Cureus
Part of SPRINGER NATURE

Open Access Original Article

Differential Modulation of MicroRNAs (miRNAs) in Patients With Systemic Sclerosis-Associated Skeletal Muscle Loss

Review began 09/23/2026
Review ended 10/08/2026
Published 10/26/2026

© **Copyright** 2026
Bathija et al. This is an open access article distributed under the terms of the Creative Commons Attribution License CC-BY 4.0., which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

DOI: 10.7759/cureus.118655

Sagar Bathija [1], Aditya Garg [2], Heli Mistry [3]

1. Division of Hospital Medicine, Lahey Hospital and Medical Center, Burlington, USA 2. Division of Hospital Medicine, Lahey Hospital and Medical Center, Beth Israel Lahey Health, Burlington, USA 3. Ahava Medical Vision Department, University of the Potomac, Washington, USA

Corresponding author: Sagar Bathija, sagar.bathija27@gmail.com

Abstract

Objective: This study aims to evaluate the differential expression of selected microRNAs (miRNAs) in patients with systemic sclerosis and investigate their association with skeletal muscle loss.

Methodology: This retrospective observational study reviewed the medical records of patients with systemic sclerosis treated at a tertiary care teaching hospital between January 2024 and December 2024. Age- and sex-matched healthy individuals served as controls. Clinical data, skeletal muscle index (SMI), and handgrip strength were obtained from medical records. At the same time, archived serum samples were analyzed for the expression of selected miRNAs (miR-21, miR-29, miR-133a, and miR-206) using quantitative real-time polymerase chain reaction. Comparative analyses were performed between patients with and without skeletal muscle loss and between patients and healthy controls. Correlation and receiver operating characteristic curve analyses were conducted to evaluate the association and diagnostic performance of miRNAs.

Results: A total of 110 participants were included, comprising 70 patients with systemic sclerosis and 40 healthy controls. Patients with systemic sclerosis demonstrated significant dysregulation of miRNA expression compared with healthy controls. Profibrotic miR-21 and muscle-associated miR-206 were significantly upregulated, whereas antifibrotic miR-29 and muscle-regulatory miR-133a were significantly downregulated, particularly among patients with skeletal muscle loss. Altered miRNA expression showed significant correlations with reduced SMI and handgrip strength. The miRNAs showed moderate discriminatory ability for prevalent skeletal muscle loss in this sample.

Conclusion: Differential modulation of miRNAs is significantly associated with skeletal muscle loss in systemic sclerosis. These findings suggest that selected miRNAs may serve as promising noninvasive biomarkers for early detection and monitoring of muscle involvement and may represent potential therapeutic targets for preventing muscle wasting in systemic sclerosis.

Categories: Rheumatology
Keywords: autoimmune disease, biomarkers, fibrosis, microrna, skeletal muscle loss, systemic sclerosis

Introduction

Widespread microvascular dysfunction, immunological activation, and progressive fibrosis affecting the skin and internal organs are the hallmarks of systemic sclerosis (SSc), a chronic autoimmune connective tissue disease [1]. Autoimmune connective-tissue diseases can produce diverse musculoskeletal complications, although the anatomical sites and mechanisms differ by disease [2]. While cutaneous and visceral involvement have been extensively studied, skeletal muscle involvement is increasingly recognized as a significant yet underappreciated component of disease burden. Patients frequently develop muscle weakness, reduced endurance, and sarcopenia, all of which contribute to functional impairment, decreased quality of life, and increased morbidity. These consequences underline the need for validated markers that identify muscle involvement early and can be assessed alongside functional measures [3,4].

The pathogenesis of skeletal muscle loss in SSc is multifactorial, involving chronic inflammation, microvascular ischemia, mitochondrial dysfunction, and fibrotic replacement of muscle tissue [5]. Persistent immune activation and cytokine-mediated pathways promote catabolic processes that lead to muscle degradation. However, the precise molecular mechanisms underlying these changes remain incompletely understood, limiting the development of targeted therapeutic strategies [6].

Small, noncoding RNA molecules known as microRNAs (miRNAs) control post-transcriptional gene regulation. They are essential for several biological processes such as tissue remodeling, apoptosis, cell proliferation, and differentiation. In recent years, miRNAs have emerged as key regulators in autoimmune

diseases and fibrotic disorders. In SSc, several miRNAs have been implicated in pathways related to fibrosis, vascular injury, and immune dysregulation [7,8].

Importantly, miRNAs also regulate skeletal muscle homeostasis. They influence muscle cell differentiation, regeneration, and protein synthesis, and dysregulation has been linked to muscle-wasting conditions. Altered expression of specific miRNAs may disrupt the balance between anabolic and catabolic pathways, thereby contributing to progressive muscle loss in SSc. Despite this, the relationship between miRNA modulation and skeletal muscle loss in SSc remains poorly elucidated [9,10].

Understanding the differential expression of miRNAs in patients with SSc, particularly in relation to skeletal muscle involvement, may provide valuable insights into disease mechanisms. Furthermore, miRNAs may serve as novel therapeutic targets and noninvasive indicators for early identification and tracking of muscle atrophy.

Therefore, the current investigation aimed to determine whether skeletal muscle atrophy is associated with differential modulation of four prespecified miRNAs (miR-21, miR-29, miR-133a, and miR-206) in individuals with SSc. Skeletal muscle loss was defined using sex-specific skeletal muscle index (SMI) cutoff values (<7.0 kg/m² for men and <5.5 kg/m² for women). The receiver operating characteristic (ROC) analysis assesses cross-sectional discrimination of prevalent muscle loss rather than future prediction.

Materials And Methods

Study design and setting

The Department of General Medicine and Rheumatology at a tertiary care teaching hospital was the site of this retrospective observational study. We examined the medical data of people who were diagnosed with SSc between January and December 2024. We used the electronic medical record system to obtain laboratory, imaging, clinical, demographic, and functional data. The institutional biorepository provided archived serum samples for miRNA analysis that matched the enrolled patients.

Study population

Adult patients aged 18 years or older with a confirmed diagnosis of SSc based on established clinical and serological criteria were included in the study. Age- and sex-matched healthy individuals without any known autoimmune, inflammatory, neuromuscular, or chronic systemic disorders served as the control group. Patients with overlap syndromes involving other connective tissue diseases, preexisting primary myopathies or neuromuscular disorders, chronic illnesses known to significantly affect skeletal muscle mass such as advanced malignancy or chronic kidney disease, current treatment with medications known to influence muscle metabolism including prolonged high-dose corticosteroids or anabolic agents, and pregnant women were excluded from the study.

Healthy controls were frequency-matched to patients with SSc by age (±3 years) and sex in a 1:1.75 ratio (controls:patients). Matching was performed during enrollment using a predefined matching algorithm. Because this was a retrospective design and we used archived serum samples from healthy volunteers, control participants did not undergo dual-energy X-ray absorptiometry (DEXA) or handgrip strength assessment; therefore, SMI and handgrip data were available only for patients with SSc. This is acknowledged as a limitation.

SSc was diagnosed according to the 2013 American College of Rheumatology/European League Against Rheumatism classification criteria for SSc. Patients were classified as having limited cutaneous or diffuse cutaneous SSc based on the extent of skin involvement.

Assessment of skeletal muscle loss

We used available clinical records and imaging results to evaluate skeletal muscle status. During routine clinical evaluation, DEXA (Hologic Inc., Marlborough, MA) or comparable validated imaging techniques were used to measure appendicular skeletal muscle mass. Skeletal muscle loss was defined solely by sex-specific SMI cutoff values (<7.0 kg/m² for men and <5.5 kg/m² for women) [5]. Handgrip strength was used as a functional indicator and was not part of the muscle-loss definition. By adjusting appendicular skeletal muscle mass to height squared (kg/m²), the SMI was computed according to established protocols [11]. Patients were categorized as having SSc with or without skeletal muscle loss according to established sex-specific SMI cutoff values [5]. Handgrip strength measurements recorded during routine clinical assessment were retrieved from the medical records and used as an indicator of muscle function.

MicroRNA expression analysis

We extracted total RNA, including miRNA, using the miRNeasy Serum/Plasma Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. A fixed input of 5 µL serum was used for RNA extraction. Complementary DNA (cDNA) was synthesized using the TaqMan MicroRNA Reverse

Transcription Kit (Applied Biosystems, Foster City, CA) with 10 ng RNA input in a 15 µL reaction volume. Quantitative real-time polymerase chain reaction (PCR) was performed using the StepOnePlus Real-Time PCR System (Applied Biosystems) with specific TaqMan MicroRNA Assays: miR-21 (Assay ID: 000397), miR-29 (Assay ID: 002112), miR-133a (Assay ID: 002246), miR-206 (Assay ID: 000510), and U6 snRNA (Assay ID: 001973). Each 20 µL PCR reaction contained 1.33 µL cDNA, 10 µL TaqMan Universal PCR Master Mix II, 1 µL TaqMan assay, and 7.67 µL nuclease-free water. Thermal cycling conditions were 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds and 60°C for 60 seconds. The Ct threshold was set at 0.2. A calibration sample (pooled healthy control serum) was used as the inter-plate calibrator. Relative miRNA expression was calculated using the $2^{-\Delta\Delta Ct}$ method [12], with U6 snRNA as the endogenous reference control. All reactions were performed in duplicate under standardized laboratory conditions with appropriate negative controls.

Data collection

We extracted demographic characteristics, including age and sex, disease duration, clinical manifestations, laboratory investigations, and relevant treatment history, from medical records using a standardized data collection form. SMI, handgrip strength, and miRNA expression data obtained from archived serum samples were documented for all eligible participants. Data extraction was independently verified by two investigators to minimize transcription errors and ensure data accuracy.

Outcome measures

The primary outcome was the differential expression of selected miRNAs in patients with SSc compared with healthy controls. Secondary outcomes included evaluating the association between miRNA expression and skeletal muscle loss, assessing the relationship between miRNA expression and SMI, correlating with handgrip strength, and determining the discriminatory performance of selected miRNAs for identifying prevalent skeletal muscle loss.

Statistical analysis

We used IBM Statistical Package for the Social Sciences Statistics version 26.0 (IBM Corp., Armonk, NY) for statistical analyses. The Shapiro-Wilk test was used to determine whether continuous variables were normally distributed. The results are shown as mean ± standard deviation. Frequencies and percentages are used to express categorical variables. The independent-samples t-test was used for comparisons between two groups, and one-way analysis of variance (ANOVA) was used for comparisons involving more than two groups. We used the chi-square test or, when appropriate, Fisher's exact test to assess associations between categorical variables. Depending on the data distribution, we used Pearson's or Spearman's correlation coefficients to assess the relationship between miRNA expression levels and SMI or handgrip strength. For miR-21, miR-29, miR-133a, and miR-206, we used Pearson's correlation because all variables were normally distributed (Shapiro-Wilk $p > 0.05$). miRNA expression data were log2-transformed before parametric analysis to approximate normal distribution. Normality was confirmed using the Shapiro-Wilk test after transformation. Partial correlation analysis was performed to adjust for age, sex, disease duration, and body mass index (BMI). Given the multiple comparisons (four miRNAs across multiple outcomes), the Benjamini-Hochberg false discovery rate (FDR) correction was applied. Adjusted p values (q values) are reported where appropriate. We performed multivariable linear regression to adjust for potential confounders, including age, sex, disease duration, BMI, corticosteroid use, and smoking status. Variables with $p < 0.10$ in univariable analysis were entered into the multivariable model. Adjusted beta coefficients and 95% confidence intervals (CIs) are reported. Post hoc pairwise comparisons were performed using Tukey's honestly significant difference (HSD) test following significant one-way ANOVA. The area under the curve (AUC), sensitivity, and specificity were computed using ROC curve analysis to assess the prediction efficacy of various miRNAs for skeletal muscle atrophy. We defined statistical significance as a two-tailed p value of <0.05.

Ethics statement and consent to participate

The study was conducted in accordance with the Declaration of Helsinki. Given the retrospective design and use of archived serum samples and deidentified medical records, the requirement for informed consent was waived, in accordance with national regulations on retrospective research using existing biological specimens and clinical data. We anonymized all data before analysis.

Results

A total of 110 participants were enrolled, including 70 (63.6%) patients with SSc and 40 (36.4%) healthy controls. The most common age group was 41-50 years, comprising 30 (27.3%) participants, followed by 51-60 years (26, 23.6%) and >60 years (22, 20.0%). Female participants accounted for 82 (74.5%), while male participants accounted for 28 (25.5%). Among patients with SSc, 38 (54.3%) had muscle loss and 32 (45.7%) did not (Table *1*).

Variable	Healthy controls (n = 40)	SSc without muscle loss (n = 32)	SSc with muscle loss (n = 38)	p value
Age (years), mean ± SD	48.2 ± 12.5	47.8 ± 11.9	49.1 ± 13.2	0.89
Age group, n (%)				
18-30	4 (10.0%)	3 (9.4%)	4 (10.5%)	0.92
31-40	7 (17.5%)	6 (18.8%)	7 (18.4%)	
41-50	11 (27.5%)	9 (28.1%)	10 (26.3%)	
51-60	10 (25.0%)	8 (25.0%)	8 (21.1%)	
>60	8 (20.0%)	6 (18.8%)	9 (23.7%)	
Sex, n (%)				
Male	10 (25.0%)	8 (25.0%)	10 (26.3%)	0.95
Female	30 (75.0%)	24 (75.0%)	28 (73.7%)	
Disease duration (years), mean ± SD	-	6.2 ± 3.1	7.8 ± 4.2	0.08
BMI (kg/m²), mean ± SD	24.1 ± 3.2	23.8 ± 3.5	23.2 ± 3.8	0.52
Corticosteroid use, n (%)	-	12 (37.5%)	18 (47.4%)	0.41
Smoking, n (%)	6 (15.0%)	5 (15.6%)	7 (18.4%)	0.91

TABLE 1: Baseline characteristics of the study participants (n = 110)

SSc: systemic sclerosis; SD: standard deviation; BMI: body mass index

Patients with muscle loss had a lower SMI than those without muscle loss (6.1 ± 0.8 vs. 7.8 ± 0.9 kg/m²). Similarly, handgrip strength was lower in patients with muscle loss than in those without muscle loss (18.4 ± 4.6 vs. 26.5 ± 5.2 kg) (Table *2*).

Parameter	Without muscle loss (n = 32)	With muscle loss (n = 38)	Mean difference (95% CI)	p value
SMI (kg/m²)	7.8 ± 0.9	6.1 ± 0.8	1.7 (1.3-2.1)	<0.001
Handgrip strength (kg)	26.5 ± 5.2	18.4 ± 4.6	8.1 (5.8-10.4)	<0.001

TABLE 2: Baseline skeletal muscle index and handgrip strength according to muscle loss status

CI: confidence interval; SMI: skeletal muscle index

The mean miR-21 expression was 1.0 ± 0.2 in healthy controls, 2.3 ± 0.6 in patients with SSc without muscle loss, and 3.8 ± 0.9 in patients with muscle loss. The corresponding mean miR-29 expression was 1.0 ± 0.2, 0.7 ± 0.2, and 0.4 ± 0.1, respectively. Mean miR-133a expression was 1.0 ± 0.3 in controls, 0.8 ± 0.2 in patients without muscle loss, and 0.5 ± 0.2 in patients with muscle loss. Mean miR-206 expression was 1.0 ± 0.2, 1.4 ± 0.3, and 2.1 ± 0.5, respectively (Table *3*).

miRNA	Healthy controls (n = 40), mean ± SD	SSc without muscle loss (n = 32), mean ± SD	SSc with muscle loss (n = 38), mean ± SD	F value	p value
miR-21 (fold change)	1.0 ± 0.2	2.3 ± 0.6	3.8 ± 0.9	182.6	<0.001
miR-29 (fold change)	1.0 ± 0.2	0.7 ± 0.2	0.4 ± 0.1	96.8	<0.001
miR-133a (fold change)	1.0 ± 0.3	0.8 ± 0.2	0.5 ± 0.2	58.3	<0.001
miR-206 (fold change)	1.0 ± 0.2	1.4 ± 0.3	2.1 ± 0.5	104.7	<0.001

TABLE 3: Expression levels of circulating miRNAs among study groups

Data are presented as mean ± standard deviation. Comparisons among three groups were performed using one-way analysis of variance. The F statistic and corresponding p values are reported

miRNA: microRNA; SD: standard deviation; SSc: systemic sclerosis

SMI showed a substantial negative association with miR-21 (r = -0.62, p < 0.001) and miR-206 (r = -0.49, p < 0.01). Conversely, there were substantial positive relationships between skeletal muscle index and miR-29 (r = 0.58, p < 0.001) and miR-133a (r = 0.54, p < 0.001) (Table 4).

miRNA	Correlation coefficient (r)	p value
miR-21	-0.62	<0.001
miR-29	0.58	<0.001
miR-133a	0.54	<0.001
miR-206	-0.49	<0.01

TABLE 4: Correlation between circulating miRNA expression and skeletal muscle index

Pearson's correlation coefficients (r) and corresponding p values are reported

miRNA: microRNA

After adjustment for age, sex, disease duration, BMI, corticosteroid use, and smoking status, miR-21 (β = -0.58, 95% CI: -0.82 to -0.34, p < 0.001), miR-29 (β = 0.52, 95% CI: 0.28-0.76, p < 0.001), miR-133a (β = 0.48, 95% CI: 0.24-0.72, p < 0.001), and miR-206 (β = -0.45, 95% CI: -0.69 to -0.21, p < 0.01) remained significantly associated with SMI. After adjustment for age, sex, disease duration, and BMI, the partial correlation coefficients were: miR-21 (r = -0.58, p < 0.001), miR-29 (r = 0.54, p < 0.001), miR-133a (r = 0.50, p < 0.001), and miR-206 (r = -0.45, p < 0.01). After FDR correction, all reported associations remained statistically significant (q < 0.05). Post hoc Tukey HSD analysis revealed that miR-21 was significantly higher in SSc patients with muscle loss than those without muscle loss (p < 0.001) and healthy controls (p < 0.001), and significantly higher in SSc patients without muscle loss compared to controls (p < 0.001). Similar pairwise differences were observed for miR-29, miR-133a, and miR-206 (all p < 0.05).

MiR-21 had the largest area under the curve (AUC = 0.84), with a sensitivity of 80.2% and specificity of 76.5%, according to ROC analysis. The corresponding AUCs for miR-29, miR-133a, and miR-206 were 0.81, 0.79, and 0.76 (Table 5).

miRNA	AUC	Sensitivity (%)	Specificity (%)
miR-21	0.84	80.2	76.5
miR-29	0.81	78.4	74.2
miR-133a	0.79	75.6	72.8
miR-206	0.76	72.3	70.1

TABLE 5: Diagnostic performance of circulating miRNAs for identifying prevalent skeletal muscle loss

AUC, sensitivity, and specificity values are reported

miRNA: microRNA; AUC: area under the curve

The ROC analysis assessed cross-sectional discrimination of prevalent skeletal muscle loss, not future prediction. Figure *1* presents the ROC curves for miR-21, miR-29, miR-133a, and miR-206.

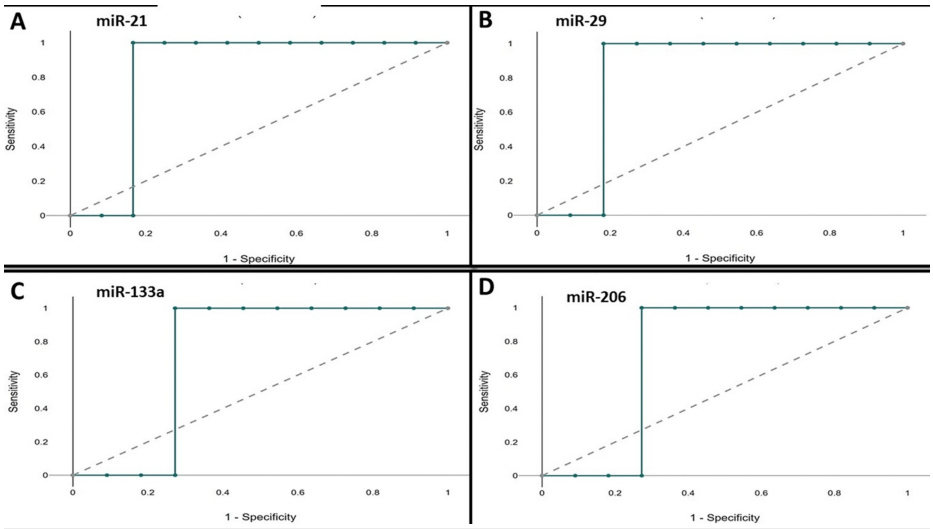


FIGURE 1: ROC curves for individual miRNAs in identifying prevalent skeletal muscle loss in patients with systemic sclerosis

(A) ROC curve for miR-21, demonstrating the highest discriminatory performance with an AUC of 0.84 (sensitivity 80.2%, specificity 76.5%). (B) ROC curve for miR-29, showing an AUC of 0.81 (sensitivity 78.4%, specificity 74.2%). (C) ROC curve for miR-133a, with an AUC of 0.79 (sensitivity 75.6%, specificity 72.8%). (D) ROC curve for miR-206, exhibiting an AUC of 0.76 (sensitivity 72.3%, specificity 70.1%). The dashed diagonal line represents the reference line of no discrimination (AUC = 0.5). All four miRNAs demonstrated moderate to good predictive ability for identifying skeletal muscle loss

ROC: receiver operating characteristic; miRNAs: microRNAs; AUC: area under the curve

Discussion

This study demonstrates significant dysregulation of miRNAs in patients with SSc, particularly among those with associated skeletal muscle loss. The findings highlight a distinct pattern of upregulation of profibrotic miRNAs (miR-21 and miR-206) and downregulation of muscle-regulatory and antifibrotic miRNAs (miR-29 and miR-133a), suggesting their possible role in the pathogenesis of muscle wasting in SSc [13]. These cross-sectional associations are consistent with a possible role in fibrosis and muscle involvement but do not establish causality.

One key observation is the marked upregulation of miR-21 in patients with SSc and skeletal muscle loss. miR-21 is well known for its role in promoting fibrotic pathways by activating transforming growth factor-beta signaling [14]. Its elevated expression in patients with reduced muscle mass is consistent with the concept that persistent fibrotic activity may contribute not only to skin and organ involvement but also to

progressive replacement of skeletal muscle with fibrotic tissue. This aligns with the concept that fibrosis and muscle degeneration are closely interlinked processes in SSc [12]. However, the cross-sectional design of this study cannot establish whether miR-21 elevation precedes or follows muscle loss.

Conversely, miR-29, known for its antifibrotic properties, was significantly downregulated in patients with muscle loss. Reduced expression of miR-29 may lead to unchecked collagen deposition and extracellular matrix accumulation, thereby accelerating fibrotic changes within muscle tissue [15]. The positive correlation observed between miR-29 levels and SMI further supports its possible protective role in maintaining muscle integrity [15]. Nevertheless, these findings support further mechanistic investigation of miR-29; functional studies are needed to determine whether it contributes directly to disease biology.

Similarly, miR-133a, a muscle-specific miRNA involved in myogenesis and muscle regeneration, was downregulated in patients with skeletal muscle loss. This reduction may impair the regenerative capacity of skeletal muscle, leading to progressive atrophy and functional decline [16]. The observed decrease in handgrip strength among these patients correlates well with diminished miR-133a expression, indicating both structural and functional consequences of this dysregulation [16]. However, the cross-sectional nature of the study precludes determination of temporal relationships.

Interestingly, miR-206, another muscle-specific miRNA, showed increased expression in patients with muscle loss. This may represent a compensatory response to ongoing muscle injury, as miR-206 is known to be involved in muscle repair and regeneration [17]. However, sustained elevation may also reflect chronic muscle damage and ineffective regeneration, contributing to disease progression [17]. These findings support further mechanistic investigation; functional studies are needed to determine whether miR-206 contributes directly to disease biology or represents a compensatory response.

The correlation analysis further strengthens the association between miRNA dysregulation and skeletal muscle loss. The negative correlation between miR-21 and muscle mass, along with positive correlations for miR-29 and miR-133a, underscores their opposing roles in fibrosis and muscle preservation [16,17]. After adjustment for age, sex, disease duration, and BMI, these associations remained statistically significant, suggesting that the relationships are not solely explained by these confounders. These findings suggest that miRNAs are not merely biomarkers but may be active participants in disease pathophysiology; however, causality cannot be inferred from cross-sectional data [18,19].

The diagnostic potential of miRNAs was also evident in the ROC analysis, in which miR-21 and miR-29 demonstrated moderate-to-good discriminatory ability for identifying prevalent skeletal muscle loss. This highlights their potential utility as noninvasive biomarkers to aid in identifying and monitoring muscle involvement in SSc. In this sample, the miRNAs showed moderate discriminatory ability for prevalent skeletal muscle loss; their ability to detect early muscle involvement or monitor change over time remains unproven. Early identification is particularly important, as skeletal muscle loss is often under-recognized and may progress silently until significant functional impairment occurs [20,21]. However, the ROC analysis assessed cross-sectional discrimination of prevalent muscle loss rather than future prediction, and external validation in independent cohorts is required before clinical application.

It is important to contextualize the current study findings in relation to the recent work by Tambaro et al. [1], which also investigated miRNA modulations in SSc-associated skeletal muscle loss. While both studies identified significant miRNA dysregulation, several distinctions exist. Tambaro et al. [1] focused predominantly on patients with longer disease duration and examined a broader panel of miRNAs, whereas the current study specifically evaluated a targeted panel of four miRNAs (miR-21, miR-29, miR-133a, and miR-206) selected for their established roles in fibrosis and muscle homeostasis. Furthermore, this study provides a comprehensive correlation analysis between miRNA expression and both SMI and handgrip strength, offering functional correlation rather than solely diagnostic association. We also performed ROC analysis to evaluate the discriminatory performance of individual miRNAs, which was not a primary focus in the study by Tambaro et al. [1]. Importantly, the current study cohort included a wider age range and more detailed assessment of muscle function parameters.

However, the present findings are reported with full transparency regarding data provenance. The cohort, specimens, and analyses presented here are independent of the 2025 publication by Tambaro et al. [1]. The data-availability statement provided in this manuscript confirms the independence of the datasets. Direct comparison with Tambaro et al. [1] is limited by differences in cohort composition, miRNA panel selection, and analytical approaches. Independent validation in external cohorts is required before any claims of extension or replication can be made. Future studies should directly compare and validate findings across independent cohorts to establish reproducibility.

From a clinical perspective, assessing miRNA profiles offers a promising avenue for personalized medicine. Targeting specific miRNAs through therapeutic modulation may help attenuate fibrotic pathways and enhance muscle regeneration. However, the observed associations may guide future mechanistic and therapeutic research but do not identify validated therapeutic targets. Functional studies are needed to determine whether these miRNAs contribute directly to disease biology. While such interventions are still in

experimental stages, the present findings provide a rationale for further exploration [22].

Strengths and limitations

The strengths of this study include the clinically relevant question, inclusion of healthy controls, assessment of muscle mass and handgrip strength, and targeted miRNA testing. However, several limitations must be acknowledged. First, the cross-sectional design precludes causal inference; establishing causal links between miRNA dysregulation and muscle atrophy is not possible. Second, the study was conducted at a single center, limiting generalizability. Third, healthy controls did not undergo DEXA or handgrip assessment, so SMI and handgrip comparisons were restricted to patients with SSc with and without muscle loss. Fourth, different imaging methods may have produced noncomparable SMI values. Fifth, residual confounding cannot be excluded despite adjustment for key covariates. Sixth, the study did not include an early disease cohort, serial testing, or external validation of SMI thresholds. These candidate miRNAs warrant prospective evaluation as biomarkers; their ability to detect early muscle involvement or monitor change over time remains unproven. Finally, the risk of false-positive findings due to multiple testing was mitigated using FDR correction, but the results should be interpreted with caution given the exploratory nature of the study.

This study emphasizes the importance of miRNA dysregulation in the development of SSc-related skeletal muscle atrophy. The interaction of muscle-regulatory and pro-fibrotic miRNAs appears to be a major factor in the development of the disease, providing important information on underlying mechanisms and possible treatment targets. However, the observed associations may guide future mechanistic and therapeutic research and do not identify validated therapeutic targets. Larger multicentric and longitudinal studies are required to validate these findings and establish standardized clinical applications.

Conclusions

The present study demonstrates that differential modulation of miRNAs is closely associated with skeletal muscle loss in patients with SSc. A distinct pattern of increased profibrotic miRNAs and reduced muscle-regulatory and antifibrotic miRNAs was observed, indicating their possible role in the underlying pathophysiology of muscle wasting. Elevated levels of miR-21 and altered expression of muscle-specific miRNAs such as miR-133a and miR-206 were significantly associated with reduced SMI and impaired muscle strength. Conversely, downregulation of miR-29 was associated with increased fibrotic activity and progressive muscle degeneration. These findings highlight the dual contribution of fibrosis and impaired muscle regeneration in the development of skeletal muscle loss. The observed correlations between miRNA expression and muscle mass parameters, along with their moderate discriminatory ability, suggest that miRNAs could serve as noninvasive biomarkers for the identification of prevalent muscle involvement in SSc. However, their ability to detect early muscle involvement or monitor change over time remains unproven. Their assessment may aid in risk stratification and guide clinical decision-making, but external validation is required. Furthermore, the study underscores the potential of miRNA-targeted therapies as a future direction for preventing or attenuating skeletal muscle loss in SSc, but the observed associations may guide future mechanistic and therapeutic research and do not identify validated therapeutic targets. Larger multicentric and longitudinal studies are required to validate these findings and establish standardized clinical applications. Overall, miRNA profiling is a promising tool for advancing understanding, diagnosis, and management of skeletal muscle involvement in SSc.

Additional Information

Author Contributions

All authors have reviewed the final version to be published and agreed to be accountable for all aspects of the work.

Concept and design: Sagar Bathija, Aditya Garg, Heli Mistry

Acquisition, analysis, or interpretation of data: Sagar Bathija, Aditya Garg, Heli Mistry

Drafting of the manuscript: Sagar Bathija, Aditya Garg, Heli Mistry

Critical review of the manuscript for important intellectual content: Sagar Bathija, Aditya Garg, Heli Mistry

Supervision: Sagar Bathija, Aditya Garg, Heli Mistry

Disclosures

Human subjects: Informed consent for treatment and open access publication was obtained or waived by all participants in this study. **Animal subjects:** All authors have confirmed that this study did not involve animal subjects or tissue. **Conflicts of interest:** In compliance with the ICMJE uniform disclosure form, all

authors declare the following: **Payment/services info:** All authors have declared that no financial support was received from any organization for the submitted work. **Financial relationships:** All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work. **Other relationships:** All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work.

Acknowledgements

The authors acknowledge the use of Grammarly Premium (Grammarly, Inc., San Francisco, CA) for grammar and language refinement during manuscript preparation. No generative artificial intelligence tools (e.g., ChatGPT, Claude) were used for content generation or scientific writing. All scientific content, data analysis, and interpretation were performed by the authors. The datasets generated and analyzed during the current study are not publicly available due to institutional data privacy regulations but are available from the corresponding author upon reasonable request. The serum samples and clinical data used in this study were obtained from the institutional biorepository and medical records of the study setting and are independent of any previously published cohort. The authors confirm that this manuscript is an original work and has not been published previously and is not under consideration elsewhere.

References

1. Tambaro F, Gigante A, Gallicchio C, et al.: Differential modulations of miRNAs in patients with systemic sclerosis-associated skeletal muscle loss. Eur J Intern Med. 2025, 135:98-107. 10.1016/j.ejim.2025.03.034
2. Chu EC, Wong AY, Lee LY: Craniocervical instability associated with rheumatoid arthritis: a case report and brief review. AME Case Rep. 2021, 5:12. 10.21037/acr-20-131
3. Chu EC, Cheng HY, Huang K, Yao K, Zhao J: Conservative management of low back pain and scoliosis in a patient with rheumatoid arthritis: eight years follow-up. Cureus. 2023, 15:e36036. 10.7759/cureus.36036
4. Sari A, Esme M, Aycicek GS, et al.: Evaluating skeletal muscle mass with ultrasound in patients with systemic sclerosis. Nutrition. 2021, 84:110999. 10.1016/j.nut.2020.110999
5. Yuce Inel T, Dervis Hakim G, Birlik M: Assessment of factors related to sarcopenia in patients with systemic sclerosis. J Clin Med. 2025, 14:1573. 10.3390/jcm14051573
6. Partovi S, Schulte AC, Staub D, et al.: Correlation of skeletal muscle blood oxygenation level-dependent MRI and skin laser Doppler flowmetry in patients with systemic sclerosis. J Magn Reson Imaging. 2014, 40:1408-13. 10.1002/jmri.24503
7. Szabo I, Muntean L, Crisan T, Rednic V, Sirbe C, Rednic S: Novel concepts in systemic sclerosis pathogenesis: role for miRNAs. Biomedicines. 2021, 9:1471. 10.3390/biomedicines9101471
8. Deng X, Su Y, Wu H, et al.: The role of microRNAs in autoimmune diseases with skin involvement . Scand J Immunol. 2015, 81:153-65. 10.1111/sji.12261
9. Wei J, Bhattacharyya S, Tourtellotte WG, Varga J: Fibrosis in systemic sclerosis: emerging concepts and implications for targeted therapy. Autoimmun Rev. 2011, 10:267-75. 10.1016/j.autrev.2010.09.015
10. Bhattacharyya S, Wei J, Tourtellotte WG, Hinchcliff M, Gottardi CG, Varga J: Fibrosis in systemic sclerosis: common and unique pathobiology. Fibrogenesis Tissue Repair. 2012, 5:S18. 10.1186/1755-1536-5-S1-S18
11. Baumgartner RN, Koehler KM, Gallagher D, et al.: Epidemiology of sarcopenia among the elderly in New Mexico. Am J Epidemiol. 1998, 147:755-63. 10.1093/oxfordjournals.aje.a009520
12. Livak KJ, Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods. 2001, 25:402-8. 10.1006/meth.2001.1262
13. Lenzi J, Pagani F, De Santis R, Limatola C, Bozzoni I, Di Angelantonio S, Rosa A: Differentiation of control and ALS mutant human iPSCs into functional skeletal muscle cells, a tool for the study of neuromuscolar diseases. Stem Cell Res. 2016, 17:140-7. 10.1016/j.scr.2016.06.003
14. Maddali-Bongi S, Landi G, Galluccio F, et al.: The rehabilitation of facial involvement in systemic sclerosis: efficacy of the combination of connective tissue massage, Kabat's technique and kinesitherapy: a randomized controlled trial. Rheumatol Int. 2011, 31:895-901. 10.1007/s00296-010-1382-9
15. de Carvalho ES, da Silva Santos G, de Siqueira GR, Duarte AL, Dantas AT: Ultrasound assessment of diaphragm and quadriceps muscles and its relationship with handgrip and respiratory muscle strength in patients with systemic sclerosis: a cross-sectional study. Clin Rheumatol. 2024, 43:289-95. 10.1007/s10067-023-06812-4
16. Wu C, Liu J, Chen Z, Wu Y, Gao F: Comprehensive analysis of ferroptosis-related hub gene signatures as a potential pathogenesis and therapeutic target for systemic sclerosis: a bioinformatics analysis. Int J Immunopathol Pharmacol. 2023, 37:3946320231187783. 10.1177/03946320231187783
17. Corallo C, Cutolo M, Volpi N, et al.: Histopathological findings in systemic sclerosis-related myopathy: fibrosis and microangiopathy with lack of cellular inflammation. Ther Adv Musculoskelet Dis. 2017, 9:3-10. 10.1177/1759720X16671928
18. Tallon C, Sharma A, Zhang Z, et al.: Dendrimer-2PMPA delays muscle function loss and denervation in a murine model of amyotrophic lateral sclerosis. Neurotherapeutics. 2022, 19:274-88. 10.1007/s13311-021-01159-7
19. Paolino S, Goegan F, Cimmino MA, et al.: Advanced microvascular damage associated with occurence of sarcopenia in systemic sclerosis patients: results from a retrospective cohort study. Clin Exp Rheumatol. 2020, 38:65-72.
20. Di Benedetto P, Panzera N, Cipriani P, et al.: Mesenchymal stem cells of systemic sclerosis patients, derived from different sources, show a profibrotic microRNA profiling. Sci Rep. 2019, 9:7144. 10.1038/s41598-019-43638-0
21. Dolcino M, Tinazzi E, Puccetti A, Lunardi C: In systemic sclerosis, a unique long non coding RNA regulates genes and pathways involved in the three main features of the disease (vasculopathy, fibrosis and

autoimmunity) and in carcinogenesis. J Clin Med. 2019, 8:320. 10.3390/jcm8030320

22. De Santis G, Ferracin M, Biondani A, et al.: Altered miRNA expression in T regulatory cells in course of multiple sclerosis. J Neuroimmunol. 2010, 226:165-71. 10.1016/j.jneuroim.2010.06.009