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Original Research Article

CLINICAL SPECTRUM AND IMMUNOLOGICAL PROFILE OF PATIENTS WITH MIXED CONNECTIVE TISSUE DISEASE PRESENTING IN A TERTIARY CARE HOSPITAL

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Abstract: *This study investigated the clinical spectrum and immunological profile of mixed connective tissue disease (MCTD) patients in a tertiary care hospital in Pakistan. This study was conducted in the Department of Rheumatology at Fatima Memorial Hospital in Lahore for six months from March to August 2021. A clinical diagnosis was made based on the Kasukawa criteria. In this study, 45 patients who fulfilled the MCTD criteria were enrolled. Their clinical presentation and laboratory findings were studied, and disease characteristics and demographics were recorded using a self-designed proforma. The analysis of the collected data was done using SPSS version 25.0. Of 45 patients, 42 were females, and 3 were males, with a mean age of 33. The most common clinical features studied were Raynaud phenomenon (found in 36 (80%) patients), sclerodactyly (in 12 (26.7%) patients), digital ulcers (in 12 (26.7%) patients), puffy hands (in 14 (31.1%) patients), skin tightness (in 21 (46.7%) patients), oral ulcers (in 8 (29.6%) patients), rash over face (in 13(28.9%) patients), rash over other areas than face (in 16 (35.6%) patients), calcinosis (in 2(4.4%) patients), diffuse hair loss (in 10(37%) patients), arthritis (in 21(46.7%) patients), proximal muscle weakness (in 9 (20%) patients), dryness of eyes and mouth (in 13 (48.1%) patients), and esophageal dysmotility (in 9 (20%) patients). Pulmonary HTN was found in 5(11.1%) patients, ILD with NSIP pattern in 7(13.7%) patients, and UIP pattern on HRCT chest was found in 2(4.4%) patients. Several antibodies were found in patients, including U1RNP in 24(53.3%) patients, ANA in 33(73.3%) patients, AntiScl 70 in 9(20%) patients, Anti-centromere in 2(4.4%) patients, Anti jo1 in 1(3.7%) patient, Anti-dsDNA in 14(31.1%) patients, LAC in 1(3.7%) patient, ACL in 1(3.7%) patient, Anti Ro in 14 (31.1%) patients, and Anti La in 2 (7.4%) patients. S/CPK was raised in 10(22.2%) patients. MCTD is an immune-mediated disorder affecting multiple systemic organs with shared features of three autoimmune disorders: scleroderma, systemic lupus erythematosus (SLE), and polymyositis. Sometimes, it can convert to a full autoimmune rheumatological condition, so if it is treated early, future complications can be prevented. Autoimmune rheumatological condition, so if it is treated early, future complications can be prevented.*

Keywords: Mixed Connective Tissue Disease, Scleroderma, Systemic Lupus Erythematosus, Polymyositis

Introduction

Mixed connective Tissue Disease is a rare broad spectrum of diseases in which different manifestations of autoimmune disorders, including the Raynaud phenomenon, malar rash, puffy hands, arthralgias, and weakness of proximal muscles. The presence of U1RNP is also a Hallmark of MCTD. Clinical manifestations of scleroderma, SLE, polymyositis, and Rheumatoid arthritis are included in MCTD. There are several classification criteria for diagnosing MCTD (Chaigne et al., 2018).

According to one survey conducted in Norway, the prevalence of adult-onset MCTD was estimated to be 3.8 per one hundred thousand individuals, and the mean annual incidence was 2.1 per 1,000,000 per annum (Gunnarsson et al., 2016).

The genetic association of MCTD was observed with HLA-DR4 and DR2 phenotypes in MCTD patients. HLA-B and DRB1 showed that risk alleles for Rheumatoid arthritis were also found in MCTD patients. Other factors, including exposure to UV radiation, drugs, toxins, infections, and chemicals, including vinyl chloride and silica, play a role in triggering the disease process (Sapkota and Al Khalili, 2019).

There are several proposed criteria for the classification of MCTD. Among these, the Kasukawa criterion has maximum sensitivity (77.5%) and specificity (92.2%). Another criterion, the Alarcón-Segovia criterion, has a sensitivity of 69.4% with a 99.4% specificity. Other criteria include the Kahn criterion (52.3% sensitivity and a specificity of 99.4%) and the Sharp criterion (57.7% sensitivity and 90% specificity (John et al., 2020).

Hand edema, hair loss, calcinosis cutis, arteriosclerosis, and telangiectasias are the skin manifestations. In MCTD, the involvement of joints is often more severe than SLE, which may result in deformities. Muscle involvement occurs in the form of myalgia and myositis (Sapkota and Al Khalili, 2019). Other manifestations may include esophageal dysmotility and pulmonary complications such as pulmonary vasculitis, pulmonary arterial hypertension, pleural effusion, pericarditis, interstitial lung disease (ILD), thromboembolic disease, and myocarditis. These may lead to cardiomyopathy (Tani et al., 2014). MCTD can rarely

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involve nervous and renal systems or sometimes secondary vasculitis.

PAH is the most common cause of death in MCTD patients. During the disease, cardiovascular complications associated with MCTD, along with thrombotic events, lead to an increase in morbidity and mortality. Additionally, the presence of antiphospholipid antibodies increased the risk of mortality (Hajas et al., 2013; Reiseter et al., 2017).

The most commonly found pattern of ANA in MCTD is speckled. To differentiate between MCTD and SLE, the use of anti-Sm-D or 70 kDa anti-U1-RNP is recommended because these markers are more specific than ANA. Anti-U1-RNP is expressed in both SLE and MCTD. However, its IgM serotype is more commonly expressed in SLE. On the other hand, MCTD is characterized by the expression of IgG serotype only (Alves and Isenberg, 2020; Carpintero et al., 2015; Dima et al., 2018). The presence of Anti Ro/ La, Sn, Sm/ RNP clusters is proposed to be involved in the disease pathogenesis of SLE.

Methodology

This prospective, descriptive, cross-sectional study was conducted at the Department of Rheumatology, Fatima Memorial Hospital, Lahore, Pakistan, between March 2021 and December 2021. A total of 45 patients who fulfilled the MCTD criterion were retrospectively included. The patients who followed up in our hospital were included in this study. Data about the patient's age, sex, marital status, qualification disease onset as well as duration, early clinical manifestations, and laboratory findings, including CBC, RFT, MCTD-associated antibodies such as ANA, ENA, anti-RNP antibody, APLAS profile, s./CPK were recorded in self-designed proformas. Also, the BMI for each patient was noted in the proformas. High-resolution chest CT scans and echocardiography were performed as a non-invasive method for estimating interstitial lung disease and pulmonary hypertension (defined as a mean pulmonary arterial pressure of ≥25 mmHg).

The patients fulfilling the Kasukawa diagnostic criterion for MCTD were included. Those patients who had overlap syndromes were also included. Patients with ages less than 14 years and those with a known diagnosis of other CTD, such as SLE, SSC, and polymyositis, were excluded. A written informed consent was obtained from each patient. Ethical approval for the study protocol was obtained from the Institutional Bioethics Review Committee (IBRC; No: FMH-09- 2019-IRB-681-M). The study was carried out according to the principles of the Declaration of Helsinki.

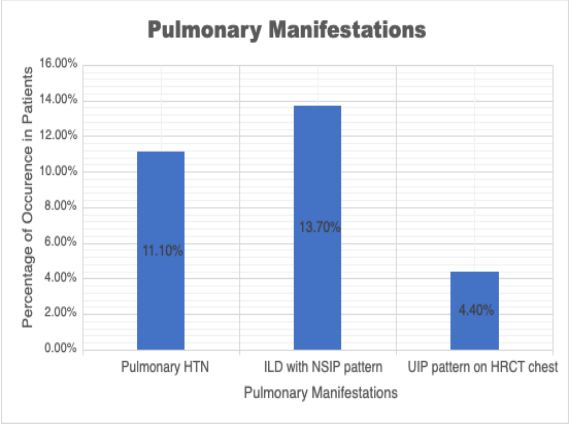
Statistical analysis of the obtained data was performed using the IBM SPSS version 25.0 software. The qualitative variables were presented as frequency and percentage, whereas quantitative variables were presented as mean ± standard deviation (SD).

Results

The mean age of the patients was 33 ± 10.3 years, with a minimum age of 18 years and a maximum of 54 years. The female-to-male ratio was 14:1. At the onset of the disease, 36 (80%) patients developed Raynaud phenomenon. The mean time from the Raynaud phenomenon to other systemic features was 3.8±1.0 years. Other clinical manifestations such as sclerodactyly was present in 12(26.7%), digital ulcers in 12(26.7%), tendon friction rubs in 5(11%), puffy hands in 14(31.1%), skin tightness in 21(46.7%,), arthritis 21(46.7), arthralgias 36(80%), malar rash in 13(28.9%), rash on other areas in 16 (35.6%), proximal muscle weakness in 9(20%), myositis 2(4.4%), calcinosis 2(4.4%), digital gangrene in 3(6.7%), telangiectasia 2(4.4%), shortness of breath in 17(37.8), sicca symptoms 25(55.6%), microstomia 18(40%), shawl sign 2(4.4%), Gottron papules 2(4.4%), regurgitation of food 8(17%), dysphagia 9(20%). Obstetric history showed an incidence of infertility in 16(35.6%) in our cohort; miscarriages were found in 14(31.1%) patients, with a higher incidence of 12(26.7%) miscarriages in the first trimester. The summary of clinical features is given in Table 1.

Table 1: Frequencies of the clinical spectrum of MCTD.

CLINICAL FEATURE	Frequencies (%)
Raynaud Phenomenon	36 (80%)
Sclerodactyly	12 (26.7%)
Digital Ulcers	12 (26.7%)
Puffy Hands	14 (31.1%)
Skin Tightness	21 (46.7%)
Oral Ulcers	8 (29.6%)
Rash over Face	13 (28.9%)
A rash over other areas other than Face	16 (35.6%)
Calcinosis	2 (4.4%)
Diffuse Hair Loss	10 (37%)
Arthritis	21 (46.7%)
Proximal Muscle Weakness	9 (20%)
Dryness of Eyes and Mouth	13 (48.1%)
Esophageal Dysmotility	9 (20%)
Pulmonary HTN	5 (11.1%)
ILD with NSIP pattern	7 (13.7%)
UIP pattern on HRCT chest	2 (4.4%) patients



Echocardiography of 20 patients was done, out of which 5 (11.1%) had moderate to severe pulmonary hypertension. 7 (13.7) patients had ILD patterns on HRCT, among which 4

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(8.9%) had NSIP patterns and 1 had bronchiectasis in 1 (2.2%) patient.

Laboratory characteristics of the cohort, including antibodies, ANA was positive in 33 (73.3%) patients, among which 14(31.1%) were homogenous, speckled were 5(11.1%), and 4 (8.8%) were nucleolar. Anti-Scl-70 antibodies were found in 9 (20%) patients, Anti centromere antibodies were found in 2(4.4%) patients, CPK raised in 10 (22.2%) patients, aldolase raised in 4(8.9%) patients, Anti RNA polymerase III in 3(6.7%) patients, Anti ds DNA positive in 14(31.1%) patients, anti-Sm antibodies in 10(22.2%) and U1-RNP positive in 24 (53.3%) patients. A summary of the immunological profiles of the patients is presented in Table 2.

Table 2: Summary of Immunological Profile in MCTD.

ANTIBODIES	OCCURRENCE
U1RNP	24 (53.3%) patients
ANA	33 (73.3%) patients
AntiScl 70	9 (20%) patients
Anti-Centromere	2 (4.4%) patients
Anti Jo 1	1 (3.7%) patient
Anti-dsDNA	14 (31.15) patients
LAC	1 (3.7%) patient
ACL	1 (3.7%) patient
Anti Ro	14 (31.1%) patients
Anti La	2 (7.4%) patients
S/CPK	Raised in 10 (22.2%) patients

Discussion

In our study Raynaud phenomenon was present in 36(80%), sclerodactyly was present in 12(26.7%), digital ulcers in 12(26.7%), tendon friction rubs in 5(11%), puffy hands in 14(31.1%), skin tightness in 21(46.7%,), arthritis 21(46.7), arthralgias 36(80%), malar rash in 13(28.9), rash on other areas in 16 (35.6%), proximal muscle weakness in 9(20%), myositis 2(4.4%), calcinosis 2(4.4%), digital gangrene in 3(6.7%). One Norwegian nationwide study reported Raynaud in 99% of patients, puffy hands in 60%, and arthritis in 79% (Gunnarsson et al., 2016). A study conducted in 2013 by Hajas A et al. described a large cohort of MCTD. They found that polyarthritis, Raynaud phenomenon, puffy fingers, and sclerodactyly were the most typical symptoms at disease onset, which were reported in 65%, 53%, 50%, and 35% of patients, respectively, at the time of diagnosis (Hajas et al., 2013).

In our cohort, anemia was found in 38(74.5%), leukopenia in 2 (3.9%), and thrombocytopenia in 3(5.8%) patients, while according to Alek, hematologic abnormalities are often seen in patients. They may have anemia, as well as leukopenia, lymphopenia, and thrombocytopenia, as observed (Alekperov, 2019).

In our cohort, PAH was found in 5 (11.1%) patients and is most commonly observed in the scleroderma pattern of MCTD. According to Fairly, PAH was more frequently seen in patients with SSc-MCTD (12.4%) than in SLE-MCTD, which is mainly similar to our cohort.12 Gunnarsson et al. conducted a study with a random cohort of MCTD patients. Their results showed that the frequency of PH in the cohort was 3.4% (5/147) over 5.6 years of observation. In 3 out of 5 cases (60%), PH was associated with interstitial lung disease (ILD). Meanwhile, PH was isolated in 2 out of 5 cases (40%) (Gunnarsson et al., 2016; Tani et al., 2014).

Peri Heckmen observed that pulmonary involvement in his cohort was present in 75% of patients, and ILD was observed in up to 50%. The rest of the patients had pleuritis/pericarditis (Fairley et al., 2021). While in our study, 7 (13.7%) patients had an ILD pattern on HRCT, among which 4 (8.9%) had an NSIP pattern and 1 had bronchiectasis in 1 (2.2%) patient. According to Kawano et al., the most common HRCT findings in MCTD patients were ground glass opacities and reticulonodular shadowing predominantly affecting the lower lobes; hence, findings are consistent with the NSIP pattern. Fibrosis was found in a few patients with longstanding disease (Kawano-Dourado et al., 2015). Another Norwegian study demonstrated similar HRCT findings in 35% of patients (Gunnarsson et al., 2016).

In our study, ANA was positive in 33 (73.3%) patients, among which 14(31.1%) were homogenous, speckled were 5(11.1%), and 4 (8.8%) were nucleolar. Anti-Scl-70 antibodies were present in 9 (20%) patients, Anti centromere antibodies in 2(4.4%) patients, Anti-RNA polymerase III in 3(6.7%) patients, Anti ds DNA in 14(31.1%) patients, anti-Sm antibodies in 10(22.2%) and U1-RNP positive in 24 (53.3%) patients. Carpintero found that among RNP-positive patients, most have lupus. Anti-U1-RNP or anti-Sm-D were studied to differentiate between SLE and MCTD. Furthermore, the IgM serotype of anti-U1-RNP was often expressed in SLE, while only the IgG serotype was found in MCTD (Carpintero et al., 2015). Tani et al.'s study showed other autoantibodies in MCTD patients. They include anti-phospholipids (aCL, antib2GPI), anti-Ro, AECA, rheumatoid factor (RF), as well as anti-cyclic citrullinated peptides (anti-CCP) (Tani et al., 2014).

In our study MCTD 33(73.3%), SSc-SLE overlap were 3(6.7%), SSc-myositis were 2(3.9%), RA-SSC overlap were 2 (4.4%) and 1(2.2%) was Sjogren's. Results of the study conducted by Pep et al. showed 49 (38.9%) patients with SSc-MCTD overlap, 17 (13.5%) with RA overlap, 22 (17.5%) patients with SLE-MCTD overlap, 3 patients (2.4%) with symptoms overlapping those of polymyositis, 43 (34.1%) patients with Sjogren syndrome, and 2 (1.6%) patients in which overlap condition was not specified.15

Long-term follow-up is essential in defining the natural disease course and evaluating the potential evolution of MCTD to other CTDs. A recent observational study has reported that in about 20% of MCTD patients, the clinical features evolved to another CTD over 5 years. In 50% of patients, the disease evolved similarly over 10 years (Alekperov, 2019). Anti-DNA antibodies were found in the MCTD patients with evolution into SLE. Meanwhile, evolution to SSc was seen in patients with esophageal dysmotility and sclerodactyly (Ahsan et al., 2018; Tani et al., 2014).

Although CNS remains characteristically uninvolved in MCTD, nearly 25% of MCTD patients have mild CNS involvement. The most frequent manifestation of CNS involvement is trigeminal neuralgia. Other manifestations may include aseptic meningitis, peripheral neuropathy, convulsion, nuchal rigidity, psychosis, headache, and sensorineural hearing loss (Sapkota and Al Khalili, 2019).

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In our study, the only CNS manifestation noted was trigeminal neuralgia, found in 2 patients (3.9%).
In our study, obstetric history showed the incidence of infertility in (Ahsan et al., 2018) (35.6%) patients, and miscarriages were found in (Kawano-Dourado et al., 2015) (31.1%) patients, with a higher incidence of miscarriages (26.7%) in the first trimester.
In addition, pain management and fatigue were still a big challenge, as pain and fatigue were not appropriately addressed and treated, as most of the patients present with fatigue as an initial symptom. Non-pharmacological modalities such as exercises, patient education, and psychological and emotional support are essential management components. Pharmacologic therapies such as hydroxychloroquine and other immunosuppressants are also required (Antunes et al., 2018).
UCTD is also one of the entities that often remain undiagnosed compared to other CTDs and can progress to full-blown connective diseases (CTD) that require regular follow-up and evaluation (Chaigne et al., 2018).

Conclusion

In conclusion, MCTD has a varied disease course. Many of the patients follow a benign course. However, in some patients, increased morbidity and mortality are seen in association with disease complications. The most severe complications are cardiopulmonary complications, especially pulmonary hypertension. Hence, both MCTD and UCTD disease spectra require further research regarding disease burden and its impact on patients' quality of life so that these can be diagnosed earlier and complications can be prevented.

Declarations

Data Availability statement
All data generated or analyzed during the study are included in the manuscript.
Ethics approval and consent to participate
Approved by the department Concerned.
Consent for publication
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Not applicable

Conflict of interest

The authors declared absence of conflict of interest.

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