Serum YKL-40 levels in rheumatoid arthritis: Correlations between clinical and laborarory parameters

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Abstract

Objective

To clarify the significance of YKL-40, also called human cartilage glycoprotein-39, in the serum of patients with RA, we studied serum YKL-40 in relation to clinical and laboratory parameters.

Methods

Seventy-two patients (16 men and 56 women) with RA and 40 age-matched healthy persons (14 men, 26 women) were included in this study. Serum levels of YKL-40, insulin-like growth factor-I (IGF-I) and interleukin-6 (IL-6) were measured by ELISA. Radiological changes reflecting joint destruction and the joint score for pain or swelling were assessed by taking into account the joint surface area. Serum CRP levels and the functional disability of patients were also determined.

Results

YKL-40 levels in the serum of patients with RA were significantly higher than those of controls ($p < 0.0001$), and showed positive correlations with serum levels of IL-6 ($r = 0.301$, $p = 0.011$) and CRP ($r = 0.326$, $p = 0.006$), but negative correlations with serum levels of IGF-I ($r = -0.340$, $p = 0.004$). The radiological score, but not joint pain, also correlated with YKL-40 levels ($r = 0.364$, $p = 0.002$). As the functional disability of patients became severe, the serum YKL levels increased.

Conclusion

Serum YKL-40 levels partially reflect the degree of inflammation and also reflect the joint destruction in patients with RA.

Key words

Rheumatoid arthritis, YKL-40, serum marker, cytokine, cartilage.

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Received on February 5, 2001; accepted in revised form on May 17, 2001.

Introduction

YKL-40, also named human cartilage glycoprotein-39 (HC gp-39), is a member of the mammalian protein family related in sequence to the bacterial chitinases (1,2). It is mainly secreted by the human osteosarcoma cell line MG-63 (3), chondrocytes (1, 2, 4), and synovial fibroblasts (5). Furthermore, YKL-40 is synthesized by activated macrophages (6) and specific granules of neutrophils (7). YKL-40 mRNA expression is found in articular cartilage from patients with rheumatoid arthritis (RA) (2) and osteoarthritis (OA) (8). The concentrations of YKL-40 in serum and synovial fluids are elevated in joint diseases such as RA and OA, suggesting that YKL-40 is a marker of inflammation and tissue remodeling or degradation (9, 10).

Recently, YKL-40 was found to be a target of the immune response in RA, having several HLA-DR4 peptide-binding motifs that are recognized by T cells from patients with RA (11). However, the significance of serum YKL-40 in the assessment of RA is not well understood. Only a few reports have shown that serum YKL-40 in patients with RA varies according to disease activity by a longitudinal study (12). Several cytokines such as IL-6, TNF- , and sIL-2r are known to increase in RA serum, and are related to the disease activity (13-16). Some of these cytokines induce degradation of cartilage matrix. On the other hand, insulin-like growth factor (IGF)-I is one of the anabolic factors for cartilage tissue and seems to be involved in arthritis (17, 18). We and others have reported increased levels of IGFs in synovial fluids of patients with RA (19, 20), and decreased levels of IGF-I in the serum of patients with juvenile RA have also been described (21). Since YKL-40 is expressed in the degradation or remodeling of cartilage, the YKL-40 levels in serum might be related to these cytokines.

In this study, to clarify the significance of YKL-40 in the serum of patients with RA, we examined whether the serum levels of YKL-40 are related to the circulating levels of specific cytokines (IL-6, IGF-I), the other marker of inflammation (CRP), and clinical indices (joint pain/swelling, radiological joint damage and functional stage).

Materials and methods

Patients

Seventy-two patients (16 men and 56 women) with RA were included in this study. All met the American College of Rheumatology criteria (22) for the diagnosis of RA. The median age was 56 years (range 20-77 years) and the median disease duration was 5 years (range 1 month to 33 years). All patients had been taking non-steroidal anti-inflammatory drugs and 49 patients were receiving disease modifying anti-rheumatic drugs (DMARDs), i.e., bucillamine (n=16), penicillamine (n=13), auranofin (n=7), salazosulfapyridine (n=5), actarit (n=2), methotrexate (n=1), gold sodium thiomalate (n=1), lobenzarit (n=1), or mizorbine (n=1). Two patients were receiving combination DMARD therapy. Actarit, lobenzarit, and mizoribine were developed in Japan for the treatment of RA by modulating the immune systems. Of the 72 patients 25 also received treatment with low-dose corticosteroids (prednisolone 2.5-5 mg/ day).

Controls

The age-matched control sera were obtained from 40 healthy persons; 14 men and 26 women with a median age of 51 years (range 33-67 years). None were taking any medicine and none had any signs or clinical symptoms of cancer, joint or liver disease.

Biochemical analysis of serum

The serum samples were collected and stored at -80°C until assayed. Serum C-reactive protein (CRP) and rheumatoid factor (IgM-RF) were analyzed with nephelometry.

Serum levels of YKL-40 were determined by a quantitative immunoassay, according to the manufacturer's instructions (Metra Biosystems, Inc. Mountain View, California, USA). Briefly, the YKL-40 assay was a sandwich immunoassay in a microtiter strip well format. The Fab fragment of a monoclonal anti-YKL-40 antibody conjugated to biotin bound to streptavidin on

the strip and captured YKL-40 in a standard control or patient sample. A polyclonal anti-YKL-40 antibody conjugated to alkaline phosphatase bound to the captured YKL-40. Bound enzyme activity was detected with p-nitrophenyl phosphate as substrate. All YKL-40 assays were performed in duplicate. The minimum detection limit was 20 ng/ml. The intra-assay and inter-assay variations were 6% and 7%.

Serum IGF-I was determined by ELISA (Fujisawa, Osaka, Japan). Serum was pre-treated with acid-ethanol to dissociate IGF-I from IGF-binding proteins. A polyclonal anti-somatomedin C antibody conjugated with peroxidase was used. The minimum detection limit was 1.56 ng/ml. The intra-assay and inter-assay variations were < 10%.

Serum IL-6 was determined using a high sensitivity ELISA kit (Amersham, Buckinghamshire, England). The minimum detectable dose was <1 pg/ml. The intra-assay and inter-assay variations were < 10%.

Clinical evaluations

The number of swollen or tender joints (total 42 joints); shoulders, elbows, wrists. metacarpophalangeal (MP) and proximal interphalangeal (PIP) joints of the hands, hips, knees, ankles, metatarsophalangeal (MTP) joints of the feet were counted. The area-weighted joint index was calculated according to Lansbury (23), where each joint was multiplied by a factor weighted for the relative joint surface area: PIP (x2), MP(x5), wrist (x15), elbow (x52), shoulder (x45), hip (x82), knee (x104), ankle (x35), 1st MTP (x8), 2nd-5th MTP (x5).

Radiological assessment included 42 joints as mentioned above. Joint changes were classified according to the method of Larsen *et al*. (24) and graded 0 (normal) to 5 (the most severe changes). The radiological score was defined as the sum of the grade of affected joints multiplied by a factor as mentioned above.

Functional status was classified as suggested by the American College of Rheumatology (25): class I – able to perform usual activities of daily living; class II – able to perform usual, but limited in avocational activities; class III – able to perform usual self-care activities, but limited in vocational and avocational activities; Class IV - limited in ability to perform usual self-care.

Statistical analysis

Comparison of serum YKL levels between groups was calculated by the non-parametric Mann-Whitney test and the Kruskal-Wallis test for unpaired differences. Correlations between the different parameters were calculated by the Spearman test and p values of 0.05 were considered significant.

Results

The clinical and laboratory parameters of patients are summarized in Table I.

The serum levels of YKL-40, IL-6 and IGF-I in patients with RA were determined by ELISA and compared to those in controls (Table II). There was no difference in age between the patients and controls, and the results were also compared among men and women. The YKL-40 and IL-6 levels in the serum of patients with RA were significantly higher than those of controls ($p < 0.0001$). In contrast, IGF-I levels in women with RA were lower than those in controls ($p < 0.05$), but no differences were seen in men.

The serum concentration of YKL-40 in the RA group showed a weak correlation with IL-6 ($r = 0.301$, $p = 0.011$) and CRP ($r = 0.326$, $p = 0.006$), but a negative correlation with serum IGF-I ($r = -0.340$, $p = 0.004$) (Fig. 1).

The radiological score, indicating destruction of joints, was also correlated with YKL-40 levels in the serum of patients with RA ($r = 0.364$, $p = 0.002$). However, the joint score, indicating pain or swelling of joints, had no relationship with YKL-40 levels (Fig. 2).

The correlations between the radiological and joint scores and IL-6, IGF-I and CRP are also shown in Table III. IGF-I and CRP levels but not IL-6 showed a weak correlation with the radiological score, and both CRP and IL-6 levels correlated with the joint score.

The serum levels of YKL-40, IL-6,

Table I. Clinical and laboratory parameters of patients with RA.

Gender (women/men)	56 / 16	
Age (yr)	56	(range 20-77)
Disease duration (yr)	5.0	(range 0.1-33)
C-reactive protein (mg/dl)	1.04	(range 0.14-17.0)
IgM RF (positive/negative)	51/21	
Bone erosion (with/without)	49/23	
Joint score	144	(0-550)
Radiological score	282	(0-3058)
Steroid (with/without)	25/47	

Table II. Comparison of serum YKL-40,IL-6 and IGF-I levels in RA patients and controls.

	YKL-40 (ng/ml) median (range)	IL-6 (pg/ml) median (range)	IGF-I (ng/ml) median(range)
All (women + men)			
RA (n = 72)	150 (7-525) *	10 (0-107) *	69 (24-215)
Controls (n = 40)	45 (8-189)	0 (0-29)	82 (27-185)
Women			
RA (n = 56)	128 (7-525) *	8 (0-41) *	62 (24-215)***
Controls (n = 26)	39 (8-189)	0 (0-11)	87 (31-185)
Men			
RA (n = 16)	169 (77-465) **	15 (0-107)*	81 (33-129)
Controls (n = 14)	49 (23-188)	0 (0-29)	78 (27-160)

* $p < 0.0001$ vs. control , ** $p < 0.0005$ vs. control, *** $p < 0.05$ vs. control.
Statistical analysis was performed with Mann-Whitney U test.

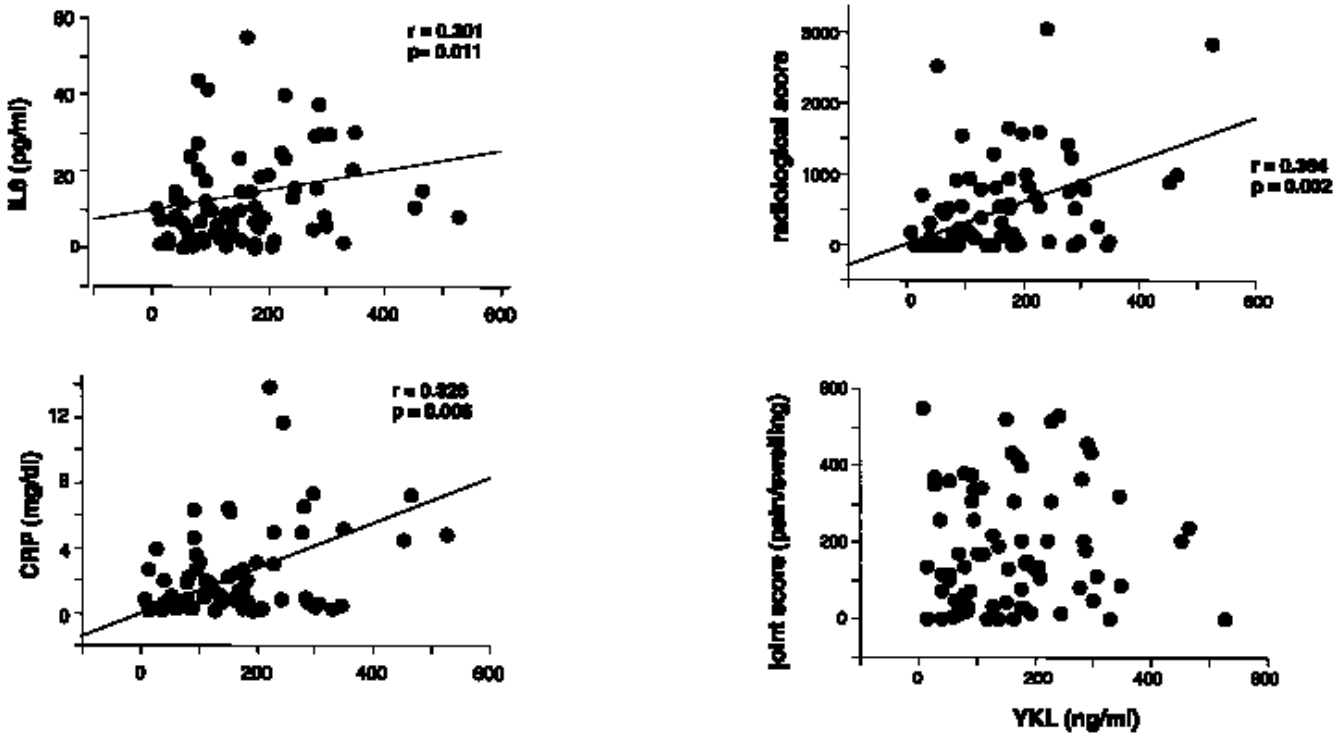

Fig. 2. Correlations between serum levels of YKL-40 and the radiological score (indicating cartilage destruction), and the joint score (indicating pain or swelling of joints) in patients with RA. The Spearman rank correlation coefficient and p value are given.

Fig.1. Correlations between serum levels of YKL-40 and interleukin-6 (IL-6),CRP, and insulin-like growth factor-I (IGF-I) in the serum of patients with RA. The linear regression line, Spearman rank correlation coefficient and p value are given.

IGF-I and CRP in the patients with bone erosions were compared to those in non-erosive RA (Table IV). YKL-40 and CRP levels were significantly higher in patients with erosive RA.

The age of the RA patients was correlated with YKL-40 ($r = 0.474$, $p < 0.0001$), IL-6 ($r = 0.246$, $p = 0.039$), IGF-I ($r = -0.458$, $p = 0.0001$), and CRP levels ($r = 0.398$, $p = 0.0008$), whereas disease duration did not correlate with these serum markers (data not shown). Furthermore, serum YKL-40 levels were significantly higher in patients treated with steroids than in those treated without steroids ($p = 0.032$). IL-6 and CRP levels in serum of patients with steroid therapy were also higher than in those without steroid therapy ($p = 0.035$, $p = 0.002$ respectively). IGF-I levels did not show a significant difference between them (Table V).

As the functional disability became

Table III. Correlations between the radiological and joint scores and the concentrations of YKL-40, IL-6, IGF-I and CRP (Spearman correlations).

	Radiological score	Joint score
YKL-40	0.364*	0.069
IL-6	0.005	0.349*
IGF-I	-0.297**	-0.014
CRP	0.252***	0.323**

*p < 0.005, **p < 0.01, * **p < 0.05

Table IV. The concentrations of YKL-40, IL-6, IGF-I and CRP in patients with/without bone erosions [expressed as median (range)].

	Bone erosions				
	+ (n = 49)		- (n = 23)		p
YKL-40 (ng/ml)	170	(26-525)	92	(7-345)	0.012
IL-6 (pg/ml)	12	(0-107)	7	(0-44)	0.052
IGF-I (ng/ml)	63	(24-201)	80	(29-215)	0.145
CRP (mg/dl)	1.9	(0.2-17)	0.6	(0.1-6. 4)	0.007

Table V. The concentration of YKL-40, IL-6, IGF-I and CRP in patients treated with/without steroid therapy [expressed as median (range)].

	Steroid therapy		
	+ (n = 25)	- (n = 47)	p
YKL-40 (ng/ml)	197 (26-525)	128 (7-450)	0.032
IL-6 (pg/ml)	15 (0-55)	7 (1-107)	0.035
IGF-I (ng/ml)	56 (24-170)	75 (29-215)	0.052
CRP (mg/dl)	3.1 (0.1-13.4)	0.8 (0.2-17)	0.002

severe, serum YKL levels increased: class I (median 87, range 7-327 ng/ml), class II (median 155, 12-347 ng/ml), class III (median 175, range 26-525 ng/ml). Although the median age of the patients in class II (58 years, range 22-74) and III (57 years, range 29-77) was slightly higher than those in class I (47 years, range 20-72), serum levels of IGF-I, IL-6 and CRP did not relate to the functional status of patients (data not shown).

Discussion

In this study we have shown that serum YKL-40 levels were increased in patients with RA and correlated with some clinical and laboratory markers. It has been reported that serum YKL-40 levels are also elevated in patients with OA, with no significant differences in concentration between RA and OA (1). Although the pathogenesis of these two diseases is different – RA is primarily an inflammatory disease of the synovium, while OA is primarily a degenerative disease of cartilage and bone – cartilage degradation is seen in both. These findings suggest that YKL-40 levels in serum may correlate with cartilage damage. Not a few reports have described radiological changes in the hands as a marker for joint destruction in RA (26). It is certain that radiological changes in the small joints are seen in most RA patients. However, large joints such as the knees and hips are also damaged in some patients. We therefore determined the degree of cartilage damage by considering the surface area of each joint. As expected, the YKL-40 levels in serum showed a positive correlation with radiological changes of the joints. These findings are also supported by a previous report in which YKL-40 levels were high in severe RA (12).

IGF-I is also an important factor for the metabolism of cartilage tissue, promoting chondrocytes to produce proteoglycan (17). Unresponsiveness of chondrocytes to IGF-I in OA was also reported (18). The effect of IGF-I on the secretion of YKL-40 by chondrocytes was examined by two groups and different results were obtained; one reported that IGF-I had no effect (2), and the other that IGF-I promoted YKL-40 secretion *in vitro* (27). In this study the concentrations of IGF-I and YKL-40 in the serum of patients with RA showed a negative correlation. Although these findings are different from the previous reports *in vitro*, we would conclude that IGF-I and YKL-40 have some interaction on the metabolism of cartilage.

Serum YKL-40 levels were also positively correlated with IL-6 and CRP levels. This is in part supported by other studies in which positive correlations between serum CRP and YKL-40 levels were obtained in RA (12), but not in OA (28). In contrast to our results, Johansen *et al.* reported that serum YKL-40 levels showed no significant correlation with serum IL-6 in patients with inflammatory joint diseases and OA, while IL-6 in synovial fluid correlated with serum YKL-40 (9). These discrepancies are probably due to differences in the diseases of the patients; we examined only patients with RA. It is also worth noting that YKL-40 levels were higher in the serum of patients treated with steroids. Considering that CRP and IL-6 levels were also high in the steroid group, all of these findings might be attributable to disease activity rather than to the use of steroids. However, joint pain and swelling were correlated not with YKL-40 levels but with CRP or IL-6 levels, suggesting that CRP or IL-6 levels could reflect disease activity better than YKL-40.

To take into account the joint surface area of each joint, we used the area-weighted joint index as explained above. By the same method Johansen *et al.* (12) found that YKL-40 correlated with the area-weighted swollen joint index during the first 6 months of RA, but not at all time points. They also showed that patients with persistently elevated serum YKL-40 levels were at risk of radiological disease progression. These findings are in part compatible with the present study. It is interesting to observe that serum YKL-40 levels increase as the functional disability of patients becomes more severe. This is confirmed by the fact that YKL-40 levels correlated with the radiological changes, reflecting the joint destruction that eventually leads to functional disability.

Various cells such as synovial cells, chondrocytes, osteoblasts, macrophages, and neutrophils are considered to produce serum YKL-40 in patients with RA, but it is difficult to distinguish which cells are responsible for the elevation of serum YKL-40 levels. In this study, we have shown that YKL-40 levels in serum correlated with both the inflammation and the radiological scores, suggesting YKL-40 may be a useful marker to monitor disease activity. However, these findings do not demonstrate that YKL-40 is superior to the conventional biochemical markers, such as CRP or ESR (29). The physiological function of YKL-40 is still unknown. Immunohistochemical analysis showed that YKL-40 staining was located mainly in the superficial and middle zones of OA cartilage but was low in normal cartilage, suggesting the involvement of YKL-40 in the remodelling or degradation of cartilage (30, 31). Recently it was reported that intranasal application of YKL-40 markedly suppressed disease activity and joint destruction in collagen-induced arthritis by the induction of cross-tolerance (32). Considering these observations, YKL-40 seems to be highly multifunctional.

In conclusion, serum YKL-40 levels in patients with RA correlated with CRP or IL-6 levels but not with the joint score, suggesting that YKL-40 levels partially reflect inflammation. On the other hand, YKL-40 levels correlated with the radiological score, erosions, and functional stage, suggesting that YKL-40 also reflects joint destruction. In addition, the negative correlation of YKL-40 with IGF-I levels may show some interaction with the IGF system in tissue repair or immune disorders in RA. Further studies are needed to clarify the significance of YKL-40.

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