) and CNS (10%) manifestations. Most common positive antibody in SLE was ds-DNA, in scleroderma it wasanti-centromere antibody while all patients of MCTD were positive to U1 RNP, in Sjogren syndrome SS-A was the most common antibody. In the present study based on ANA pattern homogenous pattern was the most common seen in (30%) followed by membranous and nucleolar pattern with (26%) in each. Membranous pattern was the most common pattern in SLE (47.6%), Nucleolar pattern was the most common pattern in scleroderma (53.84%). Homogenous and nucleolar pattern were the most common ones in MCTD with (50%) each. Homogenous pattern was the most common one in RA (100%), and fine speckled was the most common one in Sjogrens (66.66%). Conclusions: Young females were most commonly affected. Cutaneous manifestations of CTD can be an early predictor in giving a clue to impending systemic manifestations. ANA profile predicts systemic involvement, thus helping in the multidisciplinary management.***

Keywords: Connective tissue disorders, cutaneous manifestations, systemic manifestations, ANA

1. Introduction

Autoimmune disease is a disorder with destruction of the tissues due to autoimmune phenomenon. Connective tissue diseases (CTDs) are a collection of autoimmune disorders characterised by antinuclear antibodies (ANAs) in the serum of the patients. ANAs are autoantibodies directed against various cell organelles including cytoplasm, nuclei, nucleoli, subcellular structures and cell surfaces.1The antigens recognized are mainly proteins and nucleic acids. These autoantibodies are involved in the pathogenesis of the disease and also help in the diagnosing and treating the disease as well as in assessing the prognosis of the disease. CTDs relevant to dermatology are Systemic Lupus Erythematosus, drug induced lupus, Scleroderma, Dermatomyositis, Sjogren's syndrome and mixed connective tissue diseases. CTD occurs in 3-5% population worldwide. The advent of antibodies to extractable nuclear antigens (ENA) has become a reliable predictor to establish the diagnosis of CTD, sub classify patients into prognostic groups, and monitor disease activity

Systemic autoimmune diseases (as opposed to organ-specific autoimmune diseases) are characterized by the systemic involvement of autoimmune tissue destruction in various organs due to non-organ specific autoantibodies that target antigens that are present in almost all type of cell. The antibodies relevant to these CTDs are anti-dsDNA, anti-Sm, anti-RNP, antihistone, anti-Ro (SS-A), anti-L (SS-B), anti-Scl-70, anticentromere, antiribosomal P protein, antinucleolar, antinucleosomal antibodies, antiphospholipid antibodies.2

Common techniques for detecting ANA are indirect immunofluorescence (IIF) and enzyme-linked immunosorbent assay (ELISA). IIF using Hep-2 cell as substrate reveals different staining patterns and the titreis also obtained. Western blot technique (immunoblotting) is used to detect specific antibodies

Sensitivity and specificity varies with different techniques.3The availability, accessibility and cost effectiveness determine the utility of the tests and the same technique should be followed for monitoring also. Recent ACR guidelines suggest that the indirect immunofluorescence test for ANA remains the gold standard. ANA positivity should be interpreted against the clinical background since it can be positive in normal individuals and other rheumatic diseases. There are only very few studies which assess the disease activity and severity of CTD with the ANA profile pattern. This study will focus on determining the clinical findings in various CTDs and various system involvement in CTDs. This study also focuses on the patterns of antibody positivity in CTD, as well as the positivity of different antibodies in each CTD.

2. Materials and Methods

A descriptive cross sectional study was conducted in Sir T General Hospital, Bhavnagar, over a period from January 2021 to August 2021.

All patients coming to Medicine or Dermatology OPD or admitted in Sir T Hospital diagnosed with connective tissue disorder by diagnostic methods like serum ANA and ANA profile which included SLE, scleroderma, dermatomyositis, RA, MCTD, Sjogren's syndrome and overlap syndrome by their respective criteria were included in the study.

Inclusion Criteria

- Patient of age greater than or equal to 18.
- Patients giving written and informed consent having diagnosis of connective tissue disorders diagnosed by diagnostic methods like serum ANA and ANA profile.

Method of data collection

- The study included 50 patients who met the inclusion criteria who consented for further evaluation.
- A thorough history and physical examination was done which included a detailed dermatological evaluation and the findings were documented.
- All patients underwent an ANA profile. The antibodies included anti RNP, anti Sm,, anti SS-a,, anti SS-b, anti SCL-70, anti PM-scl, anti Jo-1, anti centromere, anti dsDNA, anti nuclear, anti histone, anti Rib-P, anti AMA-M2.

The positive values of the ANA profile were graded according to their titre valves:

- Borderline positive (+) – 1: 40
- Strongly positive (++) – 1: 80
- Very strongly positive (+++) – 1: 160

Data Analysis

Collected data was analysed based on frequency and percentage.

3. Observations and Results

Table 1: Age Wise Distribution

Mean Age (yrs)	No of patient
18-30	10 (20%)
31-40	17 (34%)
41-50	11 (22%)
51-60	8 (16%)
> 60	4 (8%)
Total	50 (100%)
Mean Age (yrs)	40.86 ± 12.18

In the present study, among 50 patients, 17 (34%) patients were between 31 – 40 years. The next groupof 11 (22%) patients were between 41-50years, 10 (20%) patients between 21-30 years, 8 (16%) patients between 51-60 years, 4 (8%) patients were of age > 60 yrs. The mean age of the present study was 40.86 ± 12.18 years.

Table 2: Gender Wise Distribution

Gender	No of patient
Male	11 (22%)
Female	39 (78%)
Total	50 (100%)

In the present study, Among 50 patients studied 39 (78%) patients were female and 11 (22%) patients were males. The female: male ratio is 3.54: 1.

Table 3: Types of Connective Tissue Disease

Types of CTD	No of Patients
Systemic Lupus Erythematosus	21 (42%)
Scleroderma	13 (26%)
Mixed Connective tissue disease	8 (16%)
Rheumatoid Arthritis	4 (8%)
Sjogrens Syndrome	3 (6%)
Dermatomyositis	1 (2%)
Total	50 (100%)

In the present study, with respect to type of disease, Systemic Lupuserythematosus was the most common connective tissue disease accounting for 21 (42%) cases followed by 13 (26%) cases of Scleroderma. The rest were MCTD in 5 (10%) patients, Rheumatoid arthritis in 4 patients (8%), Sjogrens Syndrome in 3 (6%) and Dermatomyositis 1 (2%) patients.

Table 4: Presenting Complaints with Type of CTD

	SLE (n=21)	scleroderma (n=13)	MCTD (n=8)	RA (n=4)	SS (n=3)	dermatomyositis (n=1)	Total (n=50)
Itching	12	3	2	2	2	1	22 (44%)
Raised skin lesion	19	1	-	-	3	-	23 (46%)
Joint pain	9	6	3	4	2	1	25 (50%)
Fever	7	6	3	2	2	1	21 (42%)
Raynaud's phenomenon	1	8	3	-	-	1	13 (26%)
Photosensitivity	14	2	-	-	-	1	17 (34%)
Oral lesions	9	3	-	-	-	1	13 (26%)
Skin wounds	1	5	2	-	-		8 (16%)
Tightening of skin	-	10	2	-	-		12 (24%)
Swelling of fingers	-	4	-	3	-	-	7 (14%)
Discoloration of skin	4	12	2	2	-	-	20 (40%)
Discoloration of digits	-	4	2	1	-	-	7 (14%)
Muscle weakness	-	-	3	-	2	-	5 (10%)
Raised nodule	-	-	-	1	-	-	1 (2%)
Difficulty in mouth opening	-	6	2	-	-	-	8 (16%)
Difficulty in swallowing	-	5	2	1	-	-	8 (16%)
Hair loss	12	6	5	-	1	1	25 (50%)
Swelling around eyelids	-	-	-	-	2	-	2 (4%)
Redness around the eyelids	-	-	-	-	3	-	3 (6%)

In the present study, Among 50 patients, 25 (50%) patients presented with joint pain and hair loss. Raised skin lesions were seen in 23 (46%) patients, majority being patients of SLE. Itching was seen in 22 (44%) patients followed by 21 (42%) patients having fever. Discolouration of the skin was seen in 20 (40%) patients. Photosensitivity was seen in 17 (34%) patients followed by Raynaud's phenomenon in 13 (26%) patients. Remaining parameters are as seen in above table.

Table 5: Systemic Manifestations Wise Distribution

Systemic manifestations	No of patients (n=50)
GI	24 (48%)
Respiratory	19 (38%)
CNS	5 (10%)
CVS	6 (12%)
Renal	13 (26%)
Musculoskeletal	22 (44%)

In the present study based on systemic manifestation, as obtained from history and clinical examination of the patients, Gastrointestinal manifestations was the predominant one seen in 24 (48%) patients followed by 22 (44%) patients having musculo skeletal manifestations. Remaining parameters were respiratory manifestations in 19 patients (38%), renal manifestations in 13 (26%), cardiovascular manifestations in 6 patients (12%) and CNS manifestations in 5 (10%) respectively.

Table 6: ANA profile in SLE

Antinuclear Antibodies	ANA Profile			No of patients (n=21)
	+	++	+++	
U1-RNP	1	1	1	3
Sm	1	2	1	4
SS-a	1	1	3	5
ANCA	1	2	2	5
SS-b	1	-	-	1
SCL-70	-	-	-	-
Jo-1	-	-	-	-
Centromere-B	-	-	-	-
ds DNA	4	4	4	12
Nucleosome	2	1	1	4
Histone	3	2	-	5
Rib-P	-	-	2	2
AMA-M2	-	-	-	-

Correlation of SLE with ANA profile: Among the antibodies positive, ds DNA was positive in 12 (57.14%) patients of which 4 patients (19.04%) each were borderline positive, strongly and very strongly positive. SS-a was positive in 5

(23.80%) patients of which 3 (14.28%) patients were very strongly positive and 1 (4.7%) patient each were strongly and very strongly positive. The other antibodies present were nRNP, Sm, ANCA, SS-b, anti nucleosome, anti histone and anti Rib-P antibodies.

Table 7: ANA profile in Scleroderma

Antinuclear Antibodies	ANA Profile			No of patients (n=13)
	+	++	+++	
U1-RNP	-	1		1
Sm	-	-	-	-
SS-a	-	-	1	1
ANCA	-	-	1	1
SS-b	-	-	-	-
SCL-70	-	-	4	4
Jo-1	-	-	-	-
Centromere-B	-	2	2	4
ds DNA	-	1	-	1
Nucleosome	-	-	-	-
Histone	-	-	-	-
Rib-P	-	-	-	-
AMA-M2	1	-	-	1

In the present study, Majority of patients having sclerodermas are positive for anti centromere antibody, among 4 patients (30.76%) with anticentromere positivity, 2 patients (15.3%) were strongly positive and 2 patients (15.37%) were very strongly positive for the same. SCL-70 is very strongly positive in 4 (30.76%) patients. The other antibodies present were U1-RNP, SS-a, ANCA, ds DNA and AMA-M2 antibodies.

Table 8: ANA profile in MCTD

Antinuclear Antibodies	ANA Profile			No of patients (n=8)
	+	++	+++	
U1-RNP	-	2	6	8
Sm	-	-	-	-
SS-a	-	-	-	-
ANCA	-	-	1	1
SS-b	-	-	-	-
SCL-70	-	-	-	-
Jo-1	-	-	-	-
Centromere-B	-	-	-	-
ds DNA	-	-	-	-
Nucleosome	-	-	-	-
Histone	-	-	-	-
Rib-P	-	-	-	-
AMA-M2	-	-	-	-

In the present study, All 8 patients with mixed connective tissue disorders were positive to U1-RNP antibody among which 2 (25%) of the patients were strongly positive and 6 (75%) of the patients were very strongly positive.

Table 9: ANA Profile in Rheumatoid Arthritis

Antinuclear Antibodies	ANA Profile			No of patients (n=4)
	+	++	+++	
U1-RNP	-	-	-	-
Sm	-	-	-	-
SS-a	-	-	-	-
ANCA	-	-	-	-
SS-b	-	-	-	-
SCL-70	-	-	-	-
Jo-1	-	-	-	-
Centromere-B	-	-	-	-
ds DNA	2	-	-	2
Nucleosome	-	-	-	-
Histone	-	-	-	-
Rib-P	2	-	-	2
AMA-M2	-	-	-	-

In the present study, Among the 4 patients having rheumatoid arthritis two were positive for ds DNA and two for Rib-P (50%) each.

Table 9: ANA Profile Insjogrens Syndrome

Antinuclear Antibodies	ANA Profile			No of patients (n=3)
	+	++	+++	
U1-RNP	-	-	-	-
Sm	-	-	-	-
SS-a	1	1	-	2
ANCA	-	-	-	-
SS-b	-	1	-	1
SCL-70	-	-	-	-
Jo-1	-	-	-	-
Centromere-B	-	-	-	-
ds DNA	1	-	-	1
Nucleosome	-	-	-	-
Histone	-	-	-	-
Rib-P	1	-	-	1
AMA-M2	-	-	-	-

In the present study, one out of 3 patients (33.3%) with Sjogrens Syndrome showed borderline positivity to anti SS – a antibody and, one (33.3%) showed strong positivity. The other antibodies present were SS-b, ds DNA and Rib-P antibodies. 1 (100%) patients with dermatomyositis showed borderline positivity to anti Jo-1 antibody. All other antibodies showed negative results.

Table 10: Percentage Distribution of ANA Patterns

S. No	ANA Pattern	NO	%
1	Homogenous	15	30%
2	Membranous	13	26%
3	Fine speckled	4	8%
4	Coarse speckled	5	10%
5	Nucleolar	13	26%

In the present study with respect to different ANA patterns it was found that homogenous pattern was the predominant one seen in 15 (30%) patients, followed by membranous and nucleolar pattern in 13 (26%) patients each. Coarse speckled patternwas seen in 5 (10%) patients and fine speckled pattern was seen in 4 (8%) patients.

Table 11: Distribution of ANA Patterns among various CTDs

S. No.	ANA Pattern	SLE, n=21	Scleroderma, n=13	MCTD, n=8	RA, n=4	Sjogrens, n=3	Dermatomyositis, n=1
1	Homogenous	5 (23.8%)	2 (15.38%)	4(50%)	4 (100%)	0 (0.0%)	0 (0.0%)
2	Membranous	10 (47.6%)	2 (15.38%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (100%)
3	Fine speckled	0 (0.0%)	2 (15.38%)	0 (0.0%)	0 (0.0%)	2 (66.66%)	0 (0.0%)
4	Coarse speckled	4 (19.04%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (33.33%)	0 (0.0%)
5	Nucleolar	2 (9.5%)	7 (53.84%)	4 (50%)	0 (0.0%)	0 (0.0%)	0 (0.0%)

In the present study it was found that majority of patients (47.6%) with SLE had membranous pattern followed by homogenous pattern in (23.8%) and coarse speckled pattern in (19.04%).

Similarly it was found that in Scleroderma the major ANA pattern was nucleolar seen in (53.84%) followed by membranous and fine speckled pattern in (15.38%) each.

MCTD had (50%) each of homogenous and nucleolar pattern. In the present study homogenous pattern was the only pattern (100%) seen in RA patients. Fine speckled was seen in (66.66%) patients of Sjogrens syndrome and coarse speckled in (33.33%). Membranous pattern was the pattern seen in (100%) of dermatomyositis.

4. Conclusion

The 'Autoimmune connective tissue disorders' also know as 'systemic autoimmune disease' can affect almost every organ of the body. These diseases have a female predilection and usually affect individuals in their 3rd to 5th decade of life. ANA profile predicts systemic involvement thus helping in multidisciplinary management and cutaneous manifestations of CTD can be an early predictor in giving a clue to impending systemic manifestation. On studying the Systemic manifestations and ANA profile, we found that every autoimmune disease has classical presentations and antibodies which are characteristic of the disease. Few antibodies are specific for certain disease, however their presence in another CTD does not confirm the diagnosis; a clinical correlation is a must.

High index of clinical suspicion is required in patients presenting with PUO, skin lesions, arthralgia, and multi system involvement and CTDs should be ruled out in such patients.

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