Serum levels of interleukins and S100A8/A9 correlate with clinical severity in patients with dermatomyositis-associated interstitial lung disease

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Research article

Keywords: Dermatomyositis, Interstitial lung disease, S100A8/A9, Interleukin

Posted Date: March 3rd, 2020

DOI: https://doi.org/10.21203/rs.3.rs-15869/v1

Version of Record: A version of this preprint was published on July 17th, 2020. See the published version at https://doi.org/10.1186/s12890-020-01226-3.

Abstract

Background: Dermatomyositis (DM) is a systemic autoimmune inflammatory disorder that affects primarily skin, muscle and lung, frequently associated with interstitial lung disease (ILD). The objective of this study is to investigate the association between serum cytokines and clinical severity in patients with DM-ILD.

Methods: Serum samples of 40 DM-ILD patients and 30 healthy controls were collected. Expressions of S100A8/A9 were analyzed by enzyme-linked immunosorbent assay (ELISA) and interleukins were analyzed by cytometric beads array (CBA).

Results: Serum IL-4, IL-6 and S100A8/A9 were observably higher in DM-ILD than those in healthy controls ($p = 0.0013$, 0.0017 and < 0.0001, respectively). Serum IL-10 level of patients was dramatically lower than that in controls ($p = 0.0001$). IL-4 ($r = 0.1171$, $p = 0.0040$), IL-6 ($r = 0.1174$, $p = 0.0040$) and IL-10 ($r = -0.1829$, $p = 0.0003$) were significantly correlated with S100A8/A9 in DM-ILD patients. S100A8/A9 was significantly correlated with high-resolution computed tomography (HRCT) ($r = 0.1642$, $p = 0.0157$) and lung function (DLCO%: $r = -0.2066$, $p = 0.0061$, FVC%: $r = -0.2156$, $p = 0.0050$).

Conclusions: Serum level of S100A8/A9 may be a valuable marker for assessing the clinical severity of DM-ILD patients. Serum IL-4, IL-6 and IL-10 levels were highly correlated with S100A8/A9, so these cytokines may play a synergistic effect on the progression of DM-ILD. Keywords : Dermatomyositis, Interstitial lung disease, S100A8/A9, Interleukin

Introduction

Dermatomyositis (DM) is a kind of idiopathic inflammatory myopathy (IIM), which mainly involves the inflammation of skeletal muscle and skin, and can cause muscle weakness and rash (1). Interstitial lung disease (ILD) is considered a common systemic complication of DM(2). DM associated with ILD is one of the major prognostic determinants, causing increased morbidity and mortality (3–5).

Autoimmune abnormalities are commonly found in inflammatory disorders and are considered to participate in the pathogenesis of DM (6). The inflammatory infiltrates are mainly composed of T lymphocytes and macrophages in muscle tissues of patients with DM. Numerous studies to date have indicated that $CD4^+$ T-helper (Th) cells may play a critical role in the pathogenesis of inflammatory myopathies (7–9). $CD4^+$ T lymphocytes are classified into type 1 (Th1) and type 2 (Th2) according to the cytokines produced. Interferon (IFN)-γ in Th1 and interleukin (IL)-4 in Th2 have been both measured in muscle biopsy specimens of patients with DM, suggesting the involvement of different Th subtypes in the disease (10).

The S100A8/A9 heterodimer is also referred to as calprotectin or MRP8/14. It is a potent inflammatory protein as an activator of the innate immune system in a Toll-like receptor (TLR) 4 dependent manner

(11). S100A8/A9 has been explored as a possible biomarker of disease severity in several autoimmune diseases, including juvenile dermatomyositis (12), systemic sclerosis (13) and rheumatoid arthritis (14).

To investigate the precise mechanisms by which cytokines affect the pathogenesis of DM-ILD, and the correlation between S100A8/A9 in the serum of DM-ILD and disease activity scores, we conducted this study to analyze serum levels of cytokines and S100A8/A9, and assessed their potency as a biomarker to predict disease activity and to aid broad implementation into clinical practice.

Methods

Participants

The present study included 40 patients with DM–ILD and 30 healthy controls. The patients were diagnosed as DM according to the classification criteria proposed by Bohan and Peter(15) (16). Diagnosis of interstitial lung disease based on clinical symptoms and high-resolution computed tomography (HRCT), with/without lung biopsy findings. The diagnosis was based on the International Consensus Statement on Idiopathic Pulmonary Fibrosis issued by the American Thoracic Society and the European Respiratory Society(17).

They were admitted to the department of Respiration and Rheumatology at our hospital from January 2017 to December 2018. Clinical and laboratory data were collected retrospectively upon admission. Exclusion criteria were immunotherapy and hormone therapy for more than two weeks or longer after rash, myopathy, and ILD symptoms. This study was approved by the Institutional Review Board of RenJi Hospital (2016075). All participants included consent the study orally.

Measurement of cytokines

Serum cytokine levels were detected by the BD^{TM} cytometric bead array (CBA) assay (BD Biosciences, San Diego, CA, U.S.A.). The Human Th1/Th2/Th17 Cytokine Kits were used to detectermine the serum concentrations of IL-2, IL-4, IL-6, IL-10, tumor necrosis factor-α (TNF-α), IFN-γ and IL-17A. Human MRP8/14 (Calprotectin) ELISA Kit (Biolegend, San Diego, CA, U.S.A.) was used to measure serum S100A8/A9 level.

Lung high-resolution CT scoring and pulmonary function evaluation

HRCT score was assessed by two pulmonary radiologists in a double-blind manner(18). Each patient was tested for lung function test, percentage of predicted forced vital capacity (FVC%) and single-breath diffusing capacity of the lung for carbon monoxide (DLCO-SB) by an experienced technician on the Jaeger platform. (CareFusion, BD Biosciences).

Statistical Analysis

Multiple unpaired t-tests were employed for comparison of serum cytokine levels between patients and healthy controls. Relationships between cytokines were investigated using Spearman's correlation coefficient test. Correlations of serum cytokines with disease severity were also using Spearman's correlation coefficient test. All data were analyzed by SPSS 23.0 and Graphpad Prism 6.0. A *P*-value of <0.05 was regarded as statistically significant in all statistical analysis. The following symbols * P < 0.05; * * P < 0.01; * * P < 0.001 were used.

Results

Baseline characteristic

The present study included 40 patients with DM–ILD (13 males and 27 females; mean age 50.66 ± 13.33 years, range 22 to 78) and 30 healthy controls (11 males and 19 females; mean age 45.37 ± 10.25 years, range 19 to 65). The mean HRCT score, FVC% and DLCO% were shown in Table 1.

Table 1
Clinical characteristic of the DM-ILD patients and healthy controls

Characteristics	**(N = 40)**	**(N = 30)**
Age at onset (mean ± SD, year)	50.66 ± 13.33	45.37 ± 10.25
Sex (female/male)	(27/13)	(19/11)
HRCT score	144.5 ± 37.21	
FVC%	67.16 ± 19.60	
DLCO%	37.36 ± 18.46	
DM, dermatomyositis; ILD, interstitial lung disease; HRCT, high-resolution computed tomography; FVC%, % forced vital capacity; DLCO%, % diffusing capacity of the lungs for carbon monoxide.		

Comparison of serum cytokine levels between patients with DM-ILD and the healthy controls.

In CBA studies, serum IL-4 (9.743 ± 0.7713 versus 6.594 ± 0.2539, P = 0.0013) and IL-6 (44.10 ± 8.109 versus 11.57 ± 1.937, P = 0.0017) levels were significantly higher in patients with DM-ILD than healthy controls. Serum IL-10 level in patients was significantly lower than controls (1.881 ± 0.09292 versus 2.809 ± 0.2445, P = 0.0001). In ELISA analysis, DM-ILD patients had significantly higher S100A8/A9 levels than controls (103.1 ± 6.692 versus 40.92 ± 4.430, P < 0.0001). No significant differences in the levels of IL-2, TNF-α, IFN-γ and IL-17A were detected between DM-ILD patients and controls (Table 2 and Fig. 1).

Table 2
Cytokine levels of the DM-ILD patients and healthy controls

	DM-ILD (n = 40) Mean ± SD, pg/mL	Controls (n = 30) Mean ± SD, pg/mL	P value
IL-2	4.569 ± 0.0424	4.635 ± 0.0551	0.3413
IL-4	9.743 ± 0.7713	6.594 ± 0.2539	0.0013**
IL-6	44.10 ± 8.1091	11.57 ± 1.9372	0.0017**
IL-10	1.881 ± 0.0929	2.809 ± 0.2445	0.0001***
TNF- α	2.366 ± 0.1400	2.525 ± 0.1301	0.4280
IFN-γ	3.505 ± 0.1612	3.604 ± 0.1496	0.6727
IL-17A	10.525 ± 0.1483	9.367 ± 0.0722	0.5725
S100A8/A9	103.1 ± 6.6925	40.92 ± 4.4302	< 0.0001***

Correlation between the levels of IL-4, IL-6, IL-10 and S100A8/A9

We next analyzed the correlation between IL-4, IL-6, IL-10 and S100A8/A9 levels. As shown in Fig. 2, significant positive correlation was found between levels of IL-4, IL-6 and S100A8/A9 (r_s= 0.1171, P = 0.0040; r_s= 0.1174, P = 0.0040). IL-10 levels were significantly negatively correlated with S100A8/A9 levels (r_s= 0.1829, P = 0.0003).

Correlation between S100A8/A9 levels and disease activity in DM-ILD patients

To assess the potency of S100A8/A9 as a biomarker to predict the disease activity of DM-ILD, we analyzed the relationship between S100A8/A9 levels and their HRCT score or lung functions in DM-ILD patients. As shown in Fig. 3, the level of S100A8/A9 was significantly positively correlated with HRCT score (r_s= 0.1642, P = 0.0157) and negatively correlated with DLCO% (r_s= 0.2066, P = 0.0061) and FVC% (r_s= 0.2156, P = 0.0050).

Discussion

DM-ILD has high morbidity and mortality. Inflammation plays a key role in the pathogenesis of DM-ILD. S100A8/A9 is mainly released by neutrophils and monocytes, and stable dimers or homodimers can be formed in vitro and in vivo. S100A8/A9 have already been verified to play an important role in the progress of inflammation. Serum S100A8/A9 levels in patients with systemic lupus erythematosus (SLE) are elevated, which may be closely related to disease activity. (19, 20). Elevated S100A9 level in sputum is a potential biomarker of neutrophilic inflammation in severe asthma (21). Andreasson K et al. found that fecal S100A8/A9 level in patients with systemic sclerosis may be a biomarker of gastrointestinal diseases (22). In Idiopathic pulmonary fibrosis (IPF), elevated level of S100A9 was observed in

bronchoalveolar lavage fluid (BALF) (23, 24). Hara A et al. reported that S100A9 level in BALF may be a biomarker of IPF fibrosis (25). Therefore, based on the above researches, we hypothesized that S100A8/A9 may play a role in the development of DM-ILD.

Interleukin-4 (IL-4) is a multifunctional and multipotent cytokine, which plays an important role in proliferation, differentiation and apoptosis of various cell types, mainly secreted by mast cells, Th2 cells, eosinophils and basophils. (26). IL-6 and IL-10 had been confirmed to be associated with A/SIP in patients with DM/CADM (27–29). IL-6, IL-8, and TNF-α were previously indicated to be associated with overall disease activity in PM/DM (30). However, the association between pulmonary disease activity and above cytokines has remained unclear.

In our cohort, Serum levels of S100A8/A9 were significantly higher in DM-ILD patients than those in healthy controls ($p < 0.0001$). Serum levels of IL-4, IL-6 were significantly higher in DM-ILD patients than those in healthy controls ($p = 0.0013, 0.0017$). Serum IL-4 ($r = 0.1171$, $p = 0.0040$), IL-6 ($r = 0.1174$, $p = 0.0040$) were significantly correlated with S100A8/A9 in patients with DM-ILD. Our findings were consistent with the previous studies that S100A8/A9, IL-4, IL-6 levels were increased in inflammatory diseases, and they may play a key role in the DM-ILD.

Different from previous studies, we found that serum IL-10 levels of DM-ILD patients were dramatically lower than controls ($p = 0.0001$). It has been indicated that IL-10 family is comprised of nine members which are powerful immune mediators with versatile functions, including reducing tissue damage caused by excess and uncontrolled inflammatory effector responses (31). Several recent studies showed that the transcription factor c-Maf up-regulated IL-10 production in vivo in CD4 + T cells from Th1, Th2, and Th17 cells in experimental disease models and Bhlhe40 repressed IL-10 production in T cells during immune responses (32, 33). Thus,we hypothesize that the development of DM-ILD may be associated with the absence of c-Maf or the overexpression of Bhlhe40 in CD4 + T cells. But it remains to be confirmed in the further research.

ILD is common among patients with DM, and is often associated with a worse prognosis (34). HRCT scoring system can be used as a parameter to evaluate the damage of ILD structure, and predict the severity and prognosis of DM-ILD (35). Furthermore, in patients with IPF, forced vital capacity (FVC) and carbon monoxide diffusion (DLCO) are considered to be the most sensitive parameters for evaluating the course of the disease. (36). Hence, here we used FVC% and DLCO% as pulmonary function impairment evaluation indicators, combined with HRCT score system, to predict the severity and prognosis of DM-ILD.

We found that serum levels of S100A8/A9 were significantly correlated with ILD structural damage (HRCT score) ($r = 0.1642$, $p = 0.0157$) and pulmonary function impairment (DLCO%: $r= -0.2066$, $p = 0.0061$, FVC%: $r= -0.2156$, $p = 0.0050$). The correlation between serum S100A8/A9 levels and HRCT and PFT impairment suggested that high serum levels of S100A8/A9 directly reflected ILD severity in patients with DM-ILD.

There are several limitations to this study. Firstly, this study was retrospectively conducted. Secondly, analysis of DM-ILD patients with anti-MDA5 antibodies was not performed, and DM patients without ILD were not enrolled in this study. Thirdly, some patients were being treated with prednisolone at the time of serum collection. These medications maybe influenced the measurement of cytokine levels.

Conclusions

The serum levels of S100A8/A9 were highly correlated with IL-4 and IL-6 and were significantly correlated with HRCT score, FVC% and DLCO%. In addition, IL-10 level was significantly decreased in serum of DM-ILD patients compared with healthy controls. These cytokines may contribute to the pathogenesis of DM-ILD. Our results suggest that the serum levels of S100A8/A9 may be useful biomarkers for assessing ILD activity in DM.

Abbreviations

Dermatomyositis (DM), Interstitial lung disease (ILD), enzyme-linked immunosorbent assay (ELISA) , cytometric beads array (CBA), High-resolution computed tomography (HRCT), ground glass opacities (GGO), idiopathic inflammatory myopathy (IIM) , Clinically Amyopathic Dermatomyositis (CADM), T-helper (Th), Interferon (IFN), interleukin (IL), Toll-like receptor (TLR) , % forced vital capacity (FVC%) , % diffusing capacity of the lungs for carbon monoxide (DLCO%), tumor necrosis factor-α (TNF-α), systemic lupus erythematosus (SLE) , Idiopathic pulmonary fibrosis (IPF), bronchoalveolar lavage fluid (BALF)

Declarations

Acknowledgements

Not Applicable.

Ethics approval and consent to participate

The study was approved by the Institutional Review Board of RenJi Hospital (2016075). All participants included consent the study orally.

Consent for publication

Not Applicable.

Availability of data and materials

The dataset used and/or analysed during the current study are available from the corresponding author on reasonable request.

Funding

This work was supported by grants from A multidisciplinary translational medicine platform of systemic autoimmune disease（No 2014MDT01-06） and Shanghai Health and Family Planning Commission Clinical Research Project (No 20184Y0189).

Competing interests

The authors have declared no conflicts of interest.

Author Contributions:

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be submitted for publication. Dr. Wei had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design: Yueyan Lou,Yu Zheng ,Xiaodong Wang, Xiaoming Tan, Qing Wei.

Acquisition of the data: Yueyan Lou, Liyan Zhang, Feng Zhu, Zhiwei Chen.

Analysis and interpretation of the data: Yueyan Lou, Bijun Fan, Yu Zheng, Qing Wei.

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Figures

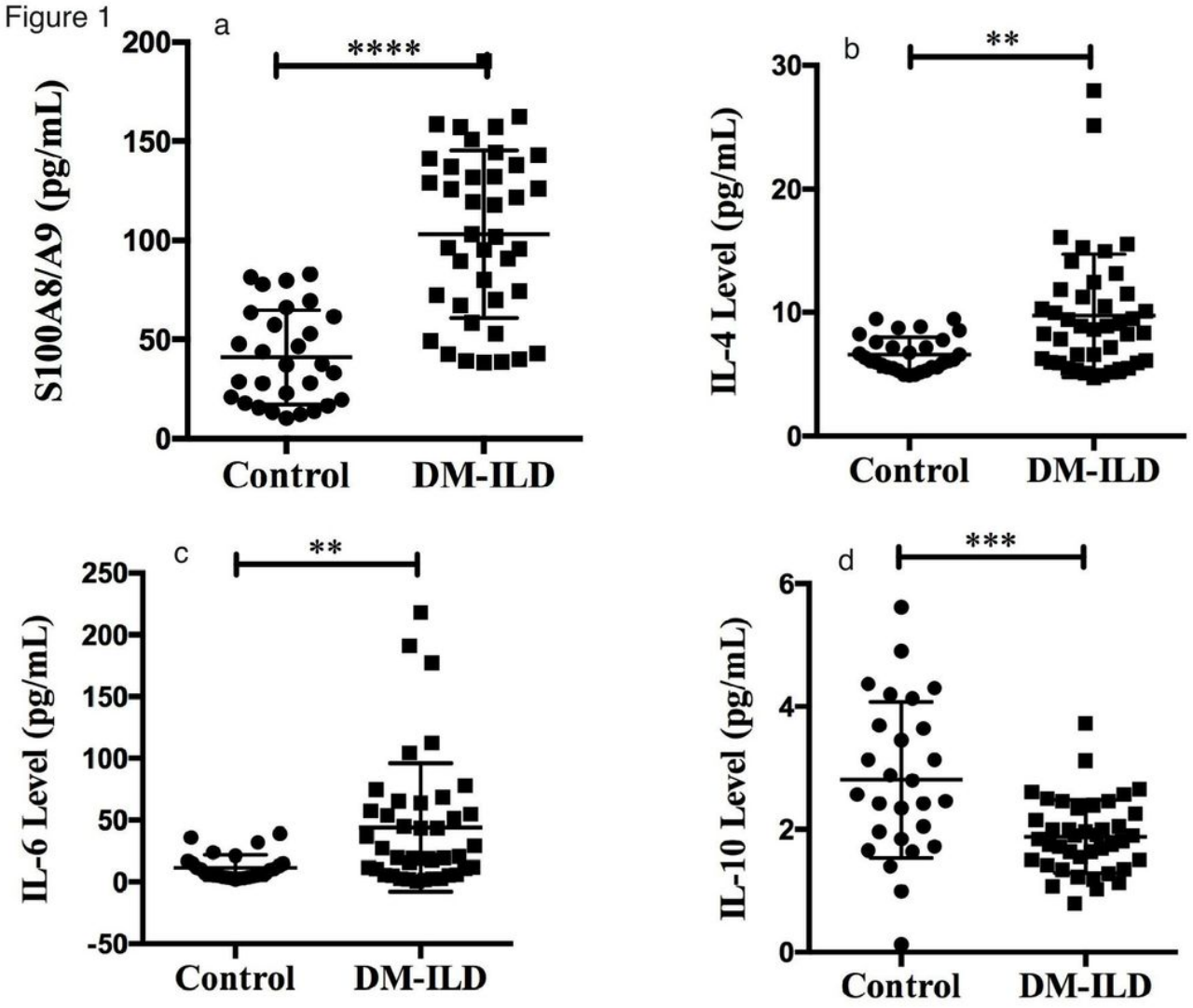

Figure 1

Comparison of serum cytokine levels between patients with DM-ILD and the healthy controls.

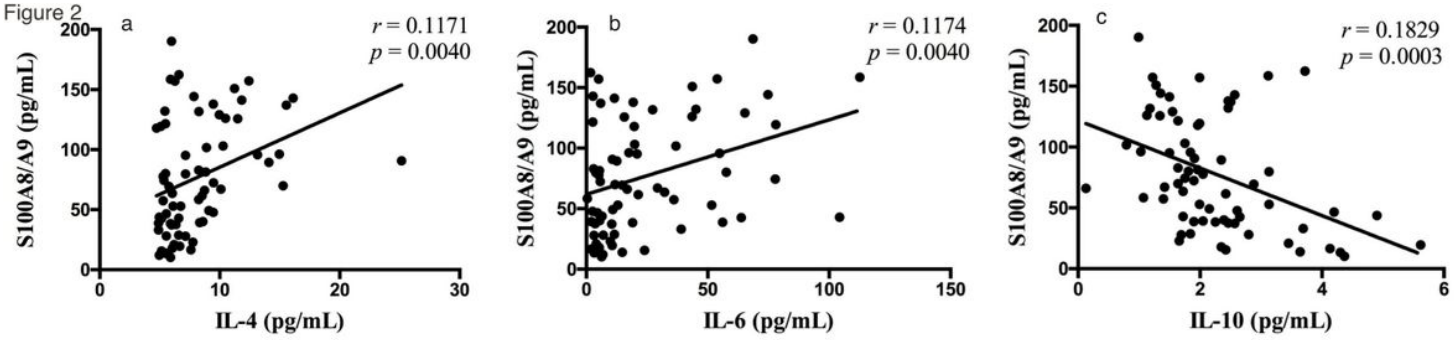

Figure 2

Correlation between IL-4, IL-6, IL-10 and S100A8/A9 levels.

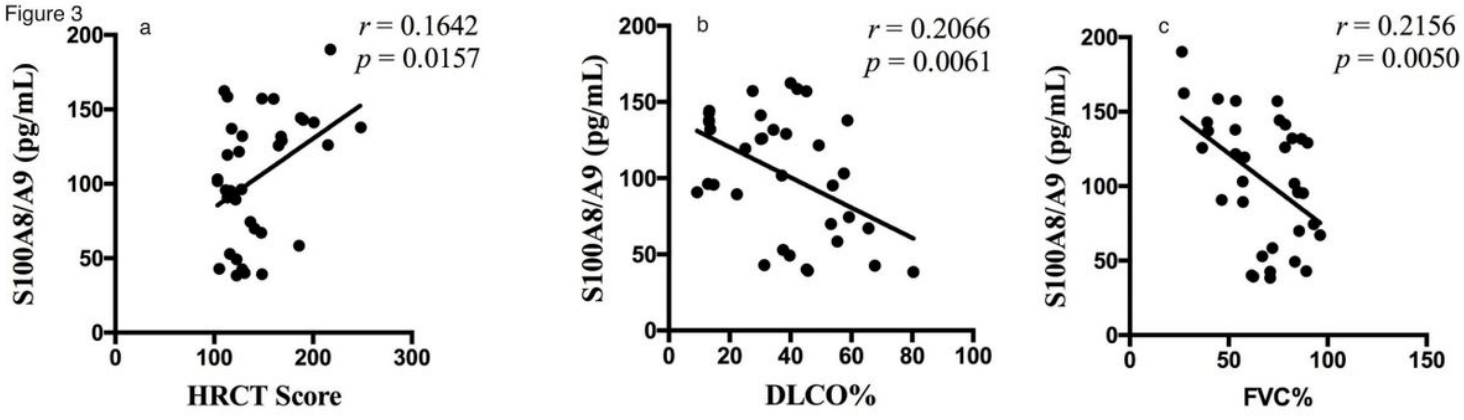

Figure 3

Correlation between S100A8/A9 levels and disease activity in DM-ILD patients.