Clinical significance of serum surfactant protein D (SP-D) in patients with polymyositis/dermatomyositis: correlation with interstitial lung disease


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Abstract

Objective. To determine the clinical significance of serum surfactant protein D (SP-D) levels in patients with polymyositis/dermatomyositis (PM/DM).

Methods. Serum SP-D levels were assayed using a sensitive enzyme-linked immunosorbent assay in 59 patients with PM/DM and in 29 healthy controls.

Results. The serum level of SP-D was significantly higher in patients with PM/DM than in healthy controls (mean ± s.d. 61.7 ± 122.6 vs 31.0 ± 12.4 ng/ml, \( P < 0.01 \)). The serum SP-D level in patients with interstitial lung disease (ILD) was significantly higher than in those without ILD (118.7 ± 220.2 vs 38.7 ± 21.0 ng/ml, \( P < 0.001 \)). Serum level of SP-D was correlated with the presence of ILD. The incidences of decreased vital capacity (%VC) and of decreased diffusing capacity of carbon monoxide (%DLCO) were also significantly greater in patients with an elevated SP-D level than in those with a normal level (64 vs 7%, \( P < 0.02 \); 73 vs 27%, \( P < 0.01 \)). Moreover, the serum SP-D level was inversely correlated with %VC (\( r = -0.452, P < 0.01 \)) and %DLCO (\( r = -0.349, P < 0.05 \)).

Conclusion. The serum SP-D level may be a useful marker for ILD in patients with PM/DM.

Key words: Interstitial lung disease, Enzyme-linked immunosorbent assay, Vital capacity.

Polymyositis/dermatomyositis (PM/DM) is an inflammatory condition characterized by subacute skeletal muscle involvement, and typical cutaneous lesions in DM. Interstitial lung disease (ILD) is one of the severe complications in patients with PM/DM and is found in 9–17% of PM/DM patients [1, 2]. The prognosis of those with ILD is poor; approximately 40% of patients with ILD die [1, 2].

Pulmonary surfactant is composed of phospholipids and associated proteins. It covers the alveolar surface and maintains alveolar gas exchange during expiration. There are four distinct proteins in pulmonary surfactant, which are designated surfactant proteins: SP-A, SP-B, SP-C and SP-D [3]. These surfactant proteins play various important roles in the lung, such as maintaining the biophysical activity of the surfactant and regulating surfactant homeostasis in the alveoli [4]. SP-D is a hydrophilic glycoprotein with a reduced molecular mass of 43 kDa. SP-D is produced and secreted by alveolar type II epithelial cells and Clara cells [3].

Recently, the SP-D levels in bronchoalveolar lavage (BAL) fluids and the systemic circulation have been measured in several lung diseases, including idiopathic pulmonary fibrosis [5–7]. It was reported that the SP-D level in BAL fluids was decreased in patients with pulmonary fibrosis, while the serum SP-D level was significantly elevated. This may be because the increased permeability of SP-D results in an increase in alveolar-to-vascular leakage of SP-D in these patients [6]. Increased serum SP-D levels were detected in patients with idiopathic pulmonary fibrosis, interstitial pneumonia with collagen diseases and pulmonary alveolar proteinosis [6, 7]. In contrast, the serum SP-D levels in patients with bacterial pneumonia were not elevated [6]. Moreover, serum SP-D levels reflected the disease activity of pulmonary fibrosis. However, previous studies have included only small numbers of PM/DM patients [6, 7]. The mechanism of serum SP-D elevation is unknown, but probably involves the combination of type II cell hyperplasia with a concomitant increase...
in the synthesis of surfactant proteins and spillover into the circulation.

SP-B and SP-C are extremely hydrophobic proteins that are rarely detected in serum. SP-D gets into the bloodstream from the alveoli more easily than SP-A, resulting in higher levels of SP-A. Because SP-D exhibited a wider range of serum concentration and was detected in patients with pulmonary fibrosis more than SP-A, SP-D is thought to be a better diagnostic marker than SP-A [6].

In this study, we investigated the serum SP-D levels in patients with PM/DM and assessed the relationships among serum SP-D level and clinical features in these patients.

Patients and methods

Patients

Serum samples were obtained from 59 Japanese patients diagnosed as having PM/DM according to the criteria of Bohan and Peter [8] or having amyopathic DM diagnosed by the clinical appearance and histology of skin biopsies (12 men and 47 women). Approval was obtained from the Tokyo University Hospital, and all subjects gave informed consent to participation in the study. At the time of serum sampling, none of the patients had received immunosuppressant agents, including prednisolone, azathioprine and methotrexate, to control myositis or lung fibrosis. There were three subgroups of PM/DM, defined by the same criteria [8]: 46 patients with primary idiopathic DM, six patients with idiopathic PM, and seven patients with DM associated with neoplasia. There were no PM/DM patients who had other collagen diseases, including systemic lupus erythematosus, systemic sclerosis and rheumatoid arthritis. The PM/DM patients who had other collagen diseases were excluded because the patients with these collagen diseases were shown to have increased serum SP-D levels [9]. Of the seven patients with neoplasia, two had lung carcinomas, two had ovarian carcinomas, two had mammary carcinomas, and one had laryngeal carcinoma. In this study, no PM/DM patients with internal malignancy showed ILD. Electromyographic examination and skin and muscle biopsies were performed at the time of diagnosis in all patients. Clinical and laboratory data, including serum creatine kinase (CK) levels, the erythrocyte sedimentation rate (ESR) and respiratory function data, were also obtained at the time of serum sampling. The diagnosis of ILD was based on the findings of chest radiography, computed tomography of the chest, lung function tests and the diffusing capacity of carbon monoxide (%DLCO). No other active lung diseases, including asthma, were found at the time of serum sampling in the PM/DM patients. Control serum samples were also obtained from 29 healthy volunteers. There were no significant differences in age and gender distributions between the healthy volunteers and the PM/DM patients.

Enzyme-linked immunosorbent assay (ELISA) for SP-D

Aliquots of serum were frozen at −20°C until assayed. Specific ELISA kits were used for the measurement of SP-D according to the manufacturer’s instructions (Yamasa, Tokyo, Japan) [5]. In brief, polystyrene cups coated with SP-D antibodies were incubated with 20 μl of 11-fold diluted serum at 4°C for 24 h. The cups were then washed and incubated at 25°C for 2 h with 100 μl of 111-fold diluted horseradish peroxidase conjugated anti-SP-D antibody. The cups were washed again and 100 μl of tetramethylbenzene was added. Incubation was then performed at 25°C for 15 min. Finally, 2 mmol/l NaNO₃ was added to terminate the peroxidase reaction and the absorbance at 450 nm was measured. Serum SP-D levels more than 2 S.D. greater than the mean level in the normal controls were regarded as elevated.

Antinuclear or anticytoplasmic antibodies

Antinuclear or anticytoplasmic antibodies were detected by the indirect immunofluorescence method using HEP-2 cells as the substrate [9].

Statistical analysis

Statistical analysis was carried out with the Mann–Whitney test for the comparison of means and Fisher’s exact probability test for the analysis of frequency. Correlations with clinical data were assessed using Spearman’s rank correlation coefficient. Two-tailed P values less than 0.05 were considered significant.

Results

Serum SP-D levels

Serum SP-D levels in samples obtained from the patients with PM/DM and healthy control subjects are shown in Fig. 1. No correlation was found between serum SP-D level and gender, age, or body weight in normal control subjects. There was no correlation between smoking and serum SP-D level. Compared with the level in the control subjects (mean ± S.D., 31.0 ± 12.4 ng/ml), that in the PM/DM patients was significantly elevated (61.7 ± 122.6 ng/ml; P < 0.01). Among the patients with PM/DM, those with ILD had a significantly higher serum SP-D level (118.7 ± 220.2 ng/ml) than those without ILD (38.7 ± 21.0 ng/ml; P < 0.001). The cut-off value (mean ± 2 S.D.) was set at 56.9 ng/ml on the basis of the data for the 29 healthy control sera. As shown in Fig. 1, elevated serum levels of SP-D were found in 11 of 59 (18.6%) patients with PM/DM. The serum SP-D level was elevated in eight of 46 (17%) idiopathic DM patients, three of six (50%) idiopathic PM patients, and none of seven PM/DM patients with neoplasia. There were no significant correlations between the serum SP-D level and the subsets of PM/DM patients.
Elevated serum SP-D levels were found in 11 of 59 (18.6%) patients without ILD. Those with ILD had significantly higher serum SP-D levels than those without (118.7 ± 22.6 vs 31.0 ± 12.4 ng/mL, \( P < 0.001 \)). Elevated serum SP-D levels were found in 11 of 59 (18.6%) patients with PM/DM. Elevated serum SP-D levels were found in eight of 11 PM/DM patients with ILD and three of 40 without ILD.

**Correlation of serum SP-D level with clinical and serological features of patients with PM/DM**

The clinical and serological features of the patients with elevated or normal levels of SP-D are shown in Table 1. There was no significant difference between these groups in terms of sex or age. The percentage of individuals with Gottron’s papules was significantly lower among patients with an elevated SP-D level than among those with a normal level (18% vs 63%, \( P < 0.01 \)). The percentage of individuals with ILD was significantly greater among patients with an elevated SP-D level than among those with a normal level (73% vs 24%, \( P < 0.01 \)). The incidences of decreased %DLCO and decreased vital capacity (%VC) were also significantly higher in patients with an elevated SP-D level than in those with a normal level (73 vs 27%, \( P < 0.01 \); 64 vs 7%, \( P < 0.02 \)). The patients with an elevated SP-D level had significantly lower %DLCO values than those with a normal level (mean ± s.d., 55.5 ± 30.1 vs 83.4 ± 20.9%, \( P < 0.01 \)).

**Longitudinal measurements of serum SP-D levels**

In 15 patients, the serum SP-D level was examined longitudinally over a period of 1–2 yr. The activity of ILD was evaluated on the basis of subjective symptoms (e.g., cough) and objective signs, including chest radiographs, computed tomography and pulmonary function tests. The cases were divided into four groups as described [9, 11]: those in whom ILD became exacerbated, those in whom ILD improved, those with unchanged ILD and those without ILD (Fig. 2).

In three of the four patients in the exacerbated ILD group, the exacerbation of ILD was accompanied by a definite increase in serum SP-D level. Moreover, all patients in this group showed an elevated serum SP-D level at the time of the second examination. In a patient whose ILD improved, the serum SP-D level decreased from elevated to normal. In two patients with unchanged ILD, the serum SP-D level was elevated at both points. In seven of the eight patients without ILD,

![Fig. 1. Serum levels of SP-D. The horizontal line represents the cut-off value (mean ± 2 s.d.), which was set at 56.9 ng/mL on the basis of the data for the 29 healthy control subjects. The patients with PM/DM had significantly higher serum SP-D levels than the control subjects (mean ± s.d. 61.7 ± 122.6 vs 31.0 ± 12.4 ng/mL, \( P < 0.01 \)). Of the patients with PM/DM, those with ILD had significantly higher serum SP-D levels than those without (118.7 ± 220.2 vs 38.7 ± 21.0 ng/mL, \( P < 0.001 \)). Elevated serum SP-D levels were found in 11 of 59 (18.6%) patients with PM/DM. Elevated serum SP-D levels were found in eight of 11 PM/DM patients with ILD and three of 40 without ILD.](https://academic.oup.com/rheumatology/article-abstract/41/11/1268/1787950/1270)
the serum SP-D level was within normal levels at both points.

Discussion
ILD is one of the most severe complications in PM/DM, and often carries a poor prognosis in spite of various therapies. The therapy for PM/DM differs widely depending on the existence or activity of ILD. Therefore, it is important to evaluate the existence and severity of ILD when treating patients with PM/DM [1, 2]. In general, chest radiography, lung function testing and blood gas analysis are usually used in the management of ILD in patients with PM/DM. However, these markers can be influenced by other factors, including bacterial and protozoan infections and muscular involvement.

In the present study, the serum SP-D level of PM/DM patients with ILD was significantly elevated compared with those without ILD. The incidences of decreased %DLCO and decreased %VC were significantly greater in patients with an elevated SP-D level than in those with a normal level. Furthermore, the serum SP-D level was inversely correlated with %VC and %DLCO. These results suggest that the serum SP-D level is a serum marker for ILD and reflects the severity of ILD in patients with PM/DM. In this study, the diagnosis of ILD was made on the basis of the findings of chest radiography, computed tomography of the chest, lung function tests and %DLCO. However, there may be patients with minor respiratory crepitations, probably due to fibrosis, who do not have abnormal values in these examinations. Further longitudinal studies of the serum SP-D level in these patients are needed to clarify the clinical significance of the serum SP-D level.

Longitudinal measurements of the serum SP-D level showed that the exacerbation of ILD was accompanied by definite increases in serum SP-D. In a patient whose ILD improved, the serum SP-D level decreased from elevated to normal. In two patients with unchanged ILD, the serum SP-D level was elevated at both points. In seven of the eight patients without ILD, the serum SP-D level was within normal limits at both points. All of these patients were treated with oral prednisolone. In particular, a patient whose ILD improved received high doses of prednisolone. Glucocorticoid treatment increased the concentrations of SP-D mRNA and protein in cultured lung fibroblasts [13]. This indicates...
that the serum SP-D level reflects the severity of ILD rather than the effect of treatment with glucocorticoid in patients with PM/DM.

Significantly fewer individuals had Gottron’s papules among the patients with an elevated SP-D level than in those with a normal level. This may be due to the fact that PM patients had ILD more frequently than DM patients in our study. In this study, four of the six PM patients (67%) and 17 of the 53 DM patients (32%) had ILD.

Recently, we showed that the serum level of KL-6, a MUC 1 mucin expressed on alveolar type II cells and also on epithelial cells in other organs, was significantly elevated in PM/DM patients with ILD compared with those without ILD, and was inversely correlated with %DLCO and %VC [13]. The sensitivity of serum KL-6 levels for ILD in patients with PM/DM was 100%. However, the specificity of serum KL-6 levels for ILD in PM/DM patients was 56%, because some patients in whom DM was associated with neoplasia had an elevated serum SP-D level. In this study, none of the PM/DM patients with neoplasia had an elevated serum SP-D level and the specificity of serum SP-D level was 93%. These results, including those of our previous study, showed that SP-D may serve as a specific marker for evaluating ILD in patients with PM/DM.

In conclusion, we have demonstrated the elevation of serum SP-D level in PM/DM patients with ILD. SP-D may be a useful marker for evaluating ILD in patients with PM/DM. However, this study was undertaken with a small patient population. Further studies are needed to clarify the usefulness of SP-D in PM/DM patients.

Acknowledgements

This study was supported in part by a grant for scientific research from the Japanese Ministry of Education (10770391) and by project research for progressive systemic sclerosis from the Japanese Ministry of Health and Welfare.

References