*Research Communication*

# Serum Levels of Tissue Inhibitors of Metalloproteinase 2 in Patients With Systemic Sclerosis With Duration More Than 2 Years: Correlation With Cardiac and Pulmonary Abnormalities

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In this study, we measured the serum concentration of TIMP-2 in patients with systemic sclerosis (SSc) and explored its possible correlation with cardiac and pulmonary lesions. We studied 42 patients with SSc, with duration equal to or more than 2 years. CT chest, ECG, echocardiography, and serum TIMP-2 concentration measurement using ELISA technique were performed in all patients and in 25 normal controls. The mean serum levels of TIMP-2 in patients was higher than in controls ($P = .005$). The mean CT score of dSSc patients with elevated TIMP-2 levels was significantly higher than dSSc patients with normal levels ($P = .013$). Four patients out of five with elevated TIMP-2 levels showed diastolic dysfunction (80%), compared to 2 out of 15 lSSc patients with normal levels (13.3%), with $P = .014$. Our research, though involving a small group of patients, points to the probable role of TIMP-2 in the development of pulmonary lesions in dSSc patients and cardiac lesions in lSSc patients with duration equal to or more than 2 years.

## BACKGROUND

Systemic sclerosis (SSc) is an autoimmunological multisystem disease affecting the skin, dermal blood vessels, and internal organs. It is characterized pathologically by the overproduction of connective tissue and fibrosis [1].

Fibrosis in the skin and internal organs in the course of systemic sclerosis is caused by the accumulation of an excessive quantity of collagen and other components of the extracellular matrix (ECM) in the affected area [2].

Fibroblasts play an important role in the metabolism of extracellular matrix and connective tissue within the skin. They produce matrix metalloproteinases (MMPs)—zinc-dependent endopeptidases—which can digest all matrix components. The fibroblast also produce the specific inhibitors of MMPs, known as tissue inhibitors of matrix metalloproteinases (TIMPs) [3]. The quantity of ECM components is thought to depend on the equilibrium between MMPs and their inhibitors [4].

The results of the research evaluating the production of collagen by the fibroblast of SSc patients have also been inconclusive. Some authors have demonstrated normal functioning in these cells, while others have found increased fibroblast production of collagen [5, 6].

The role of TIMP-1 in the pathogenesis of SSc has been well described in previous studies. Its levels in patients with diffuse SSc (dSSc) were suggested to correlate with the progressive course of dermal fibrosis seen in early disease [7–9].

TIMP-2 is an important member of the TIMPs. It was demonstrated that an increased concentration of TIMP-2 may play a certain role in the development of SSc [9]. It was also suggested that TIMP-2 may have a role in the pathogenesis of pulmonary fibrosis in SSc [10].

The fibrillar collagen matrix is an important constituent of the left ventricular myocardium. It contributes to the maintenance of left ventricular geometry and the structural alignment of adjoining myocytes. MMP activity is tightly controlled in normal myocardium by TIMPs. Increased myocardial MMP activity has been reported to occur in both clinical and experimental forms of dilated cardiomyopathy [11–16]. Higher serum levels of TIMP-2 were observed in patients with SSc and cardiovascular changes [17].

The purpose of our research was to specify the dependence between the serum concentration of TIMP-2 in patients with SSc and the pulmonary and cardiac dysfunction as detected by CT, echocardiography, and ECG.

## MATERIAL AND METHODS

### Patients

Forty-two nonsmoking patients affected by SSc, with disease duration equal to or more than 2 years, attending the Rheumatology and Rehabilitation Department, Kasr Eleini Hospital, were recruited in the recent study (thirty-three women, mean age 42.2 ± 11.3, range 19–65 years, and mean disease duration 6.5 ± 5.2 with range 2–25 years).

Twenty-two patients had diffuse form (dSSc) (17 women, mean age 39.9 ± 11.7 with range 19–58 years, and mean duration 6.4 ± 4.6 with range 2–18 years), and 20 patients had limited form (lSSc) (16 women, mean age 44.7 ± 9.9 with range 19–65 years, and mean disease duration 6.6 ± 5.8 with range 2–25 years).

All patients met the American Rheumatism Association preliminary criteria for diagnosis and classification of SSc [18], and the LeRoy et al criteria for subclassification of SSc [19].

Twenty-five normal nonsmoking volunteers (eighteen women, mean age 42.9 ± 12.0, ranging from 21–61 years) were involved as controls.

All patients had complete clinical examination including evaluation for gastrointestinal, pulmonary, cardiac, renal and muscle involvement, in addition to the routine laboratory investigations.

Antinuclear antibodies (ANA) were identified by the indirect immunofluorescence method using HEp-2 cells as a substrate. Anti-Scl-70 antibodies were identified by using ELISA.

Drug treatment—during the period of observation and collection of serum samples for testing—included calcium channel blockers (26 patinets, 14 with dSSc), vasodilators (6 patients, 2 with dSSc), corticosteroids (20 patients,16 with dSSC, 10–40 mg/day), methotrexate (16 patients, 12 with dSSc, 12.5–15 mg/week), and cyclophosphamide (4 patient, 3 with dSSc, IV pulse/month).

*Modified Rodnan skin thickness score* was used to evaluate the skin involvement. The body was divided into 17 regions (face, anterior chest, abdomen, upper arms, forearms, hands, fingers, thighs, legs, and feet). The degree of skin involvement in each region was scored from 0 to 3 (0 = normal skin, and 3 = extreme thickening) and then summing the score of all the palpated sites [20].

### Doppler echocardiography

A two-dimensional method and Doppler echocardiography technique were applied for all patients. Imaging was performed with a Sonos 1000 equipped with 2.5-mHz phased pulsed array transducers (Hewlett-Packard, USA).

### High-resolution computed tomography

Pulmonary interstitial disease was assessed by high-resolution computed tomography (HRCT) using Somatom Plus-S (Siemens, Erlangen, Germany) CT units. The HRCT images were divided into upper, middle, and lower areas. For each area of the lung, the following scores were used and then summed for each patient: 0 = no interstitial changes; 1 = thickened septal lines, subpleural lines, parenchymal bands, and subpleural cysts; and 2 = honey combing. Any HRCT scoring above 0 was considered abnormal [21].

*Serum levels of tissue inhibitor of metalloproteinases 2 (TIMP-2)* was measured by using Biotrak TM TIMP-2 human ELISA system, supplied by Amersham Pharmacia Bioteck. The assay is based on a 2-site ELISA sandwich format. Peroxidase-labeled Fab antibody to TIMP-2 is added to standard sample and the mixture is incubated in microtitre wells precoated with anti-TIMP-2 antibody. The amount of peroxidase is determined by the addition of TMB substrate, and the concentration of TIMP-2 in a sample is determined by interpolation from a standard curve.

The cutoff value at and above which the level was considered abnormal (2 SD above the mean in the control subjects) for TIMP-2 was settled at 68.7 ng/mL (mean serum level in the controls was 39.1 ± 14.8 ng/mL).

### Statistical analysis

The results were expressed as mean ± SD, and analyzed statistically by using the Mann-Witney test and Chi square with Fisher exact test when appropriate. $P$ value less than .05 was regarded as statistically significant.

## RESULTS

The serum levels of TIMP-2 in the patients ranged from 23–274 ng/mL, and the mean concentration was 78.3 ± 71.9 ng/mL, while in the controls it ranged from 23–73 ng/mL, and the mean concentration was 36.4 ± 10.5 ng/mL. The difference between the means of the patients and the controls was statistically significant ($P$ = .005).

The mean concentration of TIMP-2 in patients with dSSc was 94.6 ± 79.8 ng/mL, with range 31–274 ng/mL, with significant difference when compared to controls ($P$ = .0007). While the mean concentration in patients with lSSc was 61.3 ± 58.5 ng/mL with range 23–243 ng/mL, without significant difference when compared to the controls ($P$ = .051). The difference between the mean concentrations of the dSSc patients and the lSSc patients was nonsignificant ($P$ > .05).

Anticentromere antibodies were detected in 16 patients (38.1%); all of them had lSSc (80% of the patients with lSSc). While anti-Scl-70 antibodies were detected in 19 patients (45.2%), 18 patients had dSSc (81.8% of patients with dSSc), and 1 patient had lSSc (5% of patients with lSSc).

In patients with dSSc, a significant positive correlation was observed between the serum levels of TIMP-2 on the one hand and the age of patients ($P$ = .02) and CT score ($P$ = .05) on the other hand. In patients with lSSc, no significant

TABLE 1: Clinical and laboratory data of dSSc patients, dSSc patients with elevated levels of TIMP-2 (Group 1), and dSSc patients with normal levels (Group 2).

| Parameter | dSSc patients ($n = 22$) | Group 1 ($n = 9$) | Group 2 ($n = 13$) | P |
|---|---|---|---|---|
| Age (years)[a] | 39.9 ± 11.7 | 39.9 ± 6.1 | 39 ± 16.2 | NS |
| Duration (years)[a] | 6.4 ± 4.6 | 5.3 ± 2.7 | 7.2 ± 5.8 | NS |
| ESR[a] | 44.4 ± 23.9 | 54.3 ± 30.2 | 33.3 ± 9.9 | NS |
| TIMP-2 (ng/ml)[a] | 94.6 ± 79.8 | 168.1 ± 79.1 | 43.7 ± 10.6 | $P = .05$ |
| Rodnan skin score[a] | 19 ± 9.1 | 22 ± 11 | 17 ± 7.3 | NS |
| Raynaud's phenomenon[b] | 18 (81.8) | 7 (77.8) | 11 (84.6) | NS |
| Esophageal dysmotility[b] | 16 (72.7) | 7 (77.8) | 9 (69.2) | NS |
| Telangectasia[b] | 4 (18.2) | 1 (11.1) | 3 (23.1) | NS |
| Hypertension[b] | 9 (40.9) | 2 (22.2) | 7 (53.9) | NS |
| HRCT score[a] | 4.9 ± 4.9 | 6.1 ± 5.7 | 2.8 ± 2.9 | $P = .05$ |
| Cardiac lesion[b] | 7 (31.8) | 4 (44.4) | 3 (23.1) | NS |
| Valvular lesion[b] | 4 (18.2) | 2 (22.2) | 2 (15.4) | NS |
| Diastolic dysfunction[b] | 1 (4.5) | 1 (11.1) | 0 | NS |
| Conduction defect | 2 (9.1) | 1 (11.1) | 1 (7.7) | NS |
| Cardiomegally | 2 (9.1) | 1 (11.1) | 1 (7.7) | NS |
| Anti-Scl-70 | 18 (81.8) | 8 (88.9) | 10 (76.9) | NS |
| A.centro.[b] | 0 | 0 | 0 | — |

[a]Mean ± SD.
[b]Number (%).
dSSc: diffuse systemic sclerosis.
ESR: erythrocyte sedimentation rate.
A.centro.: anticentromere antibodies.
Mann-Whitney test, chi-square with Fisher's exact test, and Student $t$ test were used.

correlation was observed between TIMP-2 levels and any of the other variables.

Fourteen patients showed elevated serum levels of TIMP-2 above the cutoff value (9 patients had dSSc).

### TIMP-2 in patients with dSSc

Nine patients out of 22 with dSSc (40.9%) showed elevated serum levels above the cutoff value. Patients were divided into two groups: patients with elevated serum levels of TIMP-2 (group 1), and patients with normal levels (group 2).

The mean duration in group 1 was 5.3 ± 2.7 years (range 2–10 years). The mean duration of patients in group 2 was 7.2 ± 5.8 (range 2–18 years), and the difference between the two groups was nonsignificant ($P > .05$).

The mean Rodnan score of patients in group 1 was 22 ± 11 (range 8–42), while in group 2 it was 17 ± 7.3 (range 6–30), and the difference was nonsignificant ($P > .05$).

For all dSSc patients, the mean HRCT score was 4.9 ± 4.9 (range 0–14). Six patients showed normal lungs by HRCT, and the remaining 16 patients showed different types of lung lesions.

The mean CT score of the patients in group 1 was 6.1 ± 5.7 (range 0–14), and in group 2 it was 2.8 ± 2.9 (range 0–10), and the difference between the two groups was significant ($P = .013$) (Figure 1).

The difference between the frequency of cardiac abnormalities in group 1 and group 2 was statistically nonsignificant (Table 1).
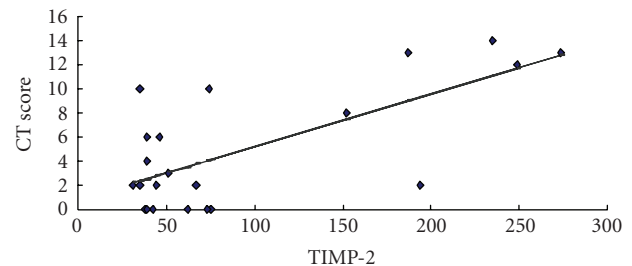


FIGURE 1: Linear regression line fit plot showing the significant relation between TIMP-2 levels and CT score in DSSc patients.

No statistically significant difference was found between the two groups concerning any of the other disease's parameters (Table 1).

### TIMP-2 in patients with lSSc

Five lSSc patients showed elevated serum levels of TIMP-2 above the cutoff value. Patients with lSSc were further divided into two groups: patients with elevated serum levels of TIMP-2 (group 3), and patients with normal levels (group 4).

The duration of disease in 5 patients in group 3 was more than 4 years, and the mean duration was 12.4 ± 7.5 years (range 6–25 years). The mean duration of patients in group 4 was 4.6 ± 3.7 (range 2–12), and the difference between the two groups was significant ($P = .014$).

TABLE 2: Clinical and laboratory data of lSSc patients, lSSc patients with elevated levels of TIMP-2 (Group 3), and lSSc patients with normal levels (Group 4).

| Parameter | lSSc patients ($n = 20$) | Group 3 ($n = 5$) | Group 4 ($n = 15$) | $P$ |
|---|---|---|---|---|
| Age (years)[a] | 44.7 ± 9.9 | 49.2 ± 10.6 | 43.1 ± 9.7 | NS |
| Duration (years)[a] | 6.6 ± 5.8 | 12.4 ± 7.5 | 4.6 ± 3.7 | $P=.014$ |
| ESR[a] | 50.3 ± 25.8 | 52 ± 40.9 | 49.7 ± 20.7 | NS |
| TIMP-2 (ng/ml)[a] | 61.3 ± 58.5 | 145.4 ± 65.6 | 33.2 ± 6.6 | $P = .05$ |
| Rodnan skin score[a] | 13.8 ± 8.9 | 10.6 ± 2.1 | 11.3 ± 4.2 | NS |
| Raynaud's phenomenon[b] | 19 (95) | 5 (100) | 14 (93.3) | NS |
| Esophageal dysmotility[b] | 15 (75) | 4(80) | 11 (73.3) | NS |
| Telangectasia[b] | 6 (30) | 1 (20) | 5 (33.3) | NS |
| Hypertension[b] | 1 (5) | 0 | 1 (6.7) | NS |
| HRCT score[a] | 3.9 ± 3.3 | 5.4 ± 3.3 | 3.3 ± 3.3 | NS |
| Cardiac lesion[b] | 8 (40) | 4 (80) | 4 (26.7) | NS |
| Valvular lesion[b] | 5 (25) | 1 (20) | 4 (26.7) | NS |
| Diastolic dysfunction[b] | 6 (30) | 4 (80) | 2 (13.3) | $P = .014$ |
| A.centro.[b] | 16 (80) | 4 (80) | 12 (80) | NS |

[a]Mean ± SD (range).
[b]Number (%).
lSSc: limited systemic sclerosis.
ESR: erythrocyte sedimentation rate.
A.centro.: anticentromere antibodies.
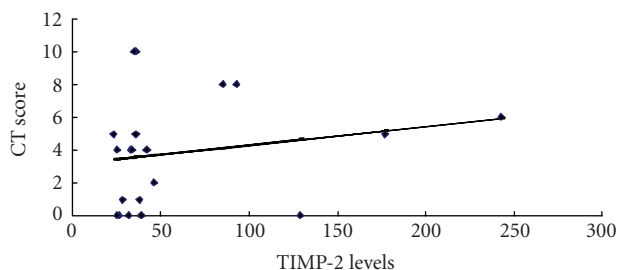Mann-Whitney test, chi-square with Fisher's exact test, and Student $t$ test were used.



FIGURE 2: Linear regression line fit plot showing the nonsignificant relation between TIMP-2 levels and CT score in lSSc patients.

The mean Rodnan score of patients in group 3 was 10.6 ± 2.1 (range 8–13), while in group 4 it was 11.3 ± 4.2 (range 6–21), and the difference was nonsignificant ($P > .05$).

For all lSSc patients the mean HRCT score was 3.9 ± 3.3 (range 0–10). Six patients showed normal lungs by HRCT, and the remaining 14 patients showed different types of lung lesions.

The mean CT score of the patients in group 3 was 5.4 ± 3.3 (range 0–8), and in group 4 it was 3.3 ± 3.3 (range 0–10), and the difference between the two groups was nonsignificant ($P > .05$) (Figure 2).

Diastolic dysfunction was the commonest echocardiographic finding found among patients with lSSc. Four patients out of five in group 3 showed diastolic dysfunction (80%), compared to1 out of 15 in group 4 (13.3%) with $P = .014$. The lSSc patient who had the highest TIMP-2 levels (243 ng/mL) had left atrial and left ventricular hypertrophy. One patient in group 3 had RBBB and LAHB.

No statistically significant difference was found between the two groups concerning any of the other disease's parameters (Table 2).

## DISCUSSION

In the recent study, we investigated the relation between the serum levels of TIMP-2 and cardiac and pulmonary involvement in SSc patients with duration equal to or more than 2 years.

There are many factors that affect the development of fibrosis in scleroderma. Many studies pointed to the role of metalloproteinases and their inhibitors in this process [22–25]. More than 20 human metalloproteinases have been described [26]. Among these metalloproteinases are collagenases, gelatinases, elastases, stromelisine, and membrane metalloproteinases [27].

The production of metalloproteinases is regulated by cytokines and growth factors. Their activity is also affected by TIMPs [28].

The quantity of ECM components is thought to depend on the equilibrium between MMPs and their inhibitors, the TIMPs. The disruption of this equilibrium is of essential significance both in the pathomechanism of physiological processes such as healing of wounds or involution of the uterus and in the process of angiogenesis as well as in certain diseases such as tumor growth and metastasis [4].

MMPs and TIMPs may play an important role in various rheumatic diseases. MMP-3 was suggested to be an important factor in the pathogenesis of systemic lupus erythematosus and rheumatoid arthritis. Serum MMP-9 activity in patients with diffuse cutaneous SSc was significantly decreased

compared with that of limited cutaneous SSc or normal controls [8]. The role of TIMP-1 in the pathogenesis of SSc has been well described in previous studies. Increased levels of both MMP-1 and TIMP-1 were found to be correlated with the disease severity [7, 8]. TIMP-1 level was reported to be a useful indicator of disease activity, especially of lung fibrosis [29].

The previous studies reported contradictory results concerning the role of TIMP-2 in SSc patients. While Yazawa et al demonstrated that Serum TIMP-2 level could be a useful marker of the extent of skin sclerosis and disease activity and the balance of TIMP-2 and MMP-2 might play an important role in the pathogenesis of the disease [9]. Young-Min et al found no difference in serum levels of TIMP-2 between patients of SSc and controls [7].

The recent study showed a significant difference between patients and controls concerning the serum TIMP-2 levels ($P = .005$), and this difference was more significant when comparing patients with dSSc and controls ($P = .0007$).

TIMPs were accused for being responsible for acceleration of dermal fibrosis in early stages of the disease. Zurita-Salinas et al reported increased TIMP-1 levels in fibroblasts only from patients with early stages SSc [30]. Young-Min have demonstrated that TIMP-1 levels were significantly higher in early disease ($< 2$ years) than in late stage disease ($> 4$ years) in patients with diffuse form of SSc [7].

In the recent study, the duration of disease in all patients was equal to or more than 2 years to avoid considering skin involvement as an associated factor. No relation was reported between the skin scoring and the serum TIMP-2 levels in this study. The duration of disease was significantly longer in lSSc patients with elevated levels of TIMP-2 (group 3) compared to lSSc with normal levels (group 4) with $P < .05$.

No relation could be found between TIMP-2 levels and anti-Scl-70 (anti-topoisomerase 1) nor anticentromere antibodies. Kikuchi et al [8] found an association between TIMP-1 levels and anti-Scl-70. Young-Min et al [7] found no relation between TIMP-1, TIMP-2, or MMP-1 and anti-Scl-70 or anticentromere antibody.

MMPs and TIMPs have been shown to exist in various tissues, including myocardium, and to be involved in collagen remodeling [13–16]. TIMP-2 and TIMP-3 were found to have important and unique roles in early cardiac development [31]. Changes in the balance between matrix deposition and matrix degradation by MMPs and their inhibitors TIMPs can lead to cardiac fibrosis [32]. It was suggested that MMPs could be responsible for induction of cardiac and pulmonary lesions in patients with sarcoidosis by causing damage to adjacent cardiac myocytes and pulmonary alveoli, leading to the interstitial fibrosis [33].

In the recent study, the mean lung CT score in dSSc patients with elevated TIMP-2 levels (group 1) was significantly more than in patients with normal levels (group 2) ($P = .013$). This finding supports the results of Dziankowska-Bartkowiak et al [10] who suggested a possible role of TIMP-2 in the development of pulmonary fibrosis.

Diastolic dysfunction was the most common echocardiographic finding found among lSSc patients. Eighty % of group 3 showed diastolic dysfunction compared to 13.3% of group 4 with $P = .014$. It has been suggested that left ventricular relaxation pattern is a feature of SSc [34, 35], and could be explained by myocardial fibrosis [36].

Cardiac involvement is not rare in SSc, and it may be primary or secondary to lung involvement [37–40]. The recent findings suggest a probable role of TIMP-2 in the development of myocardial fibrosis in SSc patients. A higher serum TIMP-2 levels were observed in a previous study in SSc patients with cardiovascular changes [17].

## CONCLUSION

This study, though involving a small group of patients, confirms the observations made by other authors regarding the probable role of TIMP-2 in inducing fibrosis in the course of SSc, and suggests a possible role of TIMP-2 in the development of pulmonary involvement in dSSc patients, and cardiac lesions in lSSc patients, with disease duration equal to or more than 2 years.

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