






High mobility box protein-1 may be a new biomarker in active interstitial lung disease of systemic sclerosis

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ABSTRACT

Introduction: To investigate the significance of high mobility group box 1 (HMGB1) levels as both an immune and inflammatory mediator in systemic sclerosis (SSC) patients with interstitial lung disease (SSC-ILD) and whether HMGB1 levels could be a biomarker for progression and disease activity.

Materials & methods: Our study included 27 patients diagnosed with SSC according to the 2013 ACR/EULAR classification criteria, along with 12 healthy controls (HC). Among the patients with a diagnosis of SSC, they were further categorized into two groups based on the presence of ILD with 19 patients having lung involvement and eight patients without. In ILD-positive group, the activity of the involvement was assessed using the simple Goh algorithm. Serum levels of HMGB1 were evaluated in all groups using ELISA method.

Results: Significantly higher serum HMGB1 levels were found in patients with SSC-ILD active disease when compared to those with inactive ILD involvement and HC (14.01 mg/dl vs. 7.87 mg/dl and 8.04 mg/dl).

Conclusions: Serum HMGB1 levels reflect the disease activity in SSC-ILD. HMGB1 could be used for a potential biomarker for detecting active lung disease.

Keywords: HMGB1, systemic sclerosis, interstitial lung disease

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INTRODUCTION

Systemic sclerosis (SSC) is an autoimmune multisystemic disease characterized by vascular damage and fibrosis of the skin and visceral organs. Although the etiopathogenesis of SSC remains unclear, the hallmarks are endothelial dysfunction and stimulated fibrogenesis caused by systemic inflammation and autoimmunity [1-3]. SSC can be presented with a wide variety of clinical presentations. Although the involvement of multiple systems can be seen like cardiac and renal sarcoidosis; interstitial lung disease (ILD) is the major cause of mortality and morbidity [4, 5]. ILD is the most common lung involvement in SSC, and 20%-30% of patients have progressive ILD [6]. Older age, male gender, reduced diffusing capacity for carbon monoxide (DLCO) and forced vital capacity, presence of anti-Scl-70 antibodies, and absence of

anti-centromere antibodies are the risk factors for progressive ILD. Early diagnosis and management of SSC-associated interstitial lung disease (SSC-ILD) is essential due to the lack of optimal treatment and its poor progressive prognosis [6-9]. Besides a large number of biomarkers proposed to predict the course and the progression of the disease, reliable prognostic biomarkers are needed for optimal disease management in SSC-ILD.

High mobility group box 1 (HMGB1) is a histone chromosome interacting protein, in the nucleus of mammalian eukaryotic cells [10, 11]. It acts as a proinflammatory mediator via binding to the receptors (advanced glycation end-products, toll-like receptor 2, TLR4) due to cell activation, injury or death. It plays an important role in the acute and chronic inflammatory process [12-14]. Recent studies reported that HMGB1, as both an immune and

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Table 1. Demographic and laboratory features of study groups

Demographic features & laboratory values	SSC without ILD (n=8)	SSC-ILD inactive disease (n=16)	SSC-ILD active disease (n=3)	HC (n=12)
Age (years, M±SD)	50±12	55±13	43±15	28±7
BMI (kg/m ² , M±SD)	24.7±6.5	28.1±5.2	23.3±2.3	23.1±4.8
WBC (per mcl, median [IQR])	5,800 (312)	6,800 (200)	8,190 (275)	7,725 (125)
HB (mg/L, M±SD)	13.1±1.3	12.7±0.5	12.2±0.2	13.1±0.7
PLT (per mcl, median [IQR])	240,000 (3,250)	269,000 (6,400)	2,820,008 (6,200)	325,000 (3,000)
CRP (mg/L, median [min-max])	4 (1.0-10.0)	3 (2.0-323.0)	3 (1.3-7.0)	2 (1.0-5.0)
ESR (mm/hour median [min-max])	12.5 (5-34)	16.0 (2-96)	9.0 (5-18)	7.0 (2-11)
HMGB-1 (mg/dL, median [min-max])	7.82 (6.91-8.54)	7.87 (6.76-9.05)	14.01 (9.96-18.07)	8.04 (6.76-9.92)

Note. BMI: Body mass index; WBC: White blood cell count; HB: Hemoglobin rate; PLT: Platelet count; FEV1: Forced the first second of expiratory volume; FVC: Forced vital capacity; DL_{CO}: Diffusing capacity of lung for carbon monoxide; & CRP: C-reative protein

inflammatory mediator, is associated with several autoimmune diseases such as rheumatoid arthritis (RA), Sjogren syndrome (SS), ankylosing spondylitis (AS), and systemic lupus erythematosus (SLE) [15-18]. Furthermore, HMGB1 was associated with systemic involvement of many autoimmune diseases like SSC-ILD, and SLE-renal involvement [19-21].

This study aimed to evaluate serum levels of HMGB1 in patients with SSC and compare levels of this biomarker between patients with and without lung involvement. HMGB1 could be a predictive biomarker for the diagnosis and progression of ILD in patients with SSC.

MATERIALS & METHODS

A total of 27 patients, eight with SSC without ILD and 19 with SSC-ILD, with a diagnosis as SSC according to ACR/EULAR 2013 classification criteria were recruited at the Rheumatology outpatient clinic between January and October 2022. SSC-ILD was diagnosed with high-resolution computed tomography (HRCT) of the thorax, pulmonary function test, DLCO, and expert opinions of rheumatology and chest disease departments. Severity of SSC-ILD was classified according to the simplified Goh algorithm [22]. 12 people with no signs of pulmonary, autoimmune, cardiovascular, or other diseases were recruited as healthy controls (HC).

This study was performed compatible with the Declaration of Helsinki and Local Ethical Committee approved the study (Ethical Approval Number: 78). Informed consent was obtained from all participants

Demographic features and clinical and laboratory values were obtained from all patients and controls. Serum samples were collected, and serum was analyzed for HMGB1 autoantibodies by using an enzyme-linked immunosorbent assay (ELISA) kit (Elabscience, E-EL-H1554c, China), levels of an anti-nuclear antibody, SSC-related autoantibodies (anti-centromere, anti-Scl-70) complete blood count, and levels of acute phase proteins were also recorded.

SPSS 22.0 version (Chicago, IL, USA) was used for the statistical analysis of data. Results were expressed as mean ± SD or median and minimum-maximum levels according to the distribution properties of data. The normality of numerical variables was examined with the Kolmogorov-Smirnov test. Because serum HMGB1 levels were not distributed normally, Kruskal-Wallis analysis is used for the comparison of levels between study groups. Post-hoc analysis of subgroups was performed with Mann-Whitney U test. p-values under 0.05 were accepted as statistically significant.

RESULTS

Demographic features and clinical and biochemical data from patients of SSC patients and HC are shown in **Table 1**. There were no significant differences between groups in body mass index, white cell blood count, hemoglobin, and platelet count.

Serum HMGB1 levels and erythrocyte sedimentation rate (ESR) were significantly different between groups ($p=0.017$ and $p=0.020$). The results are shown in **Figure 1**. SSC-ILD active disease patients showed elevated serum HMGB1 levels (14.01 mg/dl [9.96-18.07]) compared with SSC-ILD inactive disease patients (7.87 mg/dl [6.76-9.05]), patients with SSC without ILD (7.82 mg/dl [6.91-8.54]) and HC (8.04 mg/dl [6.76-9.92]) ($p=0.017$).

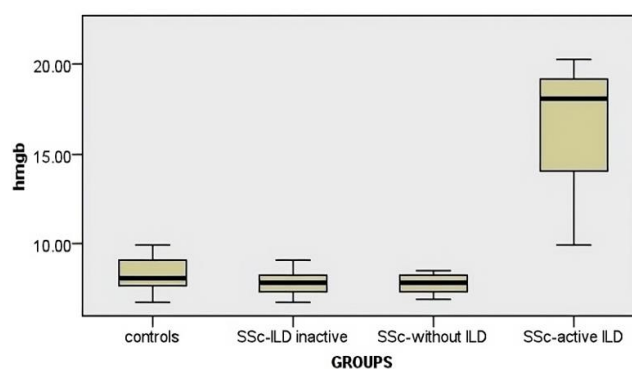


Figure 1. Comparison of serum HMGB-1 levels between study groups (Source: Authors' own elaboration)

Serum HMGB1 levels were elevated in patients with SSC-ILD active disease in comparison to patients with SSC-ILD but have stable pulmonary functions with immunosuppressive treatment (10.00 vs. 4.50 ng/ml, respectively; $p=0.007$). Serum HMGB1 levels were also higher in patients with SSC-ILD active disease than in those with HC (14.00 vs. 6.50 ng/ml, $p=0.032$). However, there were no significant differences between HMGB1 levels of patients with SSC-ILD in remission, patients without lung involvement, and HC (12.44 vs. 12.63, $p=1.000$) and (12.66 vs. 16.96 ng/ml, $p=1.000$).

Serum ESR levels were significantly higher in patients with SSC-ILD inactive disease than in SSC without ILD patients (7.30 vs. 18.40, $p=0.070$) although there were no significant differences between other subgroups.

HMGB1 level was not associated with laboratory variables such as ESH, CRP, white blood cell count, hemoglobin rate, and platelet count. In addition, there was no relationship between HMGB1 levels and the presence of SSC-related autoantibodies (anti-Scl-70 antibodies, $p=0.300$ and anti-centromere antibodies, $p=0.700$). Furthermore, serum HMGB1 levels did not show a significant correlation with pulmonary hypertension, esophageal involvement, digital ulcer, contracture, telangiectasia, myositis, and arthritis.

DISCUSSION

In this study, we investigated the role of serum HMGB1 levels in patients with SSC and SSC-ILD patients. Serum HMGB1 levels were elevated in patients with SSC-ILD compared with SSC without ILD patients and HC. In addition, compared with patients in inactive disease of SSC-ILD, patients in active disease of SSC-ILD showed higher serum HMGB1 levels.

SSC is a fibrotic disorder that is characterized by multisystemic inflammation. ILD is an important cause of both mortality and morbidity. Patients with early disease could be asymptomatic and SSC-ILD symptoms are nonspecific like dyspnea and cough. Moreover, a combination of X-ray and physical examination had sub-optimal sensitivity to detect SSC-ILD [23]. Although HRCT is very sensitive and reliable in detecting ILD, patients with very early SSC-ILD may not be able to access screening [24]. In clinically significant disease at presentation or disease progression in ILD, early and systemic immunosuppressive treatment is necessary as it improves pulmonary functions, lung scores, symptoms as well and quality of life [25]. Therefore it is important to diagnose and screen for SSC-ILD as early diagnosis and treatment may prevent the progression of pulmonary disease and may contribute to reserve pulmonary functions.

Biomarkers are recently needed to evaluate disease diagnosis, severity, prognosis, and the activity of the disease.

HMGB1 is an important non-histone nuclear protein that plays a role in inflammatory and autoimmune diseases. HMGB1 could be translocated to the cytoplasm and extracellular space, during cell death and it plays a proinflammatory role in processes by inducing proinflammatory mediators and cytokines [15]. HMGB1 is an immune biomarker reported to have an important role in the pathogenesis of several autoimmune diseases, especially in connective tissue diseases. The role of HMGB1 in the pathogenesis of RA is demonstrated in the studies showing an increase in extracellular HMGB1 expression in blood, synovial tissue, and synovial fluid [17, 21]. Also, serum HMGB1 levels were higher than osteoarthritis patients in RA patients and HMGB1 levels were correlated with disease activity scores [26-27]. Moreover, HMGB1 is associated with systemic involvements of many autoimmune diseases. It was reported that serum HMGB1 levels may reflect disease activity in SLE patients and may be associated with lupus disease activity [28]. In a study with 61 lupus patients, HMGB1 levels were elevated in active lupus nephritis, and levels were shown associated with LN [29]. HMGB1 expression was reported as a prognostic indicator in polymyositis/dermatomyositis progression for patients with ILD [30]. Ayumi et al. found a positive correlation between serum HMGB1 levels and modified Rodnan total skin thickness score, despite a negative correlation with lung functions. Serum HMGB1 levels were higher in patients with organ involvement. In addition compared with control mice, HMGB1 levels were found to be increased in the peripheral blood of the bleomycin-induced mouse scleroderma model [31]. HMGB1 levels are not only described as a new potential biomarker but also as novel therapeutic targets for the treatment of SSC or SSC-ILD [20]. In our study, SSC-ILD patients were stable for disease activity under immunosuppressive treatment, therefore their serum HMGB1 levels were not increased and there were no significant differences found between them and SSC without ILD patients. These results indicate that serum HMGB1 could be a potential biomarker in predicting SSC-associated lung disease, though ILD is considered to be the cause of mortality. Moreover, as HMGB1 levels were elevated in SSC-ILD active disease patients, HMGB1 could be used for reflecting the disease activity in the lung. Furthermore, HMGB1 was analyzed by an ELISA kit from serum samples, as it could be also an easy way to early prediction of progression and the state of the disease.

ESR is a helpful acute phase marker in monitoring chronic inflammatory conditions [32]. ESR levels are a predictor of ILD and higher ESR levels are associated with the severity of ILD [33]. Also, persistent higher ESR levels are associated with worse prognosis and higher mortality rates despite immunosuppressive treatment [34]. ESR serum levels greater than 15.00 mm/h or hemoglobin levels less than 12.50 g/dl are associated with a -2.5 to 3-fold increase in mortality in patients with SSC. Moreover, a correlation is

documented between higher ESR levels and the severity of ILD [35, 36]. In our study, patients with SSC-ILD active disease have greater ESR levels than SSC without ILD patients and controls

CONCLUSIONS

Our results suggest that serum HMGB1 levels reflect the disease activity in SSC-ILD. HMGB1 could be used as a potential biomarker for detecting active lung disease and also it could be used for reflecting the active disease in IAH patients. Therefore elevated serum HMGB1 levels could be useful in the diagnosis and exacerbation of SSC-IAH. A limitation of this study is that our patient numbers were limited. Therefore further studies with larger sample sizes and heterogeneity are needed for the generalization of our findings. Despite the limitations, our study identified HMGB1 was elevated in patients with active disease of SSC-ILD. Further studies are also needed, and our study was the first to detect active lung disease in SSC-ILD.

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Ethics statement: This study was approved by the Gazi University Local Ethical Committee with ethical approval number 78 on 14 January 2023. Written informed consents were obtained from the participants.

Declaration of interest: No conflict of interest is declared by authors.

Data sharing statement: Data supporting the findings and conclusions are available upon request from the corresponding author.

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