

Clinical significance of serum matrix metalloproteinase-13 levels in patients with localized scleroderma

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Abstract

Objectives

To investigate the clinical significance of serum matrix metalloproteinase-13 (MMP-13) levels in patients with localized scleroderma (LSc).

Methods

Serum MMP-13 levels were determined by using a peptide substrate cleavage assay in 10 patients with generalized morphea, 10 with linear scleroderma, 10 with morphea, and 10 normal controls.

Results

The serum MMP-13 levels in patients with LSc were lower than those in normal controls, but there was no significant difference (64.9 ± 19.9 versus 73.2 ± 11.5 , $p = 0.058$). Serum MMP-13 levels in patients with generalized morphea were significantly lower than those in normal controls (54.0 ± 18.7 versus 73.2 ± 11.5 ng/ml; $p < 0.01$). Serum levels of MMP-13 were comparable among normal controls, the patients with linear scleroderma, and those with morphea. The prevalence of muscle involvement was significantly greater in the LSc patients with decreased MMP-13 levels compared with those with normal MMP-13 levels (50% versus 8%, $p < 0.05$). Serum MMP-13 levels were significantly inversely correlated with the number of linear lesions ($r = 0.366$, $p < 0.05$) and the number of involved body areas ($r = 0.552$, $p < 0.005$) in patients with LSc, while there was no significant correlation between serum MMP-13 levels and the number of plaque lesions. Furthermore, there was significant inverse correlation between serum MMP-13 levels and the number of involved body areas in patients with generalized morphea ($r = 0.631$, $p < 0.05$).

Conclusions

The serum MMP-13 levels may reflect the disease severity in patients with LSc, especially generalized morphea, the severest form of this disorder.

Key words

Localized scleroderma, matrix metalloproteinase-13, systemic sclerosis, skeletal muscle.

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Introduction

Localized scleroderma (LSc) is a connective tissue disorder limited to the skin and subcutaneous tissue, often involving the muscular tissues beneath the cutaneous lesions. The absence of Raynaud's phenomenon, of acrosclerosis, and of involvement of internal organs differentiates it from systemic sclerosis (SSc) (1). Therefore, the prognosis for patients with LSc is good; however, the disfigurement and deformities of the extremities and face resulting from deep fibrosis markedly impair the quality of life. Morphologically, localized scleroderma is classified into three subsets: morphea, linear scleroderma, and generalized morphea (2). Morphea is usually characterized by one or a few circumscribed sclerotic plaques with an ivory-colored center and a surrounding violaceous halo. Linear scleroderma (LS) appears in a linear, band-like distribution, and often involves the muscle and bone underlying the skin lesions. Generalized morphea (GM) is the severest form of localized scleroderma characterized by widespread skin involvement with multiple lesions. SSc and LSc may share similar pathogenetic processes, since abnormal collagen metabolism (3-6) and autoimmunity (7, 8) are considered to be fundamental characteristics of both. Elevated collagen synthesis by skin fibroblasts derived from involved lesions is one of the common characteristics of the 2 conditions and may be closely related to their pathogenesis.

The fibrosis in this disorder is characterized by uncontrolled excessive deposition of type I collagen, a major component of extracellular matrix (ECM), which is thought to be attributed to an imbalance between production and degradation of the protein. Such process is achieved by coordinating the expression of several functionally distinct but biologically related gene products. In fact, LSc fibroblasts produce excessive amount of type I collagen (9). In addition, LSc fibroblasts show a marked decrease in matrix metalloproteinase (MMP)-1 production (10), while the expression of tissue inhibitor of metalloproteinase-3 is increased (11).

MMPs are important breakdown enzymes of various ECM components. MMPs can be divided into subgroups, which include collagenases, stromelysins, stromelysins-like MMPs, gelatinases, membrane-type MMPs, and others. MMP-13 is a member of the collagenase family, which degrades fibrillar collagens of types I, II, III, IV, X, and XIV, tenascin, fibronectin, aggrecan, versican, and fibrillin-1 (12-14). It is now accepted that MMP-13 plays a key role in the MMP activation cascade, both activating and being activated by several MMPs. The expression of MMP-13 has been well-studied in cancer and arthritic diseases. Elevated MMP-13 expression has been documented in numerous malignancies, including adenocarcinoma, squamous cell carcinoma, and basal cell carcinoma, and its association with tumor behavior and patient prognosis has been reported (12,15-19). Significant expression of MMP-13 is observed in highly invasive tumors, suggesting that MMP-13 likely plays a role in regulating tumor invasion, which needs remodeling of ECM. In rheumatoid arthritis (RA), MMP-13 is of special interest for the pathogenesis because it cleaves type II collagen of hyaline cartilage more efficiently than two other human collagenases, interstitial collagenase (MMP-1) and neutrophil collagenase (MMP-8) (20). Significant expression of MMP-13 is demonstrated by immunohistochemistry and in situ hybridization in fibroblast-like cells of the synovial membrane in RA (20, 21). Furthermore, MMP-13 production and activity in synovial fluid and serum are significantly elevated in patients with RA (22). In SSc, we recently investigated the serum levels of MMP-13 and demonstrated that MMP-13 may be involved in the fibrotic process, especially in the initiation of fibrosis, and the serum MMP-13 levels may serve as a useful marker for the severity of PF (23).

The purpose of this study is to investigate the serum levels of MMP-13 in patients with LSc and assess whether these levels can serve as a useful marker for any clinical symptoms in this disease.

Materials and methods

Patients

Serum samples were obtained from 30 Japanese patients with LSc (6 men and 24 women; age 1-65 years, mean $\pm$ SD 29.0 ± 18.6 years). Patients were classified into the following 3 subgroups: 10 patients with GM, 10 with LS, and 10 with morphea as described previously (2). The mean disease duration was 3.9 years (range 0-20 years). None of the patients with LSc received any treatment, including oral steroids or immunosuppressive drugs, when the serum samples were obtained. The number of sclerotic lesions more than 3 cm in diameter was counted in each patient with localized scleroderma when the serum samples were obtained. The sclerotic lesions were morphologically classified into plaque and linear lesions. We divided the whole body into the following seven areas: head and neck; right upper extremity; left upper extremity; anterior trunk; posterior trunk; right lower extremity; and left lower extremity. Then we counted the number of involved areas as described previously (24). As normal controls, 10 serum samples were also obtained from healthy controls. Informed consent was obtained from all subjects. Aliquots of sera were frozen at -80°C until assayed. The protocol was approved by Graduate School of Medicine, University of Tokyo and The University of Tokyo Hospital.

Measurement of serum MMP-13 levels

Aliquots of serum were frozen at -80°C until assayed. According to the manufacturer's instructions, specific kits were used for the measurement of serum MMP-13 levels (Amersham pharmacia biotech) (23). In brief, polystyrene cups coated with F(ab')_2 goat anti-mouse were incubated with mouse anti-MMP-13 antibodies at 37°C for 2 hours and subsequently incubated with $100\ \mu\text{l}$ of 10-fold diluted serum at 4°C for 24 hours. Then, the cups were washed and incubated at 37°C for 1 hour with $50\ \mu\text{l}$ of 0.5mM p-aminophenylmercuric acetate solution, which changes pro MMP-13 into its active form. Next, the detection reagent,

which includes a pro detection enzyme and a specific chromogenic peptide substrate, was added and the absorbance at 405 nm was immediately measured. Then, the cups were incubated at 37°C for 2 hours and the absorbance at 405 nm was measured again. A standard curve was generated by plotting δ Absorbance₄₀₅ (y axis) against ng/ml standard (x axis) and serum MMP-13 levels were calculated from δ Absorbance₄₀₅ of each sample using this standard curve. Serum MMP-13 levels more than 2SD lower than the mean level in the normal controls were regarded as decreased.

Detection of antinuclear antibody, anti-histone antibody, anti-ssDNA antibody, and rheumatoid factor

Antinuclear antibodies (ANA) were detected by indirect immunofluorescence using HEp-2 cells as the substrate, as described previously (25). Antihistone antibodies (AHA) and anti-ssDNA antibodies were measured with ELISAs, as described previously (26, 27). Absorbance values exceeding the mean plus 2 SD for the normal control subjects were considered positive. Rheumatoid factor was measured using a latex agglutination slide test (Eiken, Tokyo, Japan), according to the manufacturer's protocol. Rheumatoid factor was considered to be present when agglutination of latex beads was observed.

Statistical analysis

Statistical analysis was carried out with a Student's t-test for the comparison of means, and Fisher's exact probability test for the analysis of frequency. Correlations with clinical data were assessed by Spearman's rank correlation coefficient. Statistical significance was defined as a P value of less than 0.05.

Results

Serum levels of MMP-13 in patients with localized scleroderma

MMP-13 levels in serum samples from patients with LSc and normal controls were assessed by using a peptide substrate cleavage assay. Patients with LSc exhibited lower serum MMP-13 levels

than normal controls, but there was no significant difference (64.9 ± 19.9 versus 73.2 ± 11.5 ng/ml, $p = 0.058$). Serum MMP-13 levels in patients with GM were significantly lower than those in normal controls (54.0 ± 18.7 versus 73.2 ± 11.5 ng/ml; $p < 0.01$) or in patients with LS (54.0 ± 18.7 versus 72.6 ± 17.7 ng/ml; $p < 0.05$). Serum MMP-13 levels in patients with GM were also lower than those in patients with morphea, but there was no significant difference (54.0 ± 18.7 versus 68.0 ± 20.1 ; $p = 0.063$). Serum levels of MMP-13 were comparable among normal controls, the patients with LS, and those with morphea. The cut-off value (mean - 2SD) was set at 50.2 ng/ml, based on data of the 10 healthy control sera. As shown in Figure 1, decreased serum levels of MMP-13 were found in 2 of 10 patients with GM, 2 of 10 patients with LS, and 2 of 10 patients with morphea.

Correlation of serum MMP-13 levels with clinical and immunological features

The clinical and serological features in LSc patients with decreased or normal serum levels of MMP-13 are shown in Table I. There was no significant difference between these groups in terms of sex, age at onset, and disease duration. The presence of ANA, AHA, anti-ssDNA antibody, or RF was not correlated with decreased serum MMP-13 levels. Although there were also no significant differences in the number of plaque lesions, linear lesions, or involved body areas between these groups, the frequency of muscle involvement was significantly greater in patients with decreased MMP-13 levels than in those with normal levels.

We next investigated the correlation of serum MMP-13 levels with the number of linear lesions, plaque lesions or involved body areas. As shown in Figure 2, serum MMP-13 levels were significantly inversely correlated with the number of linear lesions ($r = 0.366$, $p < 0.05$) and the number of involved body areas ($r = 0.552$, $p < 0.005$), while there was no significant correlation between serum MMP-13 levels and the number of plaque lesions. Furthermore, there

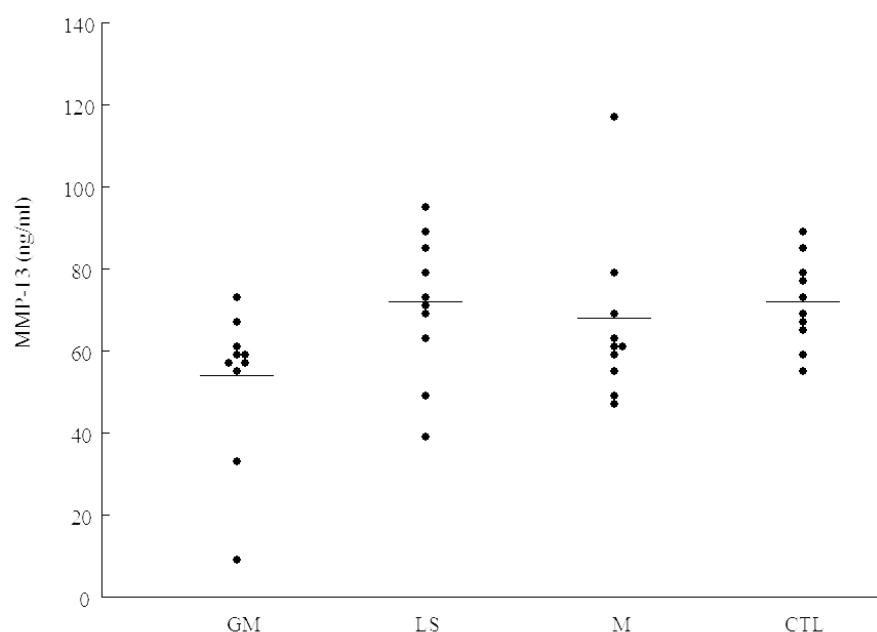


Fig. 1. MMP-13 concentrations in sera from patients with LSc. The dashed line indicates the cut-off value (50.2 ng/ml). The horizontal bars indicate the mean value in each group. Decreased serum levels of MMP-13 were found in 2 of 10 patients with generalized morphea, 2 of 10 patients with linear scleroderma, and 2 of 10 patients with morphea. GM; patients with generalized morphea, LS; patients with linear scleroderma, M; patients with morphea, CTL; normal controls.

Table I. Correlation of serum MMP-13 levels with clinical and serological features of patients with localized scleroderma.

	Patients with decreased MMP-13 levels (n = 6)	Patients with normal MMP-13 levels (n = 24)
Clinical		
Sex (M/F)	0/6	6/18
Age at onset (years)	33.0 ± 14.0	22.9 ± 17.8
Disease duration (years)	7.67 ± 6.62	2.86 ± 4.32
No. of linear lesions	1.17 ± 1.47	0.79 ± 0.88
No. of plaque lesions	1.83 ± 1.33	3.00 ± 3.23
Total No. of lesions	3.00 ± 2.28	3.79 ± 3.13
No. of involved body areas	2.16 ± 1.60	1.50 ± 0.88
Muscle involvement (%)	50*	8
Serological		
ANA (%)	83	65
AHA (%)	100	67
anti-ssDNA (%)	50	36
RF (%)	60	25

Unless otherwise indicated, values are means ± SD.

*p < 0.05 versus patients with normal MMP-13 levels.

was significant inverse correlation between serum MMP-13 levels and the number of involved areas in patients with generalized morphea ($r = 0.631$, $p < 0.05$).

Discussion

This study was undertaken to clarify the clinical significance of serum MMP-13 levels in patients with LSc.

We found that serum MMP-13 levels in patients with GM, the severest form of LSc, were significantly decreased compared with normal controls, while serum MMP-13 levels were comparable among the patients with LS, those with morphea, and normal controls. We also demonstrated that the prevalence of muscle involvement was significantly greater in LSc patients with de-

creased MMP-13 levels compared with those with normal MMP-13 levels. Furthermore, serum MMP-13 levels in patients with LSc were significantly inversely correlated with the number of linear lesions and the number of involved body areas. Moreover, there was significant inverse correlation between serum MMP-13 levels and the number of involved body areas in patients with GM. Collectively, serum MMP-13 levels serve as a useful marker to evaluate the severity of patients with LSc, especially GM. Considering the reduction of serum MMP-13 levels is around 20%, decreased MMP-13 is partially involved in the fibrotic process in LSc. Although these data are preliminary because of the small number of patients, to our knowledge, this is the first report indicating the clinical significance of serum MMP-13 levels in patients with LSc.

The function of MMP-13 has been well-studied in human fetal skin fibroblasts and human gingival fibroblasts. In these cells, the expression of MMP-13 is increased by the stimulation with TGF- β , a growth factor implicated in ECM accumulation in wound repair and fibrosis (28-31). It is therefore possible that MMP-13 may play an important role in rapid turnover of collagenous ECM of granulation tissue during normal repair of fetal and gingival wounds, resulting in minimal scar formation. By contrast, the stimulation of TGF- β showed no significant effect on the expression of MMP-13 in human neonatal skin fibroblasts (29). However, the elevated expression of MMP-13 was observed by in situ hybridization in fibroblast- and macrophage-like cells in fibrotic areas of chronic dermal wound (32). Furthermore, other reports demonstrated that the expression of MMP-13 was induced by three-dimensional culture in human neonatal and adult skin fibroblasts (32, 33) and in such situation the expression level of MMP-13 was significantly reduced by the stimulation of TGF- β 1 (33). Taken together with the evidence that LSc fibroblasts may be activated by the stimulation of autocrine TGF- β (4), these previous findings suggest that the expression levels of MMP-13 may be

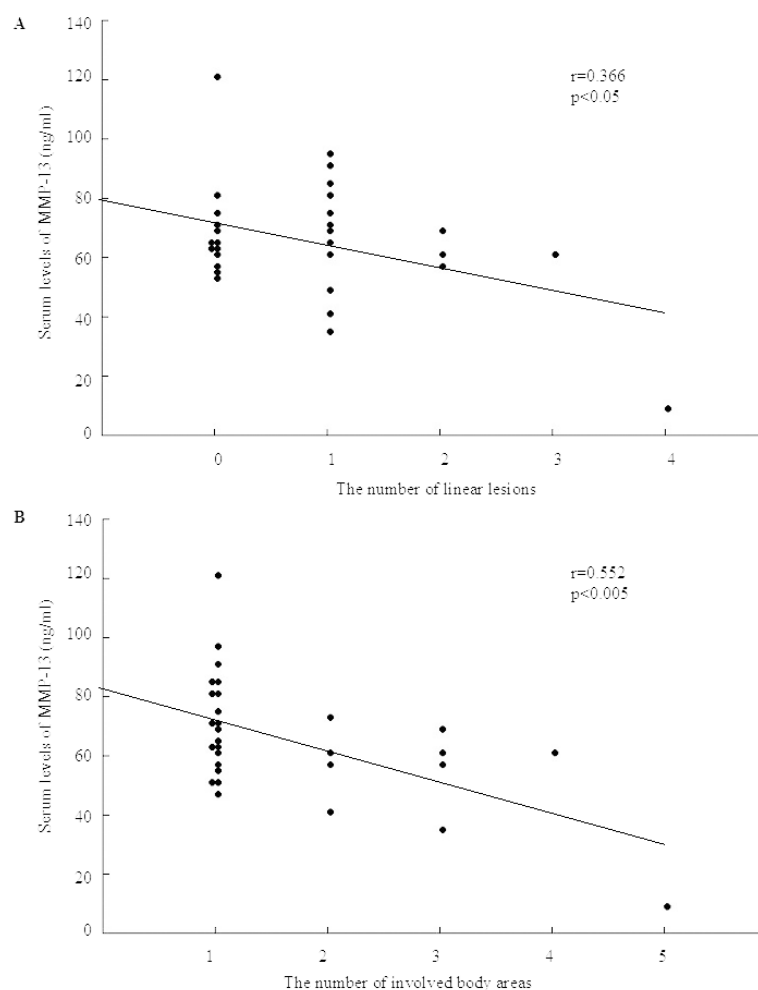


Fig. 2. Correlation of serum MMP-13 levels and the severity of LSc Serum MMP-13 levels were significantly inversely correlated with the number of linear lesions (A) and the number of involved body areas (B).

decreased in LSc fibroblasts as a result of the stimulation by autocrine TGF- β . This hypothesis clearly explains the present observation that the serum MMP-13 levels were significantly inversely correlated with the severity of LSc.

We recently measured serum MMP-13 levels in patients with SSc using the same ELISA system and demonstrated the following evidences; (i) The serum MMP-13 levels in patients with SSc were significantly lower than those in normal controls, (ii) Disease duration prior to the diagnosis was significantly shorter in SSc patients with decreased serum MMP-13 levels than in those with normal levels, and (iii) Serum MMP-13 levels were significantly correlated with the duration of the disease (23). These results indicate that MMP-13 may be involved in the fibrotic

process of SSc, especially in the initiation of fibrosis. By contrast, there was no significant difference in serum MMP-13 levels between patients with LSc and normal controls. This is probably due to the difference in the number of activated fibroblasts between SSc and LSc. In SSc, fibroblasts in skin and other organs are universally activated, which results in the decrease of serum MMP-13 levels. In LSc, the fibroblast activation is restricted to the specific areas of dermis and subcutaneous tissue. Therefore, serum MMP-13 levels are likely to decrease only in severe cases of LSc. Consistently, the prevalence of muscle involvement was significantly greater in LSc patients with decreased MMP-13 levels and serum MMP-13 levels were significantly inversely correlated with the number of linear lesions and the number of

involved body areas. Regarding disease duration, there was no significant difference between LSc patients with decreased MMP-13 levels and those with normal MMP-13 levels. Furthermore, there was no significant correlation between disease duration and serum MMP-13 levels in LSc patients and GM patients (data not shown). Based on these data, it is postulated that the role of MMP-13 in the fibrotic process may be different between SSc and LSc. In summary, the present study demonstrated that serum MMP-13 levels reflect the disease severity of LSc, especially GM. These results suggest that decreased MMP-13 is involved in the fibrotic process in LSc. Further *in vivo* and *in vitro* studies are required to clarify the significance of MMP-13 in the pathogenesis of LSc.

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