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We aimed at analyzing the serum levels of citrullinated histone H3 (CitH3) in patients with dermatomyositis (DM) and their association with disease activity.

Serum CitH3 levels were measured using enzyme-linked immunosorbent assays in serum samples obtained from 93 DM patients and 56 healthy controls (HCs). Receiver operating characteristic (ROC) curve analysis was performed to evaluate the discriminant capacity of CitH3 and other disease variables. The association between CitH3 and disease variables was analyzed using Pearson's rank correlation.

Serum CitH3 level was significantly lower in DM patients than in HCs ($p < 0.001$). The ROC curve analysis revealed that CitH3 strongly discriminated DM patients from HCs (area under the curve [AUC], 0.86), and a combination of CitH3 and the ratio of neutrophil to lymphocyte counts (NLR) showed a greater diagnostic value (AUC, 0.92). Serum CitH3 levels were markedly lower in DM patients with normal muscle enzyme levels than in HCs (all $p < 0.001$), and when compared to an elevated group, the CitH3 levels were comparable (all $p > 0.05$). The CitH3 levels showed no difference between DM in active and remission groups. However, in a paired test with 18 hospitalized DM patients, the CitH3 levels were higher in remission state than in active state. Moreover, the CitH3 levels showed no correlation with disease variables that were associated with the disease activity of DM.

Serum CitH3 level may serve as a useful biochemical marker for screening patients with DM from HCs, while its role in monitoring DM disease activity requires further research.

biochemical marker, citrullinated histone H3, dermatomyositis, disease activity

Idiopathic inflammatory myopathies (IIMs), including dermatomyositis (DM), polymyositis (PM), inclusion body myositis, and immune-mediated necrotizing myopathy, are rare systemic autoimmune diseases.[1,2] The incidence of IIMs is fairly low, as reported by several research groups.[3–5] Among IIMs, DM is the most common.[6] Both children and adults may develop DM with an overall female-to-male ratio of approximately 2:1.[7] Patients with DM are characterized by chronic proximal muscle inflammation and weakness accompanied

by characteristic skin rashes, such as periorbital purple-red edema, Gottron sign, and nail fold rigid dilated capillary erythema.[6] At present, the diagnosis of DM is mainly based on symmetrical proximal muscle weakness, pain, and tenderness, accompanied by a characteristic skin rash, as mentioned above.[8] Increased serum enzyme activity is an important serological indicator in the diagnosis of this disease.[9] Muscle enzyme testing includes serum creatine kinase (CK), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and lactate dehydrogenase (LDH) activity and has been shown to moderately correlate with disease activity in IIMs.[9,10] However, the serum levels of muscle enzymes, generally used to evaluate muscle inflammation, may be in the normal range at the early stage of the disease, or even throughout the disease process, thereby making it more difficult to establish a definite diagnosis of DM.[10,11] Moreover, patients with DM may develop serum autoantibodies, such as antinuclear antibodies and myositis-specific autoantibodies.[12] However, the positive rate of diagnosis is low, and early diagnosis is difficult.[9] Therefore, it is important to identify early novel diagnostic indicators.

The histological features of DM include infiltration of mononuclear and inflammatory cells in skeletal muscle tissue, leading to muscle damage and dysfunction by releasing cytokines, cytotoxic molecules, or proteinase.[9] In DM, most of the previous work has focused on the role of the adaptive immune system and lymphocyte subsets, and recent research on the cellular components of the innate immune system, such as neutrophils, is beginning to be investigated.[13–16] Neutrophils(o)-1 (f t)-1(3)-82.7 (–)]Ta.-

Clinical characteristics of the study subjects.

			p
Number	95	56	
Gender (female/male)	73/22	49/7	0.108
Age (years)	50 (43–58.5)	49.5 (43.5–57.25)	0.923
Disease durations (years)			
Active/remission (number)	74/39[a]		
MYOACT	1.587 (1.031–2.104)		
Muscle enzymes			
CK, uKat/L	59.7 (41.95–159.35)		
AST, uKat/L	36.7 (23.6–65.8)	22.8 (19.5–26.3)	0.000***
ALT, uKat/L	29.8 (18–64.9)	19.3 (13.9–24.4)	0.000***
LDH, uKat/L	299 (228–383.5)		
Inflammation marker			
CRP, mg/L	3.8 (2.6–9.8)		
ESR, mm/h	43.5 (27.3–73.5)		
Neutrophil, $\times 10^9$/L	4.6 (3.3–6.4)	3.2 (2.7–3.9)	0.000***
Lymphocyte, $\times 10^9$/L	1.1 (0.7–1.5)	1.8 (1.5–2.0)	0.000***
NLR	4.3 (2.4–7.0)	1.8 (1.4–2.6)	0.000***
PLT, $\times 10^9$/L	207.5 (161.5–262.8)	216.5 (189.3–260.5)	0.258

Note: Data are presented as number of patients or median (Q25–Q75). Differences between groups were assessed with the Mann–Whitney U test. A p value < 0.05 was used to indicate a statistically significant result (***$p < 0.001$).

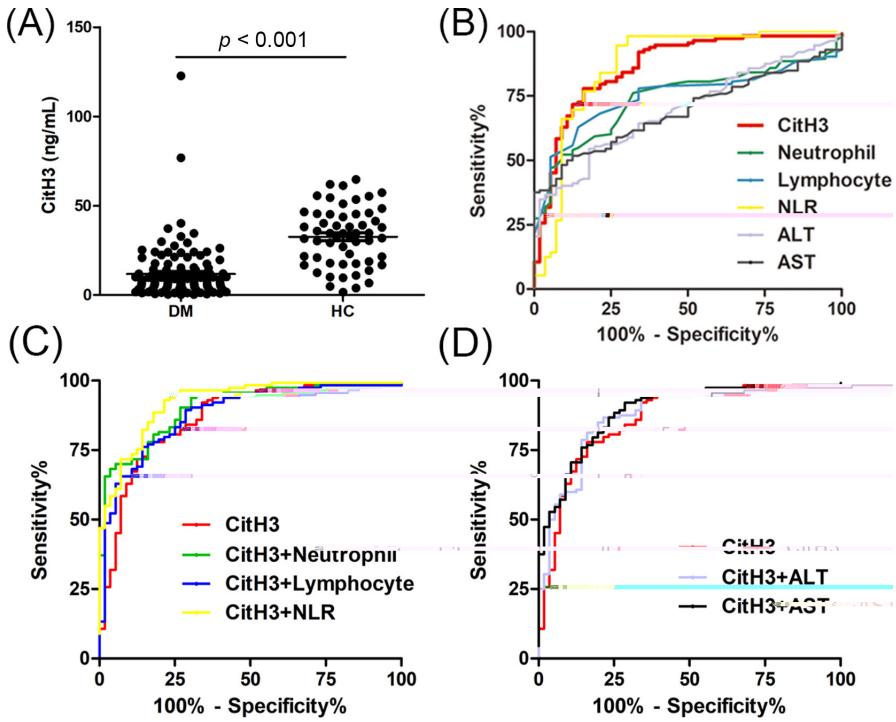
Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; CK, creatine phosphokinase; CRP, C-reactive protein; DM, dermatomyositis; ESR, erythrocyte sedimentation rate; HC, healthy controls; LDH, lactate dehydrogenase; MYOACT, myositis disease activity assessment; NLR, the ratio of neutrophil to lymphocyte counts; PLT, platelet.

[a]Among 39 remission samples, 18 of them are paired samples.

Serum samples (1 mL) were collected in Eppendorf tubes and stored at −20°C until analysis. Human CitH3 serum levels were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Cayman Chemicals, Inc.). The ELISA kit instructions showed that the minimum detection level of CitH3 was 0.1 ng/mL, and the inter- and intra-assay coefficients of variations at 1.25 ng/mL were 13.2% and 7.2%, respectively. Standards and patient samples were analyzed in duplicate, according to the manufacturer's instructions.

Data were tested for normality using the Kolmogorov–Smirnov test. Paired data collected in the experiment were compared using a paired-sample t test. Differences between unpaired groups were assessed using the Mann–Whitney U test. Data are shown as the mean ± standard deviation or median (interquartile range). Correlations between variables were evaluated using the Pearson's rank correlation. Receiver operating characteristic (ROC) curves were used to evaluate the significance of CitH3 levels and other disease variables in distinguishing patients with DM and HCs. The Youden index was calculated as a sensitivity + specificity − 1. The best critical point (cutoff value) was selected as the largest tangential point of the Youden index. Statistical significance was set at $p < 0.05$. Statistical analysis was performed using SPSS 20.0 (IBM SPSS Statistics, IBM Corporation) or Prism software (version 5.0; GraphPad Software, Inc.).

The concentrations of CitH3 in the patients with DM and HCs are shown in Figure 1A. Serum CitH3 levels in the DM patients were particularly lower than those in the HCs (6.6 [3.7–12.0] vs. 33.6 [18.5–45.3] ng/mL, median [interquartile range]; $p < 0.001$; Figure 1A). The predictive value of CitH3 in patients with DM versus HCs was studied using a univariate ROC analysis. The univariate areas under the curve (AUC) of CitH3 were 0.86 (95% CI: 0.81–0.94), sensitivity of 0.78, and specificity was 0.839 for discriminating between patients with DM and HCs (Figure 1B and Table 2).



Serum levels of citrullinated histone H3 (CitH3) in patients with dermatomyositis (DM). (A) CitH3 levels in DM patients and healthy controls (HCs). (B) The predictive values of CitH3 (area under the curve [AUC], 0.86), neutrophil (AUC 0.74), lymphocyte (AUC 0.75), the ratio of neutrophil to lymphocyte counts (NLR, AUC 0.87), alanine aminotransferase (ALT, AUC 0.70), and aspartate aminotransferase (AST, AUC 0.69) to distinguish DM and HCs. (C) The predictive values of the combination of CitH3 with neutrophil (AUC 0.90), lymphocyte (AUC 0.88), or NLR (AUC 0.92) to distinguish DM and HCs. (D) The predictive values of the combination of CitH3 with ALT (AUC 0.88) or AST (AUC 0.89) to distinguish DM and HCs. Each dot represents the CitH3 level of each individual. The horizontal lines represent the mean levels of CitH3 in each group. *p* values were calculated by Mann–Whitney U test, and a *p* value < 0.05 was used to indicate a statistically significant result.

AUC, sensitivity and specificity for CitH3, other serological markers and combinations.

					p
CitH3	0.86 (0.80–0.93)	16.095 ng/mL	78.0	83.9	<0.001***
Neutrophil	0.74 (0.67–0.82)	3.25×10^9/L	76.1	67.9	<0.001***
Lymphocyte	0.75 (0.67–0.82)	1.35×10^9/L	62.8	85.7	<0.001***
NLR	0.87 (0.80–0.94)	2.75	68.1	91.1	<0.001***
ALT	0.70 (0.62–0.78)	25.8 uKat/L	54.5	82.1	<0.001***
AST	0.69 (0.61–0.77)	29.0 uKat/L	51.8	87.5	<0.001***
CitH3+Neutrophil	0.90 (0.86–0.95)	0.22	68.1	96.4	<0.001***
CitH3+Lymphocyte	0.88 (0.82–0.93)	0.28	76.1	85.7	<0.001***
CitH3+NLR	0.92 (0.88–0.96)	0.49	92.9	78.6	<0.001***
CitH3+ALT	0.88 (0.83–0.94)	0.37	86.6	78.6	<0.001***
CitH3+AST	0.89 (0.84–0.94)	0.35	86.6	76.8	<0.001***

Note: A *p* value < 0.05 was used to indicate a statistically significant result (***$p < 0.001$).

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; AUC, area under the curve; CitH3, citrullinated histone H3; NLR, the ratio of neutrophil to lymphocyte counts; 95% CI, 95% confidence interval.

Considering the diagnostic values of muscle enzymes and inflammatory markers in patients with DM (Figure 1B and Table 2), we further tested the predictive value of the combination of CitH3 and the abovementioned markers. The results showed that a combination of CitH3 with NLR had the greatest diagnostic value for distinguishing DM from HCs, with an AUC of 0.92 (0.88–0.96), sensitivity of 0.93, and specificity of 0.79 (Figure 1C and Table 2).

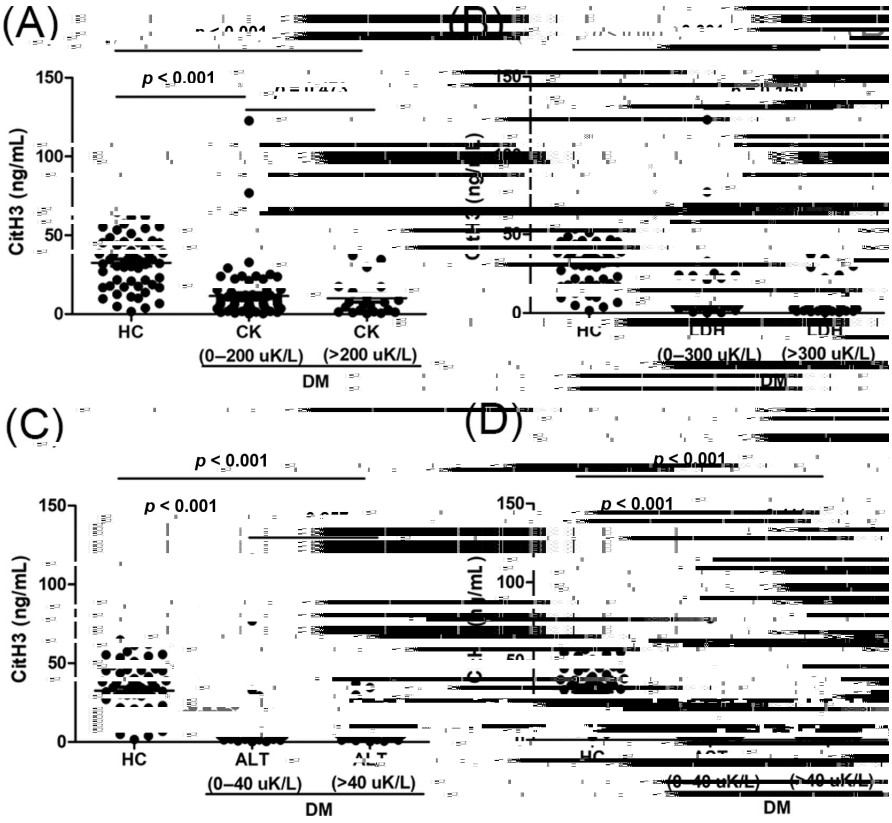
Muscle enzyme activity is an important serological indicator for the diagnosis of DM, but the levels of muscle enzymes in nearly half of DM patients are not elevated even throughout the disease process. Hence, we further compared the serum CitH3 levels in normal muscle enzyme group of the patients with DM and HC. As shown in Figure 2A, the serum CitH3 levels in a CK normal DM group (0–200 uK/L, n = 74) were markedly lower than those in the HCs ($p < 0.001$), while there was no difference in the CK elevated group (>200 uK/L, n = 21) ($p = 0.473$). Similar results were observed in an LDH normal DM group (0–300 uK/L, n = 49) versus HCs, ALT normal DM group (0–40 uK/L, n = 59) versus HCs, and AST normal DM group (0–40 uK/L, n = 59) versus HCs (both $p < 0.001$). Moreover, the serum CitH3 levels showed no differences between the LDH normal group and LDH elevated group (>300 uK/L, n = 46; $p = 0.150$), ALT normal group versus ALT elevated group (>40 uK/L, n = 35; $p = 0.257$), and AST normal group versus AST elevated group (>40 uK/L, n = 35; $p = 0.111$).

To investigate the correlation between CitH3 levels and disease activity, we initially compared the DM patients in active with those in remission. Results showed that the serum CitH3 levels were comparable in the DM patients with active disease and those in remission (6.3 [3.7–10.9] vs. 8.7 [3.9–14.0], $p = 0.479$; Figure 3A).

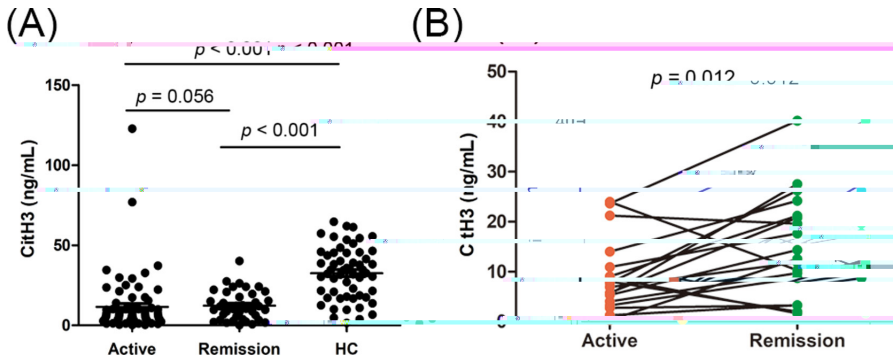
To further explore the role of CitH3 in distinguishing between patients with active DM and those in remission, paired sera from 18 hospitalized patients in a state of active and remission were collected and tested. The CitH3 levels were markedly higher in the remission state of the DM patients when compared to in active state (15.29 ± 10.36 vs. 9.04 ± 7.24, $p = 0.012$; Figure 3B).

Correlation analysis revealed no correlations between serum CitH3 levels and MYOACT (which represents the disease activity of DM), LDH, CK, AST, and ALT. Moreover, serum CitH3 levels showed no correlation with inflammatory markers, such as CRP, ESR, neutrophils, and lymphocytes. The associated data are presented in Table 3.

In patients with DM, the prevalence of ANA, anti-Ro-52, anti-MDA5, anti-TIF-1γ, anti-Mi-2, and anti-PM-Scl was 83.9%, 46.5%, 39.5%, 20.9%, 15.1%, and 12.8%, respectively. The prevalence of other autoantibodies was all less than 10%. We examined the differences in serum levels of CitH3 in DM patients with and without elevation of an autoantibody. Our data showed that the serum levels of CitH3 were higher in the anti-TIF-1γ-positive group than in the anti-TIF-1γ-negative group (13.83 ± 9.13 vs. 8.32 ± 8.23, ng/



Serum levels of citrullinated histone H3 (CitH3) in normal muscle enzymes group. (A) The serum CitH3 levels in creatine phosphokinase (CK) normal dermatomyositis (DM) group (0–200 uK/L), CK elevated DM group (>200 uK/L), and healthy controls (HCs). (B) The serum CitH3 levels in lactate dehydrogenase (LDH) normal DM group (0–300 uK/L), LDH elevated DM group (>300 uK/L) and HCs. (C) The serum CitH3 levels in alanine aminotransferase (ALT) normal DM group (0–40 uK/L), ALT elevated DM group (>40 uK/L), and HCs. (D) The serum CitH3 levels in aspartate aminotransferase (AST) normal DM group (0–40 uK/L), AST elevated DM group (>40 uK/L), and HCs. Each dot represents the CitH3 level of each individual. The horizontal lines represent the mean levels of CitH3 in each group. *p* values were calculated using Mann–Whitney U test, and $p < 0.05$ was used to indicate a statistically significant result.



Serum levels of citrullinated histone H3 (CitH3) in dermatomyositis (DM) patients with active disease and remission. (A) The level of CitH3 in DM patients with active disease and remission. (B) The level of CitH3 in paired active and remission DM patients (n = 18). Each dot represents the CitH3 level of each individual. The horizontal lines represent the mean levels of CitH3 in each group. *p* values were calculated using Mann–Whitney U and paired-sample t tests. A *p* value of <0.05 was used to indicate a statistically significant result.

Association of CitH3 levels with laboratory parameters of patients with DM.

		p
MYOACT	0.047	0.653
Muscle enzymes		
CK	−0.04	0.647
LDH	−0.08	0.386
ALT	−0.16	0.085
AST	−0.13	0.156
Inflammation marker		
CRP	−0.05	0.613
ESR	0.05	0.653
Neutrophil	−0.08	0.389
Lymphocyte	−0.02	0.836
NLR	−0.09	0.322

Note: Data were analyzed using the Pearson's rank correlation. A *p* value < 0.05 was used to indicate a statistically significant result.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; CitH3, citrullinated histone H3; CK, creatine phosphokinase; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; LDH, lactate dehydrogenase; MYOACT, the myositis disease activity assessment; NLR, the ratio of neutrophil to lymphocyte counts.

mL, $p = 0.016$) in the DM patients. In contrast, no significant differences were observed between other autoantibody-positive and autoantibody-negative groups. The prevalence of autoantibodies and association between CitH3 and autoantibodies are summarized in Table 4.

In this study, the CitH3 levels were significantly lower in the DM patients than in the HCs. The ROC curve analysis revealed that the CitH3 level strongly discriminated the DM patients from the HCs (AUC 0.86), even in the normal muscle enzymes DM group. This study is the first to focus on CitH3 as a serological biomarker to distinguish DM patients from HCs.

Extracellular histones in serum are mainly derived from NETs produced by activated neutrophils.[29] CitH3 is the product of post-translational conversion of peptidylarginine to citrulline at the N terminus of histone H3. Levels of circulating CitH3 have been reported to be significantly increased in patients with septic shock,[20] septic AP,[19] advanced cancer,[22] pneumonia,[23] and coronavirus disease 2019 (COVID-19).[24] Different from the abovementioned, our results showed that the serum level of CitH3 is markedly decreased in DM patients when compared to HCs. A similar decline in CitH3 levels was observed in oral squamous cell carcinoma (OSCC) patients, and the expression of CitH3 was statistically and significantly lower in the neutrophils of OSCC patients than in the control group.[30] A decline in CitH3 in OSCC patients was at the cellular level, which indicates that the process of NETosis in OSCC is disrupted.[30] Zhang et al.[14] reported that in DM/PM patients, excessively formed NETs cannot be completely degraded because of decreased DNase I activity, suggesting that abnormal regulation of NETs may be involved in DM/PM, which may lead to a decrease in CitH3 in the serum. In this study, we only tested the serum level of CitH3 in the DM patients; the cellular level of CitH3 in the neutrophils of DM patients remains unknown and requires further investigation. Moreover, the CitH3 level in pathological muscle tissue of DM also requires further investigation, and research has revealed that IIM NETs decreases the viability of myotubes in a citrullinated histone-dependent manner, indicating the important role of citrullinated histones in IIM.[15]

Consistent with our results, several studies have reported that muscle enzyme activity,[10] neutrophil count, and NLR[31,32] are serological indicators that distinguish patients with DM from HCs. In this study, we focused on CitH3 as a serological biomarker for DM diagnosis. The combination of CitH3 with the abovementioned markers showed superior diagnostic value, particularly the combination of CitH3 with NLR. Further, our research showed that in the DM patients with normal muscle enzymes (including CK, LDH,

Associations of CitH3 with autoantibodies in DM patients.

				p
ANA	74/15	11.81 ± 17.55	5.62 ± 4.05	0.180
Anti-Ro-52	40/48	9.38 ± 9.30	9.48 ± 8.02	0.956
Anti-MDA5	34/54	10.42 ± 9.44	10.42 ± 9.44	0.173
Anti-TIF-1γ	19/69	13.51 ± 8.98	8.31 ± 8.17	0.018*
Anti-Mi-2	14/74	9.95 ± 9.20	9.34 ± 8.51	0.806
Anti-PM-Scl	11/77	9.17 ± 8.33	9.17 ± 8.33	0.446
Anti-Jo-1	8/80	9.98 ± 13.10	9.38 ± 8.11	0.851
Anti-NXP2	6/82	12.75 ± 12.54	9.19 ± 8.27	0.330
Anti-SAE1	5/83	7.99 ± 2.69	9.52 ± 8.80	0.701
Anti-PL7	4/84	11.66 ± 12.37	9.33 ± 8.44	0.597
Anti-EJ	3/85	10.54 ± 11.95	9.39 ± 8.53	0.821
Anti-SRP	2/86	5.40 ± 5.24	9.53 ± 8.63	0.504
Anti-Ku	1/87	/	/	/

Note: Data are presented as number of patients or mean ± standard deviation. Differences between groups were assessed with the Mann–Whitney U test. A p value < 0.05 was used to indicate a statistically significant result (*$p < 0.05$).

Abbreviations: ANA, antinuclear antibody; Anti-EJ, anti-glycyl transfer RNA antibodies; Anti-Jo-1, anti-histidyl transfer RNA antibody; Anti-Ku, antibodies to Ku; anti-MDA5, antimelanoma differentiation-associated gene 5 antibodies; Anti-Mi-2, antibodies to Mi-2; Anti-NXP2, antinuclear matrix protein 2 antibody; Anti-PL7, anti-threonyl transfer RNA antibody; Anti-PM-Scl, anti-polymyositis/scleroderma antibodies; anti-Ro-52, antibodies to Ro-52; Anti-SAE1, anti-small ubiquitin-like modifier activating enzyme 1 antibody; Anti-SRP, anti-signal recognition particle antibody; anti-TIF-1γ, antitranscriptional intermediary factor 1 antibody.

ALT, and AST), serum CitH3 levels were markedly lower than those in HCs, and the serum CitH3 levels between the normal muscle enzyme group and elevated group were comparable. This means that even if the levels of the characteristic muscle enzymes of DM are normal, we can distinguish DM from HCs by the serum level of CitH3.

The discovery of new biomarkers for monitoring disease activity in patients with IIM remains a topic of great interest. Previous studies have revealed that the serum level of CitH3 is closely correlated with disease severity in patients with septic shock[20] and septic AP.[19] Moreover, Thalin et al.[22] found that high levels of circulating CitH3 strongly predicted poor clinical outcomes in a cohort of cancer patients with a twofold increased the risk of short-term mortality. However, further research showed that the level of CitH3 may have no correlation or at least a weak correlation with the disease activity of DM. This conclusion is based on two aspects of the data. First, in patients with active and remission disease, the levels of CitH3 were comparable. Further paired test of 18 hospitalized patients revealed that CitH3 levels in DM with an active state were significantly lower than DM in remission state, among four patients showed an upward trend. Second, serum CitH3 levels showed no correlation with disease activity-associated factors (MYOACT, CK, ALT, AST, and LDH) or inflammation markers (CRP, ESR, neutrophil, lymphocyte, and NLR). These results indicate that a larger sample size of the paired test is needed to further carry out this experiment.

Despite the novel and clinically relevant findings in this study, there are some limitations. The patients included in our study differed in terms of disease severity and treatment options. In order to eliminate the influence of abovementioned factors, larger sample size of the paired test was needed to confirm the correlation of serum levels of CitH3 with the disease activity of DM. Additionally, in this study, we only tested the serum level of CitH3 in DM patients; the cellular level of CitH3 in the neutrophils of DM patients and the CitH3 level in pathological muscle tissue remain unknown. Thus, further research on CitH3 is warranted in future studies.

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None.

All data generated or analysed during this study are included in this published article. If any additional information is required it may be obtained by request with the corresponding author.

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