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Original Article

Internal Medicine Section

Prevalence, Characterisation and Diagnosis of Common Connective Tissue Diseases from Coimbatore Region in Tamil Nadu

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ABSTRACT

Introduction: Systemic autoimmune diseases include conditions where the immune system fails to recognise self antigens leading to production of "auto antibodies" and subsequent damage to several organs and tissue systems. It is commonly observed worldwide, that Connective Tissue Diseases (CTDs) occur predominantly among women in childbearing age groups. The most common laboratory diagnosis done is to detect Antinuclear Antibodies (ANAs) by ELISA. Indirect Fluorescence Antibody (IFA) and ANA profile analysis are newer and slowly progressing concepts. The fewer existing studies available show that, IFA test in conjunction with ANA ELISA can provide more conclusive diagnosis. Not much data is available on the detailed diagnostic aspects of CTDs involving ELISA, IFA and ANA profile together as diagnostic tools.

Aim: To analyse the common CTDs in Coimbatore region in Tamil Nadu and to determine the classical clinical and diagnostic features of the common CTDs.

Materials and Methods: The study was conducted among 442 patients who attended PSG Hospitals, Coimbatore. ANAs were tested in patients clinically suspected to have connective tissue disease using ANA ELISA and ANA-IFA (Indirect Fluorescent Antibody). ANA profile was done on selected cases as final confirmatory test. Clinical details of patients were obtained from medical records.

Results: A total of 193 (44%) patients had CTDs. Females constituted to about 88% and 53% of the patients were between the age group of 21-40 years. The three most commonly occurring CTDs were Systemic Lupus Erythematosus (SLE), Mixed Connective Tissue Disease (MCTD) and Scleroderma or Systemic Sclerosis (SS).

Conclusion: CTDs are common in our community. Females are commonly affected. IFA was a useful diagnostic tool and ANA profile was a valuable confirmatory test. SLE was the most common CTD.

Keywords: Antinuclear antibodies, Indirect fluorescence antibody, Mixed connective tissue disease

INTRODUCTION

Systemic autoimmune diseases include conditions where the immune system fails to recognise self antigens leading to production of "auto antibodies" and subsequent damage to several organs and tissue systems. Systemic autoimmune diseases include SLE, Scleroderma, Dermatomyositis, Polyarteritis Nodosa and MCTD; the most common one being SLE with a prevalence rate of 53/100,000 in USA [1] and 14-60/100000 in India [2].

Autoimmune diseases represent a significant health burden in the developed world affecting 5-10% of the population in the west [3]. In India a study shows that 11% of causes for Pyrexia of unkown origin (PUO) as connective tissue disorders which mostly remain undiagnosed [4]. Though infectious disease are a leading cause of PUO, it is being closely followed by CTDs with SLE being predominant followed by Rheumatoid arthritis [5].

India reports much lower survival rates for CTD than the west [2]. Many Indian studies [2,6-8] show that the common CTDs like SLE, Sjogren's syndrome, MCTD or Scleroderma have a female predominance and the common age group to be affected between 20-40 years of age. They have also studied the common clinical features and IFA patterns and associated autoantibodies for each disease.

The present study was carried out to assess the prevalence and to describe the diagnostic and clinical features of the most common CTDs in Coimbatore, Tamil Nadu. As there are very few Indian studies that have studied all the above mentioned features in detail for every disease, this study is an ideal and relevant one as it comprehensively analyses the major CTDs.

MATERIALS AND METHODS

After obtaining an Institutional Ethical committee clearance, this descriptive study was carried out in the Diagnostic Microbiology Department, PSG Hospitals, Coimbatore from June 2010 to September 2011. As this test was done as part of the diagnostic package, specific patient consents were not obtained.

Inclusion criteria:

1. Patients with history and clinical examination findings suggestive of CTDs.

Exclusion criteria:

1. Established Rheumatoid arthritis patients.
2. Patients with connective tissue disease who have co-existing infectious diseases or carcinomas.

Serum samples were collected from 442 consecutive patients in whom connective tissue disorder was clinically suspected. All the serum samples were subjected to ANA-IFA test and ANA ELISA. ANA IFA test was performed by the ImmcoImmuglo kit (Immco diagnostics supplied by Anand Brothers) which use Hep 2 cells as substrate antigens. It was read using the Leica immunofluorescence microscope using the blue filter (470-550 nm). Positives were seen as apple green fluorescing patterns against a dark background. ANA ELISA was performed using Esculisa kit which uses target nuclear and cytoplasmic antigens. ELISA was read using the BioRad ELISA reader and values more than 1 were considered as positives. The positive and negative controls were regularly plotted using the LJ chart. A total of 105 Samples were subjected to the ANA profile test using the Euro immune kit. The strips were read using a Cannon Euro line digital scanner and the specific antibody bands and their intensity were noted. Clinical details of the patients were obtained

from the patient case files from the medical records department and also from questionnaires used to interview patients and collect relevant data. Forty serum samples from blood bank were tested as negative controls for both ANA ELISA and IFA.

STATISTICAL ANALYSIS

All the variables from the questionnaire and patient medical records were analysed and results were obtained in percentages.

RESULTS

During the study period, CTD was clinically suspected in 327 in-patients and 115 out-patients, a total of 442 patients attending the departments of Medicine and Dermatology. Serum samples were collected from all the patients suspected to have CTD. Out of the 442 serum samples, 105 were subjected to the ANA profile test. A total of 193 cases were found to have CTD based on ANA positivity by IFA (174), anticytoplasmic antibodies (12) and in 7 cases ANA profile alone was positive. The prevalence of CTD was found to be 44%.

Among the 193 laboratory confirmed cases of CTD, 170 (88%) were females; and 53% of the patients were between the age group of 21-40 years [Table/Fig-1].

Age in years	Number (%)
New born	2 (1)
<21	18 (9.3)
21-30	63 (33)
31-40	39 (20)
41-50	33 (17)
51-60	22 (11)
61-70	14 (7.2)
.70	2 (1)

[Table/Fig-1]: Age wise distribution of patients with CTD.

The most commonly occurring connective tissue disease was found to be SLE followed by MCTD and Scleroderma. The prevalence of the CTDs is shown in [Table/Fig-2].

Out of the 193 CTD patients 73 (38%) had SLE. The age and gender distribution is detailed in [Table/Fig-3]. Out of the 73 SLE patients 71 were positive by IFA. The 2 samples which were negative by IFA were also negative by ELISA. They were diagnosed as SLE based on ANA profile which was positive for dsDNA and histones. The common ANA IFA pattern seen in SLE patients was homogenous (44%) [Table/Fig-4]. The most common complaints patients presented with is detailed in [Table/Fig-5].

Disease	Total No/ (%)*	IFA Positive	Pattern	No. profile done	Common auto antibodies
SLE	73/(38)	71	Homogenous Speckled Anticytoplasmic (Rib P)	37	Sm, dsDNA, histones, nucleosomes.
MCTD	34/(18)	32	Speckled	34	nRNP/Sm
Sclero-derma	24/(12)	24	Speckled Centromere	18	Scl – 70 Cenp B
Sjogren's Syndrome	8/ (4)	6	Speckled	8	SSA, SSB, Ro 52
Polymyositis	6/(3)	6	Nucleolar, anticytoplasmic	2	nRNP/Sm SSA, Ro 52, Rib P
Unclassified	57/(30)	56	Speckled	5	Ro 52

[Table/Fig-2]: CTDs with IFA & ANA Profile test positivity.
*The total no. of diagnosed patients is 193. But few patients produced multiple antinuclear antibodies in ANA profile and ANA patterns in IFA. This causes a condition called 'overlap', where multiple diseases can overlap in a single patient. Therefore for the sake of specific classification they have been considered as separate disease. Therefore, the total comes up to 202.

CTD	Gender (%)		Age in years (%)			
	Male	Female	<20	20-40	40-60	>60
SLE (73)	12 (16%)	61 (84%)	10 (14%)	36 (50%)	21 (29%)	6 (8%)
MCTD (34)	3 (9%)	31 (91%)	2 (6%)	22 (65%)	10 (30%)	-
Scleroderma (24)	6 (25%)	18 (75%)	2 (8%)	10 (42%)	13 (50%)	-
Sjogren's syndrome (8)	-	8 (100%)	-	5 (63%)	2 (25%)	1 (13%)
Polymyositis (6)	-	6 (100%)	-	2 (33%)	2 (33%)	2 (33%)

[Table/Fig-3]: Gender and age wise distribution among the common CTDs.

The next commonly occurring CTD was found to be MCTD which consisted of 34 patients among a total of 193 (18%). The age and gender distribution is described in [Table/Fig-3]. The most common IFA pattern seen was speckled (79%) [Table/Fig-4] and autoantibody seen was nRNP/sm.

Scleroderma was seen in 24 (12%) of the CTD patients. The age and gender distribution is described in [Table/Fig-3]. All 24 patients were positive by IFA. The common pattern was speckled (50%) followed by centromere (38%) [Table/Fig-4].

Out of the 193 CTD patients, 8 (4%) were diagnosed to have Sjogren's syndrome based on auto antibodies seen in ANA profile. All the affected patients were females. The age group between 20-30 years had 5 patients. Out of the 8, 6 were positive by IFA and showed speckled pattern.

Auto immune disease	Homogenous	Speckled	Anticytoplasmic	Peripheral	Centromere	Nucleolar	Nucleolar +Anticy-toplasmic	Combination	Total
SLE (73)	31 (44%)	13 (18%)	12 (17%)	4 (6%)	-	-	-	11 (15%)	73
MCTD (34)	1 (3%)	26 (79%)	1 (3%)	-	-	-	-	6 (12%)	34
Scleroderma (24)	2 (8%)	12 (50%)	-	-	9 (38%)	-	-	1 (4%)	24
Sjogren's syndrome (8)	-	6 (75%)	-	-	-	-	-	-	6
Polymyositis (6)	-	-	-	-	-	1 (17%)	5 (83%)		6

[Table/Fig-4]: IFA patterns seen in the common CTDs.

Disease	Clinical features						
	Fever	Skin hyperpigmentation/ rash/ ulcers	Joint swelling/ arthralgia	Respiratory/ cardiovascular/ renal involvement	Dysphagia	Others (epilepsy, malignancy, weakness, BOH, jaundice)	Unknown (proper clinical history unavailable)
SLE (73)	9 (12%)	5 (7%)	8(11%)	10(13%)	3 (4%)	4 (5%)	35 (48%)
MCTD (34)	5 (15%)	6 (17%)	2 (6%)	3 (9%)	1 (3%)	-	17 (50%)
Scleroderma (24)	4 (17%)	8 (33%)	-	3 (13%)	-	2 (8%)	7 (29%)

[Table/Fig-5]: Common clinical features in patients with CTDs.

A total of 6 (3%) patients were diagnosed to have Polymyositis based on IFA patterns. Nucleolar pattern was seen in 1 patient and nucleolar with anticytoplasmic pattern was seen in 5 patients. All the affected were females. The age group between 20-30 and 30-40 years had 2 patients each and 2 patients were between 60-70 years.

Interestingly, 57 (30%) patients out of the 193 could not be classified into any particular type of disease. IFA was done in all of them and 56 of them were positive and showed speckled pattern. The one IFA negative sample was positive by ANA profile for Ro 52 antibody.

DISCUSSION

CTD are manifested as a systemic autoimmune response where in, auto-antibodies are produced against nuclear self antigens like Smith, Ro 52, SSA, SSB, centromere, Scl 70 etc., leading to host tissue destruction and organ malfunction.

The most common CTDs in our study was SLE which had a prevalence rate of 38%. A comparison is shown in [Table/Fig-6].

	Our study	Kosaraju K et al., [6] (Mangalore, Karnataka	Paul BJ et al., [7] (Calicut, Kerala)
Common age affected	20-40 years	21-30 years	16-25 years
Gender affected	Females (84%)	Female: male is 15:1	Females (93%)
Common IFA patterns	Homogenous (31%)	Homogenous (55%)	--
	Peripheral (17%)		--
	Speckled (18%)	Speckled (45%)	--
Most common clinical features	Fever, arthralgia, skin lesions, systemic	Similar findings	Arthritis, myalgia, fever, skin lesions

[Table/Fig-6]: SLE, comparison of features.

The homogenous and the peripheral pattern in IFA are specific for SLE [9]. A combination of both homogenous and peripheral patterns were seen in 6% of patients as was observed by White RH et al., [10]. Speckled pattern can be suggestive of SLE depending on the auto antibodies produced [9]. Patients with RNP auto antibodies and Smith antibodies will show a Speckled pattern [10,11]. Anticytoplasmic pattern, which is also seen in SLE due to antibodies against Ribosomal P protein, was seen in 17% of the patients [9]. Egner W (Sheffield, UK) in his article suggests Ribosomal P titers are elevated in SLE [12]. Hence, apart from nuclear patterns, cytoplasmic patterns should also be interpreted for a diagnosis [13].

The next commonly occurring CTD observed in our study was MCTD (18%), which is a combination of diseases like Scleroderma, SLE and Polymyositis-Dematomyositis [9]. As there are not many Indian studies to compare this disease with, we drew most of our analysis from foreign articles. Presence of speckled patterns in IFA and the presence of Anti RNP antibodies are the diagnostic criteria of MCTD [9]. Our study showed that 79% of the MCTD individuals have speckled pattern and 59% patients had Anti RNP as the sole antibody. As observed, MCTD is found to occur less commonly than SLE but more frequently than SS which also correlates with the study done by Gaubitz M [14]. (Oxford University) Females (91%) were most commonly affected. The age group between 21-30 years had the maximum number of patients (44%), which again forms the child bearing age group. Gunnarsson R et al., (Norway) report a similar female predominance but the predominantly affected age group was the 4th decade [15]. Gunnarsson R et al., report a female: male ratio of 3:1 to 16:1 [15]. Greidinger EL et al., (Miami) observed that the common age group affected was between 15-25 years [16]. The most common presenting illness was fever and skin rashes which correlated with observations by Narasimulu (Hyderabad, Telengana) [17].

Systemic sclerosis was found to be the next commonly occurring disease (12%) [Table/Fig-7].

	Our study	Pradhan V et al., [8] (Western India)
Common age affected	41-50 years	Mean age 34.7+/-10.7
Gender affected	Females (75%)	Females (91%)
Common IFA patterns	Speckled (50%)	Speckled (63.9%)
	Centromere (38%)	Centromere (7%)
	Homogenous (8%)	Nucleolar (18%)
Most common clinical features	skin lesions, fever and systemic	Similar findings

[Table/Fig-7]: Systemic Sclerosis/Scleroderma, comparison of features.

The unusual feature is that Scleroderma commonly occurs between the age group of 30-50 years and declines after menopause [14,18].

The next common CTD was Sjogren's syndrome which is diagnosed based on the presence of SSA/Ro 52 and SSB/La autoantibodies [9,18]. All the patients showed Speckled pattern which is seen when SSA and SSB auto antibodies are present [9]. All the affected individuals in our study were females which correlates with other studies (Female: Male ratio 9:1) [14]. Also, the age group between 20-30 years had maximum number of patients, where as other studies suggest the predominance of Sjogren's syndrome in middle age or during the 4th and 5th decade of life [9,14,18].

Polymyositis was seen in 3% of the patients. Diagnosis was made based on the nucleolar staining pattern seen where auto antibodies are produced against Pm-Scl, combined with coarse cytoplasmic staining produced due to auto antibody against Jo-1 antigens [9,18]. Presence of nucleolar pattern alone could be suggestive of Polymyositis or Scleroderma. Hence, anticytoplasmic pattern plays a role in the diagnosis of Polymyositis.

Another important group of patients (30%) were those who were not classified into any specific disease. This was because all of them showed a speckled pattern which can be suggestive of SLE/Sjogren's syndrome/Scleroderma/MCTD/Polymyositis. Clinical history or examination findings were not specific for any CTD in these patients and none of them were subjected to the ANA Profile test. Hence, a speckled pattern can indicate any one of the above diseases or an overlap of the above diseases. Thus, in case of a patient showing a speckled pattern, diagnosis cannot be made only by IFA and here ANA profile holds its importance in being a confirmatory test where it can indicate specific auto antibodies seen in the patients.

LIMITATION

If ANA profile could be done at different stages of the disease, it would be more useful to analyse the pattern and clinical associations of the autoantibodies produced.

CONCLUSION

Connective tissue diseases are quite common worldwide. Though SLE is the most commonly occurring CTD, others like Scleroderma, MCTD, Sjogren's syndrome and Polymyositis are also prevalent. Females and patients between the third and fourth decade of age are most commonly affected. High index of clinical suspicion and knowledge to request the appropriate diagnostic test is mandatory in patients presenting with PUO, skin lesions, arthralgia, systemic involvements and even with bad obstetric history. ANA detection is the key laboratory diagnostic feature for CTD. IFA is a good diagnostic tool where a clear pattern correlates with clinical features as well. But where there is confusion due to mixed patterns or a non specific pattern, the diagnosis has to be made by the ANA Profile test.

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