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Association between Localized Scleroderma Cutaneous Assessment Tool and clinicopathologic characteristics in patients with morphea

Morfealı hastalarda Lokalize Skleroderma Kutanöz Değerlendirme Aracı ve klinikopatolojik özellikler arasındaki ilişki

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Abstract

Background and Design: Morphea is also known as localized scleroderma. It is a rare autoimmune skin disease characterized by inflammation and sclerosis in the dermis and sometimes in the subcutaneous tissue. Laboratory findings, imaging, and histopathological features facilitate diagnosis and provide sufficient information about disease severity. Clinicopathologic correlations and severity factors in morphea are poorly described. Thus, this study aimed to review the clinical and histopathological features and treatment responses of patients with morphea and compare these features with disease activity and damage scores to identify new tools for assessing disease severity other than clinical findings. The applicability of the Localized Scleroderma Cutaneous Assessment Tool in clinical practice was also evaluated.
Materials and Methods: This study reviewed data of 41 patients who had a histopathologically confirmed diagnosis of morphea and had been followed up regularly for at least 6 months. The modified Localized Scleroderma Skin Severity Index (mLoSSI), Localized Scleroderma Skin Damage Index (LoSDI), Physician Global Assessment-Activity (PGA-A), and Physician Global Assessment-Damage (PGA-D) were calculated at baseline and final treatment.
Results: Among morphea subtypes, superficial morphea had significantly more sclerosis in the papillary dermis and plaque-type morphea had significantly more sclerosis in the reticular dermis ($p<0.05$). When positive antinuclear antibody (ANA) and high levels of thyroid autoantibodies were compared with mLoSSI, LoSDI, PGA-A, and PGA-D scores at baseline, no significant correlation was found. Comparison of the subgroups according to the initial mLoSSI and LoSDI scores revealed no significant histopathological differences between the groups.
Conclusion: Although the mLoSSI, LoSDI, PGA-A, and PGA-D scores can be successfully used for the follow-up and treatment of patients with morphea, no correlation was found between positive ANA, high levels of thyroid autoantibodies, and histopathological features.
Keywords: Localized scleroderma, morphea, Localized Scleroderma Cutaneous Assessment Tool, pathology, autoantibodies

Öz

Amaç: Lokalize skleroderma olarak da bilinen morfea, dermiste ve bazen subkütan dokuda enflamasyon ve skleroz ile seyreden, nadir görülen, otoimmün bir deri hastalığıdır. Laboratuvar bulguları, görüntüleme yöntemleri ve histopatolojik özellikler tanıyı kolaylaştırmakla birlikte hastalığın şiddeti hakkında yeterince bilgi vermemektedir. Hastalığın klinikopatolojik özellikleri ile şiddeti arasındaki ilişki nadir olarak gösterilmiştir. Bu çalışmanın amacı morfealı hastaların klinik ve histopatolojik özelliklerini ve tedavi yanıtlarını gözden geçirmek ve bu özelliklerle hastalık aktivite ve hasar skorlarını karşılaştırmak ve klinik bulgular dışında hastalık şiddetini değerlendirmek için yeni araçlar geliştirmektir. Ayrıca Lokalize Skleroderma Kutanöz Değerlendirme Aracının klinik pratikte uygulanabilirliği de değerlendirilmiştir.
Gereç ve Yöntem: Bu çalışma, histopatolojik olarak morfea tanısı doğrulanmış, en az altı ay düzenli takip edilen, tedavi başlangıcında ve bitiminde Modifiye Lokalize Skleroderma Deri Şiddet İndeksi (mLoSSİ), Lokalize Skleroderma Deri Hasar İndeksi (LoSDİ), Doktorun Global Değerlendirmesi Aktivitesi (PGA-A) ve Doktorun Global Değerlendirmesi Hasarı (PGA-H) hesaplanmış olan 41 hastanın mevcut verileri gözden geçirilerek tasarlanmıştır.
Bulgular: Morfea alt tipleri arasındaki karşılaştırmada, yüzeyel tip morfealı hastaların papiller dermisinde ve plak tip morfealı hastaların alt retiküler dermisinde anlamlı olarak daha fazla skleroza sahip oldukları görüldü ($p<0,05$). Antinükleer antikor (ANA) pozitifliği ve troid

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otoantikorlarının yükseliği ile tedavi başlangıç mLoSSI, LoSDI, PGA-A ve PGA-H skorları karşılaştırıldığında anlamlı bir korelasyon bulunamadı. Başlangıç mLoSSI ve LoSDI skorları göz önüne alınarak oluşturulan alt gruplar arasında anlamlı bir histopatolojik farklılık gözlenmedi.
Sonuç: Morfealı hastaların takip ve tedavisinde mLoSSI, LoSDI, PGA-A ve PGA-D skorları başarılı bir şekilde kullanılabilmekle birlikte, pozitif ANA, yüksek tiroid otoantikor düzeyleri ve histopatolojik özellikler arasında bir ilişki bulunamadı.
Anahtar Kelimeler: Lokalize skleroderma, morfea, Lokalize Skleroderma Kutanöz Değerlendirme Aracı, patoloji, otoantikorlar

Introduction

Morphea, also known as localized scleroderma, is a rare fibrosing connective tissue disorder that affects the skin and/or subcutaneous tissue[1,2]. Although its etiology and pathogenesis are not fully understood, the natural course of morphea consists of several phases: An early inflammatory stage characterized by hyperemia and edema, followed by progressive fibrosis and sclerosis, and finally atrophy or scarring[3].

To assess the progression of morphea, Arkachaisri et al.[4,5] developed the Localized Scleroderma Cutaneous Assessment Tool (LoSCAT). The LoSCAT is used to measure the severity of localized scleroderma in the active phase and assess the extent of damage during the disease course[6]. The modified Localized Scleroderma Skin Severity Index (mLoSSI), which is the first part of LoSCAT, measures disease severity in active and inflammatory stages. The mLoSSI score is based on disease extent (enlargement of existing lesions and new lesion development) in 18 anatomical skin regions of the body: Head, neck, chest, abdomen, upper back, lower back, and the right and left arms, forearms, hands/fingers, thighs, legs, and feet). It is calculated by adding points between 0 and 3, depending on the disease extent (extent of existing lesions/emergence of new lesions) and intensity (erythema and skin thickness). The total score ranges from 0 to 162, and higher scores are associated with greater disease activity[4,5].

The Localized Scleroderma Skin Damage Index (LoSDI), the second part of the LoSCAT, was developed to determine the presence and extent of skin damage changes in the same anatomical regions of the skin, as mLoSSI. In this assessment, skin atrophy, subcutaneous atrophy, and hypo-/hyperpigmentation are scored from 0 to 3. The total scores range from 0 to 162, and higher scores indicate more severe damage[5,6]. The authors recommend combining the mLoSSI and LoSDI with the Physician Global Assessment-Activity (PGA-A) and Physician Global Assessment-Damage (PGA-D)[5]. PGA-A and PGA-D are 100 mm visual analog scales set at 0 and 100, respectively. Higher scores indicate more activity or damage[7].

Certain histopathological features have been associated with morphea activity[8]. Plasma cells and eosinophils are rarely seen along with lymphocytes in the early stage of active morphea[8,9]. In the late stage, inflammatory infiltration disappears. The collagen bundles in the reticular dermis are eosinophilic, thickened, and densely packed[9,10]. These morphological changes are found in the papillary and upper reticular dermis in superficial morphea and subcutaneous tissue in deep morphea[2,8].

Three principles guide the therapeutic decision-making process in morphea, i.e., evidence of clinical disease activity, depth of lesion involvement, and disease extent. Patients with limited involvement of active lesions confined to the dermis (no evidence of subcutaneous atrophy or deep inflammation) may be treated initially with topical medications[11]. Phototherapy or systemic treatment with methotrexate (MTX) should be initiated for more common lesions limited to the dermis. MTX is considered the first-line treatment for lesions with deep involvement[8]. In addition, mycophenolate mofetil should be considered the second-line treatment if MTX is not tolerated, if contraindications to MTX exist, or if MTX does not elicit a response[9,11].

Biopsy of skin lesions helps in the diagnosis and assessment of disease activity and depth of involvement[1]. The histologic features of morphea are well defined in the literature[10,12,13]; however, only a few studies have provided information on clinicopathologic correlations[1,14-16]. In this retrospective study, we aimed to evaluate the clinical and laboratory features, treatment regimens, treatment response, and histopathologic features of patients diagnosed with morphea. We also evaluated the applicability of LoSCAT in clinical practice. In addition, we investigated the correlation between patients' clinicopathological features and severity of morphea.

Materials and Methods

Study design

This study was conducted as a retrospective records review of patients who presented to the Dermatology and Venereal Diseases Department of Trakya University Faculty of Medicine between January 2017 and January 2021 and were diagnosed with morphea.

Patients who were diagnosed with histopathologically confirmed morphea and had not received topical or systemic therapy were included in the study. Patients who attended regular monthly follow-up visits and were followed up for at least 6 months were evaluated to assess clinical response[1,17].

At each visit, all patients were examined, clinically scored, and classified into subtypes by the same dermatologist. Each case was classified into subtypes according to the criteria defined by Kreuter et al.[18]. The authors considered eosinophilic fasciitis as a distinct type in this spectrum. Images of the clinical subtypes of morphea are shown in Figure 1, 2.

The LoSCAT was used to clinically grade the cases. The scores of mLoSSI, LoSDI, and PGA activity and damage calculated at baseline and final treatment were recorded. Patients were divided into three subgroups-mild, moderate, and severe-to indicate disease severity according to their scores. Mild, moderate, and severe disease activity corresponded to mLoSSI scores of 0-4, 5-12, and ≥13, respectively. Moreover, mild, moderate, and severe disease damage corresponded to LoSDI scores of 0-10, 11-15, and ≥16, respectively[6].

The same dermatologist retrospectively assessed the clinical outcomes at the last visit using the classification criteria suggested by Kumar et al.[19]. Complete response (CR) was considered >95% improvement and partial response (PR) >50% improvement.

Data collection

Clinical data were obtained from electronic medical records. Patient demographic and clinical characteristics, presence of concomitant

autoimmune diseases, clinical symptoms, and concomitant lichen sclerosus et atrophicus (LSA) were recorded. Laboratory results, detailed treatment modalities, treatment duration, and treatment responses were assessed. The same dermatologist performed a biopsy of the erythematous rim of the inflammatory lesions or central sclerosis of the lesions that did not clinically show inflammation using a 4 mm punch biopsy instrument. Incisional biopsy was performed in patients with a provisional diagnosis of linear morphea, deep type morphea, or eosinophilic fasciitis.

The study was approved by Local Ethics Committee of Trakya University Faculty of Medicine (approval number: 07/07, date: 15.03.2021). Informed consent was obtained.

Histopathological examination

All specimens were examined at Trakya University Medical Pathology Laboratories, Edirne, by the same pathologist.

Each slide was fixed in 10% neutral buffered formalin for 8 h. Paraffin blocks were prepared, and five serial sections of 4 µ were made. The sections were stained with hematoxylin and eosin, Masson's trichrome, and Verhoeff's elastic stains. For histochemical staining with Masson's trichrome, the slide containing the 4 µ section was kept in an incubator at 63 °C for 20 min. Then, it was placed in an automatic stainer and stained with trichrome (v3.00.0052, BenchMark Special Stains Module). The same procedure was used for staining with Verhoff's elastic (v1.00.0021, BenchMark Special Stains Module).

Epidermal atrophy, perivascular lymphoplasmocytic infiltration, sclerosis (thickening and homogenization of collagen fibers with Masson's trichrome), hyalinization (glassy, homogeneous appearance of collagen fibers in the dermis), atrophy of skin appendages, and loss of elastic fibers were examined histomorphologically in sections stained with hematoxylin-eosin. Loss of elastic fibers in the papillary or reticular dermis was noted by Verhoff's elastic staining. In addition, sclerosis was classified into papillary dermis, reticular dermis, and hypodermis depending on the location. The severity of inflammation was recorded as absent, mild, moderate, or prominent and classified into perivascular, periadnexal, interstitial, and dermal subcutaneous nodule formations depending on the location. Assessment was based on the classification by Walker et al.[16]. The proportion of lymphocytes, plasma cells, and eosinophils in the inflammatory infiltrate was classified as absent (at x40 magnification, <5 cells) or present (at x40 magnification, >5 cells)[1].

Statistical Analysis

Statistical analysis of data was performed using IBM SPSS Statistics version 22 software. Fisher's exact test was used to compare categorical data between groups. Since continuous variables were not parametric (Kolmogorov-Smirnov and Shapiro-Wilk tests, $p<0.05$),

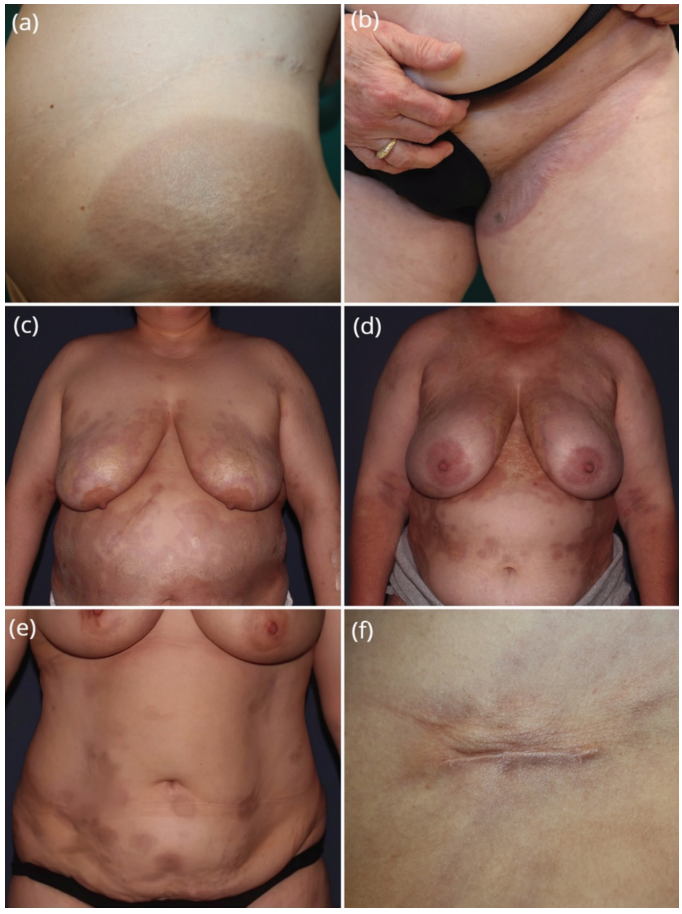

Figure 1. (a, b) Plaque morphea (classical plaque-type), **(c, d)** generalized morphea, **(e)** atrophoderma idiopathica of Pierini and Pasini (superficial morphea), and **(f)** deep type (deep morphea)

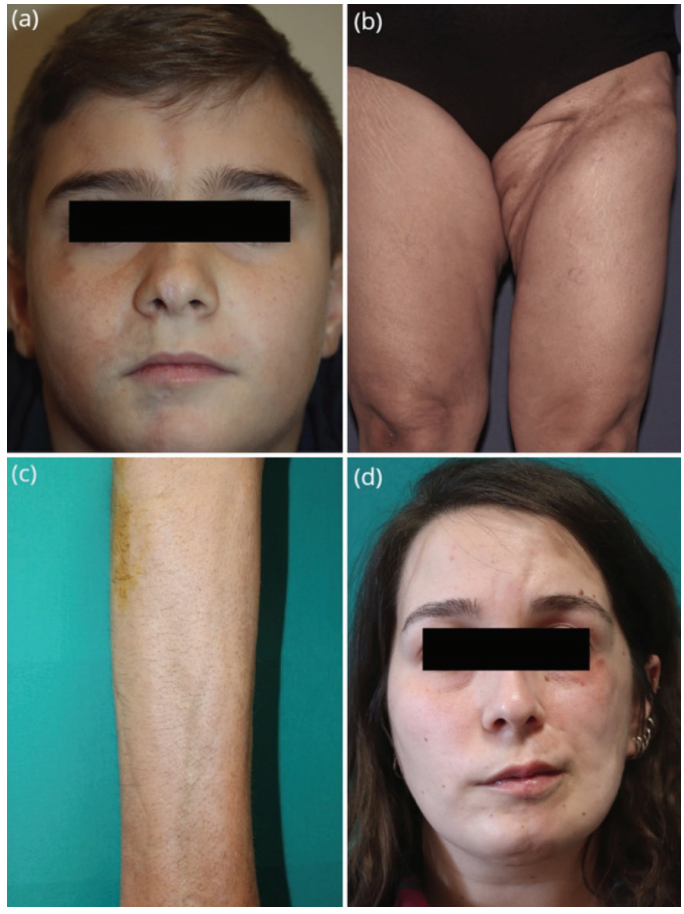

Figure 2. (a) Linear morphea "en coup de sabre," **(b)** linear morphea of the extremities, **(c)** groove sign in eosinophilic fasciitis (Shulman syndrome), and **(d)** progressive facial hemiatrophy (Parry-Romberg syndrome)

the Mann-Whitney U test was used for comparisons between the two groups, and Wilcoxon signed-ranks statistical analysis was used to compare baseline and final measurement results; $p<0.05$ was considered significant.

Results

In total, 41 patients were enrolled in the study. Of these patients, 36 (87.8%) were female, and the mean age at baseline was 43.2 years. The lower extremities were most commonly affected (n=23, 56.1%), and plaque-type was the most common form of morphea (n=19, 46.3%). In addition, 13 (31.7%) patients had concurrent autoimmune diseases such as vitiligo, bullous pemphigoid, diabetes mellitus, and autoimmune thyroiditis. Antinuclear antibodies (ANAs) with a titer of at least 1:80 were present in 20 (48.8%) patients. Rheumatoid factor was negative in all patients (n=41, 100%). Regarding the treatments of 40 patients, tacrolimus was the most commonly used topical agent (n=34, 82.9%) and hydroxychloroquine was the most commonly used systemic agent (n=15, 36.6%) (Table 1).

In 29 treated patients, the mLoSSI score decreased from 6.8 (±6.6) to 0.7 (±2.8), whereas the LoSDI score increased from 2.8 (±3.7) to 6.5 (±6.5). Furthermore, patients were classified into three subgroups according to the disease severity and damage. Based on this classification, the change in the severity of morphea in the subgroups at baseline and final treatment is illustrated in Figure 3. Based on the mLoSSI score at baseline, 43.9% (n=18) of the cases were classified as mild, 41.5% (n=17) as moderate, and 14.6% (n=6) as severe. When the LoSDI score was evaluated at the end of treatment, 82.8% (n=24) of the cases with mild damage improved. Patient outcomes showed that 23 (79.3%) patients had CR and 6 (20.7%) had PR. The median follow-up time of the treated patients was 10 (range; 4.3-25.3) months.

The most common histopathologic findings were sclerosis (n=37, 94.9%), perivascular lymphoplasmacytic infiltration (n=32, 82.1%), and coarsening of sclerosis in the lower reticular dermis (n=31, 79.5%) (Table 2).

As regards the distribution of sclerosis in morphea subtypes, a significant change was observed in the papillary dermis in superficial morphea and reticular dermis in plaque-type morphea ($p<0.05$) (Table 3).

The mean mLoSSI, LoSDI, PGA-A, and PGA-D scores of ANA-positive and ANA-negative patients are shown in Table 4. No significant differences were found in any of the scores between the two groups ($p<0.05$).

In the study group, the mean levels of thyroid autoantibody parameters in relation to disease severity are presented in Table 5. No significant difference was found between high thyroid autoantibody levels and mLoSSI, LoSDI, PGA-A, and PGA-D scores at baseline ($p<0.05$).

Differences in histopathologic features were also noted depending on patients' baseline mLoSSI and LoSDI scores (Table 6). Patients were classified into three subgroups according to their baseline mLoSSI and LoSDI scores, and comparative analysis of histopathologic findings revealed no significant differences between the groups. No significant difference in histopathologic features was noted between active and inactive lesions ($p<0.05$).

Discussion

The demographics, clinical subtype distribution, and involvement sites in the present study are similar to previous reports[1,20-23]. In this study, the proportion of linear morphea is much lower than that of linear scleroderma in the general population (approximately 30%)[22]. It may be because only one pediatric patient had morphea in our study and linear morphea is more common in pediatric patients. Joint involvement and positive rheumatoid factor can be observed in linear morphea with extremity involvement[9]. However, in this study, rheumatoid factor was negative in all patients, as the linear type was present in only three patients and seven patients had extracutaneous symptoms.

Morphea is an autoimmune disease, but the major antigen has not yet been identified. The presence of an autoimmune disease may be associated with a higher risk of another disease[24]. In this study, we also investigated autoimmune diseases associated with morphea and their laboratory findings.

Leitenberger et al.[25] reported that 30% of adult patients with morphea had concurrent autoimmune disease. This rate was 31.7% in our study,

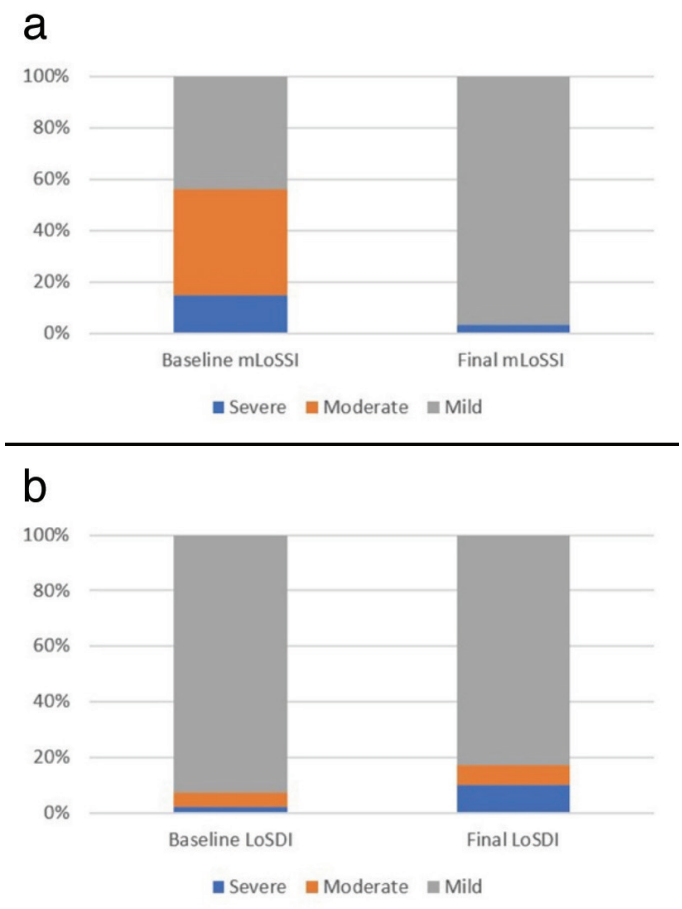

Figure 3. (a) Changes in mLoSSI score at baseline and final treatment. mLoSSI: Modified Localized Scleroderma Skin Severity Index (mild, moderate and severe disease activity groups correspond to the mLoSSI scores of 0-4, 5-12, and ≥13, respectively). **(b)** Changes in LsoDI score at baseline and final treatment. LoSDI: Localized Scleroderma Damage Index (mild, moderate and severe disease damage groups correspond to the LoSDI scores of 0-10, 11-15, and ≥16, respectively)

Table 1. Summary of patient characteristics, clinical and laboratory findings, and treatment regimens	
Feature	**n=41 (%)**
Sex	
Male	5 (12.2)
Female	36 (87.8)
Age at diagnosis, years (mean ± SD)	43.2±22.3
Duration of the lesions, months (mean ± SD)	45.2±85.5
Subtypes of morphea	
1A- Plaque morphea (classical plaque-type)	19 (46.3)
1B- Guttate morphea	0
1C- Atrophoderma idiopathica of Pierini and Pasini (superficial morphea)	8 (19.5)
2A- Generalized morphea	7 (17.0)
2B- Disabling pansclerotic morphea	0
3A- Linear morphea of the extremities	1 (2.4)
3B- Linear morphea "en coup de sabre"	2 (4.9)
3C- Progressive facial hemiatrophy (Parry-Romberg syndrome)	1 (2.4)
4- Deep type (deep morphea)	2 (4.9)
5- Mixed type	0
6- Eosinophilic fasciitis (Shulman syndrome)	1 (2.4)
Anatomical location	
Scalp-face and neck	3 (7.3)
Trunk	21 (51.2)
Back	18 (43.9)
Upper extremities	12 (29.3)
Lower extremities	23 (56.0)
History of autoimmune disease	13 (31.7)
Clinical symptoms	
Pain or arthralgia of the affected joints	7 (17.1)
Lichen sclerosus et atrophicus	3 (7.3)
Laboratory findings	
Peripheral eosinophilia (>500/UI)	2/41 (4.8)
Elevated LDH	2/41 (4.8)
Elevated ESR	2/41 (29.2)
Elevated CRP	10/41 (24.3)
Rheumatoid factor	0/41 (0.0)
Thyroid autoantibodies	
Anti-thyroglobulin antibody	3/41 (7.3)
Anti-peroxidase antibody	8/41 (19.5)
Elevated IgG	4/41 (9.7)
ANA ≥1:80	20/41 (48.7)
ENA	
Anti-SSA antibody	1/41 (2.4)
Anti-DFS 70 antibody	5/41 (12.1)
Treatment regimens (n=40)	
Topical corticosteroid	32 (78)
Topical tacrolimus	34 (82.9)
Hydroxychloroquine (400 mg/day)	15 (36.6)
Methotrexate (20-25 mg/week)	8 (19.5)
Narrow-band UVB	6 (14.6)
Systemic corticosteroid	3 (7.3)
Mycophenolate mofetil	2 (4.9)

LDH: Kactate dehydrogenase, ESR: Erythrocyte sedimentation rate, CRP: C-reactive protein, IgG: Immunoglobulin G, ANA: Antinuclear antibody, ENA: Extractable nuclear antigen antibodies, anti-SSA: Anti-Sjögren's-syndrome-related antigen A autoantibodies, anti-DFS 70: Anti-dense fine speckled 70, UVB: Ultraviolet B, SD: Standard deviation

Table 2. Histopathological features of the specimens	
Features	**n=39 (%)**
Epidermal atrophy	26 (66.7)
Degree and location of inflammatory infiltrate	
None	13 (33.3)
Mild/perivascular	9 (23.1)
Moderate/perivascular	8 (20.5)
Prominent/perivascular	4 (10.3)
Prominent/dermal/subcutaneous nodule formation	5 (12.8)
Inflammatory cell type present*	
Lymphocytes	23 (59)
Plasma cells	1 (2.6)
Eosinophils	17 (43.6)
Perivascular lymphoplasmacytic infiltrates	32 (82.1)
Sclerosis	37 (94.9)
Location of sclerosis	
Papillary dermis	11 (28.1)
Reticular dermis	31 (79.5)
Hypodermis	9 (23.1)
Hyalinization	5 (12.8)
Loss of elastic fibers	
Upper reticular dermis	3 (7.7)
Lower reticular dermis	6 (15.4)
Atrophy of skin appendages	13 (33.3)
*The proportion of lymphocytes, eosinophils, and plasma cells in the inflammatory infiltrate was categorized into present (>5 cells in small x40 magnification area)	

and autoimmune thyroiditis was the most common concomitant disease.

In large cohort studies, positivity to ANA was found in 23-68% of patients with morphea[23]. In the present study, this rate was 48.8%. ANA is a potential biomarker of the depth of tissue involvement and extracutaneous manifestations[24]. It can be also used as an indicator, similar to scoring systems used in the treatment of the disease[26]. We found no association between autoantibodies and clinical markers of disease severity. This result is in agreement with the findings of Dharamsi et al.[26].

Dańczak-Pazdrowska et al.[27] did not find clinical correlation in the incidence of thyroid autoantibodies and disease activity. In the present study, antithyroglobulin antibodies were positive in 7.3% (n=3) and antiperoxidase antibodies in 19.5% (n=8) of the patients. Although thyroid antibodies might be elevated in patients with active morphea, no elevation of thyroid autoantibodies was detected in patients with active morphea in our study, which is consistent with the findings of Dańczak-Pazdrowska et al.[27].

Morphea progresses with relapses and remissions and can lead to permanent disfigurement and functional impairment if the disease activity is not controlled[28]. The LoSCAT, which is used to measure disease activity, has been used since 2009, and its validity and reliability have been demonstrated in many studies[6,29]. In this study, changes in mLoSSI, LoSDI, PGA-A, and PGA-D scores of our cases at baseline and final treatment were significant ($p<0.05$). Most of our patients had mild and moderate disease activities, and most patients reached CR and recovered after treatment with mild damage. Literature data on the LoSCAT used in the treatment and follow-up of patients with morphea are summarized in Table 7. The LoSCAT has been found to be safe to use[7,29-40].

Table 3. Distribution of sclerosis according to the subtype of morphea						
	Plaque morphea	**Superficial morphea**	**Generalized morphea**	**Other**	**χ^2**	**p**
	n (%)	**n (%)**	**n (%)**	**n (%)**		
Papillary dermis						
Absent	18 (94.7)	0 (0)	6 (85.7)	4 (80)	3.008	0.332
Present	1 (5.3)	8 (100)	1 (14.3)	1 (20)	23.842	**<0.001**
Reticular dermis						
Absent	1 (5.3)	3 (37.5)	1 (14.3)	3 (60)	8.445	**0.017**
Present	18 (94.7)	5 (62.5)	6 (85.7)	2 (40)	-	-
Hypodermis						
Absent	13 (68.4)	8 (100)	5 (71.4)	4 (80)	3.375	0.351
Present	6 (31.6)	0 (0)	2 (28.6)	1 (20)	-	-
Fisher's exact test						

Table 4. Comparison of ANA positivity and baseline mLoSSI, LoSDI, PGA-A, and PGA-D scores				
	ANA-negative	**ANA-positive***	**Z**	**p**
	(Mean ± SD) (min-max)	**(Mean ± SD) (min-max)**		
Baseline mLoSSI	6.29±6.43 (0-26)	7.5±6.39 (0-23)	-0.565	0.572
Baseline LoSDI	2.76±4.01 (0-16)	2.85±3.53 (0-12)	-0.287	0.774
Baseline PGA-A	50.95±30.32 (0-100)	65±31.37 (0-100)	-1.565	0.118
Baseline PGA-D	24.29±31.55 (0-100)	25±25.85 (0-80)	-0.269	0.788
Mann-Whitney U test. ANA: Antinuclear antibody, mLoSSI: Modified Localized Scleroderma Skin Severity Index, LoSDI: Localized Scleroderma Damage Index, PGA-A: Physician Global Assessment of Disease Activity, PGA-D: Physician Global Assessment of Disease Damage. *Titer of 1:80 or higher, SD: Standard deviation, min.: Minimum, max.: Maximum				

O'Brien et al.[29] followed up patients with morphea over 5 years and emphasized that the change in mLoSSI should be used to assess treatment response. They reported that the LoSDI either remained stable or worsened over time. The sclerosis component of the LoSCAT decreased, whereas the atrophy and subcutaneous atrophy components increased. The mLoSSI is important for assessing short-term response of disease to treatment, but the LoSDI response appears to change over the long term after treatment cessation[29]. Although the LoSDI increased after treatment in our study, with a median follow-up of 10 months, it is too early to evaluate these data. In addition, 19.5% of our patients had superficial morphea. Mosbeh et al.[2] emphasized that superficial morphea responds poorly to both topical and ultraviolet

Table 5. Comparison of thyroid autoantibody levels and baseline mLoSSI, LoSDI, PGA-A, and PGA-D scores

	Thyroid autoantibody levels		Z	p
	Normal	High		
	(Mean ± SD) (min-max)	(Mean ± SD) (min-max)		
Baseline mLoSSI	6.65±6.76 (0-26)	7.6±5.17 (2-15)	-0.811	0.418
Baseline LoSDI	3.26±4.05 (0-16)	1.4±2.12 (0-6)	-1,337	0.181
Baseline PGA-A	54.52±32.02 (0-100)	68±27.81 (30-100)	-1,041	0.298
Baseline PGA-D	28.39±29.79 (0-100)	13±21.63 (0-50)	-1,449	0.147

Mann-Whitney U test. mLoSSI: Modified Localized Scleroderma Skin Severity Index, LoSDI: Localized Scleroderma Damage Index, PGA-A: Physician Global Assessment of Disease Activity, PGA-D: Physician Global Assessment of Disease Damage, SD: Standard deviation, min.: Minimum, max.: Maximum

Table 6. Distribution of histopathologic features according to patients' baseline LoSDI and mLoSSI scores

		Mild*	Moderate*	Severe*	χ^2	p
Epidermal atrophy	Baseline LoSDI	25	1	-	2,758	0.293
	Baseline mLoSSI	11	12	3		
Lymphocytes**	Baseline LoSDI	21	2	-	2,540	0.309
	Baseline mLoSSI	9	10	4		
Plasma cells**	Baseline LoSDI	15	2	-	2,434	0.642
	Baseline mLoSSI	7	7	3		
Eosinophils**	Baseline LoSDI	1	-	-	-	-
	Baseline mLoSSI	-	1	-		
Perivascular lymphoplasmacytic infiltration	Baseline LoSDI	30	2	-	2,540	0.309
	Baseline mLoSSI	12	14	6		
Sclerosis	Baseline LoSDI	34	2	1	2,434	0.642
	Baseline mLoSSI	15	16	6		
Location of sclerosis						
Papillary dermis	Baseline LoSDI	4	1	-	5,000	0.200
	Baseline mLoSSI	1	4	-		
Reticular dermis	Baseline LoSDI	29	1	1	2,991	1,000
	Baseline mLoSSI	14	11	6		
Hypodermis dermis	Baseline LoSDI	8	1	–	2,001	1,000
	Baseline LoSDI	4	4	1		
Hyalinization	Baseline LoSDI	5	-	-	-	-
	Baseline mLoSSI	3	2	-		
Loss of elastic fibers						
Upper reticular dermis	Baseline LoSDI	3	-	-	-	-
	Baseline mLoSSI	1	2	-		
Lower reticular dermis	Baseline LoSDI	6	-	-	-	-
	Baseline LoSDI	2	3	1		
Atrophy of skin appendages	Baseline LoSDI	11	2	-	2,758	0.293
	Baseline mLoSSI	5	5	3		

Fisher's Exact test. mLoSSI: Modified Localized Scleroderma Skin Severity Index, LoSDI: Localized Scleroderma Damage Index. *Mild, moderate, and severe disease activities correspond to the mLoSSI scores of 0-4, 5-12, and ≥13, respectively. *Mild, moderate, and severe disease damage groups correspond to the LoSDI scores of 0-10, 11-15, and ≥16, respectively. **The proportion of lymphocytes, eosinophils, and plasma cells in the inflammatory infiltrate was classified as absent (at x40 magnification, <5 cells) or present (at x40 magnification, >5 cells)

Table 7. A summary of previous studies that used LoSCAT during the treatment and follow-up of patients with morphea*

Author	Study design	Number of patients	Patient population	Morphea subtype	Treatment	Conclusions
Kelsey and Torok[7]	Prospective	29	Pediatrics	Linear, generalized, mixed, plaque, deep	TS, SS, MTX, and MMF	mLoSSI is a sensitive and valid measure of activity in morphea.
Kim et al.[30]	Cohort	113	Adults	Pansclerotic, generalized	TS, phototherapy, MTX, and SS	During the 3-year follow-up of patients with pansclerotic morphea, there was a gradual decrease in LoSAI and PGA-A scores, whereas there was permanent damage related to the initial skin lesions in LoSDI and PGA-D.
Condie et al.[31]	Cohort	302	Adults	Linear, plaque, generalized, and others	Phototherapy, MTX, SS, topical treatments, and other systemic therapies	In patients with active disease pediatric-onset morphea, mLoSSI and PGA-A scores were less severe. In patients previously treated with MTX, LoSDI and PGA-D scores were higher.
Ferguson et al.[32]	Case report	1	Pediatrics	Mixed	Infliximab and leflunomide	Significant improvement was observed in mLoSSI and PGA-A scores among predetermined scores.
Martini et al.[33]	Case report	2	Pediatrics	Pansclerotic	Tocilizumab	A significant decrease in the LoSCAT score was observed.
Fage et al.[34]	Case series	13	Adults	Generalized, linear, deep	Abatacept	During the follow-up of patients with linear or deep type morphea, LoSDI was found to be decreased in some and increased in some.
Noakes[35]	Case series	4	Adults	Plaque, linear, generalized morphea, and limited scleroderma	MTX, HCQ, TS, and topical tranilast	Worsening of LoSDI was observed only in TS-applied regions. A decrease in other LoSCAT subscores was found in TS and tranilast treatment combined with TS.
Agazzi et al.[36]	Cohort	47	Pediatrics	Linear, plaque, generalized, mixed	MTX, MMF, and SS	In patients with juvenile localized scleroderma, variation in mLoSSI scores in different clinical subtypes was consistent. LoSDI was unreliable in damage assessment and follow-up.
Shahidi-Dadras et al.[37]	Prospective	33	Pediatrics, adults	Linear, generalized,	MTX and IVPS	Significant improvement was seen in both LoSSI and LoSDI scores. LoSCAT parameters were not sensitive enough in the En coup de sabre, one of the subtypes of morphea.
Teske and Jacobe[38]	Cohort	120	Pediatrics, adults	Linear, plaque, generalized, indeterminate	Unspecified	Among patients at the lower end of the LoSCAT score range, the ability to detect clinically significant differences is limited.
Kunzler et al.[39]	Cohort	581	Pediatrics, adults	Linear, generalized, plaque, mixed	TC, ILS, topical immunomodulators, topical vitamin D analogs, IVPS, SS, HCQ, MTX, MMF, UVA1, UVB, and PUVA	During the first-year follow-up of patients with linear morphea, the median mLoSSI regressed to 0. At 3-year follow-up, LoSDI scores increased but were not significant.
O'Brien et al.[29]	Cohort	130	Pediatrics, adults	Linear, Generalized, and others	MTX, SS, UVA1 phototherapy, and TS	As a result of a 5-year follow-up of 50 patients, LoSDI and PGA-D were stable. However, improvement in sclerosis and worsening of atrophy contributed to the stability of the LoSDI.
Malewska-Woźniak et al.[40]	Retrospective	31	Adults	Generalized	PUVA, UVA1	Significant reductions in LoSCAT scores were observed using both UVA1 and PUVA therapy.

Table 7. Continued

Author	Study design	Number of patients	Patient population	Morphea subtype	Treatment	Conclusions
Present study	Retrospective	41	Pediatrics, adults	Plaque, superficial, generalized, and others	TS, topical tacrolimus, HCQ, MTX, NB-UVB, SS, and MFF	A decrease in mLoSSI scores and an increase in LoSDI scores were observed in 29 patients.

TS: Topical steroid, SS: Systemic steroid, MTX: Methotrexate, MMF: Mycophenolate mofetil, HCQ, Hydroxychloroquine, IVPS: Intravenous pulse steroids, ILS: Intralesional steroid, UVA1: Ultraviolet A1, UVB: Ultraviolet B, PUVA: Psoralen and ultraviolet, NB-UVB: Narrow-band ultraviolet B. *Alternative treatments (laser treatments, autologous dermal fat graft, physical therapy and surgical treatments etc.) are not included in this table

A1 treatment. The increase in the LoSDI in our patients may be related to the inadequate response of our patients with superficial morphea. Because our patients had mild and moderate activities at baseline, topical agents were preferred in the first-line treatment. In cases that did not respond to topical treatment, hydroxychloroquine was preferred. Kumar et al.[19] investigated the efficacy of hydroxychloroquine, and observes CR and PR in 42.9% and 38.1%, respectively, of the patients, suggesting that it could be an alternative treatment. In our study, 39.1% of the patients taking hydroxychloroquine had CR.

The histopathological results of our study were consistent with those of previous reports. Sclerosis could be observed at all microanatomical levels, from the papillary and superficial reticular dermis to the deep dermis and hypodermis[16]. Similar to Walker et al.[16], we found differences between the microanatomical locations of sclerosis detected in the morphea subtypes. Kim et al.[1] did not find differences between the sclerosis patterns in collagen fibers depending on the clinical subtypes of morphea. Regarding the inflammatory infiltrates, lymphocytes (n=23, 59%) and plasma cells (n=17, 43.6%) were the most common cells[9,18]. The co-existence of these two cell types suggests fibrosis associated with a T-helper 2-mediated immune response[41].

In common practice, the preservation of elastic fibers in morphea compared with the loss of elastic fibers in LSA is used as a distinguishing feature[42]. McNiff et al.[43] reported in their study that elastic fibers in papillary and reticular dermis did not show significant changes in morphea lesions. Walters et al.[44] described the pattern of elastic fibers but did not address the loss of elastic fibers. Mosbeh et al.[2] found that in patients with superficial morphea, elastic fibers in the upper reticular dermis are generally lower than those in the deep reticular dermis. In our study, nine (23.1%) patients had loss of elastic fibers without histopathological findings of LSA. Six of these patients had plaque-type morphea, and the loss of elastic fibers was localized in the reticular dermis. Based on these findings, we hypothesized that changes in elastic fibers may occur in patients with morphea due to the thickening and hyalinization of collagen bundles. However, further studies are needed to confirm this theory.

For histomorphological evaluation of disease severity, these results were compared with the mLoSSI and LoSDI scores at baseline. Similar to the study by Chiu et al.[45], no significant histopathological difference was found between active and inactive morphea. These authors emphasized that moderate and severe inflammation was persistent in 56% of the lesions that appeared clinically inactive[45]. In our study and study by Chiu et al.[45], histomorphological findings were not parallel with disease activity.

Study Limitations

The study is limited by the small number of patients and the lack of a control group. The correlation between clinical and histopathological features of morphea will be better understood by further studies with larger cohorts.

Conclusion

Although the mLoSSI, LoSDI, PGA-A, and PGA-D scores can be successfully used for the follow-up and treatment of patients with morphea, no correlation was found between positive ANA, high levels of thyroid autoantibodies, and histopathological features.

Ethics

Ethics Committee Approval: The study was approved by Local Ethics Committee of Trakya University Faculty of Medicine (approval number: 07/07, date: 15.03.2021).

Informed Consent: It was obtained.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: Y.G.Ü., E.U.K., Design: Y.G.Ü., E.U.K., Data Collection or Processing: Y.G.Ü., E.U.K., Analysis or Interpretation: Y.G.Ü., E.U.K., Literature Search: Y.G.Ü., E.U.K., Writing: Y.G.Ü., E.U.K.

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