Clinical Significance of Serum Hyaluronan Levels in Systemic Sclerosis: Association with Disease Severity

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ABSTRACT. Objective. To determine any clinical association of serum levels of hyaluronan in patients with systemic sclerosis (SSc).

Methods. Hyaluronan levels in serum samples from patients with SSc (n = 66) and hyaluronan expression in skin were assessed.

Results. Serum hyaluronan levels in SSc patients were higher than those in healthy controls. SSc patients with elevated hyaluronan levels had more frequent involvement of several clinical manifestations and immunological abnormalities compared to those with normal levels. Thus, hyaluronan levels correlated with disease severity. Hyaluronan expression in the sclerotic skin from SSc patients was more intense, relative to expression in normal skin.

Conclusion. These results suggest that elevated serum hyaluronan levels are associated with disease severity and immunological abnormalities in patients with SSc. (First Release Aug 1 2008; J Rheumatol 2008;35:1825–9)

Key Indexing Terms:
SYSTEMIC SCLEROSIS HYALURONAN FIBROSIS IMMUNE SYSTEM

Systemic sclerosis (SSc) is a multisystem disorder of the connective tissue characterized by excessive accumulation of extracellular matrix in the skin and various internal organs1. Hyaluronan, a component of extracellular matrix, has both structural and regulatory functions2. Toll-like receptors (TLR) and their ligands are critical components of the innate immune system and contribute to defensive antimicrobial immune responses3. Breakdown products of the extracellular matrix, especially hyaluronan, stimulate TLR as endogenous ligands for TLR and thereby regulate inflammatory responses, including autoimmune disorders4. Although the pathogenesis of SSc remains unknown, breakdown products of hyaluronan may play a role in immunological abnormalities of SSc by activating TLR on various immune cells.

Elevated serum hyaluronan levels have been described in various disorders including SSc2,5. Although hyaluronan regulates inflammatory immune responses as an endogeous TLR ligand and thereby affects immunological abnormalities6, any correlation of serum hyaluronan levels with immunological measures has not been previously investigated in patients with SSc. Further, previous studies did not assess association of hyaluronan levels with the involvement of each organ. We investigated the clinical and immunological correlation of serum hyaluronan levels in patients with SSc.

MATERIALS AND METHODS

Serum samples. Serum samples were obtained from 66 Japanese patients with SSc (59 women, 7 men). All patients fulfilled the criteria proposed by the American College of Rheumatology7 for a diagnosis of SSc. Patients were grouped according to the classification system proposed by LeRoy, et al8. The study population and clinical and biochemical data are given in Table 1. No patient with SSc had been treated with oral corticosteroid, D-penicillamine, or other immunosuppressive therapy prior to the evaluation. Twenty-two age- and sex-matched healthy Japanese individuals were enlisted as controls. Fresh venous blood samples were centrifuged shortly after clot formation. All samples were stored at –70°C prior to use.

Clinical assessment. Complete medical histories, physical examinations, and laboratory tests including vital capacity (VC), diffusion capacity for carbon monoxide (DLCO), and the modified Rodnan total skin thickness score (modified Rodnan TSS)9 were conducted for all patients. Organ involvement was defined as described10.

The protocol was approved by Kanazawa and Nagasaki University Graduate School of Medicine and Kanazawa and Nagasaki University Hospital, and informed consent was obtained from all patients.

Hyaluronan staining. Formalin-fixed and paraffin-embedded tissues were obtained from 10 SSc patients [5 diffuse cutaneous SSc (dSSc) and 5 limited cutaneous SSc (lSSc)] and 5 controls. Hyaluronan staining was performed11 using the biotinylated hyaluronan-binding protein (Sigma-Aldrich Inc., St. Louis, MO, USA). The reaction products were visualized using diaminobenzidine with methyl green as the counterstain.

ELISA for serum hyaluronan. ELISA for serum hyaluronan levels was per-
RESULTS

Serum hyaluronan levels by ELISA. Serum hyaluronan levels in ISSc and dSSc patients were significantly higher than those in controls (Figure 1A). Serum hyaluronan levels in dSSc patients were significantly elevated relative to those in ISSc patients. In this assay system, the cutoff value was 216.1 ng/ml, which was calculated as the mean + 2 SD for controls.

Hyaluronan expression in fibrotic skin. Hyaluronan expression was only faintly detected in the dermis of controls (Figure 1B). SSc patients had remarkably higher expression of hyaluronan in the dermis compared with controls (Figure 1C). Hyaluronan expression in sclerotic skin of ISSc patients was similar to that of dSSc patients (data not shown).

Clinical correlation. SSc patients with elevated hyaluronan levels had significantly higher modified Rodnan TSS points, higher frequency of dSSc and decreased %VC and %DLCO, and more frequent involvement of pitting scar/ulcer, diffuse pigmentation, pulmonary fibrosis, esophagus, heart, kidneys, joints, and muscle than did those with normal levels (Table 1). Consistent with these findings, hyaluronan levels were significantly elevated in SSc patients with pitting scar/ulcer, diffuse pigmentation, pulmonary fibrosis, heart involvement, kidney involvement, or arthritis/arthralgias compared with those without each clinical measure (Figure 2A). Hyaluronan levels also correlated inversely with %VC or %DLCO (Figure 2B). Further, hyaluronan levels correlated positively with modified Rodnan TSS and renal vascular resistance, which was determined as the pulsatility index value in the renal interlobar arteries by color-flow Doppler scans. However, hyaluronan levels did not correlate with any other clinical measures, including disease duration. Indeed, hyaluronan levels were increased in patients with early-stage SSc with disease duration ≤ 2 years (233.1 ± 146.5 ng/ml) and in patients with late-stage SSc (272.1 ± 202.2 ng/ml), relative to controls (p < 0.001); however, there was no significant difference between them. Further, when 51 paired serum samples obtained at the time of diagnosis and 2 years after diagnosis were compared, there was no significant difference between them (239.4 ± 105.7 ng/ml compared to 269.2 ± 108.7 ng/ml).

SSc patients with elevated hyaluronan levels had significantly higher frequency of elevated levels of serum IgG, IgA, C-reactive protein (CRP), and erythrocyte sedimentation rates (ESR) and more frequent presence of anti-topoisomerase I antibody compared to those with normal hyaluronan levels (Table 1). Moreover, hyaluronan levels were positively correlated with levels of serum IgG and IgA (Figure 2B). Thus, elevation of serum hyaluronan levels was associated with the severity of skin and pulmonary fibrosis, with involvement of many other organs, and with various immunological abnormalities in patients with SSc.

DISCUSSION

We observed that serum hyaluronan levels were elevated in patients with SSc, especially dSSc, and hyaluronan expression in the fibrotic skin from SSc patients was increased relative to expression in normal skin. Although studies have shown that hyaluronan levels are generally higher in proximal scleroderma than in distal scleroderma, their direct correlation with the extent of skin fibrosis using a skin score system was not evaluated. In our study, we observed that hyaluronan levels correlated positively with modified Rodnan TSS. Moreover, consistent with the previous finding that hyaluronan levels were associated with decreased %DLCO in SSc, we also showed that elevation of hyaluro-
Hyaluronan levels was accompanied by the presence of pulmonary fibrosis and decreased %VC and %DLCO, indicating that hyaluronan levels correlated with the severity of lung fibrosis. Remarkably, increased hyaluronan levels were also associated with the involvement of many other organs such as the esophagus, heart, kidneys, joints, and muscles. Finally, hyaluronan levels also correlated with renal vascular resistance and the presence of pitting scar/ulcer, suggesting that they reflect vascular damage in SSc. Collectively, these results suggest that the hyaluronan level is a useful serological marker for evaluating disease severity in patients with SSc.

A previous study reported that hyaluronan levels were elevated in early-stage SSc, in patients with disease duration ≤ 2 years, but not in late-stage SSc. By contrast, in our study, there was no significant difference in hyaluronan levels between early-stage and late-stage SSc patients. The reasons for this discrepancy are unknown: this may be due to differences in the patient populations studied or the assay system used to detect serum hyaluronan. Our results suggest that serum hyaluronan levels reflect the disease severity rather than the disease activity in SSc. However, to clarify the clinical significance of hyaluronan levels in SSc, prospective studies with larger numbers of patients will be needed.

In this study, serum hyaluronan levels correlated with various immunological measures, including the presence of anti-topoisomerase I antibody and serum levels of IgG, IgA, CRP, and ESR. Hyaluronan fragments are endogenous ligands for TLR2 and TLR4, which are also expressed by various immune cells, such as macrophages, B cells, and T cells. Stimulation through TLR2/TLR4 induces B cell differentiation to Ig-secreting plasma cells and enhances the production of cytokines, including interleukin 6 (IL-6), suggesting that B cell stimulation by hyaluronan is related to the correlation of hyaluronan levels with IgG and IgA levels. Further, TLR4 signaling stimulates macrophages to produce various cytokines, such as IL-6. Since IL-6 is responsible for CRP production, IL-6 production by hyaluronan-stimulated B cells and macrophages may contribute to elevated CRP and ESR levels in SSc patients with increased hyaluronan levels. Further, aberrant regulation or expression of TLR is suggested to predispose an individual to autoantibody production by rendering B cells hyperresponsive to autoantigens, which may explain the higher prevalence of anti-topoisomerase I antibody in SSc patients with elevated hyaluronan levels. Thus, hyaluronan may play a role in immunological abnormalities associated with SSc.

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REFERENCES

Figure 1. A. Hyaluronan levels in serum samples from patients with ISSc and dSSc and healthy controls (CTL). Serum hyaluronan levels were determined by ELISA. The short bar indicates the mean value in each group; broken line indicates mean + 2 SD levels of controls. Representative hyaluronan expression in normal skin (B) and sclerotic skin from patient with SSc (C). Hyaluronan was stained by biotinylated hyaluronan-binding protein (original magnification x100).
Figure 2. A. Serum hyaluronan levels in the presence (+) and absence (–) of pitting scar/ulcer, diffuse pigmentation, pulmonary fibrosis, heart involvement, kidney involvement, and arthritis/arthralgia in SSc patients. Serum hyaluronan levels were determined by ELISA. B. Correlations of serum hyaluronan levels with modified Rodnan TSS, %VC, %DLco, the pulsatility index (PI) value, and serum levels of IgG and IgA in patients with SSc. PI value is a measure for renal vascular resistance determined by color-flow Doppler ultrasonography of the renal interlobar arteries of both kidneys. Serum hyaluronan levels were determined by ELISA.


