Serum KL-6 in adult patients with polymyositis and dermatomyositis

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Abstract

Objective. To determine the serum levels of KL-6, a mucin-like high-molecular-weight glycoprotein, in polymyositis/dermatomyositis (PM/DM) patients.

Methods. Serum samples from 42 adult PM/DM patients and 38 healthy control subjects were examined using specific enzyme-linked immunosorbent assay (ELISA) systems.

Results. The serum levels of KL-6 in the PM/DM patients were significantly higher than those of the healthy controls. The elevated serum KL-6 levels correlated with the presence of interstitial lung disease (ILD) and decreased percentage diffusing capacity of carbon monoxide (%DLco) in the PM/DM patients. The serum KL-6 levels inversely correlated with %DLco and percentage vital capacity. Additionally, the serum KL-6 levels were elevated in five of the seven DM patients with malignant neoplasia.

Conclusions. These results suggest that the serum KL-6 level might be a useful serum marker for ILD and internal malignancy in PM/DM.

KL-6 is a mucin-like high-molecular-weight glycoprotein and is classified as ‘Cluster 9 (MUC1)’ of lung tumour and differentiation antigens according to the findings of immunohistochemical and flow cytometry studies [1–3]. The molecule consists of multiple heterogeneous sub-molecules. KL-6 is detected by a murine monoclonal antibody which recognizes a sialylated sugar chain on the molecule. It is expressed on alveolar type II pneumocytes and bronchiolar epithelial cells in normal human lungs, pyloric gland cells in the stomach, epithelial cells in mammary glands and ductal epithelial cells in the pancreas.

In fibrosing lung diseases, type II pneumocytes are regenerated and express KL-6 strongly on the surface [4]. This has also been detected in alveolar macrophages by immunohistochemistry [5]. Serum KL-6 levels have been reported to be elevated in various internal malignancies and benign pulmonary diseases, including interstitial lung disease (ILD), hypersensitivity pneumonitis, radiation pneumonia, pulmonary tuberculosis, lung cancer and pulmonary sarcoidosis [2, 5–10].

Polymyositis/dermatomyositis (PM/DM) is an uncommon inflammatory condition involving skeletal muscles. There is skin involvement in DM. ILD is one of the severe complications in adult PM/DM patients found in 9–17% of PM/DM patients [11, 12], and the prognosis of those with ILD is quite poor so that approximately 40% of patients with ILD die [11, 12]. ILD is more commonly found in adult PM/DM than in juvenile PM/DM [12].

In addition, adult PM/DM is associated with various malignancies, which increase the mortality [12–14]. Monoclonal antibodies to KL-6 were previously reported to react strongly to many kinds of malignant tissues, including lung cancer, and the serum levels of KL-6 are elevated in patients with malignant diseases [2].

In the present study, we investigated the serum concentrations of KL-6 in patients with adult PM/DM to clarify the value of KL-6 in the detection of the severe complication of ILD and associated malignancy.

Materials and methods

Patients

Serum samples were collected from 42 Japanese patients definitively or probably diagnosed as having PM/DM according to the criteria of Bohan and Peter [15] or having amyopathic DM diagnosed by clinical appearance and histology of skin biopsy (11 men and 31 women; age range, 25–72 yr; mean age, 51 yr). All patients had not received immunosuppressant agents, including prednisolone, azathioprine and methotrexate to control myositis or lung fibrosis at the time of serum sampling. There were three subgroups of PM/DM...
patients, as defined by the same criteria [15]: primary idiopathic DM (30 patients), idiopathic PM (five patients), and DM associated with neoplasia (seven patients). There were no PM/DM patients who had other collagen diseases, including systemic lupus erythematosus, systemic sclerosis or rheumatoid arthritis. Of the seven patients with DM accompanied by neoplasia, two had ovarian carcinoma, two had mammary carcinoma, two had lung carcinoma, and one had laryngeal carcinoma. Five of the seven patients died of metastasis of the cancer. Seventeen of the 35 patients with adult PM/DM had ILD, while it was absent in all seven patients with internal malignancy. 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, erythrocyte sedimentation rate (ESR) and respiratory function tests, were also obtained at the time of serum sampling. Diagnosis of ILD was made based on the findings of chest radiography, chest computed tomography, lung function tests and diffusioning capacity of carbon monoxide (DLCO) with careful ruling out of differential diagnoses including pneumoeytis carinii pneumonia and tuberculosis by sputum cultures and bronchoalveolar lavage findings. No other active lung diseases, including tuberculosis or carinii pneumonia, were found at the time of serum sampling among the PM/DM patients. Furthermore, no patients were treated with drugs that could induce interstitial pneumonitis or have lung toxicity, including bleomycin hydrochloride, popleomycin sulphate and methotrexate, and were not exposed to silica in their disease courses before serum sampling. Control serum samples were also obtained from 38 healthy volunteers (18 men and 20 women; age range, 24–70 yr; mean, 43 yr). There were no significant differences in age and gender distributions between the healthy volunteers and the PM/DM patients. All serum samples were stored at −80°C prior to use.

In 10 patients with PM/DM, we collected serial serum samples to determine the changes in serum KL-6 levels in the course of treatments such as corticosteroid therapy, immunosuppressant therapy, or surgical resection of internal malignancies.

Measurement of serum KL-6 levels
The serum levels of KL-6 were determined using sandwich enzyme-linked immunosorbent assay (ELISA) kits using KL-6 antibody, obtained from Eisai Co. Ltd (Tokyo, Japan), according to the manufacturer’s protocol. Briefly, polystyrene cups coated with KL-6 antibody were incubated with 120 μl of 1200-fold diluted serum at 25°C for 2 h. Then the cups were washed with 0.9% NaCl and incubated at 4°C for 1 h with 100 μl of diluted horseradish peroxidase-conjugated KL-6 antibody. The cups were then washed again, 100 μl of ABTS solution (2,2’-azino-bis 3 ethyl-benz-thiazoline-6 sulphonic acid) and 0.02% H₂O₂ were added, and incubated at 25°C for 30 min. Finally, 100 μl of stop solution (0.013% sodium azide) was added to inhibit the peroxidase reaction. The absorbance at 405 nm was measured.

The intra- and interassay coefficients of variation of the kit were under 10%.

Antinuclear or anticytoplasmic antibodies
Antinuclear or anticytoplasmic antibodies were detected by the indirect immunofluorescence method using human laryngeal tumour (HEP-2) cells as the substrate [16]. The antinuclear or anticytoplasmic antibodies of each serum sample were defined as positive when fluorescence was found in the serum diluted at 1:250.

Specific autoantibodies to PM/DM
RNA immunoprecipitation, 35S protein immunoprecipitation or double immunodiffusion as previously described identified specific autoantibodies to PM/DM, including antibodies to anti-tRNA synthetase, antibodies to signal recognition particles, and anti-Ku antibodies and anti-Mi-2 antibodies [17].

Statistics
Values more than twice the standard deviation (s.d.) higher than the mean of control values were considered elevated. The Mann–Whitney U-test assessed the significance of differences and the comparisons of patients’ subgroups were assessed by the χ²-test. Spearman’s rank correlation coefficient assessed correlations with clinical data. Two-tailed P values less than 0.05 were considered significant. Data are shown as mean ± standard error (s.e.).

Results
Serum levels of KL-6
The serum KL-6 levels were significantly higher in the PM/DM patients than in the healthy controls (1183 ± 310 vs 383 ± 9 U/ml; P < 0.01) (Fig. 1). No correlation was found between serum KL-6 levels and gender or age distribution in normal control subjects. The cut-off value (mean + 2 s.d.) was set at 493 U/ml, based on the data from the 38 healthy control sera. Elevated serum KL-6 levels were found in 28 PM/DM patients (67%). Serum KL-6 levels were elevated in 20 of the 30 idiopathic DM patients (67%), three of the five patients with idiopathic PM (60%), and five of the seven PM/DM patients with neoplasia (71%). There were no significant correlations between the serum KL-6 levels and the subsets of PM/DM patients. Elevated serum KL-6 levels were found in all 17 idiopathic PM/DM patients with ILD, and six of 18 without ILD. The sensitivity of serum KL-6 levels for ILD in patients with adult idiopathic PM/DM was 100% and that for internal malignancy was 71%. The specificity of serum KL-6 levels for internal malignancy or ILD was 67%.

Changes in serum KL-6 levels by therapies
In three cases of DM associated with malignancy, the serum KL-6 levels were decreased slightly after surgical resection (cases 1 and 2 in Table 1). On the other hand,
In PM/DM patients with ILD, no significant changes in serum KL-6 levels were observed in cases 4–6 in Table 1). No improvement of the ILD was observed in patients with DM with ILD in spite of immunosuppressive therapies.

In PM/DM patients without ILD or internal malignancy, no remarkable changes in serum KL-6 levels were found in spite of immunosuppressive therapies for PM/DM (cases 7–10 in Table 1).

Correlation of serum KL-6 levels with clinical features and laboratory findings in PM/DM patients

We compared the clinical and laboratory findings of the adult PM/DM patients with their serum KL-6 levels (Table 2). No significant differences were found in gender distribution between the patients with elevated serum KL-6 levels and those with normal levels. No correlation was found between age and serum KL-6 levels.

Patients with internal malignancy were excluded from the following analyses between serum KL-6 levels and clinical findings because serum levels of KL-6 were previously reported to be elevated in patients with various kinds of internal malignancies [2]. We found significant correlation between the elevated levels of serum KL-6 and the presence of interstitial pneumonia in patients with idiopathic PM/DM (P < 0.001) by $\chi^2$ analysis. As for variables associated with interstitial pneumonia, elevated serum KL-6 levels showed a significant correlation with decreased %DLco ($P < 0.001$) by $\chi^2$ analysis. However, no significant correlations were found between elevated serum KL-6 levels and decreased percentage vital capacity (%VC), probably because %VC decreases only in cases with progressed ILD. Significant correlations were found between serum KL-6 levels and %DLco ($P < 0.05$) or %VC ($P < 0.01$) by Spearman’s rank correlation coefficient. There were no correlations between elevated serum KL-6 levels and other clinical features or laboratory findings, including history of smoking, duration of the disease, skin rashes, muscle weakness, serum CK activity or the presence of antinuclear or anticytoplasmic antibodies. Although the serum KL-6 levels of all six patients with anti-tRNA synthetase antibodies including anti-Jo-1 antibodies, anti-EJ antibodies and anti-PL-12 antibodies were elevated, we could not find the significance because of the low prevalence of the antibodies especially in DM patients [18].

Discussion

ILD is one of the most severe complications in PM/DM, and often induces poor prognosis in spite of therapies. The initial therapy for PM/DM differs widely depending on the existence or activity of ILD because of its poor prognosis. Thus, it is quite important to evaluate the existence and the severity of interstitial pneumonia in treating patients with PM/DM [11, 12]. 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 often be influenced by other factors, including bacterial or protozoan infections, liver damage by drugs or autoimmune hepatitis and muscular involvement. Thus, these variables are not specific, and their range is relatively narrow.

Serum levels of KL-6 were previously reported to be
Table 2. Clinical and laboratory findings of the adult PM/DM patients without internal malignancy (n = 35) with elevated or normal serum KL-6 levels

<table>
<thead>
<tr>
<th>No. (%) with</th>
<th>Patients with elevated KL-6 level (n = 23)</th>
<th>Patients with normal KL-6 level (n = 12)</th>
<th>$\chi^2$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/female (n)</td>
<td>8/15</td>
<td>2/10</td>
<td>1.26</td>
</tr>
<tr>
<td>Age (yr; mean ± s.d.)</td>
<td>49 ± 12</td>
<td>52 ± 6</td>
<td>0.13</td>
</tr>
<tr>
<td>Disease duration (months; mean ± s.d.)</td>
<td>5 ± 3</td>
<td>4 ± 4</td>
<td>1.53</td>
</tr>
<tr>
<td>Proximal muscle weakness</td>
<td>18 (78)</td>
<td>10 (83)</td>
<td>0.09</td>
</tr>
<tr>
<td>Dysphagia</td>
<td>1 (4)</td>
<td>2 (17)</td>
<td>0.14</td>
</tr>
<tr>
<td>Skin eruption</td>
<td>20 (87)</td>
<td>10 (83)</td>
<td>0.70</td>
</tr>
<tr>
<td>Gottron’s papules</td>
<td>10 (43)</td>
<td>6 (50)</td>
<td>0.68</td>
</tr>
<tr>
<td>Heliotropic coloration</td>
<td>10 (43)</td>
<td>7 (58)</td>
<td>0.68</td>
</tr>
<tr>
<td>Joint involvement</td>
<td>11 (48)</td>
<td>4 (33)</td>
<td>0.00</td>
</tr>
<tr>
<td>Heart involvement</td>
<td>2 (9)</td>
<td>1 (8)</td>
<td>1.26</td>
</tr>
<tr>
<td>Lung fibrosis</td>
<td>17 (74)</td>
<td>0 (0)</td>
<td>17.25*</td>
</tr>
<tr>
<td>Decreased %VC</td>
<td>6 (26)</td>
<td>0 (0)</td>
<td>3.78</td>
</tr>
<tr>
<td>Decreased %DLco</td>
<td>17 (74)</td>
<td>1 (8)</td>
<td>13.58*</td>
</tr>
<tr>
<td>Positive ANA</td>
<td>12 (52)</td>
<td>1 (8)</td>
<td>4.69</td>
</tr>
<tr>
<td>Positive ACyA</td>
<td>15 (65)</td>
<td>6 (50)</td>
<td>0.76</td>
</tr>
<tr>
<td>Anti-rRNA synthetase antibodies</td>
<td>6 (26)</td>
<td>0 (0)</td>
<td>3.78</td>
</tr>
<tr>
<td>Anti-Jo-1 antibodies</td>
<td>3 (13)</td>
<td>0 (0)</td>
<td>1.71</td>
</tr>
<tr>
<td>Anti-EJ antibodies</td>
<td>2 (9)</td>
<td>0 (0)</td>
<td>1.11</td>
</tr>
<tr>
<td>Anti-PL12 antibodies</td>
<td>1 (4)</td>
<td>0 (0)</td>
<td>0.53</td>
</tr>
<tr>
<td>Anti-SRP antibodies</td>
<td>0 (0)</td>
<td>1 (8)</td>
<td>1.97</td>
</tr>
<tr>
<td>Elevated ESR</td>
<td>13 (57)</td>
<td>7 (58)</td>
<td>0.01</td>
</tr>
<tr>
<td>Elevated CK</td>
<td>16 (70)</td>
<td>11 (92)</td>
<td>2.18</td>
</tr>
</tbody>
</table>

ANA, antinuclear antibodies; ACyA, anticytoplasmic antibodies; SRP, signal recognition particles.

* $P < 0.01$ by $\chi^2$ analysis.

elevated in ILD including hypersensitivity pneumonia, pneumonitis related to collagen diseases and idiopathic interstitial pneumonia [19]. This report showed the elevation of serum KL-6 levels especially in patients with active ILD in comparison with patients with inactive ILD. Furthermore, the serum KL-6 levels in patients with active ILD were reported to be elevated remarkably in comparison with patients with other benign lung diseases including sarcoidosis or tuberculosis. However, there was little information about the status of the patients in the report. In this study, we compared serum KL-6 levels between 17 PM/DM patients with ILD and 18 PM/DM patients without ILD. The strong correlation between ILD and elevated serum KL-6 levels was found. Furthermore, there was a significant inverse correlation between serum KL-6 levels and %VC or %DLco. These results suggest that serum KL-6 level might be a useful marker for ILD in patients with PM/DM.

Internal malignancy is another critical complication associated with adult PM/DM [12–14]. KL-6 was reported to be strongly expressed in various kinds of malignant tissue, including lung adenocarcinoma, lung squamous cell carcinoma, oesophageal squamous cell carcinoma, bile ductal adenocarcinoma and pancreas adenocarcinoma [2]. Furthermore, serum levels of KL-6 were reported to be remarkably elevated in 30–80% of patients with these internal malignancies [2]. The serum levels of KL-6 in patients with internal malignancy were previously reported to be remarkably higher than in patients with benign lung diseases, including lung tuberculosis and sarcoidosis, and it was suggested that serum KL-6 levels were useful in the differential diagnosis between benign lung diseases and lung carcinoma [2].

In this study, we measured serum KL-6 levels in the seven PM/DM patients with internal malignancy. Although interstitial pneumonia was not detected in all these patients, the serum KL-6 levels of five patients were elevated. Additionally, remarkable elevation (11 300 U/ml) of the serum KL-6 level was found in a patient with ovarian carcinoma without lung metastasis or interstitial pneumonia. The elevation of serum KL-6 levels in PM/DM patients with various kinds of internal malignancy may suggest that KL-6 could be a relatively non-specific tumour marker for various kinds of internal malignancy.

There were no significant changes in serum KL-6 levels in longitudinal analysis, probably because of the small number of cases with successive serum collection. Further longitudinal studies of serum KL-6 levels before and after the beginning of lung fibrosis in patients with PM/DM are required to confirm the significance of serum KL-6 levels.

In summary, the serum KL-6 levels were measured in patients with PM/DM, and the results suggested that KL-6 might be useful in PM/DM patients as an indication of an internal malignancy or the presence of ILD.

Acknowledgement

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References