Original Article

Clinical Significance of Antibodies to Soluble Extractable Nuclear Antigens (Anti-ENA) in Autoimmune Connective Tissue Disorder

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ABSTRACT

Introduction: Autoimmune diseases occur in 3-5% of the population. Alteration in immune system occurs due to various genetic & environmental factors leading to development of auto reactive phenomena that can be detected.

Aims & objectives: 1) Clinical diagnosis & its serological correlation. 2) To diagnose the diseases at its earliest stage and to intervene before serious end organ damage occurs. 3) To assess the prognosis of the disease.

Method: A group of 90 patients were studied who clinically showed signs of systemic autoimmune disease. All patients were subjected to Anti-ENA profile to see the possible correlation between clinical diagnosis and serological markers.

Result: 90 patients enrolled in study presented with predominant clinical manifestation of polyarthritis, Raynaud's phenomenon, fever and skin manifestations. Based on clinical examination various connective tissue disorder diagnosis was confirmed with serological testing in 63% cases while deferred in 37% cases. According to clinical diagnosis out of 90 patients, diagnoses were kept of Systemic Lupus Erythematosus (SLE) (43), Systemic sclerosis (SS) (34), Dermatomyositis (DM) (01), Overlap syndrome (12). After serological testing diagnosis found to be SLE (28), SS (22), Mixed connective tissue disease (MCTD) (29), DM (01), undifferentiated connective tissue disease (UCTD) (08), Overlap syndrome (02).

Conclusion: From this study patients with one or more than one connective tissue disorder where diagnosed early. It is important to correlate clinical diagnosis with serological testing (Anti-ENA) so that disease can be treated early before end organ damage occurs and prognostic outcome is also determined.

Key words: Autoimmune,Connective tissue, Nuclear antigen,Aantibody, Polyarthritis, Raynaud's phenomenon

INTRODUCTION

Autoimmune diseases occur in 3-5% of population [6].The autoimmune connective tissue diseases (AI CTDs) are a group of polygenic disorders often having heterogeneous and overlapping clinical features. Certain features are common to all of them viz. autoimmunity, vascular abnormality, arthritis/ arthralgia and cutaneous manifestations[1].Alteration in immune system occurs due to various genetic and environmental factors leading to development of auto reactive phenomenon by a loss of immunological tolerance to self-antigens that can be detected in the peripheral blood in form of the autoantibodies. In 1947, Klemperer used the term "diseases of the collagen system" to refer to conditions in which alterations of the extractable portions of the connective tissue were prominent. The diseases included were lupus erythematosus, scleroderma, rheumatic fever and polyarteritis nodosa [3, 4]. It is now customary to group systemic & discoid lupus erythematosus, systemic sclerosis, localized & generalized morphea, dermatomyositis, rheumatoid arthritis, mixed connective tissue disease and Sjogren's syndrome together [3,5]. The terms used to describe these diseases collectively include "collagen vascular disease", "connective tissue disease", "autoimmune connective tissue disease" because of their common autoimmune basis. Defining specific pathogenic mediators that may trigger the development or progression of an autoimmune disease remains a focus of intense research [3]. Hallmark of these disorders are presence of various circulating autoantibodies that have been identified by various immunochemical techniques. In 1948, Hargraves described the LE cell phenomenon to be highly specific for systemic lupus erythematosus (LE). A decade later, the

classic ANA assay was described by Friou in 1958 and the lupus band by Burnham in 1963[2].

MATERIAL AND METHODS

A group of 90 patients were included in this study who presented in our Dermatology outpatient department at Pandit Deendayal Upadhyay Medical College & Civil Hospital Rajkot with one or other skin and articular manifestations of autoimmune connective tissue disorder. All patients from Dermatology department were referred to Medicine department for systemic evaluation. The patient's preliminary details, chief complaints and its duration, blood investigations viz. complete blood count, blood sugar, ESR(erythrocyte sedimentation rate), RFT(renal function test), LFT (liver function test), Anti-nuclear antibody(ANA), ECG, X-ray chest, x-ray of joint, abdominal sonography and urine routine and microscopic examination were done. Certain special investigations like pulmonary function test, barium swallow, echocardiography and renal biopsy were done. Clinical diagnosis of various connective tissue disorders were made as per various guidelines recommended by Rheumatology society [E.g. American college of Rheumatology, European Society of Rheumatology- EUROPEAN LEAGUE AGAINST RHEUMATOLOGY (EULAR) criteria etc.].

Above all data related to patients were recorded in a pre-validated case record form. All patients had ANA positive except one and hence all were subjected to Anti-ENA profile testing [11] to see the serological correlation of clinical diagnosis. Presence of various autoantibodies in Anti-ENA profile testing, can detect various other coexisting autoimmune connective tissue diseases and throw light on its prognosis too (Table I).

Table1: percentages of anti-ENA antibodies in connective tissue disorders.

ANTI-ENA ANTIBODY	SLE	SJS	MCTD	DM/PM	SS
ANTI-SSA	24-40	60-75	17	8	4
ANTI-SSB	9-15	40-60	0	0	0
ANTI-Sm	30-40	0	3	0	0
ANTI-RNP	23	4	100	14	3
ANTI-Jo-1	<1	0	3	25-31	0
ANTI Scl 70	0	0	0	0	15-20

Among 90 patients enrolled in study, 18 Males (20%) and 72 females (80%) were seen making incidence ratio of 1:4 respectively. Autoimmune connective tissue disease is commonly seen in middle age with average age of occurrence ranging from 20-50 years.

Table 2: distribution of prticipants according to clinical features

Clinical Feature	No. Of Patients (Total 90)	Percentage (%)
Raynaud's Phenomenon	48	53.3
Joint Pain	77	85.5
Photosensitivity	44	48.8
Oral Ulcers	52	57.7
Fever	45	50
Muscle Weakness	22	24.4
Dry Mouth, Eyes	10	11.1
Diffuse Hairfall	31	34.4
Swollen Fingers	45	50
Thickening Of Skin	36	40

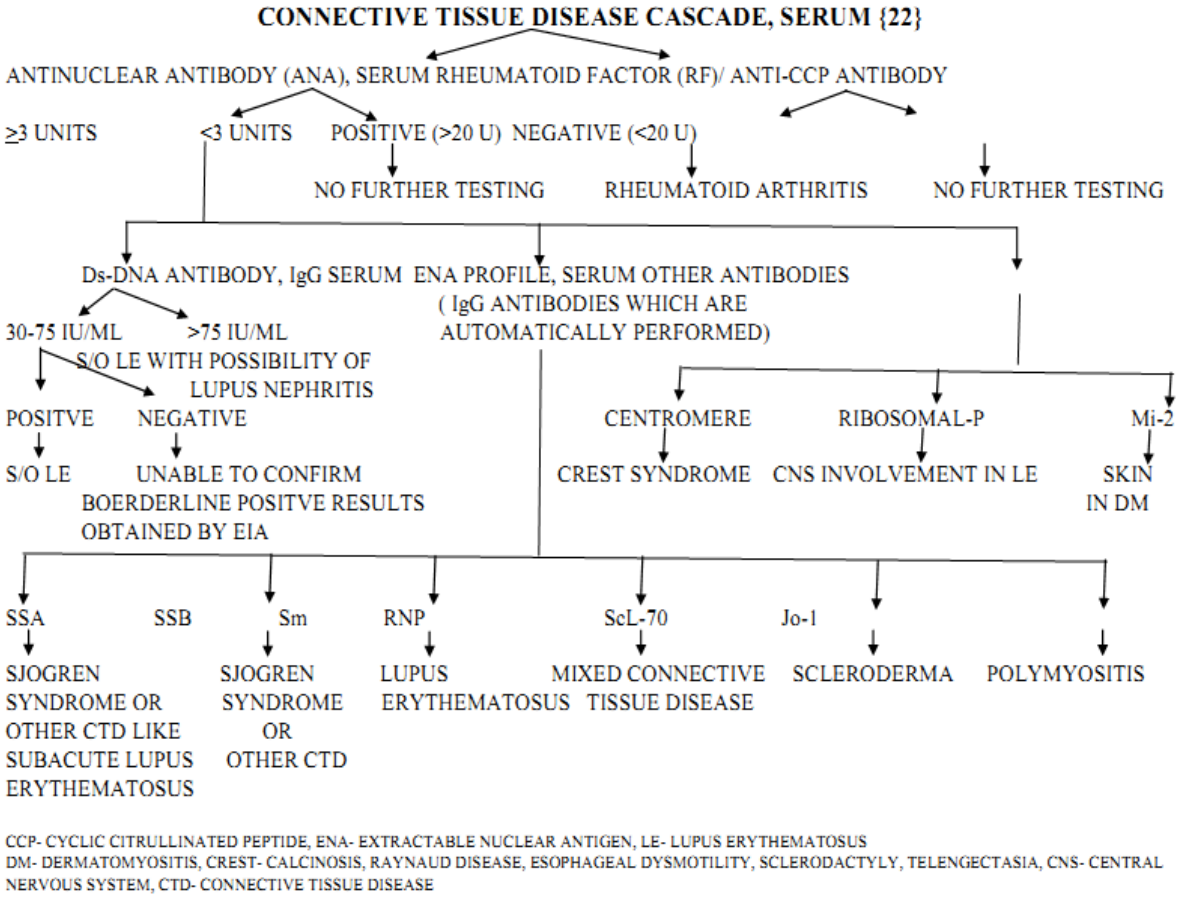
GENERAL RESULT: The majority of the positive sera contained anti-SS-A (40%) & anti-Sm/RNP (35.53%). Other multiple antibodies were also positive in 40% of cases. SEROLOGICAL & CLINICAL CORRELATIONS: All of them presented with predominant clinical manifestation of polyarthritis (85.5%), Raynaud's phenomenon (53.3%), fever (50%), swollen fingers (50%) and skin manifestations (98.8%) (Table II). Based on clinical examination and clinical criteria provisional diagnosis of connective tissue disease were made and later confirmed with serological testing (anti ENA profile) in 63 cases (70%) while diagnosis deferred in 27 cases (30%).

According to clinical diagnosis out of 90 patients, diagnosis kept were SLE (43), SS (34), DM (01), Overlap (12) and UCTD (00). After serological testing diagnosis found to be SLE (28), SS (22), MCTD (29), DM (01), UCTD (08) and Overlap (02).

DISCUSSION

The rising incidence of autoimmune connective tissue disorder has occurred due to availability of new molecular technologies of detecting autoantibodies. Various diseases include under group of autoimmune connective tissue disorder are systemic and discoid lupus erythematosus, localize and generalized morphea, systemic sclerosis, dermatomyositis, mixed connective tissue disorder /overlap syndrome / undifferentiated connective tissue disorder and Sjogren's syndrome together[3]. All of these diseases are characterized by B cell hyperactivity (polygonal B cell activation) resulting into over production of auto antibodies against cytoplasmic, nuclear and surface antigens and immune complex formation. All AICTDs are differentiated on basis of various clinical

manifestations, extent of tissue involvement (localized or generalized), according to clinical criteria by different colleges of Rheumatology and their possible serological correlation with presence of specific auto antibodies for that particular disease. The majority of autoantibodies are targeted against intracellular antigens of cell nucleus (double & single stranded DNA), histones and extractable nuclear antigens (ENAs) **(Flow Chart).**



CCP- CYCLIC CITRULLINATED PEPTIDE, ENA- EXTRACTABLE NUCLEAR ANTIGEN, LE- LUPUS ERYTHEMATOSUS
DM- DERMATOMYOSITIS, CREST- CALCINOSIS, RAYNAUD DISEASE, ESOPHAGEAL DYSMOTILITY, SCLERODACTYLY, TELENGECTASIA, CNS- CENTRAL NERVOUS SYSTEM, CTD- CONNECTIVE TISSUE DISEASE

Newer technology in clinical immunology has enabled screening for multiple autoantibodies hence making it possible (i) to diagnose the autoimmune disease in its earliest stage (ii) to intervene before serious end organ damage occurs (iii) to diagnose presence of more than one connective tissue disorder (iv) to assess the prognosis [12].

Diffuse connective tissue diseases (DCTD) are usually associated with autoimmunity to spliceosomal component (uridine ribonuclear protein particles U1 RNP), heterogeneous (RNPs), nucleosomal components (nucleosomes, DNA, histones) or proteosomal components (LMP2) of autoantigens. There are six DCTDs: - 1) Systemic Lupus erythematosus (SLE) 2) Scleroderma 3) Polymyositis 4) Dermatomyositis 5) Rheumatoid arthritis and 6) Sjogren's syndrome [9].

Lupus erythematosus (LE) is autoimmune disorder with wide clinical spectrum ranging from mild cutaneous lesions to life threatening systemic diseases with multiple organ involvement. It is diagnosed according to American College of Rheumatology (ACR) criteria 1997[7]. Specific skin lesions of LE are classified as chronic, sub-acute and acute lupus erythematosus. Photo protection, topical steroids, antimalarial and lastly immunosuppressant form the mainstay of treatment. Systemic sclerosis is a multi-system connective tissue disorder characterized by thickening and induration of the skin and involvement of other organs like kidney, lung, gastrointestinal system and heart. Excessive accumulation of collagen & extracellular matrix and damage to endothelium of the small vessels resulting into hyperplasia, tissue ischemia and activation of the immune system. Morphea is localized scleroderma which primarily affects the skin. Kreuter et al gave its various subtypes out of which linear type is more prevalent in children [8]. The 2013 EULAR criteria for classification of systemic sclerosis are more sensitive (91% vs. 75%) and specific (92 % vs. 72%) than the American College of Rheumatology criteria (ACR) [10]. Indicators of poor prognosis in scleroderma are male gender, wide extent of skin sclerosis and presence of major visceral organ involvement (heart, lung and kidneys). Dermatomyositis (DM) is an idiopathic inflammatory dermatomyopathy (IIDM) affecting skin, muscle and blood vessels, hence characterized by muscle weakness and cutaneous eruptions. More than 3 decades ago, Bohan & Peter proposed a set of 5 criteria to facilitate the diagnosis of IIDM. They classified into 5 groups:

1) Primary idiopathic Polymyositis 2) Primary Idiopathic Dermatomyositis 3) Idiopathic Inflammatory Myositis (IIMs) associated with malignancy 4) Childhood IIMs associated with vacuities 5) IIMs associated with collagen vascular diseases. Drawback of this classification is over diagnoses of Polymyositis. Poor prognostic factors in DM are older age, fever, progressive disease, cardiac and pulmonary involvement, dysphagia, extensive skin lesions, leukocytosis and associated malignancy [9]. Mixed connective tissue disorder was first described by Sharp et al [12] in 1972 as a distinct entity due to its clinical features and specific presence of an antibody (u1 RNP). It's a special form of overlap syndrome having overlapping features of systemic sclerosis, SLE and Dermatomyositis associated with antibodies against polypeptides on U1 ribonuclear protein (U1-RNP) component of spliceosome. The overlap features of MCTD seldom occur concurrently, it usually takes several years before enough overlapping features have appeared. The most common clinical association with in these patients with U1 RNP antibodies in the early phase of disease are Raynaud's phenomenon, dactylitis (sausage digits), inflammatory muscle disease, low grade fever and arthritis. Over a period, these patients develop manifestations more consistent with classic systemic lupus erythematosus or systemic sclerosis. There are no ACR criteria for diagnosis of MCTD, but a comparative study reported 2 criteria sets viz. Alarcon-Segovia and Kahn, having the best sensitivity and specificity of 62.5% & 86.2% respectively [9].Kausukawa's criteria can also diagnose MCTD when all 3 of the following are fulfilled: 1) common symptoms- a) Raynaud's phenomenon b) swollen fingers 2) Anti-U1-RNP antibody 3) mixed findings- [one or more positive findings in 2 or 3 disease categories of A, B & C] [A] SLE like features [B] Systemic sclerosis like features [C] IIDM like features [17]. Raynaud's phenomenon is seen in all patients with MCTD and if it's absent than diagnosis should be reconsidered. In comparison to pure isolated SLE patients, MCTD patients have lower incidence of renal disease (25%) but have higher incidence of pulmonary disease (pulmonary arterial hypertension- PAH is the most common cause of death)[9]. Overlap syndrome is defined as a combination of major clinical & serological features of more than one autoimmune disease in same patient. The autoimmune diseases are present in their entirety. Any autoimmune connective tissue disease can be partner and 2^{nd} or 3^{rd} disease can appear while 1^{st} disease is still in active stage even with adequate treatment [9].Diagnosis depends on for which diseases the patient shows the symptoms and positive antibodies in their lab serology. In overlap syndrome, features of the following diseases are found (most common listed): - 1) SLE 2) Systemic sclerosis 3) Polymyositis and dermatomyositis 4) Rheumatoid arthritis 5) Sjogren's syndrome 6) Churgstrauss syndrome 7) Autoimmune thyroiditis 8) Ant phospholipid antibody syndrome [16]. Its treatment is by use of corticosteroids and immunosuppressant. Biological drugs anti-TNF alpha and anti-CD 20 (monoclonal antibodies), recently introduced as alternative medicine for refractory cases. Undifferentiated Connective Tissue Disease (UCTD) is defined as a case in which few symptoms of more than one autoimmune connective tissue disease are present, without meeting the full criteria for any one of them. Some of these diseases criteria overlap further complicating the diagnostic workup in these patients. The diagnosis of UCTD is usually made in patients with Raynaud's phenomenon in combination with an unexplained synovitis or inflammatory myopathy. About 55% of patients with UCTD fail to differentiate into classic diffuse connective tissue disease (DCTD). Unclassifiable symptoms, physical examination finding or serological results lead to diagnosis such as incomplete lupus, latent lupus, overlap syndromes or undifferentiated connective tissue diseases. In 1980, Le Roy et al and in 1999, Mosca et al gave proposed preliminary classification criteria of which Mosca et al criteria is increasingly accepted [14,15]. Hence UCTD is defined as those in which sign and symptoms are consistent with connective tissue disease but that do not fulfill the classification or diagnostic criteria of other defined diseases (AI-CTD).

All patients were subjected to Anti-ENA profile, which detects the various autoantibodies present in blood. Extractable Nuclear Antigens are over 100 different soluble cytoplasmic and nuclear antigens. Autoantibodies to these antigens have a partial marker function for the individual disease; hence they are associated with particular connective tissue disorders. The main six antigens in immunological laboratories for detection are Ro, La, Sm, RNP, Scl-70 and Jo1 (ENA-6 Profile) [6, 13], which are screened by Ouchterlony double immuno diffusion techniques and confirmed by immunoblotting. On anti-nuclear antibody (ANA) tests, these antigens have a speckled pattern [14]. ENAs originally referred to proteins found in saline extract of cell nuclei. Its components have been clearly identified and include many cytoplasmic molecules. The misnomer however has stuck. These proteins are intimately associated with various RNA molecules and are thus called ribonucleoproteins, but the nomenclature used for them is confusing e.g. Sm, Ro and La were named after first 2 letters of the surnames of the patients in whom they were first found. Two proteins associated with Sjogren's syndrome were independently described as antigens A and B, but are now known to be identical to Ro and La respectively .i.e. SS-A= Ro and SS-B= La antibodies used to screen the specific disorders. Sensitivity and specificity of these tests depends on the type of assay employed and will therefore vary by lab. The following table illustrates the sensitivity and specificity of various antibodies in various diseases. Certain auto antibodies have considerable disease specificity and thus can be of great diagnostic value viz. anti-ds DNA & Sm for SLE, Mi-2 for classic Dermatomyositis, jo-1 for antisynthetase syndrome, topoisomerase- 1(Scl-70) & centromere for differentiating clinical forms of systemic sclerosis and particular organ involvement, c-ANCA(cytoplasmic antineutrophil cytoplasmic antibody) for Wegner's granulomatosis. However

most autoantibodies fall into the disease nonspecific category. The aim of this study was to correlate the clinical diagnosis with its serological markers, hence the disease can be diagnosed at early stage and its prognosis can be assessed too.

Our study result showed that autoimmune disorders are common in female compared to male, ratio M: F = 1:4. This result is in accordance with reports from various authors viz. Malaviya et al [M: F= 1:8] and Lee et al [M: F= 1:6] [18, 2]. The median age of onset in present study is 40 years while study by Malaviya et al had 24years [18] and Masi et al had 31 years [19] as a median age. Possible reason for difference in result might be due to small study group and our group had more number of systemic sclerosis patients. In present study we found following correlation between clinical diagnosis and its serological markers (Table III).

Table 3: Correlation between clinical diagnosis and its serological markers

Antibody	SLE (28)	SS (22)	MCTD (29)	DM (1)	OVERLAP(2)	UCTD (8)
Sm	10 (35.7)	0 (0)	9 (31.03)	0 (0)	1 (50)	3 (36.6)
RNP	2 (7.14)	1 (4.5)	7 (24.1)	0 (0)	0 (0)	0 (0)
Sm/RNP	11 (39.3)	5 (22.7)	29 (100)	0 (0)	0 (0)	0 (0)
SCL-70	2 (7.1)	5 (22.7)	1 (34.5)	0 (0)	0 (0)	1 (12.5)
ds-DNA	8 (28.6)	0 (0)	3 (7.7)	0 (0)	0 (0)	0 (0)
SSA	13 (46.4)	4 (18.2)	10 (34.5)	0 (0)	2 (100)	2 (25)
RO-52	13 (46.4)	5 (22.7)	11 (37.9)	0 (0)	0 (0)	1 (12.5)
OTHERS	18 (64.3)	1 (4.54)	7 (24.1)	1 (100)	2 (100)	4 (50)

SLE diagnosis was made in 43 cases and 08 cases (28.6%) had anti ds-DNA antibodies. Some patients (02 cases + 11 cases) (46.4%) had anti-U1-RNP antibodies, hence diagnosis of MCTD was kept in such patients. All patients were followed up for progression of disease or appearance of new connective tissue disease on long run.

CONCLUSION AND RECOMMENDATIONS

The incidence of autoimmune connective tissue disorder is increasing day by day due to availability of modern techniques to detect them in its earlier stage. From this study it can be emphasized that more than one connective tissue diseases do exist in patient suspected of having one connective tissue disorder. Hence there is need to correlate clinical diagnosis with serological testing (Anti-ENA profile) so that early diagnosis, intervention and prognostic outcome of illness can be determined.

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