"CLINICAL SPECTRUM AND PULMONARY MANIFESTATIONS OF PATIENTS WITH ANTI U1RNP ANTIBODY STATUS"

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CERTIFICATE

This is to certify that this dissertation "**Clinical Spectrum and Pulmonary Manifestations of Patients with Anti U1RNP Antibody Status"** presented here is the original work done by Dr.N.Thilagavathi, D.M Postgraduate in the Department of Rheumatology, Madras Medical College and Rajiv Gandhi Government General Hospital, Chennai 600003 in partial fulfilment of the university rules and regulation for the award of D.M. Branch IX - Rheumatology, under my guidance and supervision during the academic period from 2011-2014.

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DECLARATION

I, **Dr.N.THILAGAVATHI** hereby solemnly declare that this dissertation entitled "**Clinical Spectrum and Pulmonary Manifestations of Patients with Anti U1RNP Antibody Status"** was done by me in the Department of Rheumatology, Madras Medical College and Rajiv Gandhi Government General Hospital, Chennai 600003 during January 2012 to December 2013 under the guidance and supervision of Dr.S.Rajeswari M.D., D.M. This dissertation is submitted to the Tamil Nadu Dr.M.G.R.Medical University towards the partial fulfilment of requirement for the award of D.M. degree in Rheumatology.

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INDEX

ABBREVIATIONS

MCTD	:	Mixed Connective Tissue Disease
PM	:	Polymyositis
DM	:	Dermatomyositis
RA	:	Rheumatoid Arthritis
SLE	:	Systemic Lupus Erythematosus
SSc	:	Systemic Sclerosis
PM/Scl	:	Polymyositis/Scleroderma
U1RNP	:	Uridine Rich Ribonuclear Protein
ANA	:	Anti Nuclear Antibody
RNA	:	Ribonucleic Acid
DNA	:	Deoxyribo Nucleic Acid
snRNP	:	Small Nuclear Ribonuclear Protein
hnRNP	:	Heterogenous Nuclear Ribonuclear Protein
PDGF	:	Platelet Derived Growth Factor
PGE1	:	Prostaglandin E1
PHT	:	Pulmonary Hypertension
ILD	:	Interstitial Lung Disease
TGF	:	Transforming Growth Factor
DLCO	:	Diffusing Capacity for Carbon Monoxide
FITC	:	Fluorescein Isothiocyanate
Pro BNP	:	Pro Brain Natriuretic Peptide

INTRODUCTION

Overlap syndrome is defined in a patient when a combination of more than one major feature of connective tissue disease is present with a specific serological test[1]. The common symptoms of overlap syndrome include sclerodactyly, arthritis and Raynaud's phenomenon. A distinguishable feature in patients with autoimmune disease is the presence of non organ specific auto antibodies to RNA, DNA and to proteins that bind them. For MCTD the well known serological marker is anti U1RNP antibody.

The ribonuclear proteins are auto antigens located on small nuclear RNP particles. They are uridine rich. The major action is splicing of pre messenger RNA. Anti U1RNP is associated with features of scleroderma, including Raynaud's phenomenon. There has been a close association of anti U1RNP antibody with pulmonary fibrosis and negative correlation with renal involvement.

MCTD, although an overlap syndrome, does not have any distinctive clinical feature. The components of MCTD (systemic sclerosis, systemic lupus erythematosus and idiopathic inflammatory myositis) do not occur simultaneously but consecutively over years. Raynaud's phenomenon occurs in almost all patients with MCTD.

AIM OF THE STUDY

1) To correlate anti U1RNP antibody with the clinical spectrum of Mixed connective tissue disease (according to Kasukawa's criteria) and Overlap Syndrome.
2) To analyse the association of anti U1RNP antibody with the pulmonary manifestations among Mixed connective tissue disease and Overlap Syndrome.

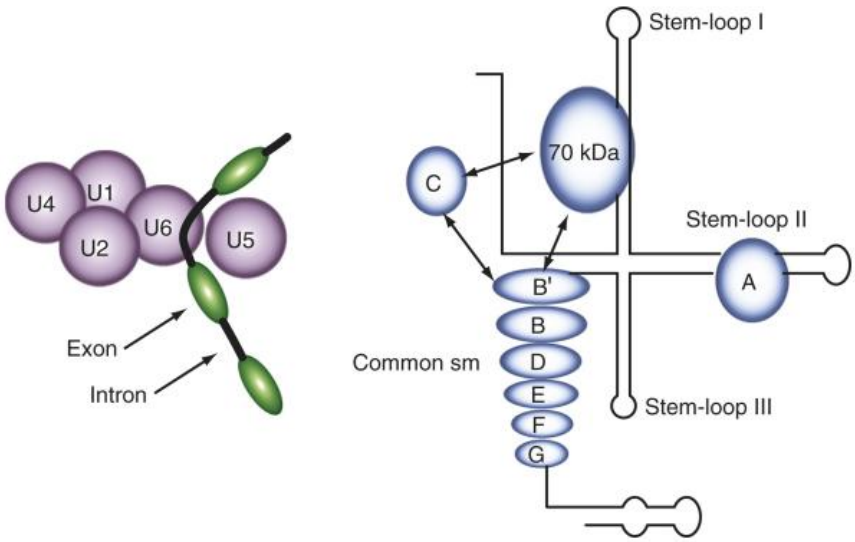
REVIEW OF LITERATURE

We find that patients diagnosed as autoimmune rheumatic disease were not classified with ease into one of the well defined clinical syndromes such as systemic lupus erythematosus, systemic sclerosis or idiopathic inflammatory myositis. The issue is complicated by such diseases with a tendency to overlap with one another which results in continuous manifestations of clinical features among rheumatic diseases. Now, there is an increased awareness in diagnosing such patients as 'Overlap Syndrome'. In literature, any of the combinations of rheumatic diseases has been reported. The very purpose of identifying Overlap Syndrome is useful in prognostication and facilitating disease management.

Autoimmune diseases are heterogenous and the role of auto antibodies in the immuno pathogenesis of disease process is not certain. As a normal physiological process auto antibodies are formed but when in excess become injurious to self. These auto antibodies act either directly against a particular tissue or by the formation of immune complexes.

AUTOIMMUNITY IN OVERLAP SYNDROME WITH REFERENCE TO U1RNP

To process pre messenger RNA to mature RNA, spliceosomes are needed[2]. Spliceosomes are made up of 300 different proteins and 5 RNAs. Their different parts are targets of immunogenecity in several auto immune connective tissue diseases. By increasing the post translational modification in spliceosomal components, as occurring during apoptosis, the immunogenic potential is heightened[3]. The major antigenic determinants of spliceosomal components are small nuclear ribonuclear protein (snRNP) and heterogenous nuclear RNP (hnRNP)[4].



Small RNAs in the range of 80-350 nucleotides combined with proteins constitute small nuclear ribo nuclear protein. There is a high quantity of uridine in these RNAs. Hence these are called as U-RNAs. Researchers, by immuno precipitating these U-RNAs were able to identify five different types namely U1, U2, U4, U5 and U6[5]. The protein components of these complexes are the target antigens to auto antibodies. The back bone of U1-RNP is double stranded U1-RNA molecule.

Non specific proteins and specific proteins constitute U1-RNP complex. The specific proteins with their corresponding molecular weights include U1-A' (33,000), U1-C (22,000) and U1-70K (68,000)[6]. In SLE the main target antigen is Sm protein[7] and the clinical features unique to MCTD ares correlated with 70kD specificity with an immuno dominant epitope embracing amino acid residue at positions 119-126.

The hnRNPs contain pre-mRNA and structurally related proteins with molecular weight ranging between 33 to 43 kD. There are nine hnRNP core proteins that have been identified and they include A1, A2, B1a, B1b, B1c, B2, C1, C2 and C3[8]. Anti –RA33 against 33-kD hnRNP-A2 is present in nearly a third of patients with rheumatoid arthritis, systemic lupus erythematosus and mixed connective tissue disease[9]. There have

been associations of anti RA33 with erosive arthritis in lupus, systemic sclerosis and mixed connective tissue disease[10]. This antibody also foretells the onset of RA in patients with very early polyarthritis[11].

Uniquely there is no correlation of anti-RA33 in polymyositis or overlap of PM/DM or PM/Scl. The hnRNP-A2 has antigenic epitopes that contain two RNA binding sites, one at the N-terminal region and the other at the glycine rich C-terminal end. The significance of these sites is that, certain rheumatic diseases target the two binding sites differently. RA and SLE sera selectively target the second RNA binding site and MCTD sera reacts with both RNA binding domains[12].

GENERATION OF AUTOIMMUNITY (U1RNP)

Spliceosomes may be the target antigen for auto antibody but the autoimmune response will result against the entire particle by antigen presenting cells. Hence all the components of the spliceosomal complex will be subjected to antigen processing and finally presented to HLA class type 2. HLA molecule polymorphism will result in diversified antibody response to recruit other antigens also and this is called as 'epitope spreading'. This process is important in the pathogenesis of auto immune connective tissue disorders[13]. As a result of epitope spreading, the immune pathogenesis and auto antibody response becomes altered with time and this correlates well with the change in the clinical scenario[14].

The role played among the proteins presented by HLA and the T cell receptors are crucial in the development of autoimmune reaction. HLA DR4 and DR2 are linked with 70kD and anti-U1-RNP antibodies[15]. The presence of DR2 and DR4 have a common set of peptide acids at positions 26, 28, 30, 31, 32, 70 and 73 in beta position for binding of antigen[16]. The most commonly found sequence of the polypeptide 70kD,

having different epitopes is KDK DRD RKR RSS RSR[17]. This site is the preferred target of mixed connective tissue disease but not by the systemic lupus erythematosus sera[18]. Varying degrees of epitope spreading characterize the different types of auto immune connective tissue disorders. SLE and MCTD patients have antibodies to both snRNP and hnRNP but MCTD patients have more restricted anti spliceosomal antibody representation. RA sera have anti spliceosomal antibodies only against hnRNP[19]. Conserved molecules also act as source of auto antigens against which auto antibodies form. The mechanism of these auto antigen formation is postulated by two theories namely apoptotic modification[20] and molecular mimicry[21].

To overcome the tolerance to self antigens the peptides get altered during apoptosis and then presented to the immune system. The self antigens are crowded onto the blebs on the surface of apoptotic cells. Broken down pieces of endoplasmic reticulum, ribonuclear protein Ro and ribosomes are contained in the smaller blebs. The apoptotic bodies which form the larger blebs encompass snRNP, Ro, La and nucleosomal DNA[17]. Many enzymes are up regulated which result in post translational changes in the breakdown of proteins. These alterations include transglutamination, citrullination, dephosphorylation, phosphorylation and finally the linkage to ubiquitin, makes the molecule more antigenic. Caspase 3[22] acts on the U1-70K and converts it into a C-terminal fragment and this contains the major epitope B cell, which is targeted by the antibodies.

Anti U1-RNP antibodies may form as a result of molecular mimicry. Recognizing non self protein is the first step of auto antibody formation. There has been increased apoptosis under certain situations like toxins, drugs, infections and ultra violet radiation. Antigens that closely resemble U1-RNP produce cross reactive antibodies. The

extraneous antigens get cleared with time but the endogenous antigenecity of U1-RNP persists. Some evidence does exist for the hypothesis of molecular mimicry in connective tissue diseases but still needs further evaluation.

Multiple other antibodies have been identified in patients with MCTD and they include anti-Ro/SS-A, antiphospholipid, anti-hn-RNP-A2 and antibodies against other components of U1-RNP complexes. The reason for the development of other antibody specificity is not known and epitope spreading may play a role. The role of U1-RNP in the pathogenesis of MCTD is not known although it is needed for the diagnosis of the disease.

OVERLAP SYNDROME

Overlap of systemic sclerosis and myositis

There has never been a uniform spectrum identified in patients with systemic sclerosis/ myositis overlap. Systemic sclerosis patients have pronounced overlap features with other connective tissue disorders. Myositis/Systemic Sclerosis Overlap Syndrome occurs as frequently as the tRNA synthetase syndrome. Many of these patients do not present with prominent skin changes (sine scleroderma) or have only a limited form of the disease. Muscle weakness may occur either concurrently, before the onset or after the establishment of systemic sclerosis.

The foremost clinical manifestation of systemic sclerosis overlap cases is the presence of Raynaud's phenomenon and it should be differentiated from primary Raynaud's. The effect on the gastrointestinal tract is more prevalent in SSc/Myositis overlap. Pneumatosis intestinalis and pseudo-obstruction as complications of the syndrome were recorded to be severe[23].

HLA-DR3 is found to be positive in more than 90% of patients with systemic

sclerosis and myositis overlap; however the frequency of this HLA is not common in Japanese population as in North American and European population. The genetic impact of the disease is suggested by the high frequency of HLA DQA *0501[24]. These genetic influences alone are not the sole cause of the disease as multiple independent factors contribute to the pathogenesis of the disease.

The presence of anti nucleolar antibody, which is usually done by indirect immunofluroscence gives an idea to the presence of Myositis/ Systemic Sclerosis overlap. By using the double immunodiffusion technique and indirect immunofluorescence, this antibody was first reported. A homogenous nucleolar pattern with poor staining of nucleoplasm is produced by Pm/Scl antibodies. The exact function of Pm/Scl antigen is not known, although it has some role in the maturation of ribosomes. This antigen is sited at the nucleolar granular component, the place where the assembly of ribosomes occur. Pm/Scl is a protein complex of around 16 different types of polypeptides with arrange of molecular weight of 20-110 kDa[25,26]. Auto-antibodies against two main molecules are 75kDa (Pm/Scl-75 protein) and 100kDa (Pm/Scl-100 protein). Nucleolar macromolecular complex are the target antigens for these auto-antibodies. These Pm/Scl auto-antibodies are seen in 6% of patients with dermatomyositis/polymyositis. In patients with systemic sclerosis alone it is found in 2% of individuals but in overlap syndrome of systemic sclerosis and myositis overlap, Pm/Scl auto-antibodies are identified in 24% of patients. Among individuals with these auto-antibodies, 43% to 88% have sclerodermatomyositis. The specific auto antibodies associated with this overlap are anti PM/Scl, anti-U2-RNP, anti-Ku and anti-U5snRNP[27].

In a study conducted in Japan, anti-Ku antibodies were associated with systemic sclerosis myositis overlap in 50% of patients. In these Japanese patients anti-Pm/Scl antibodies ware found to be very rare whereas anti-Ku antibodies were documented in only 10% of North American cases of scleromyositis[28]. The significance of the anti-PM/Scl auto antibodies is the relatively benign course of the interstitial lung disease and the good response to corticosteroids. Furthermore there is no association of this autoantibody with malignancy. Joint symptoms had significant association with these anti bodies. There were no reported cases on the presence of anti-Jo-1 in systemic sclerosis myositis overlap. Anti –Ku antibodies had no correlation to malignancy in patients with PM/Scl syndrome, as is the case with idiopathic inflammatory myositis. In 4% of these overlap patients, there is presence of antibodies to signal recognition particle (SRP) and they show poor prognosis. SRP in PM/Scl is recognized with distinctive necrosis of muscle fibers and the rapidity with which the muscle weakness occurs is very severe.

The treatment target in SSc/myositis overlap is the management of skin damage, muscle weakness and alveolitis. As high dose steroids may precipitate a renal crisis in patients with systemic sclerosis, high dose steroids should be used with caution for myositis component in systemic sclerosis myositis overlap[29]. The drugs commonly used include corticosteroids, cytotoxic drugs like azathioprine, methotrexate, mycophenolate mofetil, cyclophosphomide and certain biologicals.

The effective management of skin, joint and muscle weakness does not necessarily imply the good control of alveolitis of systemic sclerosis/myositis overlap. Methotrexate and anti-TNF alpha therapy is associated with high incidence of aggravating ILD in these cases[30]. Uncontrolled myositis responds to rituximab[31,32] and

mycophenolate mofetil[33,34] is found to be useful in improvement of skin and muscle features. Persistent and severe Raynaud's may need the addition of prostaglandins and antagonists to endothelin receptors.

MIXED CONNECTIVE TISSUE DISEASE (MCTD)

MCTD is another overlap syndrome, first identified by Sharp et al in 1971[35]. It consists of SLE, systemic sclerosis and polymyositis/dermatomyositis and with the association of U1-RNP antibodies. The recognition of mixed connective tissue disease is characterized by the presence of specific antibodies and the associated clinical features.

Nuclear antigenic pool that was soluble in normal saline, termed extractable nuclear antigen was used for complement fixation test, as the initial serologic test for MCTD. After the introduction of hemagglutination, where the antigenic mixture is treated by enzymatic action, ribonuclease (RNase) sensitive part of the antigen was identified as the target antigen for auto antibodies in mixed connective tissue disease. The differentiating factor between SLE and MCTD is that, auto antibodies in SLE reacted with RNase resistant fragment of the extractable nuclear antigen, whereas MCTD with that of RNase sensitive fragment[36]. By using immuno diffusion[37] technique, RNase resistant component has been recognized as Sm antigen and RNase sensitive as RNP. Both Smith and RNP antigens are destroyed by trypsin, indicating the presence of protein in them. Immunoprecipitation studies showed that Sm and RNP had ribonucleoproteins in them. This was done using [^{32} P] labelled cells.

Epidemiology and HLA of MCTD

The exact prevalence of mixed connective tissue disease is not known and may be approximately 10/100,000. This disease is more common in females, like any other connective tissue disease and the female to male ratio is 9:1. 80% -90% of MCTD occurs

in the third decade. The disorder has also been reported in children and in persons above 80 years of age. No known environmental factor has been reported in the etiopathogenesis of the disease, except the exposure to the chemical agent vinyl chloride[38].

HLA associated with MCTD is DR4. DR3 and DR5 have no associations, although they have been reported in SLE and systemic sclerosis respectively[39]. The presence of DR4, could give the link for the occurrence of joint erosions in these patients.

Clinical features

The onset of clinical features in mixed connective tissue disease may be that of manifestations of a lupus, systemic sclerosis, polymyositis or rheumatoid arthritis. Raynaud's phenomenon, inflammatory polyarthritis, sclerodactyly, swollen hands and gastro esophageal reflux symptoms are some of the most common clinical features of MCTD. Malar rash, hair fall, glandular enlargement and renal damage occur less frequently. Constitutional features like arthralgia, myalgia, fever and fatigue are also common in individuals with MCTD.

Proposed classification criteria for MCTD

There are no single commonly accepted criteria for MCTD. Four different criteria are available at present for MCTD and they include Sharp, Alarcon-Segovia, Kahn and Kasukawa[40,41,42,43].

SHARP's Criteria

This consists of 5 major and 11 minor criteria to differentiate among certain MCTD from probable MCTD. To diagnose probable MCTD anti U1RNP antibody is not essential, but its absolute presence is needed for establishing a definitive diagnosis.

Major criteria	Minor criteria
1. Severe myositis 2. Lung involvement with a DLCO < 70 % and/or PAH and/or proliferative vascular lesions on biopsy 3. Raynaud's phenomenon and/or oesophageal hypomobility 4. Swollen hands and/or sclerodactyly 5. anti-ENA ≥ 1 : 10000, positive for anti-U1-RNP antibodies and negative for anti-Sm-antibodies	1. Alopecia 2. Leucopoenia 3. Anaemia 4. Pleuritis 5. Pericarditis 6. Arthritis 7. Trigeminal neuralgia 8. Malar rash 9. Thrombocytopenia 10. Mild myositis 11. History of swollen hands
• Certain MCTD: 4 major criteria, no anti-Sm-antibodies, anti-U1-RNP-antibodies > 1 : 4000 • Probable MCTD: 3 major criteria and no anti-Sm-antibodies or 2 major criteria plus 1 minor criterion plus anti-U1-RNP-antibodies > 1 : 1000	

ALARCON-SEGOVIA Criteria

This consists of five clinical criteria plus the presence of high titer anti-RNP antibodies.

Clinical criteria	Serologic criterion
1. Swollen hands 2. Acrosclerosis with or without proximal SSc 3. Raynaud's phenomenon 4. Myositis: biologically or histologically proven 5. Synovitis	Anti-RNP-antibodies with a titer of > 1 : 1600 at the hem-agglutinin assay
Diagnosis of MCTD, if the serologic criterion is present and ≥ 3 clinical criteria (if 1, 2 and 3 are present, 4 and 5 are also required to distinguish MCTD from SSc)	

KAHN's Criteria

This has 4 clinical criteria besides the need for the presence of high titer anti-RNP antibodies. It almost mimics Alarcon-Segovia criteria.

Clinical criteria	Serologic criterion
1. Raynaud's phenomenon 2. Swollen fingers 3. Myositis 4. Synovitis	High titres of anti-RNP-antibodies, corresponding to a speckled ANA titre of ≥ 1 : 2000
Diagnosis of MCTD, if the serologic criterion is present plus Raynaud's phenomenon plus ≥ 2 of the other three clinical criteria	

KASUKAWA'S Criteria

Common symptoms	Symptoms of SLE, SSc and PM	Serologic criterion
1. Raynaud's phenomenon 2. Swollen fingers	SLE: 1. polyarthritis 2. adenopathies 3. malar rash 4. serositis 5. leucopoenia and/or thrombocytopenia SSc: 1. sclerodactyly 2. pulmonary fibrosis and/or restrictive changes and/or reduced DLCO 3. oesophageal hypomobility or dilatation PM: 1. muscle weakness 2. elevated muscle enzymes 3. myogenic changes in EMG	anti-snRNP-antibodies
Diagnosis of MCTD, if ≥ 1 common symptom plus ≥ 1 symptom of the mentioned signs of ≥ 2 of the defined connective tissue diseases SLE, SSc and PM plus the serologic criterion		

Joints and muscles

Patients with MCTD may present with minimal arthralgia to joint erosions. Small and large joint involvement, erosive arthritis and sometimes arthritis mutilans can occur[44]. In erosive joints, RF is found to be positive in nearly 70% of individuals[45]. Common arthritic symptom in MCTD is polyathralgia and it is also one of the early features, occurring in nearly 60% of patients. On clinical examination of the forearm, peritendinous nodules, usually small in size have been documented.

Muscular involvement has been reported in 80-90% of MCTD individuals. The pattern of muscle involvement is like any other connective tissue disease, with the proximal group of muscles being more commonly affected. Muscle enzyme analysis show elevated creatinine kinase and electromyography recording gives a picture of inflammatory myopathy. Usually the presentation of muscle weakness is acute, with or without fever. The onset can also be insidious. Some cases have persistent myopathy[46,47].

Skin manifestations

One of the most consistent skin manifestations of mixed connective tissue disease is Raynaud's phenomenon. The percentage of occurrence of Raynaud's varies between 75-90% of patients with MCTD. This clinical feature may antedate other manifestations by months or even years. Vascular pathogenesis of middle sized arteries correlates with Raynaud's phenomenon. Puffy hands and sausage digits occur in 70% of individuals with MCTD. The histopathological appearance of skin and nailfold capillaroscopy changes of digits simulates that of systemic sclerosis. Bushy appearance of capillaries in nailfold capillaroscopy is a distinct feature of mixed connective tissue disease[48]. Sicca symptoms, skin rash simulating dermatomyositis, oral ulcers and genital ulcers have been recorded. Urticarial vasculitis with low complements, were seen in patients,

although this presentation is not common. Features of SLE, like malar rash, photosensitivity and oral ulcers are also generally seen in these patients.

Raynaud's phenomenon

This phenomenon was initially identified by A.G.Maurice Raynaud, who was a physician in France. Raynaud's phenomenon is further categorised into Raynaud's disease and Raynaud's syndrome. When there is no identifiable cause it is called as Raynaud's disease and if it occurs, secondary to some other connective tissue disorder, this phenomenon is called as Raynaud's syndrome.

Raynaud's phenomenon usually occurs in young females and can be familial sometimes. It is shown that Raynaud's syndrome may precede the onset of systemic disorder by even twenty years. 85% of individuals with MCTD have this syndrome. Regarding the pathogenesis of Raynaud's phenomenon, the following theories are put forth and they include interaction between blood and blood vessel, neurogenic mechanism and role of inflammation. Vascular endothelial cells function in regulating the blood flow by secreting many substances. There is imbalance in the secretion of vasodilators like nitric oxide and prostacyclin (PGI_2) and vasoconstrictors like endothelin -1, which causes constriction of the blood vessels, inflammatory state and a procoagulant vascular endothelium. The role of platelets in pathogenesis of Raynaud's phenomenon has been studied and shows that platelets exhibit increased clumping. The platelets release thromboxane A2, growth factors like TGF-beta and PDGF.

Hyperexcitability of sympathetic neurons cause increased constriction of blood vessels as proposed by the neurogenic theory as one of the mechanism for Raynaud's phenomenon. Role of estrogen and stress, causing vasoconstriction has been studied and reported in pathogenesis of this phenomenon. Macrophages, TNF, lymphotoxins and

proteins of T- cell origin play an important part in the immune response of Raynaud's phenomenon.

The clinical findings of Raynaud's include three phases and they are a phase of pallor, cyanosis followed by redness of fingers. These three phases of colour change do not typically present in all individuals but the stage of pallor must occur to make a diagnosis of Raynaud's phenomenon. Emotional stress, elderly age group, female sex, frequent severe attacks, persistent vasospasm are some of the markers of progression of Raynaud's phenomenon. Microscopic examination of the nail fold helps in determining the structural and functional changes of the circulation in the microvasculature system. Radioisotope clearance, thermography and Doppler flowmetry using laser technology are some of the methods to diagnose Raynaud's phenomenon.

Calcium channel blockers have become the mainstay of medical management of Raynaud's phenomenon. This acts through the vasodilatory mechanism but also has antithrombotic and antiplatelet effect. Other vasodilators like inositol nicotinate, prazosin, losartan[49], phosphodiesterase inhibitors like sildenafil[50] and endothelin blockers like bosentan[51] have been tried in the medical treatment of Raynaud's phenomenon. Prostaglandin analogues, iloprost infusion helps in patients with ulcers of the digits and painful ischemia[52]. This is found to be more helpful in healing ulcers than the calcium channel blockers. Alprostadil, PGE1 and PGE1 alpha, cyclodextrin were also used in Raynaud's, in patients not tolerating iloprost. Oral prostaglandin analogues like cisaprost, oral iloprost and limaprost have been tried in patients with Raynaud's phenomenon.

Cardiovascular disease

The incidence of involvement of the heart ranges from 11% to 85% and this depends on the technique used and how the cardiac involvement was defined. The commonest cardiac presentation is pericarditis and it is usually mild[53]. Myocardial involvement, left ventricular diastolic dysfunction, conduction defects and mitral valve prolapse have been reported in patients by using echocardiography. Vasculopathy in the form of proliferation of the intima and hypertrophy of the media, affecting small and medium arteries, similar to systemic sclerosis is seen. Vitamin D level and cardiovascular disease have indirect relationship and vitamin D level has been found to be low in patients with mixed connective tissue disease[54].

Lung manifestations

In 85% of individuals with mixed connective tissue disease, lung abnormalities are made out. Varied lung features have been described including pulmonary hypertension, interstitial lung disease and fibrosis. The common causes of mortality in these patients include pulmonary arterial hypertension, congestive cardiac failure and infections. Individuals with mixed connective tissue disease and PAH have a higher rate of pericardial effusions, although good hemodynamic and right ventricular echo findings were made out compared to other connective tissue diseases, by REVEAL (registry to evaluate early and long term pulmonary arterial hypertension disease management) study. Natriuretic peptide B type was found to be elevated in MCTD patients and DLCO was decreased. Breathlessness and chest pain, usually pleuritic are the common features made out clinically. Effusions and thickening of the pleura, lung infiltrates and interstitial changes are reported by radiographic examination. Rare cases of pulmonary haemorrhage and acute onset interstitial pneumonia have been documented[55,56,57].

INTERSTITIAL LUNG DISEASE (ILD)

Interstitial lung disease is a group of heterogenous parenchymal lung diseases that have some common clinical features, pathological and radiological features. ILD can be symptom free and diagnosed by high resolution computerised tomogram of the chest and lung function tests. Interstitial lung disease can be divided into idiopathic interstitial pneumonia and diffuse parenchymal lung disease. Idiopathic pulmonary fibrosis is one of the types of the first subset and connective tissue diseases causing ILD constitute the second type. The commonest connective tissue disease associated with ILD is systemic sclerosis. Other diseases like mixed connective tissue disease, undifferentiated connective tissue disease, rheumatoid arthritis, Sjogren's syndrome, idiopathic inflammatory myositis-namely polymyositis/dermatomyositis and systemic lupus erythematosus also cause interstitial lung disease, although to a lesser extent.

The incidence of ILD in mixed connective tissue depends on the selection of patients and the method used for diagnosing the lung disorder. The prevalence of interstitial lung disease is found to be higher than that was previously reported. Nearly 66% of patients with ILD in MCTD have decreased diffusing capacity for carbon monoxide. The main lung function abnormality is of a restrictive pattern and about 50% of patients have this problem[58]. On high resolution computed tomography, the prominent radiological changes noted were ground glass opacity with thickening of the septa. Histological pattern is that of non specific interstitial pneumonia. These changes were predominant in the lower lobes. This feature simulates systemic sclerosis-ILD picture.

Based on the histological type and radiological pattern, the different types of interstitial lung disease include non specific interstitial pneumonia (NSIP), usual interstitial pneumonia (UIP), diffuse alveolar damage, acute interstitial pneumonia,

cryptogenic organising pneumonia, desquamative interstitial pneumonia and lymphocytic interstitial pneumonia.

Pathogenesis of ILD

The exact mechanism of lung fibrosis is not known but endothelial and epithelial damage predate lung inflammation and the end result is fibrosis. There is contribution from inflammatory cytokines, vascular endothelial injury and autoimmunity in the pathogenesis of interstitial pulmonary involvement. Profibrotic and proinflammatory mediators have a role in the immunopathogenesis and they include growth factors, cytokines, lipids and prostanoids. There is strong evidence for the role of transforming growth factor beta, endothelin-1 and the platelet derived growth factors. Hence targeting the profibrotic cytokines is an important area of research for better therapeutic management.

TGF beta response mediators

Canonical Smad signalling

The main function of this signalling pathway is the phosphorylation of the type 1 transforming growth factor beta receptors. Phosphorylation is of the sequential nature and the activin-like kinase-5 of TGF receptors are exclusively involved. Apart from this, Smad proteins, intracellular signalling elements are also phosphorylated. Activated TGF beta, bind to the receptor and send signals to the Smad proteins in the cytoplasm, through phophorylation. These signals are then transmitted to the nucleus of the cell and then genetic transcription begins, whereby collagen type 1, fibronectin, actin and connective tissue growth factors are generated. All these play an important role in the fibrosis of lung parenchyma[59]. Blocking the phosphorylation of Smad proteins and signals of TGF beta are future targets in the management of fibrosis.

c-Abelson tyrosine kinase

c-Abelson tyrosine kinase is a member of the Src family of tyrosine kinases. This gets activated by transforming growth factor beta[60]. In 95% of patients with chronic myeloid leukemia, mutations of c-Abl have been documented. This is indeed responsible for increased proliferation of myeloid cell lineage. The role of c-Abl in non myeloid cells has demonstrated that inhibiting the kinase activity of c-Abl in vitro, abolished the genetic expression of collagen stimulation. In vivo studies of animal models showed the prevention of fibrosis of skin and lung. Imatinib mesylate is found to be the c-Abl inhibitor. It blocked the synthesis of collagen and transformation of myofibroblast induced by transforming growth factor beta.

Egr-1

Egr-1 is a transcription element that is produced at the site of injury. Egr-1 is responsible for cell survival, differentiation and proliferation of cells and plays an important role in the acute phase of response of tissues to injury[61]. In lung the abnormal expression of Egr-1, was well correlated with the progression of fibrosis of lung[62]. It is one of the main elements of activation of fibroblast in lung which is induced by binding protein of insulin like growth factor. By inhibiting the expression of Egr-1 or blocking the activity by imatinib mesylate, the ongoing fibrosis can be controlled.

Peroxisome Proliferator-Activated Receptor Gamma

(PPAR Gamma)

Peroxisome proliferator-activated receptor gamma is a transcription factor and a receptor in the nucleus. Initially this was identified only in adipocytes but recently described in many tissues. The predominant role played by PPAR gamma is adipogenesis, but it has roles in immunity and inflammation. One of the newer functions

that has been indentified for PPAR gamma is the anti-fibrotic activity. Activated PPAR gamma inhibited the transforming growth factor beta response in skin and fibroblasts of lung[63]. Studies show that PPAR gamma is decreased in the biopsy tissues taken from lungs of patients with interstitial lung disease[64]. Prostaglandins and fatty acids act as natural agonists of PPAR gamma. Synthetic drugs like rosiglitazone, was found to decrease the skin fibrosis caused by bleomycin and also inflammation in vivo. Apart from this, it also inhibits collagen synthesis induced by transforming growth factor beta, differentiation of myofibroblasts and cellular migration among fibroblasts.

Endothelin-1

Endothelin-1 is an effective constrictor of blood vessels that is secreted by the cells of the endothelium, mesenchymal cells and epithelial cells. It has two receptors namely, endothelin-1A and endothelin-1B. Production of matrix is increased and fibroblasts are pooled in at the site of injury, by endothelin-1[65]. It also triggers the secretion of transforming growth factor beta in the fibroblasts of lung tissues. Research on the utility of bosentan, antagonist of endothelin-1, for the management of idiopathic pulmonary fibrosis and systemic sclerosis associated interstitial lung disease is going on.

Chemokines and growth factors

Lysophosphatidic Acid

Platelets and fibroblasts are activated and they produce lysophosphatidic acid. It is a phospholipid that is active biologically, and induces various effects in tissues subjected to injury. Recent studies show that lysophosphatidic acid has a role in the pathogenesis of idiopathic pulmonary fibrosis[66]. The recruitment of fibroblasts during the fibrosis of lungs is mediated by the lysophosphatidic acid, by binding to the receptor LPA1. The receptor LPA1 is found on epithelial, endothelial cells and fibroblasts. The

futuristic approach would be in blocking the pathway of LPA-LPA1, for patients with systemic sclerosis associated with lung fibrosis.

Insulin like Growth Factor

In systemic sclerosis related lung fibrosis, the role of insulin like growth factor and their proteins have been identified. In BAL study and in serum of patients with interstitial lung disease, the level of insulin like growth factor-1 is found to be elevated[68]. In animal models of lung fibrosis, by blocking IGF pathway, there was resolution of fibrosis.

Connective tissue growth factor

It is a matricellular element that is rich in cysteine. Connective tissue growth factor plays an important role in the synthesis of connective tissue and formation of new blood vessels. It helps in the synthesis of extracellular matrix, differentiation of myofibroblasts and mediates transforming growth factor beta[69]. In patients with systemic sclerosis, lungs and skin show increased levels of connective tissue growth factors. Monoclonal antibodies are prepared against these growth factors, which would be a therapeutic target for patients with lung fibrosis.

PULMONARY HYPERTENSION

Pulmonary hypertension may be asymptomatic or present with respiratory or cardiac failure. When the mean arterial pressure of the pulmonary vasculature is more than 25mmHg it is defined as pulmonary hypertension. This reference value is valid when the patient is at rest and measured by catheterising the right side of the heart[70]. In this setting of pulmonary arterial hypertension, the capillary wedge pressure of the pulmonary vasculature should be less than 15mmHg. Right heart catheterisation, apart from identifying hemodynamic abnormalities also helps in discriminating arterial

hypertension from venous hypertension due to occlusive venous disease and left heart failure.

Symptoms of pulmonary arterial hypertension include breathlessness on exertion, fatigability, syncopal attacks or precordial pain. In early stages of pulmonary hypertension the clinical examination may be normal, but later stages show a tricuspid regurgitation murmur, loud pulmonary sound and features of right heart failure. Patients can die suddenly due to hypoxia and congestive cardiac failure.

Doppler echocardiography is used to diagnose suspected pulmonary hypertension. Pulmonary arterial pressure is estimated by this modality of investigation. Ventricular dysfunction, atrial enlargements, valvular disease, intra cardiac shunt and pericardial effusion are identified by Doppler echocardiography. The sensitivity of this imaging modality to evaluate pulmonary arterial pressure is 79-100%[71].

Diffusing capacity for carbon monoxide

A decrease in diffusing capacity for carbon monoxide in the absence of restrictive or obstructive lung disease indicate an error in the exchange of gases secondary to pulmonary arterial hypertension. The fall in DLCO may progress for years before the establishment of pulmonary arterial hypertension.

Pro BNP

The presence of pulmonary arterial hypertension and right heart strain can be diagnosed by increased levels of serum pro brain natriuretic peptide[72]. Even moderate elevation (>395 pg/ml) of pro brain natriuretic peptide (N terminal) may help in diagnosing pulmonary hypertension.

6 minutes walk test

It is an exercise testing to assess cardio pulmonary system. This can give us the baseline functional idea for assessing therapeutic response. 6 minutes walk test cannot precisely estimate the severity of pulmonary arterial hypertension.

Gastrointestinal disease

The incidence of gastrointestinal manifestations in mixed connective tissue disease is around 66% to 74%[73]. This feature may be a prominent clinical finding, in the setting of overlap with systemic sclerosis. Among the gastrointestinal presentations, dysfunction of esophagus is more common. This symptom may not manifest clinically at the onset and the commonest symptom is dysphagia. Gastrointestinal reflux symptoms and dysmotility of esophagus occur at increased frequency in individuals with features related to systemic sclerosis than to systemic lupus erythematosus. Esophageal manometry study revealed that MCTD patients have comparatively less severe abnormality than that of systemic sclerosis[74]. The manometric abnormality did not correlate with skin disorder. There was decrease in the sphincter pressure of esophagus and also the reduction in the peak of peristalsis in the lower end of esophagus. Rarely, the sphincter pressure in the upper esophagus was also noted to be reduced. Patients with mixed connective tissue disease can also present as acute abdomen due to mesenteric vasculitis, acute pancreatitis, hemoperitoneum or colonic perforation. Diarrhoea, protein losing enteropathy and chronic active hepatitis are some of the other gastrointestinal manifestations[75-78]. The features of gastrointestinal system correlate well with the duration of disease course.

Renal manifestations

One of the important complications of mixed connective tissue disease is the involvement of the kidneys. Usually the kidneys are involved in an asymptomatic manner and the frequency of presentation is found to be 25%[79]. U1RNP antibodies protect against the development of proliferative glomerulonephritis[80]. Rarely the kidneys are affected severely. Common types of renal involvement include membranous glomerulonephritis and mesangial glomerulonephritis. Proliferative glomerulonephritis both focal and diffuse can be present although rare. There are case reports of immune complex mediated nephritis in some studies. Vascular and interstitial involvement of the kidneys is not common in mixed connective tissue disease patients as seen with systemic sclerosis. Children with MCTD have higher a rate of nephritis compared to that of adult counterparts. Decreased levels of complements are seen in these patients indicating a higher rate of glomerulonephritis, which includes membranous lesions as well. Children with mixed connective tissue disease are more frequently subjected for renal biopsy, because of the increased incidence of sub clinical nephritis in them[81]. Histological findings similar to renal crisis of scleroderma may be seen sometimes in MCTD patients. The other renal features include accelerated hypertension, acute kidney injury and microangiopathic haemolytic anemia[82].

Haematological manifestations

The common haematological findings in mixed connective tissue disease patients are decrease in total leucocytes count, anaemia of chronic disease, increased in gamma globulin levels and positivity for Coomb's test but without the evidence for haemolysis[83]. Nearly 75% of individuals have anemia but with lower grade. Lymphopenia relating to the activity of the disease is commonly reported. Less frequent haematological presentations include decrease in platelet count, aplastic red cells and thrombotic

thrombocytopenic purpura. None of the haematological features are specific for mixed connective tissue disease, although decreased leucocyte count and anaemia have correlation with the activity of the disease course and show improvement to treatment when other clinical manifestations are treated.

Neurological manifestations

Central and peripheral nervous system manifestations are less common in patients with mixed connective tissue disease compared to other clinical features. Recent studies indicate that the neurological involvement in MCTD individuals is higher than that reported earlier. The commonest neurological presentation is trigerminal neuralgia and this may be one of the early features of clinically undiagnosed mixed connective tissue disease[84,85]. Headaches usually due to vascular involvement and peripheral neuropathy of the same cause have been reported[86,87]. Patients with MCTD can present with aseptic meningitis as one of the features of central nervous system. The cerebrospinal fluid analysis of these patients shows higher concentration of interferon gamma and interleukin -6. The level of anti U1RNP antibodies in cerebrospinal fluid is found to be increased compared to serum and correlate with the activity of the disease process[88]. Anti U1RNP antibodies are found in both serum and cerebrospinal fluid analysis of patients with neuropsychiatric symptoms of MCTD and systemic lupus erythematosus. These anti U1RNP antibodies serve as markers of central neuropsychiatric features in individuals with the antibodies. The production of the anti U1RNP antibodies intrathecally is prominent in patients who have features of neuropsychiatric symptoms. The other rare neurological manifestations are intracranial bleed, vasculitis of the retinal vessels, optic neuropathy, transverse myelitis and cauda equina syndrome[89-93].

Auto antibodies

Rheumatoid factor is found to be positive in nearly 50-70% of individuals with mixed connective tissue diseases[94]. The percentage of positivity for anti citrullinated peptide antibody in mixed connective tissue disease is aroud 50%. The other antibodies that have reported are nucleosomes, fibrillin-1 and heterogenous nuclear ribonuleoprotein-A2. The frequency with which the antibodies to phospholipid occur is less common compared to systemic lupus erythematosus and their presence is related to hypertension of the pulmonary vasculature and decreased platelets[95,96]. No association has been linked either with abortions or thrombosis. The prevalence of antibodies against beta 2 glycoprotein is not common in mixed connective tissue disease and their clinical significance is the association with pulmonary hypertension. In nearly 50% of patients with MCTD, antibodies to endothelial cells are seen and they are linked to the development of vascular injuries of kidneys and lungs.

Pregnancy

The effect of mixed connective tissue disease on pregnancy and the foetus is conflicting. There are studies which document nearly 40% increase in flare of disease during antenatal period and also higher incidence of loss of foetus[97]. The reason for complications during pregnancy is thought to be due to autoimmunity against the tissues of placenta. On immunostaining the basement membrane of trophoblast, immunoglobulins like IgA, IgM and IgG, complements and fibrinogen are shown to be deposited. Spontaneously occurring abortions in mixed connective tissue disease have been related to the presence of anti endothelial antibodies[98]. Children born with lower birth weight are common among patients with severe Raynaud's and this correlation has been documented in individuals with mixed connective tissue diseases.

MATERIALS AND METHODS

STUDY DESIGN

Prospective cross sectional study

DURATION OF STUDY

January 2012-December 2013

ETHICAL COMMITTEE APPROVAL

Obtained before starting the study

CONSENT

Informed consent for all patients in their own language obtained

SELECTION OF STUDY SUBJECTS

All patients with features of suspected overlap syndrome and mixed connective tissue disease (according to Kasukawa's criteria) attending the Department of Rheumatology, Rajiv Gandhi Government General Hospital, were enrolled.

NUMBER OF STUDY CASES

43 patients

METHODOLOGY

All patients who satisfied the inclusion criteria were chosen and a detailed history was obtained and complete clinical examination was done. All the enrolled patients were subjected to laboratory investigations (haemogram, biochemical parameters and urine routine), Electrocardiogram, Echocardiography, Ultrasonogram, Upper gastrointestinalscopy and Immunological investigations (C-reactive protein, Rheumatoid

factor, anti nuclear antibody and ANA profile 3). Chest x-ray, high resolution computerised tomogram of the chest and pulmonary function tests were done to asses pulmonary involvement of the cases under study.

ANA profile 3 was done using Immunoblot method.

INCLUSION CRITERIA

Patients with clinical features suggestive of overlap syndrome (systemic lupus erythematosus, systemic sclerosis and myositis) and mixed connective tissue disease were included.

EXCLUSION CRITERIA

1) Patients with well defined isolated connective tissue diseases like Rheumatoid arthritis, Systemic lupus erythematosus, Systemic sclerosis, Idiopathic inflammatory myositis, Sjogren's syndrome.

2) Children below 16 yrs.

STATISTICAL METHODS

Descriptive statistical analysis has been carried out in the present study. Results on continuous measurements are presented on Mean SD and results on categorical measurements are presented in percentage. Chi-square test has been used to find the significance of study parameters on categorical scale between two groups. All analyses were two tailed and $p<0.05$ was considered significant. SPSS version 16.0 was used for data analysis.

ANA ELISA

Principle:

In the ANA screen ELISA kit, purified nuclear antigens are being coated on to wells of micro titre plate. If IgG type of ANA specific antibody is present it binds to the antigen. Washing is done to remove all the unbound materials. If any antigen antibody complex is there, it binds to the added enzyme conjugate. Again washing is done to remove excess enzyme conjugate and then substrate is added. On incubation of the plate there is hydrolysis of the substrate by the enzyme.

Test procedure:

- Universal precautions are followed during the procedure. All the reagents are dispensed in the centre of the well and the tip of the pipette should not touch the wall of the micro well.
- Prepare work sheet and remove the kit from the refrigerator and leave it at room temperature for 30 minutes.

Sample:

- Use only serum as specimen for the test

Preparation of wash buffer:

- Check the buffer concentrates for the presence of salt crystals.

  50ml of buffer is prepared for each strip
- Mix 20ml 25x wash buffer concentrate with 480 ml of distilled water

Procedure:

- Samples to be brought to room temperature
- Samples arranged so that well A1 is negative control and well B1 is positive control and well C1 & D1 is calibrator.
- To 200 µl of sample diluents, 10 µl of test sample is added to make a 1:21 dilution and mixed nicely.
- Dispense 100 V of diluted sera in E1 well & other diluted samples in their appropriate wells. Tap the holder to remove air bubbles from the liquid and mix well gently and cover with a seal.
- At room temperature incubation is done for 20 minutes
- The seal is removed and wash buffer of 300 µl is used to wash the wells thrice.
- An absorbent paper is used for blotting.
- In each well enzyme conjugate of 100 µl is added.
- The plate is sealed with a cover and is incubated for 20 minutes at room temperature.
- The seal is removed and wash buffer of 300 µl is used to wash the wells thrice.
- An absorbent paper is used for blotting.
- Dispense 100 µl of TMB substrate and incubate for 10 minutes at room temperature.
- Add 100 µl of stop solution.
- Read at 450 nm using ELISA reader

ANA – INDIRECT IMMUNOFLUORESCENCE (Hep-2)

Principle:

The antibodies present in the sample bind the relevant antigens. Once bound, the antigen-antibody complex is shown with an antibody conjugated with fluorescein and is visualized under a fluorescent microscope.

Test procedure:

- To keep the slides at room temperature for 30 minutes before performing the assay.
- The phosphate buffer saline is prepared before performing the assay.
- According to the slide to be used, prepare a screening dilution.

Reagents	1/10 dilution	1/40 dilution
PBS buffer	450 µl	300 µl
Serum	50 µl	100 µl

- With diluted samples and diluted controls the reactive areas are covered.
- Incubation is done at room temperature for a period of 30 minutes. Perform a quick wash with PBS.
- Three washings should be performed of 5 minutes each, putting the slides in the coplin jar containing PBS, shaking softly.
- Take the slides off the PBS, shake the excess on absorbent paper and keep the reactive areas wet.
- Diluted Anti IgG FITC is used to cover the reactive areas immediately and kept for incubation at room temperature in a moist chamber for 30 minutes.

- Steps for washing to be repeated.
- Cover the reactive areas with Evan’s blue.
- Wash the excess stain with PBS, keeping the reactive areas wet.
- Put the mounting medium immediately on the cover slide.
- Results are interpreted based on the pattern and the intensity of fluorescence in 1/40 dilution. Positive reaction in 1/10 dilution but negative in 1/40 dilution is reported as negative.

ANA profile 3

Procedure:

To take the strip provided by the manufacturer and place it on the tray.

To add 10 µl serum sample to 100 µl diluents (30 minutes Incubation)

Thrice washed with 1.5 ml wash buffer (incubate for 5min / wash)

Add 100 µl conjugate (30 minutes Incubation**)**

Thrice washed with 1.5 ml wash buffer (incubate for 5 min / wash)

Add 100 µl substrate (10 minutes Incubation)

Wash with 1.5 ml distilled water

Add 100 µl stop solution (5 minutes Incubation)

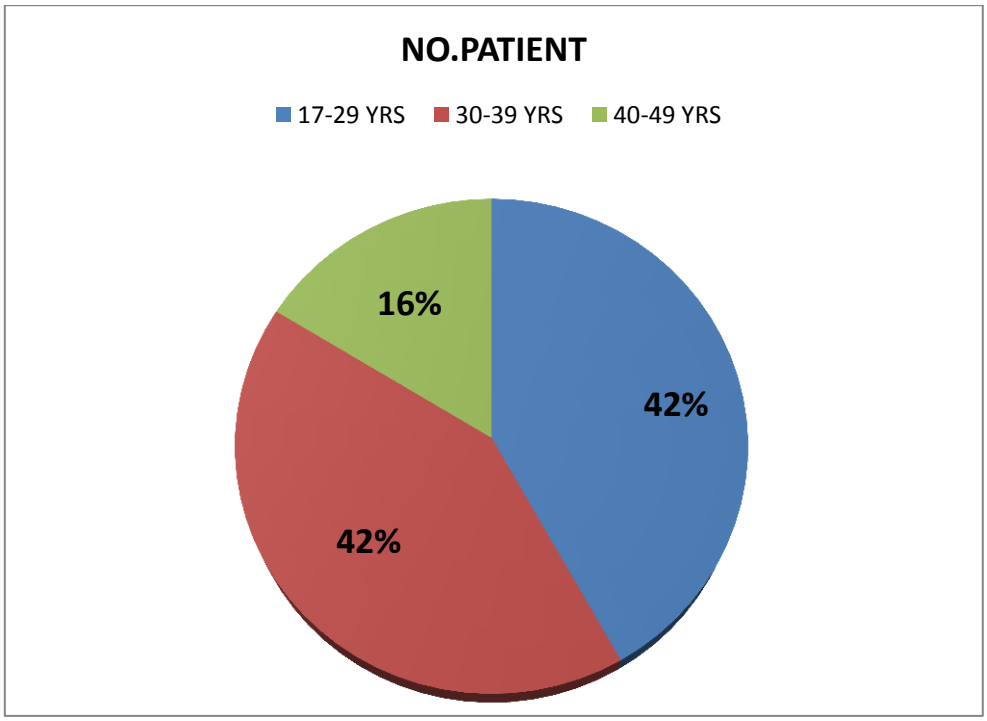
Read results

RESULTS AND ANALYSIS

Table 1: AGE DISTRIBUTION

S.NO	AGE (IN YEARS)	NUMBER OF PATIENTS
1	17-29	18
2	30-39	18
3	40-49	7

Figure 1: AGE DISTRIBUTION

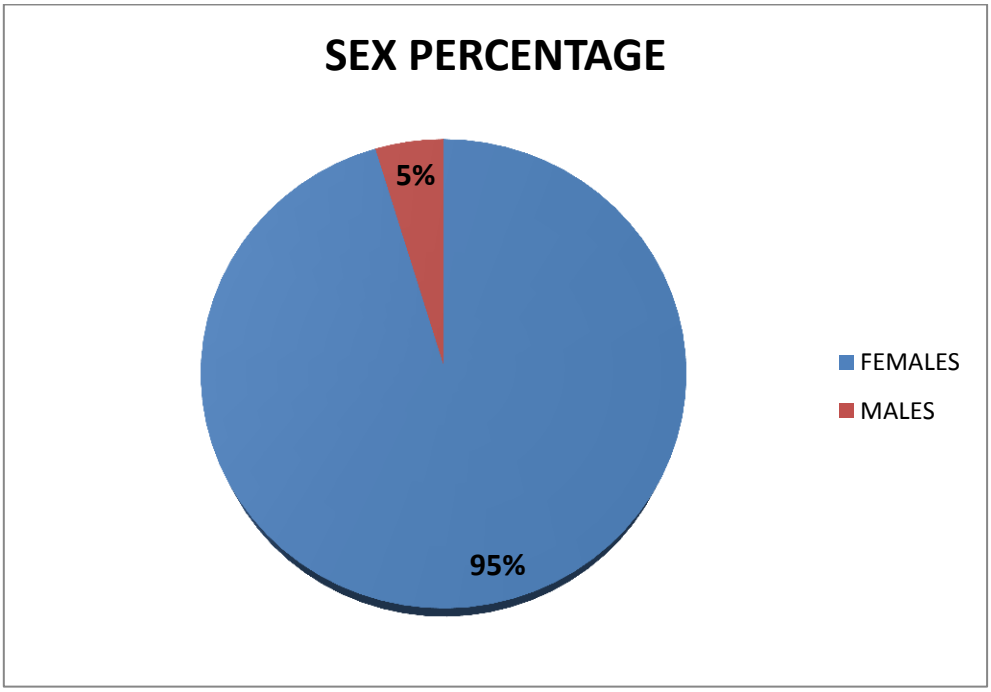


Most number of patients are between second and third decade.

Table 2: SEX DISTRIBUTION

	Frequency	Percent	Valid Percent	Cumulative Percent
Male	2	4.7	4.7	4.7
Female	41	95.3	953	100.0
Total	43	100.0	100.0	

Figure 2: SEX DISTRIBUTION

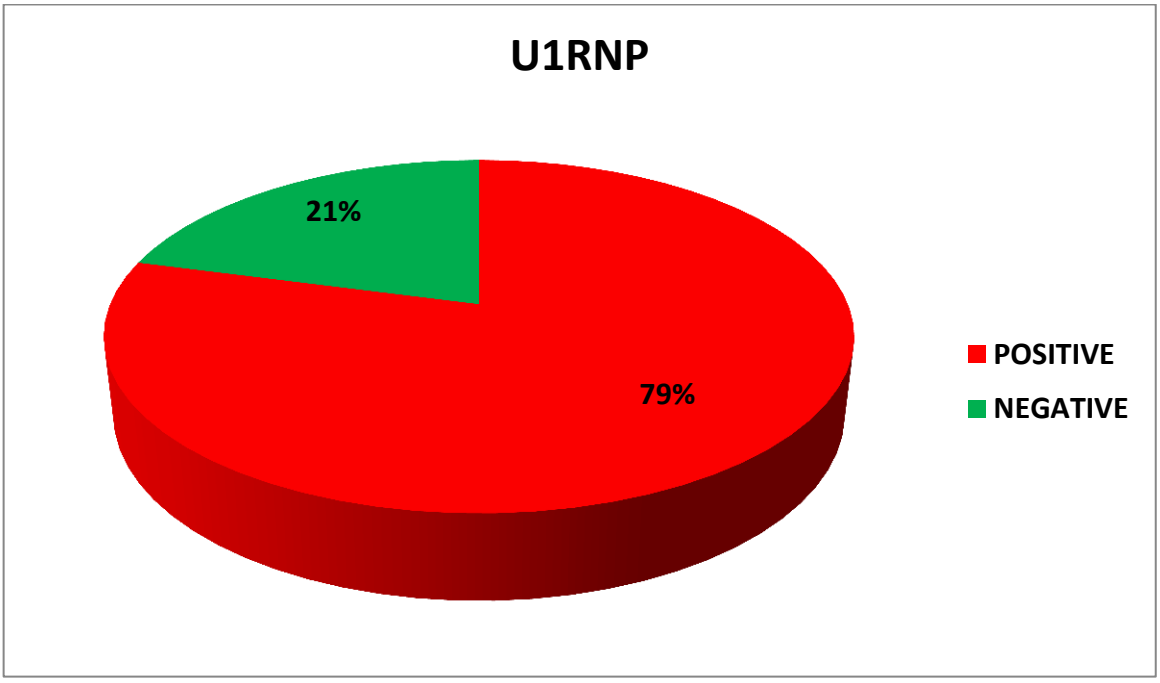


Proportion of females with overlap syndrome/mixed connective tissue disease was higher and the ratio of females to males was 20.3:1.

Table 3: U1RNP

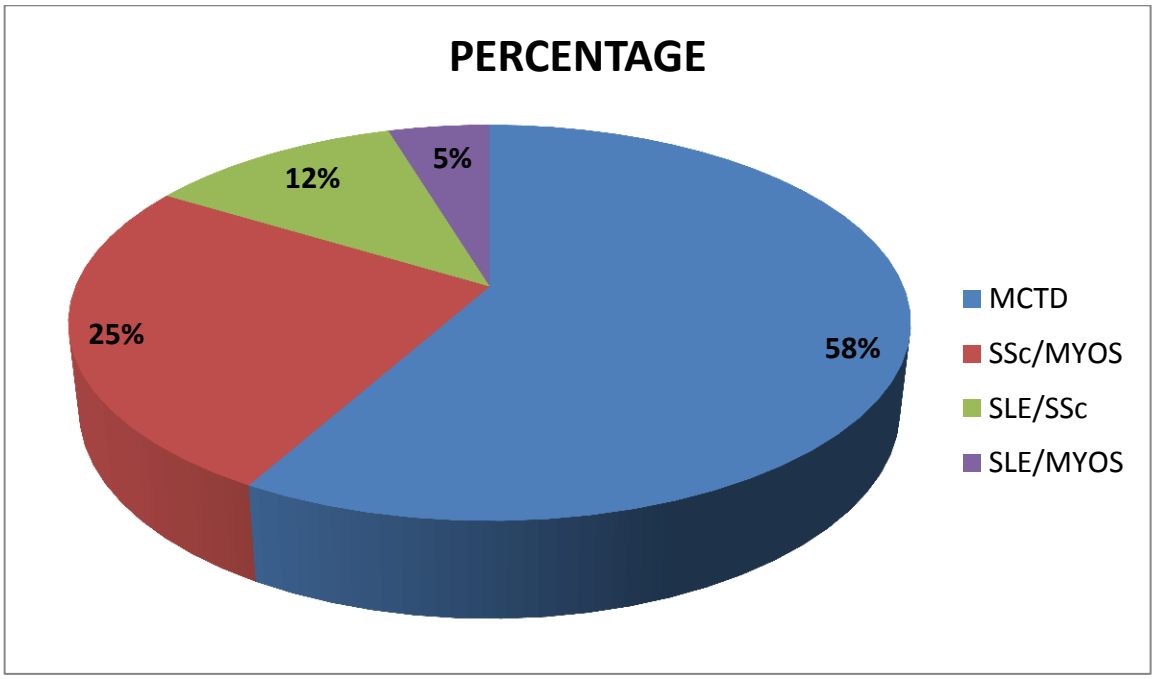
	Frequency	Percent	Valid Percent	Cumulative Percent
Positive	34	79.1	79.1	79.1
Negative	9	20.9	20.9	100.0
Total	**43**	**100.0**	**100.0**	

Figure 3: U1RNP



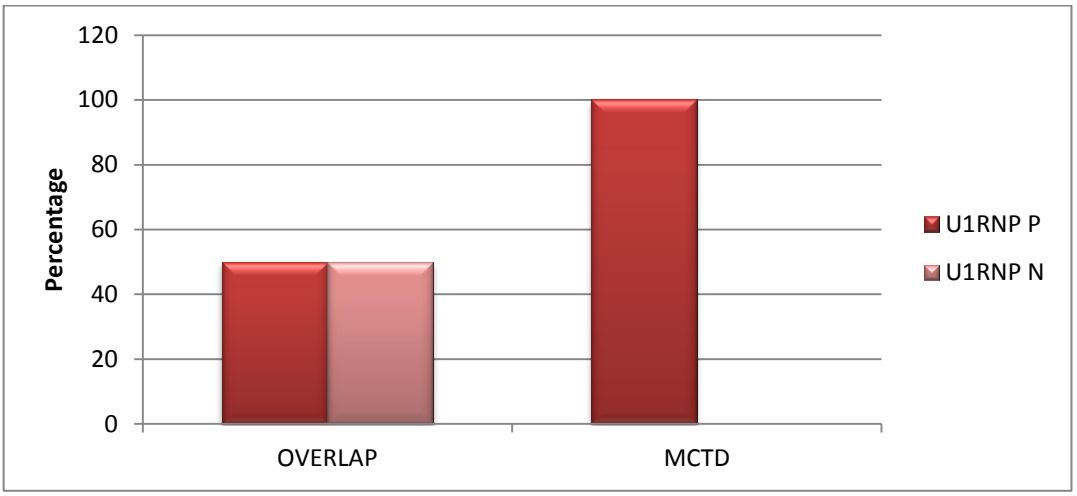
Percentage of anti U1RNP antibody positivity was 79.1% and 20.9% of patients were found to be negative for anti U1RNP.

Figure 4: SPECTRUM OF OVERLAP DISEASES



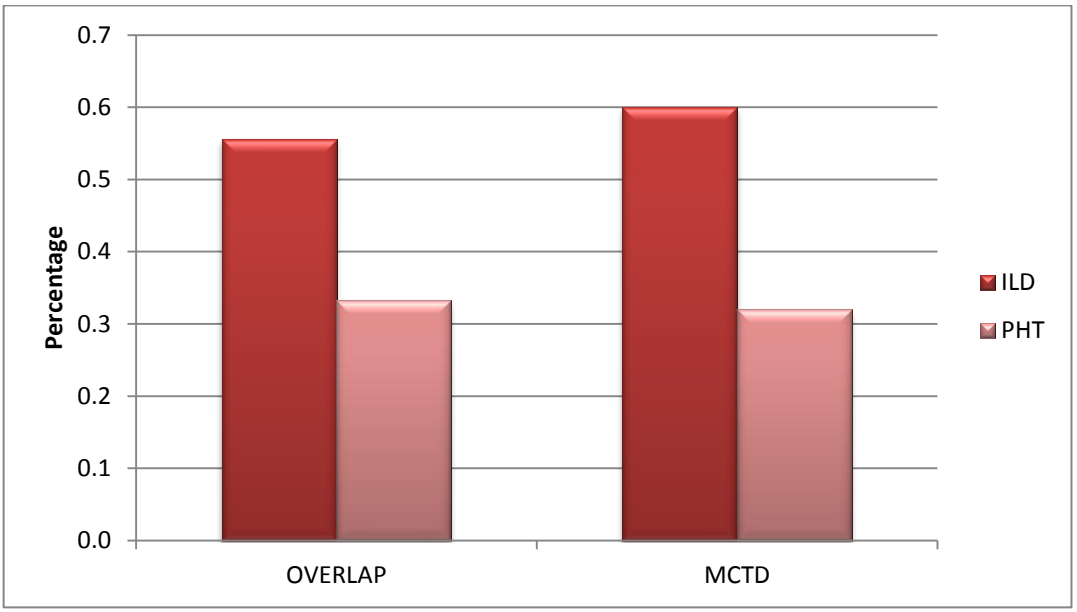
Mixed connective tissue disease	-	58.14% (n=25)
Systemic sclerosis/myositis	-	25.58% (n=11)
Systemic sclerosis/SLE	-	11.66% (n=5)
SLE/myositis	-	4.66% (n=2)

Figure 5: ANTI U1RNP ANTIBODY STATUS AMONG OVERLAP SYNDROME AND MCTD



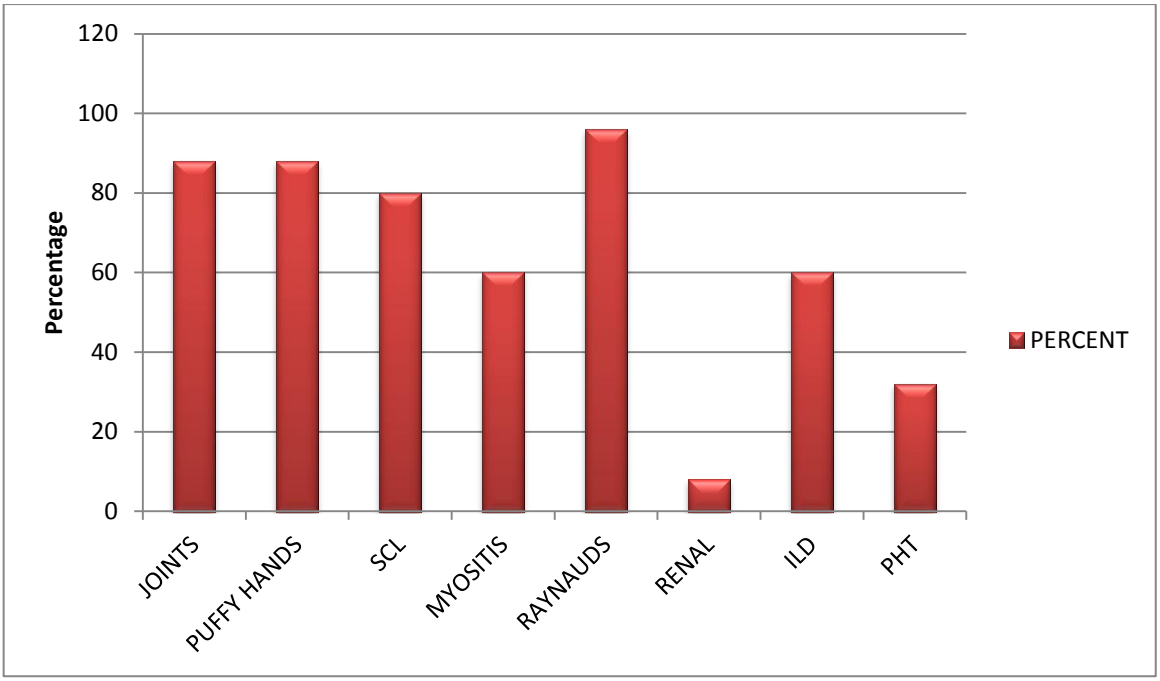
50% of patients with Overlap Syndrome are positive for anti U1RNP antibody

Figure 6: PULMONARY MANIFESTATIONS AMONG OVERLAP SYNDROME AND MCTD



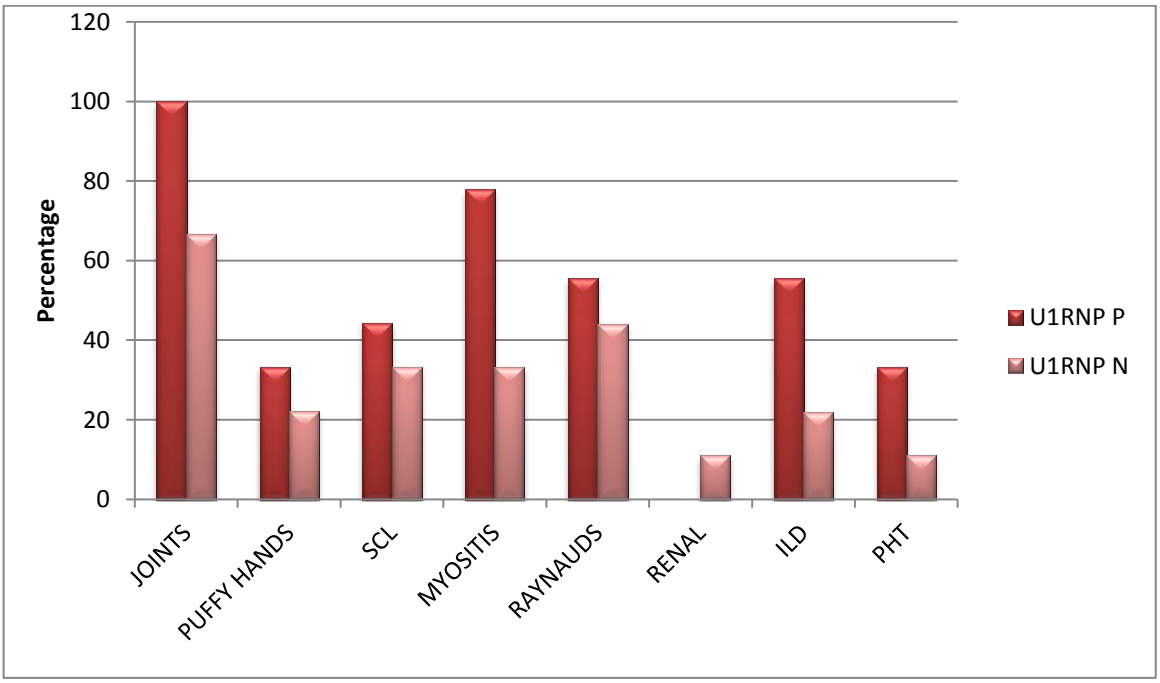
ILD is more common among MCTD patients compared to Overlap syndrome. The prevalence of PHT is almost equal in both the groups.

Figure 7:CLINICAL FEATURES OF MCTD PATIENTS



Raynaud's phenomenon, the commonest and renal involvement, the least common manifestation in MCTD patients.

Figure 8:CLINICAL FEATURES OF OVERLAP SYNDROME CORRELATING WITH ANTI U1RNP ANTIBODY STATUS



Arthritis is the commonest feature and renal manifestations scored the least, among Overlap Syndrome group.

Figure 9: CLINICAL MANIFESTATIONS OF PATIENTS WITH OVERLAP SYNDROME and MIXED CONNECTIVE TISSUE DISEASE.

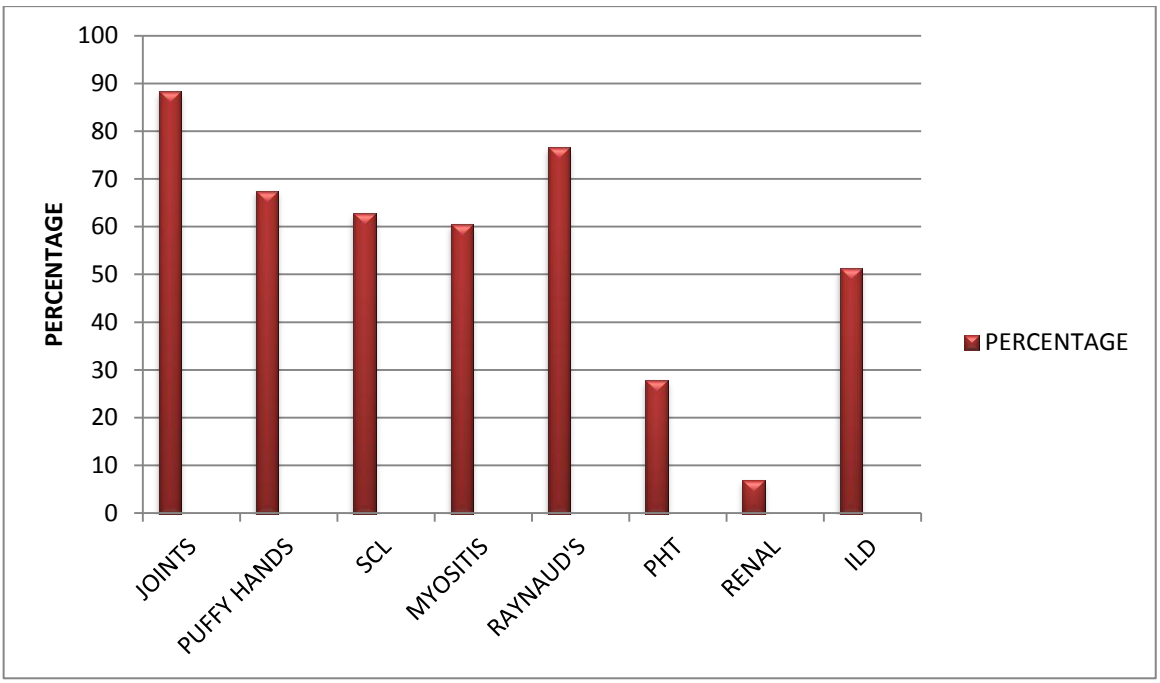


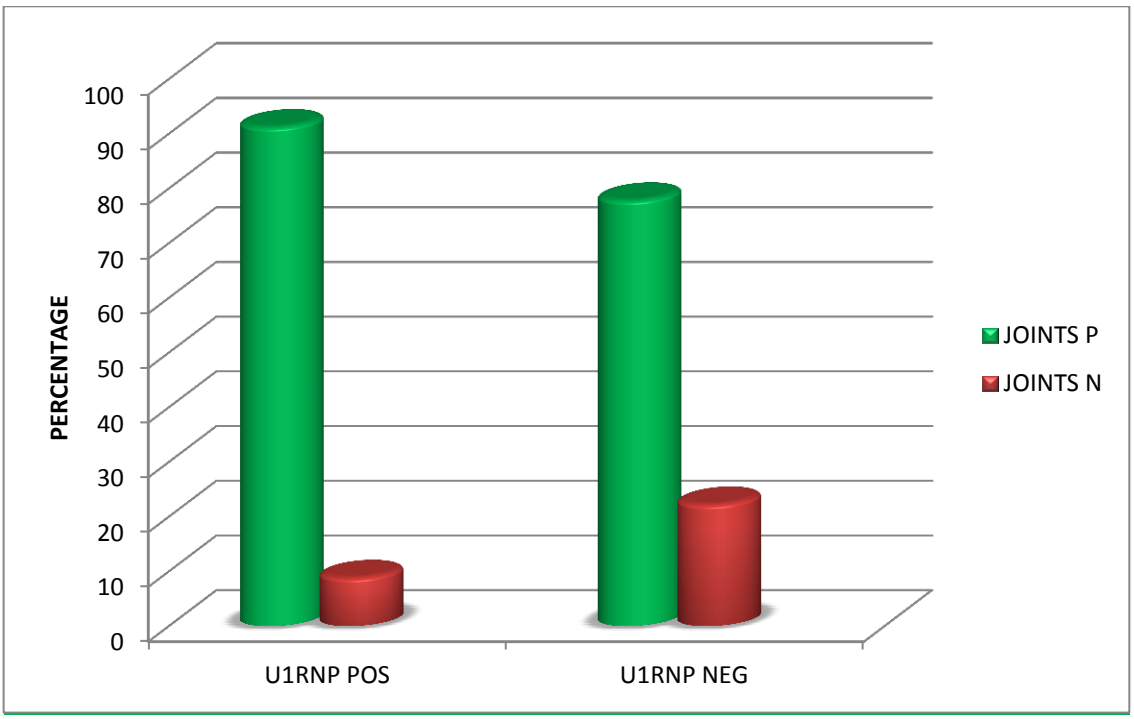
Table 4: P Value of Clinical Features correlating with anti U1RNP antibody

S.NO	CLINICAL FEATURES	P VALUE
1.	Puffy hands	.12
2.	Arthritis	.27
3.	Raynaud's	**.02**
4.	Renal	.52
5.	PHT	.41
6.	ILD	.06
7.	Sclerodactyly	.06
8.	Myositis	.45

Table 5: U1RNP – ARTHRITIS

			ARTHRITIS		Total
			Positive	Negative	
U1RNP	Positive	Count	31	3	34
		% within U1RNP	91.2%	8.8%	100.0%
	Negative	Count	7	2	9
		% within U1RNP	77.8%	22.2%	100.0%
Total		Count	38	5	43
		% within U1RNP	88.4%	11.6%	100.0%

Figure 10: U1RNP – ARTHRITIS

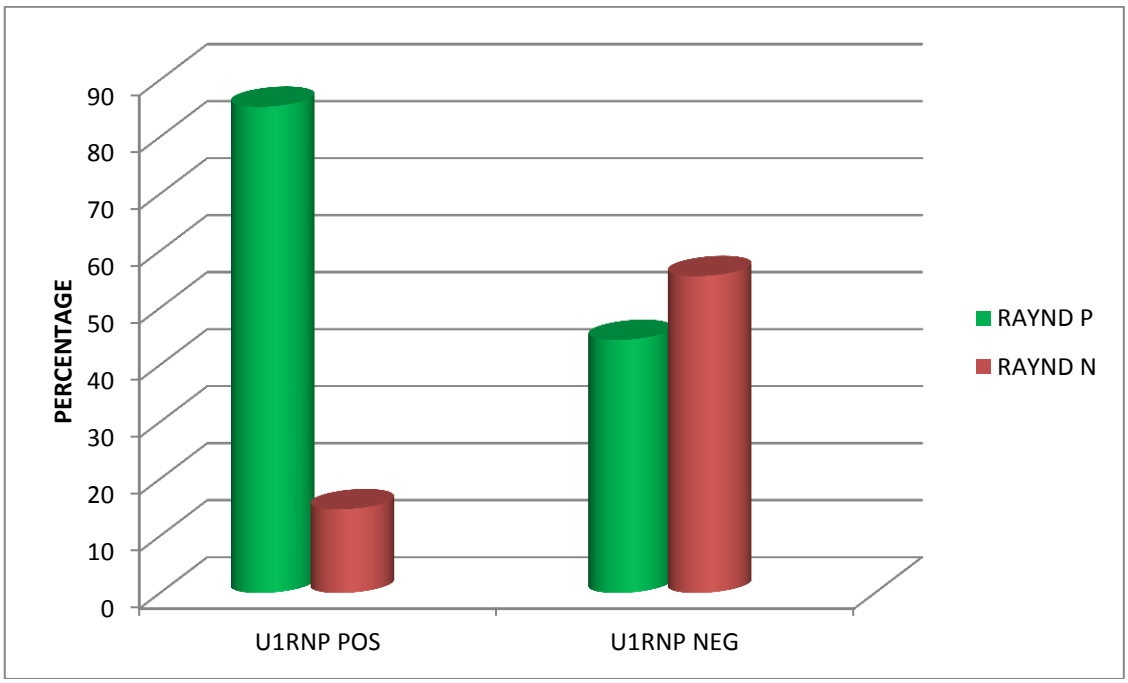


Proportion of arthritis is higher in anti U1RNP positive group compared to anti U1RNP negative group (91.2% Vs 77.8%) though statistically insignificant (p value>0.05).

Table 6: U1RNP – RAYNAUD'S

			Raynaud's		Total
			Positive	Negative	
U1RNP	Positive	Count	29	5	34
		% within U1RNP	85.3%	14.7%	100.0%
	Negative	Count	4	5	9
		% within U1RNP	44.4%	55.6%	100.0%
Total		Count	33	10	43
		% within U1RNP	76.7%	23.3%	100.0%

Figure 11: U1RNP – RAYNAUD'S

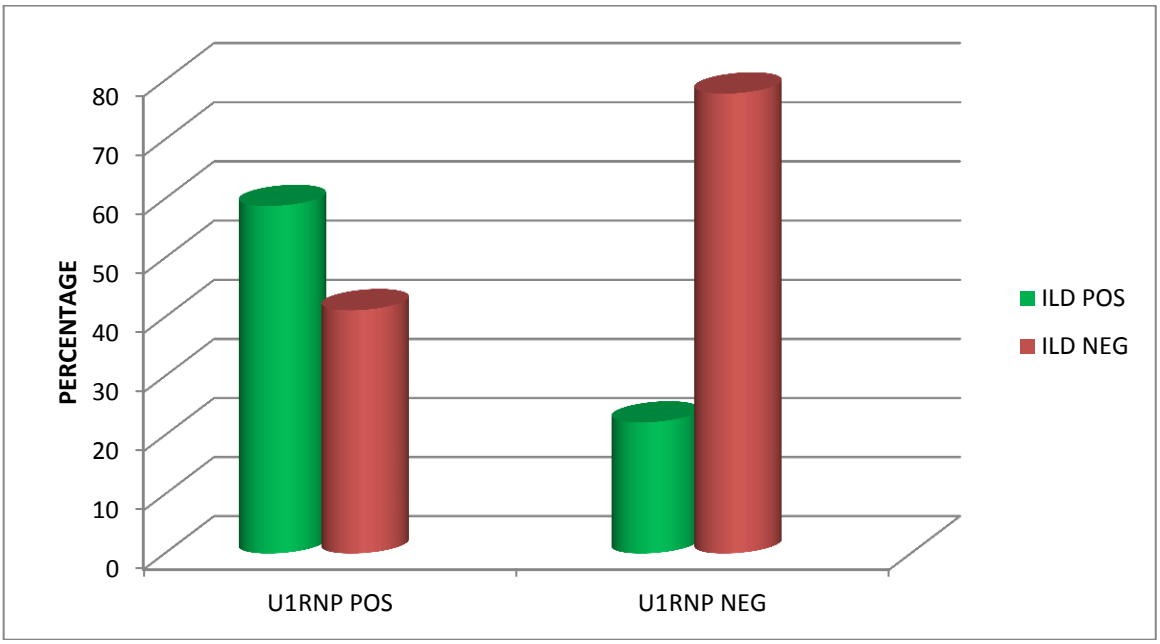


Proportion of Raynaud's phenomenon positivity is higher in anti U1RNP antibody positive group compared to anti U1RNP antibody negative group (85.3% Vs 44.4%) and had statistical significance with p value <0.05.

Table 7: U1RNP - ILD

			ILD		Total
			Positive	Negative	
U1RNP	Positive	Count	20	14	34
		% within U1RNP	58.8%	41.2%	100.0%
	Negative	Count	2	7	9
		% within U1RNP	22.2%	77.8%	100.0%
Total		Count	22	21	43
		% within U1RNP	51.2%	48.8%	100.0%

Figure 12: U1RNP - ILD

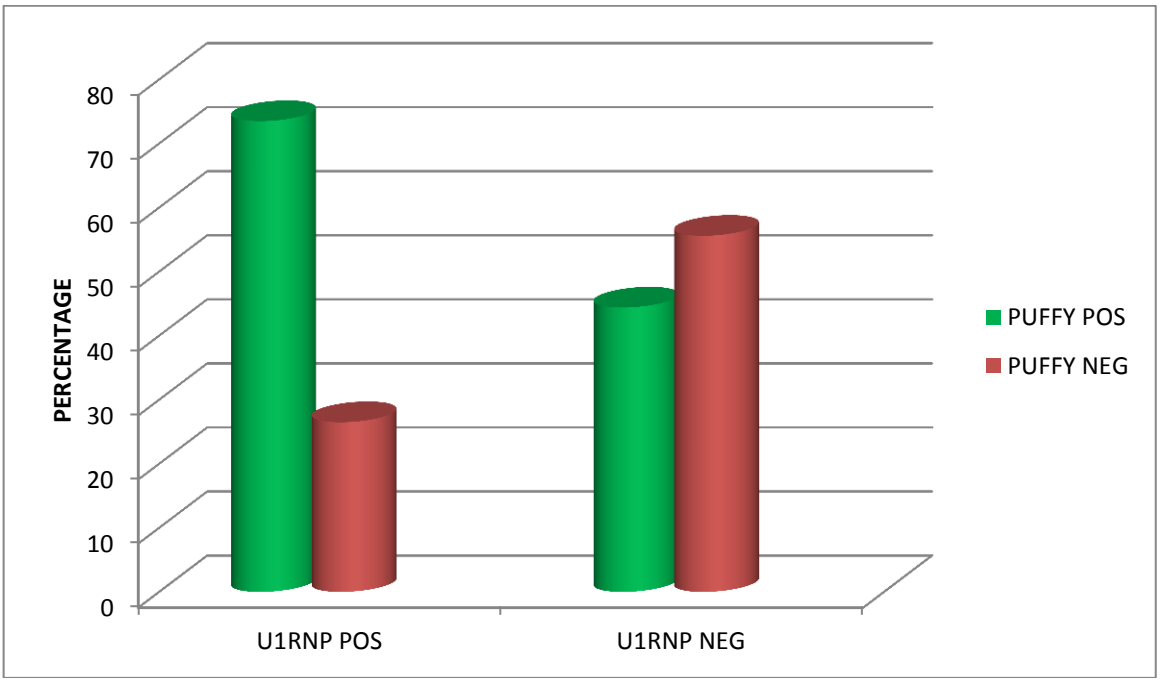


Proportion of interstitial lung disease is higher in anti U1RNP antibody positive group compared to anti U1RNP antibody negative group (58.8% Vs 22.2%) although statistically not significant (p value >0.05).

Table 8: U1RNP – PUFFY HANDS

			PUFFY HANDS		Total
			Positive	**Negative**	
U1RNP	**Positive**	Count	25	9	34
		% within U1RNP	73.5%	26.4%	100.0%
	Negative	Count	4	5	9
		% within U1RNP	44.4%	55.6%	100.0%
Total		Count	23	20	43
		% within U1RNP	67.4%	32.6%	100.0%

Figure 13: U1RNP – PUFFY HANDS

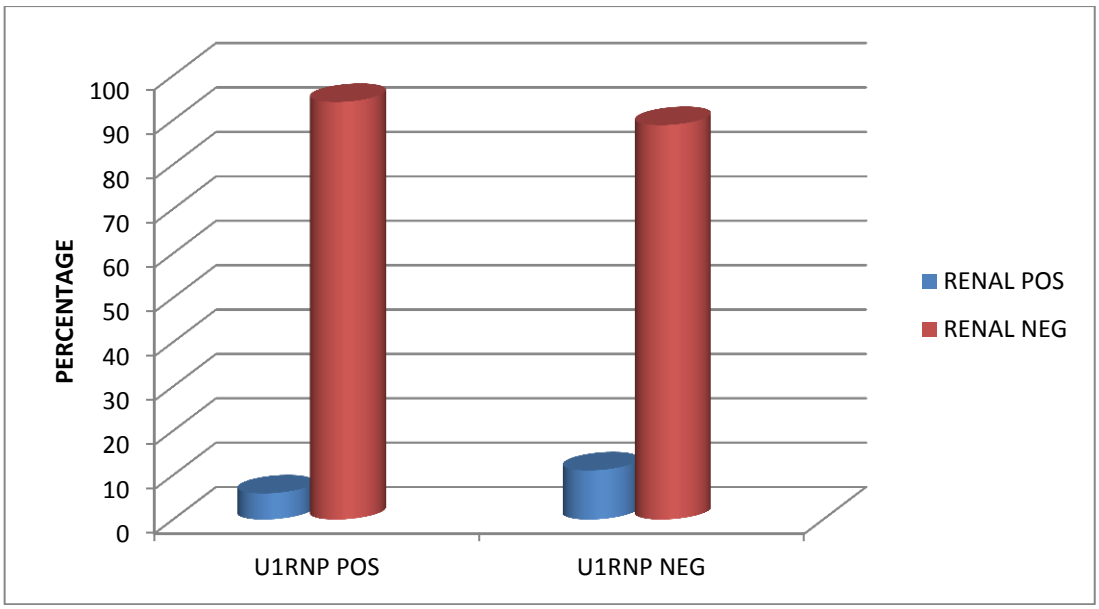


Proportion of patients with puffy hands is higher in anti U1RNP positivity compared to anti U1RNP negativity (73.5% Vs 44.4%) although statistically not significant (p value>0.05).

Table 9: U1RNP – RENAL

			RENAL		Total
			Positive	**Negative**	
U1RNP	**Positive**	Count	2	32	34
		% within U1RNP	5.9%	94.1%	100.0%
	Negative	Count	1	8	9
		% within U1RNP	11.1%	88.9%	100.0%
Total		Count	3	40	43
		% within U1RNP	7.0%	93.0%	100.0%

Figure 14: U1RNP – RENAL

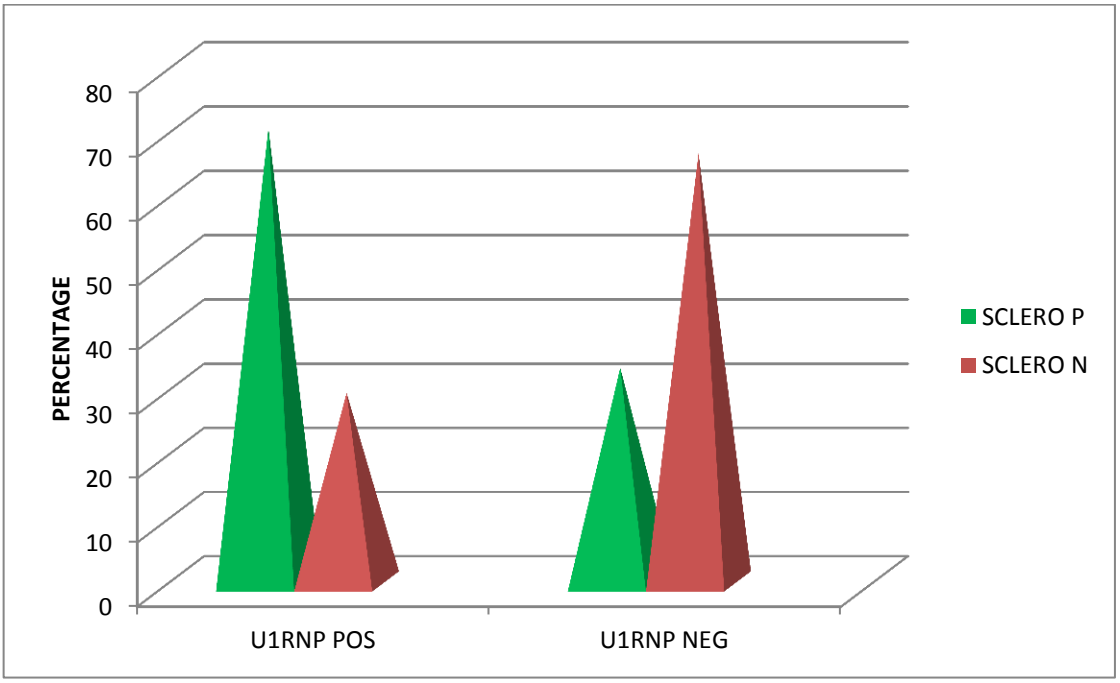


Proportion of individuals without renal involvement is higher in anti U1RNP antibody positive group compared to U1RNP negative group (32 patients Vs 8 patients).

Table 10: U1RNP – SCLERODACTYLY

			SCLERODACTYLY		Total
			Positive	Negative	
U1RNP	Positive	Count	24	10	34
		% within U1RNP	70.6%	29.4%	100.0%
	Negative	Count	3	6	9
		% within U1RNP	33.3%	66.7%	100.0%
Total		Count	27	16	43
		% within U1RNP	62.8%	37.2%	100.0%

Figure 15: U1RNP – SCLERODACTYLY

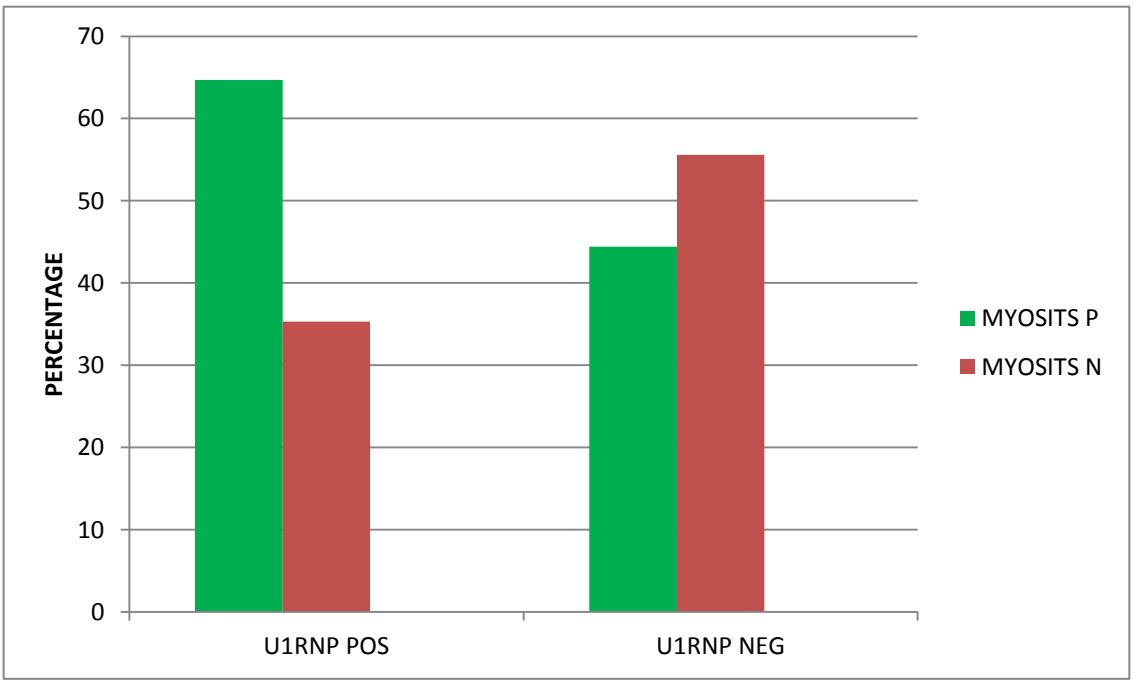


Proportion of patients with sclerodactyly was higher in anti U1RNP antibody positive group compared to the negative group, though statistically not significant (p value>0.05)

Table 11:U1RNP – MYOSITIS

			MYOSITIS		Total
			Positive	Negative	
U1RNP	Positive	Count	22	12	34
		% within U1RNP	64.7%	35.3%	100.0%
	Negative	Count	4	5	9
		% within U1RNP	44.4%	55.6%	100.0%
Total		Count	26	17	43
		% within U1RNP	60.5%	39.5%	100.0%

Figure 16: U1RNP – MYOSITIS

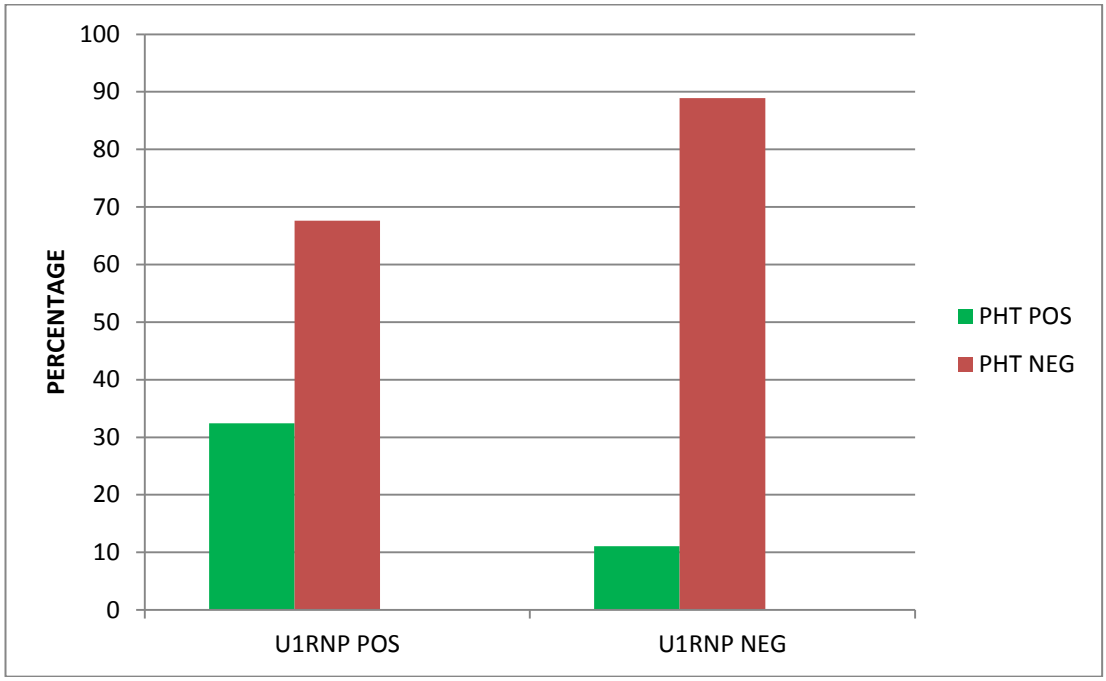


Anti U1RNP antibody positivity had no statistically significant association with myositis (p value >0.05).

Table 12: U1RNP – PHT

			PHT		Total
			Positive	Negative	
U1RNP	Positive	Count	11	23	34
		% within U1RNP	32.4%	67.6%	100.0%
	Negative	Count	1	8	9
		% within U1RNP	11.1%	88.9%	100.0%
Total		Count	12	31	43
		% within U1RNP	27.9%	72.1%	100.0%

Figure 17: U1RNP – PHT



There is no statistically significant association between anti U1RNP antibody positivity and pulmonary hypertension.

Table 13: Chi-Square Test – Raynaud's Phenomenon

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided) P value	Exact Sig. (1-sided)
Pearson Chi-Square	6.654[a]	1	.010		
Continuity Correction[b]	4.562	1	.033		
Likelihood Ratio	5.882	1	.015		
Fisher's Exact Test				**.020**	.020
Linear-by-Linear Association	6.499	1	.011		
N of Valid Cases[b]	43				

Statistically significant association was found (p value<0.05) between Raynaud's phenomenon and anti U1RNP antibody.

Table 14: Chi-Square Test - Renal

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided) P value	Exact Sig. (1-sided)
Pearson Chi-Square	.300[a]	1	.584		
Continuity Correction[b]	.000	1	1.000		
Likelihood Ratio	.269	1	.604		
Fisher's Exact Test				**.515**	.515
Linear-by-Linear Association	.293	1	.588		
N of Valid Cases[b]	43				

There is no positive correlation between renal involvement and anti U1RNP antibody (p value>0.05)

DISCUSSION

Our study is a cross sectional analysis, to find out the correlation between anti U1RNP antibody with various clinical manifestations of overlap syndrome and mixed connective tissue diseases.

This study selected mixed connective tissue disease patients according to Kasukawa's criteria and the reason for choosing is that, this criterion does not exclude patients with Sm positivity or demand the need for the titre of U1RNP and the presence of U1RNP is sufficient enough for serological criterion.

The mean age of onset of disease in our study population is 32.16 years and the mean duration of disease is 27.79 months.

In our study of 43 cases, 25 cases were diagnosed to have MCTD and the rest as overlap syndrome. Of the 18 overlap syndrome patients, 9 were found to be positive for anti U1RNP antibody (not fulfilling the Kasukawa's criteria for MCTD).

The foremost clinical feature of MCTD patients, in our analysis was Raynaud's phenomenon and this was present in 96% of individuals. This correlated well with the study by Sharp et al[36], which points to the prevalence of Raynaud's phenomenon to be around 75-90%. 88% of patients had arthritis and puffy hands, and these rank the second common manifestation among MCTD. Sclerodactyly was present in 80% and myositis in 60% of patients with MCTD. The least identified feature was the renal involvement, occurring only in 8% of the cases. This coincides with the study by Lemmer et al[80] on the renal involvement in MCTD patients. More than half (60%) the patients had ILD and only 32% of individuals with MCTD had PHT as right heart catheterisation was not done.

Of the 18 overlap syndrome cases, arthritis was present in all the anti U1RNP antibody positive patients (100%). More than 2/3 of the patients with anti U1RNP antibody positivity (77.8%) presented with myositis and the study by Yoshihide Asano et al[99] correlates with our finding. One patient without anti U1RNP antibody had focal segmental glomerulosclerosis on renal biopsy. None of the anti U1RNP antibody positive patients had renal involvement among overlap syndrome. About half the patients had Raynaud's phenomenon (55%) and sclerodactyly (44%). Puffy hands were present in 1/3 (33%) of anti U1RNP antibody positive patients[99]. 7 patients (38.9%) of the overlap syndrome [5 patients (55.6%) with and 2 patients (22.2%) without anti U1RNP antibody] had ILD. 33.3% (anti U1RNP antibody positive) and 11.11% (anti U1RNP antibody negative) had PHT[99].

In our study, we find that higher proportion of patients with anti U1RNP antibodies had joint involvement (91.2%), although statistically not significant (p value>0.05). The presence of IgG anti 70kDa and IgM anti-B/B' antibodies are largely associated with the presence of arthralgia/arthritis. This study correlated well with the reports of Ihn et al 1999[100] and Lundberg et al 1992[101]

The prevalence of Raynaud's phenomenon among our patients with anti U1RNP antibody positivity was higher compared to that of negative individuals (85.3% Vs 44.4%) and statistically significant (p value<0.05). This correlated well with other international studies, like Spanish study by López-Longo FJ et al[102], which links the association of U1RNP with Raynaud's phenomenon due to anti 70kD-U1-RNP and anti A U1RNP by immunoblotting.

Our study showed only two patients (5.9%) with anti U1RNP positivity to have renal involvement. Both had albuminuria and the spot urine protein creatinine ratio was less than 0.5, hence renal review at quarterly interval was advised and biopsy was not

done. We are aware of the fact that U1RNP antibodies are protective against the occurrence of diffuse proliferative glomerulonephritis[80]. Munves et al quotes in his study that patients with anti U1RNP antibodies have lower incidence of renal disease[103]. A study conducted by Migliorini et al in 2005[104] Italy also states that U1RNP antibodies are associated with milder renal involvement.

There were a statistically significant number of patients with interstitial lung disease among U1RNP positive individuals (58.8% Vs 22.2%). The majority of pattern recognised was nonspecific interstitial pneumonia pattern. In 1992 Lundberg et al[100] found an association between the combination of presence of anti-A, anti-C, IgG-anti 70 KDa and IgM-anti B/B' antibodies with pulmonary fibrosis. In 1999, Ihn et al[99] stated that patients with positive anti U1RNP had significantly more pulmonary fibrosis than in those who were negative (72% Vs 36% p value <0.01). Since HRCT chest was done in all patients, we were able to diagnose subclinical interstitial lung disease.

The prevalence of pulmonary hypertension among anti U1RNP positive antibodies, in our study was 32.4%. In a study conducted in Japan[105], majority of pulmonary arterial hypertension-connective tissue disease group patients, suffered from mixed connective tissue disease or systemic lupus erythematosus with anti U1RNP antibodies. Anti U1RNP antibody was the most prevalent antibody (61%) in their study population. In a different study in Japan[106], systemic sclerosis and mixed connective tissue disease had the largest prevalence of pulmonary arterial hypertension. Anti U1RNP antibody positivity and the presence of Raynaud's phenomenon correlated with pulmonary arterial hypertension in that study. The true prevalence of PAH may not have been brought out in our analysis as PAH was diagnosed only by 3D echocardiography and right heart catheterisation was not done.

Our analysis shows higher prevalence of sclerodactyly, puffy hands and myositis, although none had statistical significance. These findings have good correlation with the study by Lundberg et al[100]. The occurrence of puffy hands, sclerodactyly and myositis, were highly associated with the combined presence of anti-A, anti-C, IgG anti-70 kDa and IgM anti-B/B' antibodies[100].

Our centre, being a tertiary referral centre, there can be sampling bias, as patients may not be truly representative of the population at large. Therefore these results cannot be applied to the community in general.

The main drawback of our study is that it is a cross sectional study. It cannot define the cause effect relationship between U1RNP antibodies and the various clinical manifestations. Hence we need larger a cohort and a longer time period to establish the causal relationship between anti U1RNP antibodies and the varied clinical spectrum.

Although with its own limitations, we believe that our study will be a stepping stone for our future research on anti U1RNP antibodies among patients with overlap syndrome and mixed connective tissue disease on a larger scale.

CONCLUSION

- Mixed connective tissue disease and Overlap Syndrome is more common among females.
- Second and third decades are the susceptible age group for MCTD and Overlap Syndrome.
- Arthritis is the most prevalent symptom among the anti U1RNP antibody positive Overlap Syndrome group.
- Raynaud's phenomenon is the most common clinical feature of MCTD.
- Statistically significant association was found between Raynaud's phenomenon and anti U1RNP antibody positivity (p value <0.05).
- Puffy hands, sclerodactyly and myositis are proportionately higher in anti U1RNP antibody positive group of MCTD and Overlap Syndrome (statistically not significant p value>0.05).
- Interstitial lung disease is more common among anti U1RNP antibody positive patients in both MCTD and Overlap Syndrome (statistically not significant p value>0.05).
- Renal manifestations are uncommon in both MCTD and Overlap Syndrome patients.
- Renal involvement and PHT had no association with the presence of anti U1RNP antibody.

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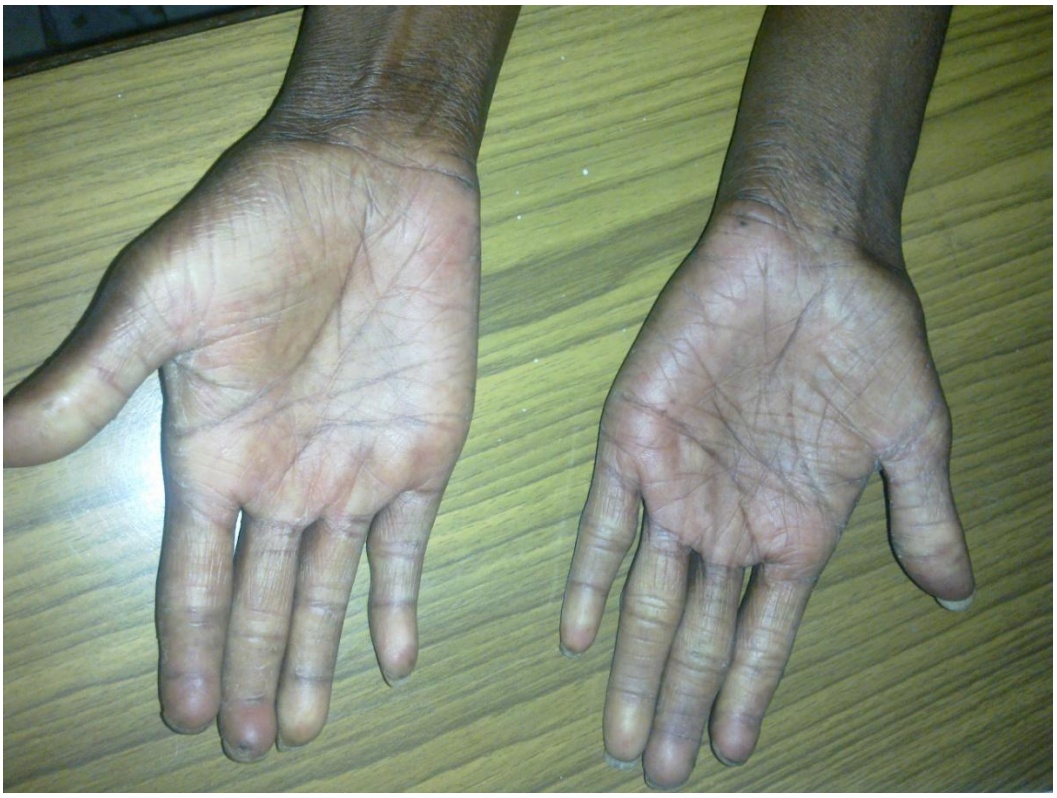
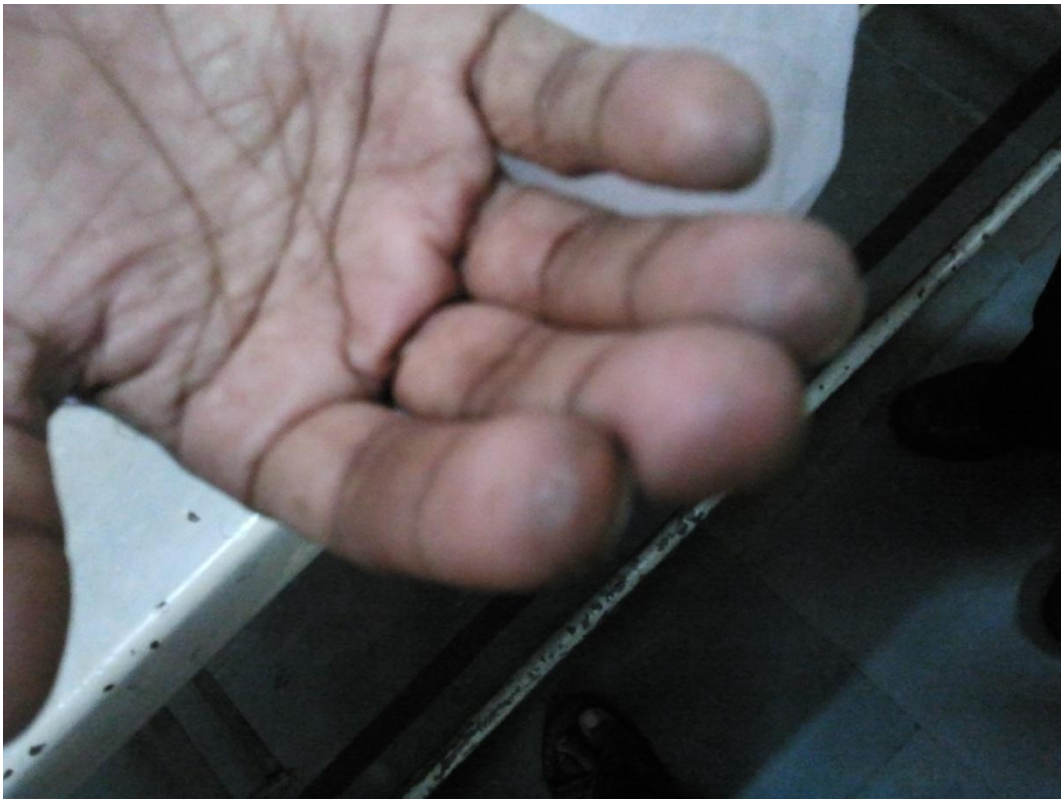


RAYNAUD'S PHENOMENON



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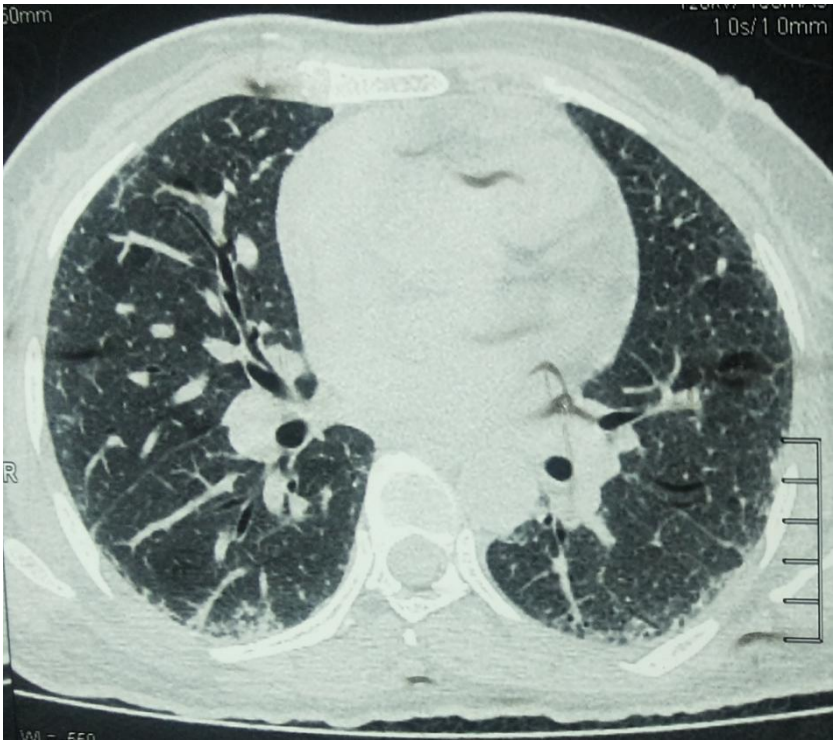

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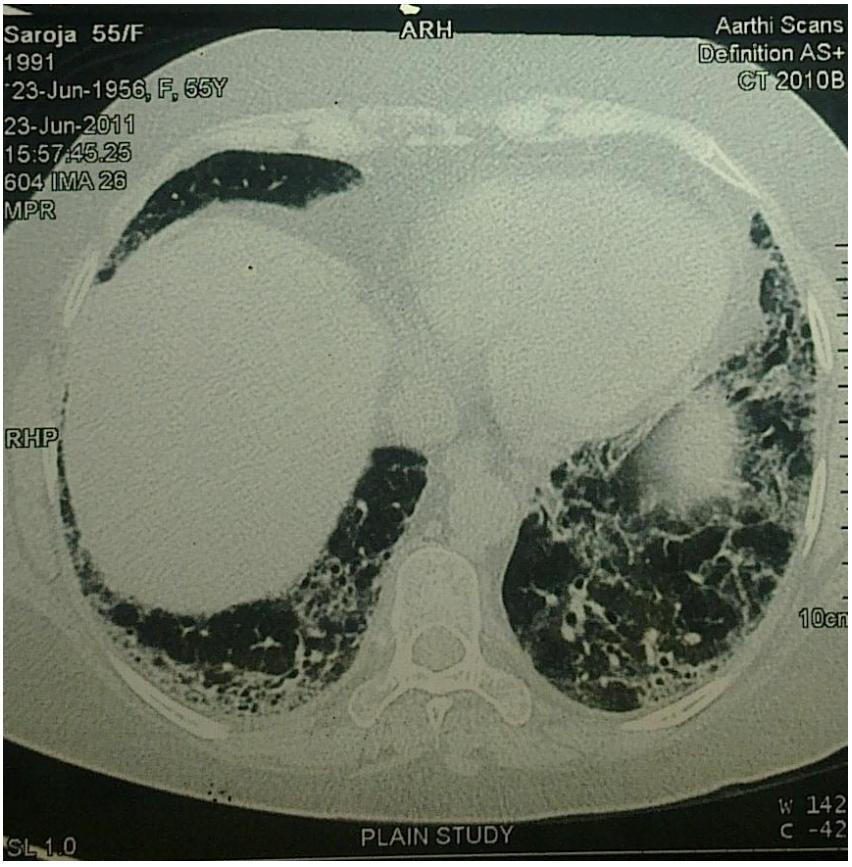

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PROFORMA

Name: Age: Sex: Date:

RCC No:

Complaints:

H/o. Present Illness:

Past History:

Personal History:

Treatment History:

Family History:

GENERAL EXAMINATION

Pallor: Icterus Cyanosis

Clubbing: Lymphadenopathy Pedal Edema

Raynaud's Phenomenon

Skin Thickening

Swollen Hands

Hair

VITAL SIGNS

Blood Pressure Pulse Rate

SYSTEM EXAMINATION

Cardiovascular System Respiratory System

Abdomen Central Nervous System

Musculoskeletal System Examination

INVESTIGATION

Haemogram

Hb: TC: DC

Platelet: ESR:

Urine Routine

Immunological

CRP

RF

Anti-CCP

ANA ANA profile 3 IgG

Biochemical

Sugar: Urea: Creatinine:

Bilirubin: AST: ALT:

ALP: Total Protein: Albumin:

Triglycerides: Cholesterol: LDL:

HDL: Uric Acid:

Radiography

CXR

HRCT Chest

USG Abdomen

Miscellaneous

PFT

ECHO

Referral

Thoracic Medicine

Cardiology

Gastroenterology

Neurology

Nephrology

MASTER CHART

S. NO	AGE	SEX	DURATION	JOINT	PUFFY HANDS	SCL	FEVER	MALAR RASH	MYOSITIS	RAYNAUD'S	GERD	PHT	RENAL	ILD	U1RNP
1	23	F	2 MONTHS	P	P	P	P	P	N	P	P	N	N	P	P
2	27	F	6 MONTHS	P	P	P	P	P	P	P	P	P	N	P	P
3	27	F	7 YEARS	P	P	P	N	N	N	P	P	N	N	N	P
4	42	F	1 YEAR	P	P	P	N	N	P	P	P	N	N	P	P
5	20	F	2 YEARS	P	P	P	P	P	N	P	P	N	N	P	P
6	18	F	1 YEAR	P	P	P	P	P	N	P	P	N	N	P	P
7	42	F	10 YEARS	P	P	P	N	N	P	P	P	P	N	N	P
8	38	F	4 YEARS	P	P	P	P	N	P	P	N	P	N	P	P
9	48	F	2 YEARS	P	P	N	N	N	N	P	P	N	N	P	P
10	19	F	3 MONTH	P	P	P	N	N	N	P	P	P	N	N	P
11	35	F	8 MONTH	P	P	N	P	N	P	P	P	N	N	N	P
12	20	F	1 YEAR	P	P	P	P	N	N	P	N	N	N	N	P
13	40	F	8 MONTH	N	P	N	N	N	P	P	N	N	N	P	P
14	27	F	6 MONTHS	P	P	N	N	N	N	P	P	P	N	P	P
15	34	F	1 YEAR	P	P	P	P	N	P	N	P	P	N	P	P
16	27	F	1 YEAR	P	P	P	P	N	N	P	P	N	P	P	P
17	35	F	2 YRS	P	P	P	P	P	N	P	P	N	N	N	P
18	21	F	1 YEAR	N	P	P	P	N	P	P	P	N	N	N	P
19	40	F	10 YEARS	P	N	P	N	N	P	P	N	P	N	N	P
20	22	F	3 MONTH	P	P	N	P	N	N	P	P	P	p	P	P
21	17	F	1 YEAR	P	P	P	P	N	N	P	P	N	N	N	P
22	26	F	1 YEAR	P	P	P	P	P	N	P	P	N	N	P	P
23	38	F	3 MONTHS	N	P	P	P	P	P	P	P	N	N	P	P
24	27	M	5 MONTHS	P	N	N	P	P	P	N	N	N	N	N	N
25	40	F	3 MONTHS	P	N	N	N	N	P	N	P	P	N	P	P
26	49	F	3 YEARS	P	N	P	N	N	N	N	P	N	N	P	P
27	24	F	10 MONTHS	P	N	N	N	P	N	N	N	N	N	P	P
28	35	F	1 YEAR	P	P	P	P	P	N	P	P	N	N	P	N
29	36	F	6 MONTHS	N	P	P	P	P	N	P	N	P	N	N	N
30	35	F	2 YEARS	P	N	P	P	N	P	N	P	N	N	P	P
31	33	F	7 MONTHS	P	P	N	N	P	P	P	N	N	N	N	P
32	35	F	1 YEAR	P	N	P	P	N	P	N	P	N	N	P	P
33	44	M	7 YEARS	P	N	N	N	N	P	P	N	N	N	N	P
34	30	F	2 MONTHS	P	N	N	P	N	P	P	N	P	N	N	P
35	47	F	3 YEARS	N	N	N	N	N	P	P	P	N	N	N	N
36	22	F	6 MONTHS	P	P	P	P	P	P	P	P	N	N	N	N
37	42	F	7 MONTHS	P	N	N	N	N	P	N	N	N	N	N	N
38	20	F	2 YEARS	P	N	N	P	N	P	N	N	N	N	N	N
39	32	F	3 YEARS	P	N	N	N	N	P	N	N	N	P	N	N
40	44	F	2 YEARS	P	P	N	P	N	P	N	P	N	N	P	N
41	32	F	8 MONTHS	P	P	P	N	N	P	P	P	N	N	N	P
42	42	F	2 YEARS	P	P	P	N	N	P	P	P	N	N	P	P
43	28	F	10 MONTHS	P	N	P	N	P	N	N	P	P	N	N	P

P - Positive, N- Negative, SCL-Sclerodactyly, PHT- Pulmonary Arterial Hypertension, ILD- Interstitial Lung Disease, GERD- Gastro Esophageal Reflux Disorder

PATIENT CONSENT FORM

Study Details: **Clinical spectrum and pulmonary manifestations of patients with anti U1RNP antibody status**

Study Centre: Department of Rheumatology,
Rajiv Gandhi Government General Hospital,
Madras Medical College, Chennai-600 003.

Patient may check (✓) these boxes

I confirm that I have read and understood the Information Sheet for the above study. I have- had the opportunity to ask questions and all my questions and doubts have been answered to my complete satisfaction. ☐

I understand that my participation in the study is voluntary and that I am free to withdraw at any time, without giving any reason, without my legal rights being affected. ☐

I understand that the Clinical study personnel, the Ethics Committee and the Regulatory Authorities will not need my permission to look at my health records both in respect to the current study and any further research that may be conducted in relation to it, even if I withdraw from the study. I agree to this access. However, I understand that my identity will not be revealed in any information released to third parties or published, unless as required under the law. I agree not to restrict the use of any data or results that arise from this study. ☐

I agree to take part in the above study and to comply with the instructions given during the study and to faithfully co-operate with the study team, and to immediately inform the study staff if I suffer from any deterioration in my health or well being or any unexpected or unusual symptoms. ☐

I hereby give permission to undergo complete clinical examination and diagnostic tests including hematological, biochemical, radiological tests. ☐

I hereby consent to participate in this study. ☐

Signature of Investigator Thumb Impression of Patient

Patient Name/ Address

Name of the Investigator

Institution

INFORMATION SHEET

- We are conducting a study on **"Clinical spectrum and pulmonary manifestations of patients with anti U1RNP antibody status"** at Department of Rheumatology, Madras Medical College and Rajiv Gandhi Government General Hospital, Chennai-600003.

- The privacy of the patients in the research will be maintained throughout the study. In the event of any publication or presentation resulting from the research, no personally identifiable information will be shared.

- Taking part in this study is voluntary. You are free to decide whether to participate in this study or to withdraw at any time; your decision will not result in any loss of benefits to which you are otherwise entitled.

- The results of the special study may be intimated to you at the end of the study period or during the study if anything is found abnormal which may aid in the management or treatment.

Signature of the Participant **Signature of the Investigator**

Institution
Date :

<u>ஆராய்ச்சி ஒப்புதல் படிவம்</u>

ஆராய்ச்சி தலைப்பு

கலப்பு இணைப்புத்திசு நோய்

ஆராய்ச்சி நிலையம் : முடக்குவாதவியல் துறை,
சென்னை மருத்துவக் கல்லூரி மற்றும்
ராஜீவ் காந்தி அரசு பொது மருத்துவமனை, சென்னை.

பங்கு பெறுவரின் பெயர் :

பாலினம் :

பங்குபெறபவரின் எண் :

பங்கு பெறுபவர் இதனை (✓) குறிக்கவும்

மேலே குறிப்பிட்டுள்ள மருத்துவ ஆய்வின் விவரங்கள் எனக்கு விளக்கப்பட்டது. என்னுடைய சந்தேகங்களை கேட்கவும், அதற்கான தகுந்த விளக்கங்களை பெறவும் வாய்ப்பளிக்கப்பட்டது. ☐

நான் இவ்வாய்வில் தன்னிச்சையாகதான் பங்கேற்கிறேன். எந்த காரணத்தினாலோ எந்த கட்டத்திலும் எந்த சட்ட சிக்கலுக்கும் உட்படாமல் நான் இவ்வாய்வில் இருந்து விலகி கொள்ளலாம் என்றும் அறிந்து கொண்டேன். ☐

இந்த ஆய்வு சம்பந்தமாகவோ, இதை சார்ந்த மேலும் ஆய்வு மேற்கொள்ளும் போதும் இந்த ஆய்வில் பங்குபெறும் மருத்துவர் என்னுடைய மருத்துவ அறிக்கைகளை பார்ப்பதற்கு என் அனுமதி தேவையில்லை என அறிந்து கொள்கிறேன். நான் ஆய்வில் இருந்து விலகிக் கொண்டாலும் இது பொருந்தும் என அறிகிறேன். ☐

இந்த ஆய்வின் மூலம் கிடைக்கும் தகவல்களையும், பரிசோதனை முடிவுகளையும் மற்றும் சிகிச்சை தொடர்பான தகவல்களையும் மருத்துவர் மேற்கொள்ளும் ஆய்வில் பயன்படுத்திக்கொள்ளவும் அதை பிரசுரிக்கவும் என் முழு மனதுடன் சம்மதிக்கின்றேன். ☐

இந்த ஆய்வில் பங்கு கொள்ள ஒப்புக்கொள்கிறேன். எனக்கு கொடுக்கப்பட்ட அறிவுரைகளின்படி நடந்து கொள்வதுடன் இந்த ஆய்வை மேற்கொள்ளும் மருத்துவ அணிக்கு உண்மையுடன் இருப்பேன் என்று உறுதியளிகிறேன். எனது உடல் நலம்பாதிக்கப்பட்டாலோ அல்லது எதிர்பாராத வழக்கதிற்கு மாறான நோய்க்குறி தென்பட்டாலோ உடனே அதை மருத்து அணியிடம் தெரிவிப்பேன் என உறுதி அளிக்கிறேன். ☐

இந்த ஆய்வில் எனக்கு மருத்துவ பரிசோதனை, இரத்தப் பரிசோதனை செய்துகொள்ள நான் முழு மனதுடன் சம்மதிக்கிறேன்.

பங்கேற்பவரின் கையொப்பம் ..... இடம்..... தேதி.....

கட்டைவிரல் ரேகை

பங்கேற்பவரின் பெயர் மற்றும் விலாசம் .....

ஆய்வாளரின் கையொப்பம் ..... இடம்..... தேதி.....

ஆய்வாளரின் பெயர் .....

<u>ஆராய்ச்சி தகவல் தாள்</u>

சென்னை இராஜீவ்காந்தி அரசு பொது மருத்துவனை முடக்குவாதவியல் துறைக்கு வரும் நோயாளிகளிடம் கலப்பு இணைப்புத்திசு நோய் பற்றிய ஆராய்ச்சி.

நீங்களும் ஆராய்ச்சியில் பங்கேற்க விரும்புகிறோம். இந்த ஆராய்ச்சியில் கதிர்வீச்சு சிகிச்சை அளித்து சில சிறப்பு பரிசோதனைக்கு உட்படுத்தி அதன் தகவல்களை ஆராய்வோம். அதனால் தங்களின் நோயின் ஆய்வறிக்கையோ, சிகிச்சையோ பாதிப்பு ஏற்படாது என்பதை தெரிவித்துக் கொள்கிறோம்.

முடிவுகளை அல்லது கருத்துகளை வெளியிடும்போதோ அல்லது ஆராய்ச்சியின் போதோ தங்களது பெயரையோ அல்லது அடையாளங்களையோ வெளியிடமாட்டோம் என்பதையும் தெரிவித்துக் கொள்கிறோம்.

இந்த ஆராய்ச்சியில் பங்கேற்பது தங்களுடைய விருப்பத்தின் பேரில் தான் இருக்கிறது. மேலும் நீங்கள் எந்நேரமும் இந்த ஆராய்ச்சியிலிருந்து பின்வாங்கலாம் என்பதையும் தெரிவித்துக் கொள்கிறோம்.

இந்த சிறப்பு பரிசோதனைகளின் முடிவுகளையும் நோயின் தன்மை பற்றியும் ஆராய்ச்சியின்போது அல்லது ஆராய்ச்சியின் முடிவின் போது தங்களுக்கு அறிவிப்போம் என்பதையும் தெரிவித்துக் கொள்கிறோம்.

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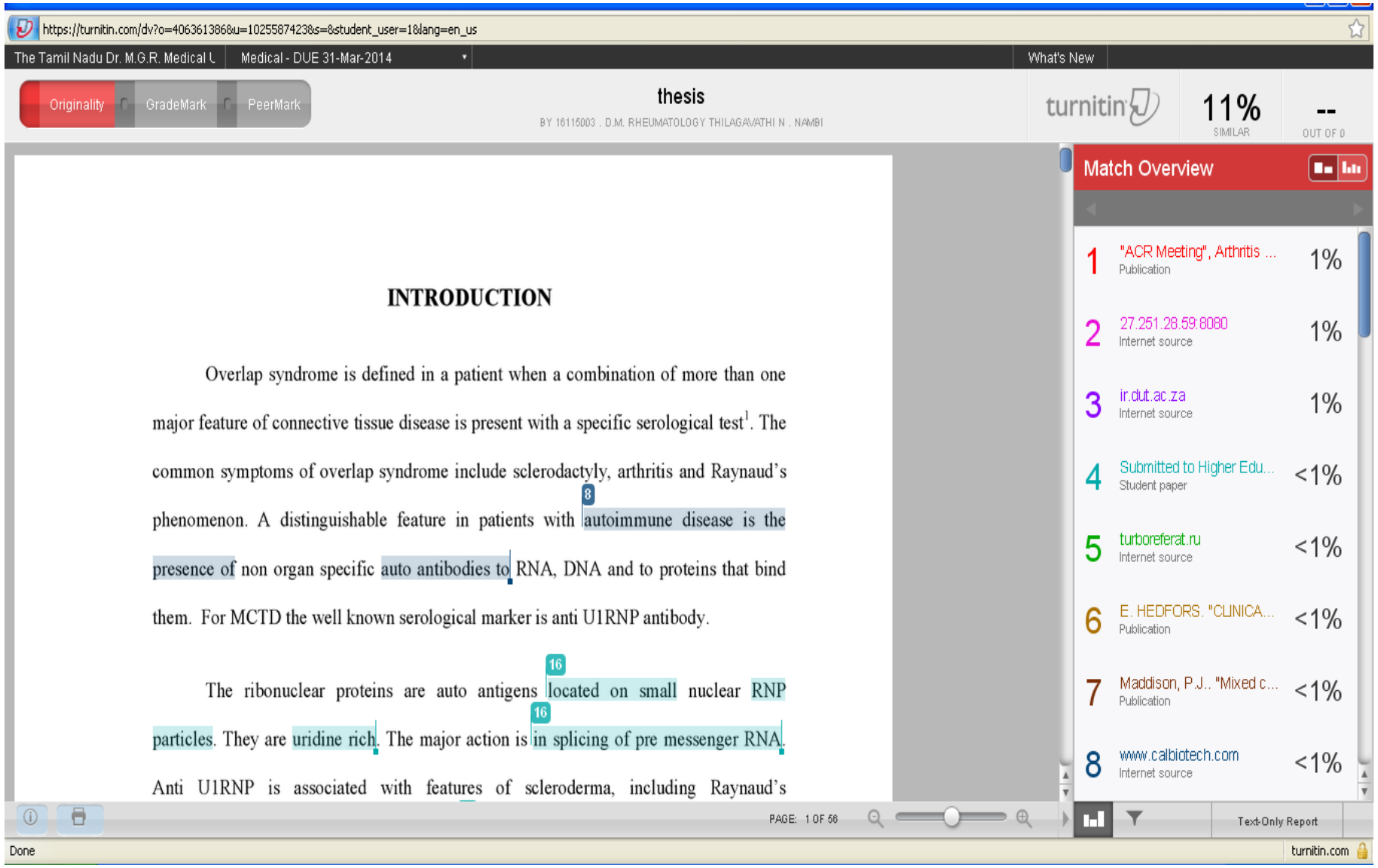
ஆராய்ச்சியாளர் கையொப்பம் பங்கேற்பாளர் கையொப்பம்

தேதி: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INTRODUCTION
Overlap syndrome is defined in a patient when a combination of more than one major feature of connective tissue disease is present with a specific serological test[1]. The common symptoms of overlap syndrome include sclerodactyly, arthritis and Raynaud's phenomenon. A distinguishable feature in patients with autoimmune disease is the presence of non organ specific auto antibodies to RNA, DNA and to proteins that bind them. For MCTD the well known serological marker is anti U1RNP antibody.
The ribonuclear proteins are auto antigens located on small nuclear RNP particles. They are uridine rich. The major action is in splicing of pre messenger RNA. Anti U1RNP is associated with features of scleroderma, including Raynaud's
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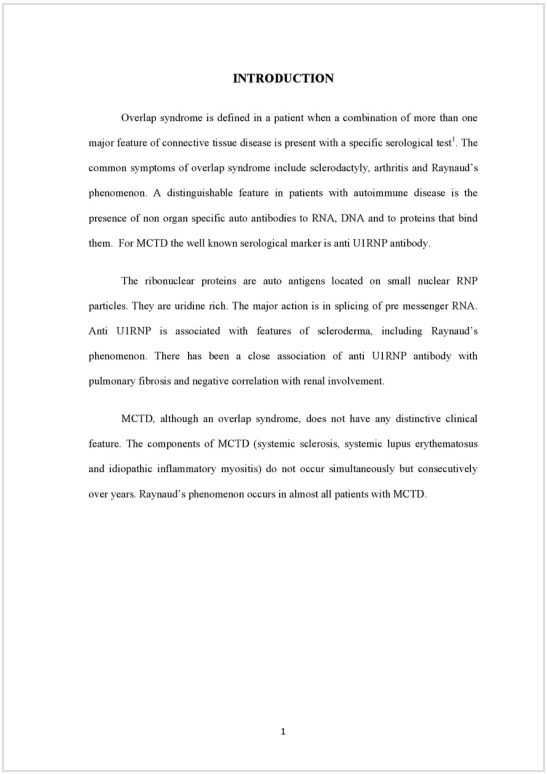

INTRODUCTION

Overlap syndrome is defined in a patient when a combination of more than one major feature of connective tissue disease is present with a specific serological test[1]. The common symptoms of overlap syndrome include sclerodactyly, arthritis and Raynaud's phenomenon. A distinguishable feature in patients with autoimmune disease is the presence of non organ specific auto antibodies to RNA, DNA and to proteins that bind them. For MCTD the well known serological marker is anti U1RNP antibody.

The ribonuclear proteins are auto antigens located on small nuclear RNP particles. They are uridine rich. The major action is in splicing of pre messenger RNA. Anti U1RNP is associated with features of scleroderma, including Raynaud's phenomenon. There has been a close association of anti U1RNP antibody with pulmonary fibrosis and negative correlation with renal involvement.

MCTD, although an overlap syndrome, does not have any distinctive clinical feature. The components of MCTD (systemic sclerosis, systemic lupus erythematosus and idiopathic inflammatory myositis) do not occur simultaneously but consecutively over years. Raynaud's phenomenon occurs in almost all patients with MCTD.

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