Correlation Between Serum Hyaluronan and Disease Activity and Severity in Thai Patients with Rheumatoid Arthritis

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Abstract

Serum hyaluronan (HA) concentration was quantified using an ELISA-based assay with biotinylated hyaluronan binding proteins, and correlated with the clinical and laboratory variables in 100 consecutive rheumatoid arthritis (RA) patients (mean ± SD age and duration of disease of 50.1 ± 12.5 and 7.9 ± 6.6 years respectively). Thirty-four patients received prednisonole at an average dose of 5.0 mg/day. The correlations were good between the serum HA level and the joint swollen scores ($r = 0.26$, $p = 0.04$), joint space narrowing scores ($r = 0.25$, $p = 0.03$), joint erosion scores ($r = 0.26$, $p = 0.03$), and erythrocyte sedimentation rate ($r = 0.31$, $p < 0.01$) in RA patients who did not take prednisolone. These correlations were diminished in those who received prednisolone, although their disease was more severe. It might be possible that corticosteroids could decrease inflammation of the joint, thus interfering with the correlations. It was concluded that the serum HA level is a useful marker for the activity and severity of disease in patients with RA.

Key word : Rheumatoid Arthritis, Disease Activity, Hyaluronan, Hyaluronic Acid

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Rheumatoid arthritis (RA) is a chronic inflammatory arthritis with a significant morbidity and mortality, particularly in patients with polyarticular diseases[1]. The disease has a variable clinical course. Assessment of the activity and severity of disease in these patients is difficult and usually requires multiple clinical and laboratory parameters. However, there is no single clinical, laboratory test

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or biochemical marker that is specific in RA. The disease activity measurement, which was developed by the American College of Rheuamtology[2], or Paulus[3], or the Disease Activity Index[4], includes both clinical and laboratory parameters. Acute phase reactants, erythrocyte sedimentation rate (ESR) and/or C-reactive protein (CRP) are often used, but they are non-specific.

Hyaluronic acid or hyaluronan (HA) is a high molecular weight polysaccharide that is produced mainly by type B synoviocytes in the joint [5]. The synthesized HA enters the circulation and is cleared by endothelial cells of the liver[6]. In liver disease, the elimination process of HA is impaired resulting in an increased HA level in the circulation[7]. Thus, the serum HA level can reflect HA synthesis and joint inflammation in individuals with normal liver function. The HA synthesis is increased in many arthropathies including osteoarthritis, rheumatoid arthritis, psoriatic arthritis and juvenile chronic arthritis[8-14]. Although many studies have shown that there is a good correlation between serum HA level and disease activity in RA[9,10,12,15], some studies have failed to confirm this association[8,11,13,14].

In this report, we studied the correlation between serum HA, determined by ELISA-based assay with biotinylated hyaluronan binding proteins, and the disease activity and severity in Thai patients with RA.

PATIENTS AND METHOD

One hundred consecutive patients diagnosed with RA, according to the American College of Rheumatology criteria[16], were seen at the Chiang Mai University Hospital, and enrolled in this study. All patients were enrolled without regard to disease activity or rheumatic medications. In addition to non-steroidal anti-inflammatory drugs (NSAIDs), disease modifying anti-rheumatic drugs (DMARDs) were used at the therapeutic dosage. These included chloroquine (< 250 mg/day), methotrexate (5-7.5 mg/week) and sulfasalazine (2,000 mg/day). Patients were excluded if they had a history of significant liver diseases, abnormal liver function tests, hepatitis or cirrhosis, or had renal impairment (serum creatinine > 1.5 mg/dL).

The RA disease activity measurement included 68 joint counts for tenderness and swelling, joint swelling scores, Ritchie articular index, rheumatoid factor (RF) and ESR. Each swollen joint was graded 0-3 (0 = no joint swelling, 1 = minimal swelling, 2 = moderate joint swelling, 3 = marked joint swelling), which was the sum for the joint swelling scores. Blood samples were collected for the routine laboratory tests, ESR and RF. The serum was also kept at $-70^{\circ}C$ for HA analysis. Rheumatological evaluation was performed by WL. Rheumatological evaluation, blood collection and radiographs of the hand were completed in the morning of the same day (between 8.00-10.00 a.m. or 3-5 hours after waking up in the morning). The serum HA level was performed by PK. Scoring of the hand joint radiographs, as described by Fuchs et al [17], was determined by CS. Each investigator did not know the results of the others.

Measurement of HA

An ELISA-based assay with biotinylated hyaluronan binding proteins was used to determine the serum HA level[18]. In brief, serum samples were each placed in a plastic tube containing biotinylated HA binding proteins (1:200 in 0.05 M Tris-HCl buffer, pH 8.6). After incubation, the serum samples were transferred to a microplate precoated with HA, which was blocked with 1 per cent bovine serum albumin. After reincubating the microplate, the serum samples were washed with phosphate buffered saline (PBS) -Tween 20 buffer, and the peroxidase conjugated anti-biotin antibody (1: 2000 dilution, 100 uL/well in PBS) was added. The bounded enzyme conjugated antibody was monitored by adding enzyme substrate, and read by using the microplate reader at 492/690 nm. The amount of HA could be calculated against the standard curve. The serum HA level determined by this technique was shown to have a good correlation (r = 0.8) with that of the commercial kit (Pharmacia HA test)[18].

Statistical analysis

The SPSS version 9.0 microcomputer statistical program was used for data analysis. The correlation between variables was calculated using the Pearson correlation coefficient. A p-value of < 0.05 was considered statistically significant.

RESULTS

There were 93 females and 7 males with a mean $\pm$ standard deviation (SD) age of 50.1 $\pm$ 12.5 years (range 24-78), and duration of disease of 7.9 $\pm$ 6.6 years. The functional classes were class I in

Table 1. Mean ± SD of the disease activity parameter and HA level.

Parameter	Prednisolone N = 34	Non-prednisolone N = 66	P
Number of swollen joints	19.0 ± 10.4	12.8 ± 8.3	<0.01
Joint swollen scores	21.4 ± 11.3	14.3 ± 11.0	<0.01
Number of tender joints	9.6 ± 8.4	9.0 ± 8.1	0.74
Ritchie articular index	9.7 ± 6.8	7.9 ± 5.5	0.17
Joint space narrowing scores	4.3 ± 2.3	3.3 ± 1.9	0.03
Joint erosion scores	2.2 ± 1.9	1.3 ± 1.6	0.02
Malalignment scores	0.9 ± 1.3	0.6 ± 1.3	0.37
Mean RF (u/L) (n=70)	323.4 ± 213.3	231.7 ± 223.5	0.11
ESR (mm/h)	46.9 ± 18.9	42.0 ± 14.6	0.17
Serum HA level (ng/mL)	750.6 ± 1018.8	345.4 ± 361.9	0.06

Table 2. Correlation between log serum HA level and disease activity variables (adjusted to age).

Disease activity	Prednisolone N = 34		Non-prednisolone N = 66	
	r	p	r	p
Number of swollen joints	0.33	0.11	0.16	0.18
Joint swelling scores	0.23	0.19	0.26	0.04*
Number of tender joints	0.15	0.94	0.16	0.17
Ritchie articular index	-0.09	0.70	0.18	0.13
Joint space narrowing scores	0.31	0.15	0.25	0.03*
Joint erosion scores	0.41	0.06	0.26	0.03*
Malalignment scores	0.43	0.04*	0.22	0.06
ESR	-0.66	0.76	0.31	<0.01**
RF	0.05	0.80	0.07	0.50

* = $p < 0.05$, ** = $p < 0.01$

24 cases, class II in 70 and class III in 6. None were in functional class IV. Seventy patients (70%) were RF positive. Thirty-four patients took prednisolone (< 10 mg/day) with an average dosage of 4.9 ± 2.3 mg/day. Patients were taking chloroquine, methotrexate and sulfasalazine in 76, 53 and 2 cases, respectively.

As corticosteroid therapy has been shown to reduce serum HA level[14,19], patients were subdivided into groups based on concurrent corticosteroid treatment. The mean ± SD of the HA level and disease activity variables of the patients studied (prednisolone group, non-prednisolone group), which included the number of swollen and tender joints, joint swollen scores, Ritchie articular index, radiological scores, RF and ESR, are shown in Table 1. Patients who received prednisolone had significantly more active and severe disease (number of swollen joints, joint swollen scores, and radiological scores) than those of the non-prednisolone group. The serum HA level tended to be higher in the prednisolone group, but this did not show a statistically significant difference.

As the HA level did not show a normal distribution, it was transformed to the log serum HA (logHA) level and used for statistical analysis. An initial analysis showed that age significantly correlated with logHA level ($p = 0.03$), therefore, the correlation between logHA levels and the disease activity variables was adjusted to age, and is shown in Table 2. In the non-prednisolone group, the logHA level significantly correlated with the joint swollen scores, radiological scores and ESR, but not with the number of swollen and tender joints, the Ritchie articular index, and RF titer. Only joint erosion and malalignment scores showed significant correlations with the logHA in the prednisolone group. The logHA also showed no correla-

tion to the duration of the disease, the RF titer and the dosage of prednisolone received.

DISCUSSION

In this study, we determined the serum HA level using the ELISA-based assay with biotinylated hyaluronan binding proteins in RA patients. Our previous study showed that the results from this technique had a good correlation to those of the commercial kit[18]. Several techniques have been developed to determine serum HA level[20], and the assay results have shown the same magnitude with a slight deviation from each other. These differences do not impair the comparison of the clinical data.

In our pervious study, the mean ± SD serum HA of healthy people at the age of 50-60 years old (mean age of this study) was 58.0 ± 36.4 ng/ml (range 2.2 ± 126.7). Using a mean ± 2 SD or 130.8 ng/ml as the upper limit of normal HA serum concentration at this age group, 72 per cent of our RA patients had elevated HA levels. None of these patients had liver diseases, although many of them had been given methotrexate. A great variation of HA levels was observed as determined by the large number of standard deviations. As transient elevation of serum HA level has been reported after physical activity[21], it might be better if the blood collection was done early in the morning before the patients have physical activity. As the blood collection was taken between 8.00-10.00 a.m. (approximately 3-5 hours after waking up), the time when most patients arrived at the hospital; it was, therefore, assumed that these patients had had the same amount of physical activity. Thus, the variation of HA level should reflect the degree of joint inflammation, as indicated by the variation of the number of swollen joints and joint swollen scores.

The serum HA level tended to be higher in patients in the prednisolone group when compared to those in the non-prednisolone group. This might reflect the more severe and active disease in these patients (as indicated by a higher disease activity parameter in Table 1), necessitating treatment with corticosteroids. Up to one third of our patients were taking corticosteroids during the study, but none took more than 10 mg/day of prednisolone. Corticosteroid therapy has been shown to reduce serum HA level[14,19]. The drop in HA level has been attributed to the decrease of HA synthesis by rheumatoid synovium[22]. However, the magnitude of the effect of corticosteroids in reducing the serum HA level in our patients was not known.

A significant correlation between age and HA level (or logHA) was observed in this study. The result agreed with our previous studies and others that the serum HA level increased with age [15,18,19]. Unfortunately, we did not have controls in this study. Moreover, the patients with RA were enrolled to the study consecutively. Therefore, it was difficult to have patients in the same age group. We found a good correlation between the logHA and the articular inflammation (joint swollen scores) and radiological scores in the non-prednisolone group. These findings were in-line with the previous reports where the HA correlated with the disease activity[9,10,12,15], and the radiological progression in RA[23].

The lack of a strong correlation between logHA and the articular inflammation and radiological scores in the prednisolone group was beyond expectation. Compared to the non-prednisolone group, patients in the prednisolone group had more severe disease and tended to have a higher HA level. Therefore, the correlation between the logHA and these parameters should be strengthened. The reason for these diminished correlations was not clear. It might be possible that corticosteroids not only decreased the serum HA level, but also the inflammation of the joint (both the number of swollen joints and the joint swollen scores), to a degree where the correlations diminished.

It should be noted that the number of tender joints and the Ritchie articular index in our patients were less than the number of swollen joints. The reason for less joint pain in our patients was not clear.

In conclusion, we confirmed earlier work, by a different method, demonstrating an increase in serum HA level in RA patients. The serum logHA showed a significant correlation with disease activity, particularly the joint swollen scores and radiological scores in RA patients without corticosteroids therapy. These correlations diminished in patients receiving corticosteroids. Thus, serum HA could be used as a marker of disease activity and severity in corticosteroids-free RA.

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ความสัมพันธ์ระหว่างระดับซีรั่มฮัยอาลูโรแนนกับความรุนแรงของโรคข้ออักเสบรูมาตอยด์ในคนไทย

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ได้ทำการวัดระดับซีรั่มฮัยอาลูโรแนน (HA) ด้วยวิธี ELISA-based assay with biotinylated binding protein และศึกษาความสัมพันธ์ระหว่างระดับซีรั่ม HA กับลักษณะทางคลินิกและการความผิดปกติทางห้องปฏิบัติการในผู้ป่วยโรคข้ออักเสบรูมาตอยด์จำนวน 100 ราย (ค่าเฉลี่ย ± ค่าเบี่ยงเบนมาตรฐานของอายุผู้ป่วยและระยะเวลาของโรค เท่ากับ 50.1 ± 12.5 และ 7.9 ± 6.6 ปี ตามลำดับ) ผู้ป่วย 34 รายได้รับยาเพรดนิโซโลนขนาดเฉลี่ย 5.0 มก./วัน ในกลุ่มผู้ป่วยที่ไม่ได้รับยาเพรดนิโซโลนพบความสัมพันธ์ทางสถิติชัดเจนระหว่างระดับซีรั่ม HA กับคะแนนของข้อที่บวม ($r = 0.26$, $p = 0.04$) คะแนนการเปลี่ยนแปลงทางภาพรังสี คะแนนของช่องข้อที่แคบ ($r = 0.25$, $p = 0.03$) และคะแนนการกัดกร่อนของข้อ ($r = 0.26$, $p = 0.03$) และอัตราการตกตะกอนของเม็ดเลือดแดง ($r = 0.31$, $p < 0.01$) แต่ไม่พบความสัมพันธ์ระหว่างระดับซีรั่ม HA กับข้ออักเสบหรือความผิดปกติทางภาพรังสีในกลุ่มผู้ป่วยที่ได้รับยาเพรดนิโซโลนทั้งที่ผู้ป่วยกลุ่มนี้มีความรุนแรงของโรคและความผิดปกติทางภาพรังสีมากกว่า อาจเป็นไปได้ที่ยาเพรดนิโซโลนซึ่งมีคุณสมบัติในการลดการอักเสบและลดระดับซีรั่ม HA จึงทำให้ความสัมพันธ์นี้เสียไป ดังนั้นซีรั่ม HA สามารถใช้เป็นตัวชี้บ่งความรุนแรงและการอักเสบในโรคข้ออักเสบรูมาตอยด์ได้ดีในผู้ป่วยที่ไม่ได้รับยาคอร์ติโคสเตียรอยด์

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