HYALURONIC ACID EXCRETION LEVELS IN PATIENTS WITH RHEUMATOID ARTHRITIS AND OSTEOARTHRITIS

Jale TAN, M.D., Fatma Atalay GENER, M.D., Nilüfer BALCI, M.D.,
C.Öznur ONGUN*, M.D., Gülçin KAYMAK, M.D.

Gazi University, Faculty of Medicine, Departments of Physical Medicine and Rehabilitation and Biochemistry*, Ankara, Turkey
Gazi Medical Journal 2 : 81-88, 1993

SUMMARY : Hyaluronic Acid (HA) excretion levels in 24 hour urine samples of rheumatoid arthritis (RA) and osteoarthritis (OA) patients were measured by a spectrophotometric method. We compared the results of each patient group with the normal controls. The mean excretion level of the control group showed a statistically significant difference with either of the patient groups. There was no significant difference between RA and OA groups. The clinical and laboratory parameters of disease activity showed no correlation with the HA excretion levels.

In this study, HA did not act as an acute - phase reactant but our data supported the suggestion that in synovial inflammatory rheumatic diseases, HA levels increase in circulation.

***Key Words :** Hyaluronic Acid, Rheumatoid Arthritis, Osteoarthritis.*

INTRODUCTION

Hyaluronic acid (HA) is a high molecular weight polysaccaride that is widely distributed in loose connective tissues such as synovial membrane and synovial fluid (Balazs et al. 1967). This glycosaminoglycan is synthesized by fibroblasts and degraded by a specific hydrolase, hyaluronidase. Hyaluronic acid enters the blood circulation by the lymph vessels and is eliminated by the endothelial cells of liver sinusoids (Fraser et al. 1981, 1984). Increased serum levels of HA have been detected in patients with cirrhosis (Engström-Laurent et al. 1985), mesothelioma (Dahl and Laurent, 1988), Wilms' tumor (Wu et al. 1984), psoriasis (Lundin et al. 1985) and rheumatoid arthritis (Engström-Laurent and Hallgren 1985, 1987). HA is synthesized in the joint by the B synoviocytes, a specialized type of fibroblasts (Balazs, 1974). Elevated levels of HA in osteoarthritis (OA) was also established and this elevation was related to the disruption of the normal structure of the joint (Engström-Laurent and Hallgren, 1987; Lindquist et al. 1988).

The practical value of serum HA measurement is still uncertain, because the results on the correlation of serum HA levels and the parameters indicating the activity of the disease are still controversial.

In this study, we have measured the urinary excretion levels of HA in patients with RA, OA and in a healthy non-arthritic control group. Our purpose was to compare the results of the patient groups with the control group, and to investigate the correlation between the parameters indicating the activity of the disease and the excretion levels of HA in order to discuss whether HA acts as an acute phase reactant or not.

MATERIALS AND METHODS

Patient Data : Eighteen patients with RA (14 F, 14 M) and 20 patients (16 F, 4 M) with OA were studied. A group of 17 healthy subjects (9 F, 8 M) with no symptoms or signs of rheumatoid disease were used as normal controls. Patients gave their informed consent prior to participation and the study conformed to the Decleration of Helsinki (1964) as modified by the 35th World Medical Assembly, Venice, Italy, 1983.

The mean age of patients with RA was 48 years within a range of 23 to 70 years. The mean duration of disease activity was 7 years (ranged from 2 to 15 years). The mean age of OA patients was 62 years within a range of 46 to 79 years. 15 patients with RA were seropositive and 3 patients were seronegative for Rheumatoid Factor (RF) which was measured by the latex agglutination test (Klein et al. 1976). All of the RA patients fulfilled American Rheumatism Association criteria (Ropes et al. 1958) for definite or classical RA.

Medication : In the RA patients group, 8 patients (patients 2, 3, 4, 6, 8, 9, 11, 12, 13 and 14 in Table 1) were on nonsteroidal anti inflammatory drugs (NSAIDs), 1 patient was on sulphasalazine (patient 18), 3 were on chloroquine and low dose prednisone (patients 1, 5, 17), 4 patients were on chloroquine (patients 7, 10, 15, 16). Two RA patients (3 and 4) also had diabetes mellitus whereas 5 and 7 had systemic hypertension. The diagnosis of OA was established according to the presence of clinical symptoms, crepitus, bony tenderness, bony enlargement in the absence of warmth, joint stiffness and radiological findings (Moskowitz, 1985).

In the OA group 10 patients (Table 2) were taking no medications (patients 6, 8, 9, 11, 12, 14, 15, 18, 19, 20) 2 patients were on NSAIDs (patients 16, 17) and 8 patients (patients 1, 2, 3, 4, 5, 7, 10 and 13) were on medication for diabetes mellitus and hypertension.

Disease activity was evaluated by biochemical and clinical parameters including sedimentation rate (Westergreen, 1920), C-Reactive protein levels (McCarthy, 1982), duration of morning stiffness, Ritchie articular index (RAI) (Ritchie et al. 1968) and grip strength of both hands (Table 3). Erithrocyte sedimentation rates were determined using the standart Westergreen method (Westergreen, 1920). Grip strength was evaluated by a vigorimeter by which strength could be measured between 0-160 kPa. Duration of morning stiffness was decided by the subjective observation of patients, and the quantitative objective evaluation of the articular surface affected by the disease was made by Ritchie Articular Index (RAI). The patients with liver and renal diseases were excluded from the study.

The radiological features were evaluated according to Steinbrocker's classification. Steinbrocker et al. (Steinbrocker et al. 1949) classified the radiologic findings on a 4 point scale:1- possible osteopenia, 2- osteopenia + minimal subchondral bone destruction + minimal joint space narrowing, 3- definite cartilage and bone destruction and joint deformity + subluxation and ulnar deviation without bony or fibrous ankylosis, 4- bony or fibrous ankylosis.

Joint functions were scored on a 3 point scale: 1- little or no discomfort or impairment of normal; daily activities, 2- moderate discomfort with some restriction of activities, 3- marked discomfort with marked decrease in functioning. For both RA and OA patients, functional capacity of daily activities were evaluated by subjective and objective observations (Steinbrocker et al. 1949). Objective functional capacity was scored as : 1- normal activities, 2- mild restriction in daily activities, 3- marked restriction of daily activities, 4- bed or chair bound. Subjective evaluation of patients for the functional capacity was scored as : 1- doing well and/or improving with little or no discomfort, 2- no improvement and/or have moderate discomfort, 3- worsened or marked discomfort and/or impairment. In patients with RA, disease stage or severity was evaluated by Steinbrocker functional joint and radiological scores. Some clinical and biochemical details of the patients are summarized in Tables 1 and 2.

Biochemical analysis of Hyaluronic Acid : Twenty-four hour urine sample was collected from each patient and healthy control subject and stored at -20°C without preservative until the assay. Sample storage time did not affect glycosaminoglycan (GAG) content according to the literature (Chuck et al. 1986). The method used was a modification of that developed by Whiteman (Whiteman, 1973) and involves complex formation with the cationic dye alcian blue 8GX. In this procedure, a standart hyaluronic acid solution (10% HA) (200 µl) or centrifuged urine (200 µl) was mixed with 4 ml of freshly prepared 0.05 % W/V alcian blue 8GX and 50 mM $MgCl_2$ in a 50 mM sodium acetate buffer (pH 5.8). The mixture was equilibrated for two ho-

Patient	Age/Sex	Urine HA ng/ml	ESR mm/hr	RAI	Joint Func	Score Radiol	Functional Subj	Functional Obj
1	52/F	119.34	49	32	2	1	2	2
2	65/F	144.84	131	33	3	4	2	3
3	60/F	147.17	180	32	2	2	2	2
4	45/F	175.55	82	61	3	4	2	3
5	65/F	104.78	43	27	2	1	2	3
6	70/F	110.80	88	13	1	3	1	2
7	50/F	154.09	49	8	1	1	2	2
8	54/M	186.04	65	21	1	3	2	2
9	52/M	113.60	24	3	1	1	1	2
10	29/F	77.00	103	55	3	4	3	3
11	38/F	106.84	32	7	1	1	1	2
12	65/F	84.91	53	49	2	1	2	3
13	44/F	104.40	39	22	2	2	2	3
14	35/F	77.48	25	21	2	1	2	3
15	32/F	128.34	36	26	2	1	2	3
16	36/F	89.58	7	9	2	4	2	2
17	49/M	135.34	14	12	1	1	1	2
18	23/M	223.84	39	6	3	4	3	4

HA : Hyaluronic Acid
ESR : Erithrocyte Sedimentation Rate
RAI : Ritchie Articular Index
Func : Functional
Radiol : Radiologic
Subj : Subjective
Obj : Objective

Table - 1 : Characteristics of the rheumatoid arthritis patients.

urs at room temperature and an alcian blue-HA complex was seperated by centrifugation at 2000 g for 20 minutes. The precipitate was washed twice with ethanol followed by centrifugation and then dissociated with a 5% solution of sodium dodecyl sulphate in distilled water (4 ml). The absorbance of the resulting solution was measured in 1 cm micro-cuvettes at 620 nm.

RESULTS

HA Levels in RA and OA patients :

HA excretion levels were measured in a group of 18 well defined RA patients and 20 OA patients. The results of the patient group were compared with the values in normal controls. The mean values of the patient groups showed statistically significant difference when compared with the control group, but the difference between the patient group means were statistically insignificant (Table 4).

Relationship of Urine Excretion Levels of HA to Clinical and Biochemical Markers of Inflammation :

HA excretion levels in the 24 hr. urine samples did not correlate with the levels of ESR, CRP and Ritchie Articular Index scores in both groups. Urine HA levels of RA and OA patients are plotted against sedimentation rates (Fig 1a, 2a) and Ritchie articular indexes (Fig 1b, 2b) of these patients.

Several clinical and laboratory parameters of rheumatoid disease activity showed a correlation when compared with each other whereas urine HA did not correlate with any of them in RA patients (Table 5).

Disease duration showed a statistically signifi-

Patient	Age/Sex	Urine HA ng/ml	ESR mm/hr	RAI	Functional Subjective	Capacity Objective
1	62/F	121.42	10	8	2	2
2	70/F	41.23	13	5	3	3
3	69/F	73.77	24	3	2	2
4	61/F	90.14	16	8	2	2
5	59/F	107.77	29	5	2	2
6	60/F	97.83	13	24	2	2
7	60/M	202.34	8	4	1	1
8	46/F	94.00	9	17	2	2
9	60/F	160.85	20	13	1	2
10	56/F	134.91	13	16	1	2
11	56/F	158.67	13	9	1	2
12	66/F	110.06	8	10	2	1
13	79/M	158.49	4	1	1	1
14	65/F	84.57	20	7	1	2
15	60/F	171.17	22	7	1	2
16	60/F	113.33	40	16	2	3
17	60/F	110.83	25	19	2	2
18	60/M	174.67	8	5	1	2
19	74/M	61.75	12	14	2	3
20	58/F	89.25	13	9	1	2

HA : Hyaluronic Acid
ESR : Erithrocyte Sedimentation Rate
RAI : Ritchie Articular Index

Table - 2 : Characteristics of the Osteoarthritis patients.

	DMS (min)	GSR (kPa)	GSL (kPa)	RAI	ESR (mm/hr)	CRP (mg/dl)
RA patients	73.1 ± 48.6	26.22 ± 26.26	25.33 ± 26.30	24.28 ± 17.15	56.06 ± 38.84	31.06 ± 36.84
OA patients	8.5 ± 7.96*	51.65 ± 12.29*	50.30 ± 12.07*	10.00 ± 6.03*	16.00 ± 8.71*	1.0 ± 2.61*

* A statistically significant difference when compared with the RA patients ($p<0.01$)
DMS : Duration of morning stiffness
GSR : Grip strength (right hand)
GSL : Grip strength (left hand)
RAI : Ritchie Articular Index
ESR : Erythrocyte sedimentation rate
CRP : C-reactive protein

Table 3 : Mean values of the clinical and laboratory parameters of the disease (Data is presented as mean ± SD).

cant correlation ($p<0.01$) with Ritchie articular index scores, joint function scores and objective functional levels of the RA patients. Similarly Ritchie Articular Index and Steinbrocker joint function scores of the RA group showed a correlation with grip strength of both hands. The comparison of Ritchie Articular Index with sedimentation rate and the comparison of Steinbrocker joint scores with objective-subjective functional levels of daily activities showed a significant correlation with each other (Table 5).

	Urine HA levels (ng/ml) (mean ± SD)
RA Patients (n : 18)	126.89 ± 39.69*
OA Patients (n : 20)	117.85 ± 42.01*
Normal Controls (n : 17)	68.04 ± 35.41

RA : Rheumatoid Arthritis
OA : Osteoarthritis
HA : Hyaluronic Acid
* : A statistically significant difference when compared with the normal controls ($p<0.01$).
Table 4 : Urine levels of HA in patients with OA, RA and normal controls.

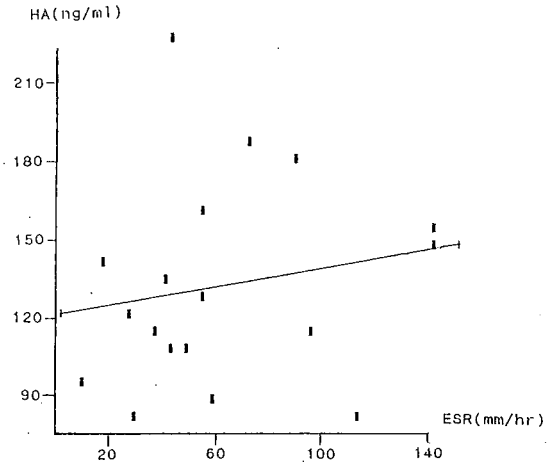

Fig 1 a : Regression analysis between urine hyaluronate level and sedimentation rate (r=0.18, p=0.485)

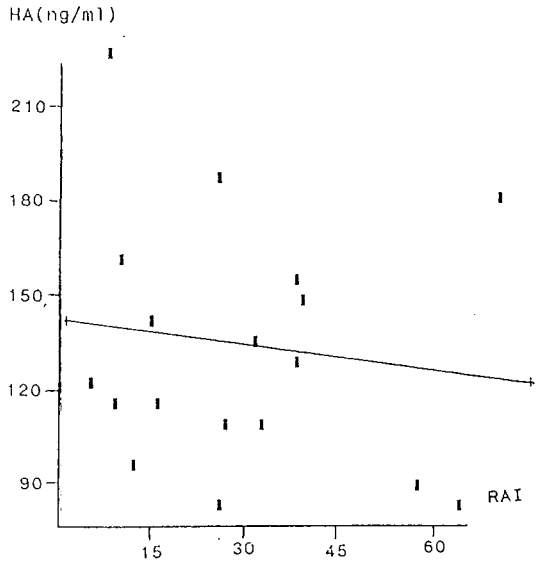

Fig 1 b : Regression analysis between urine hyaluronate level and Ritchie articular index in patients with rheumatoid arthritis (r=-0.13, p=0.631).

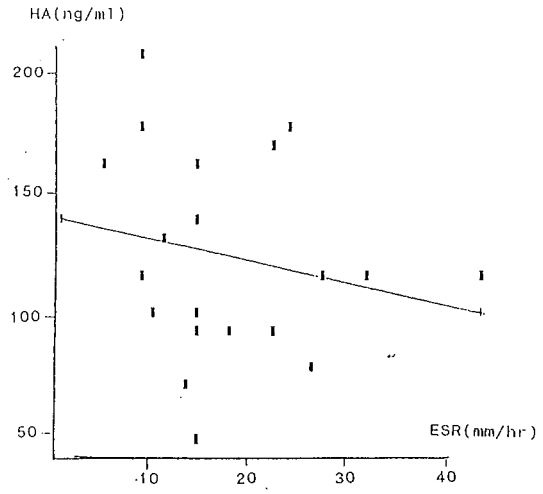

Fig 2 a : Regression analysis between urine hyaluronate level and sedimentation rate (r=-0.18, p=0.464).

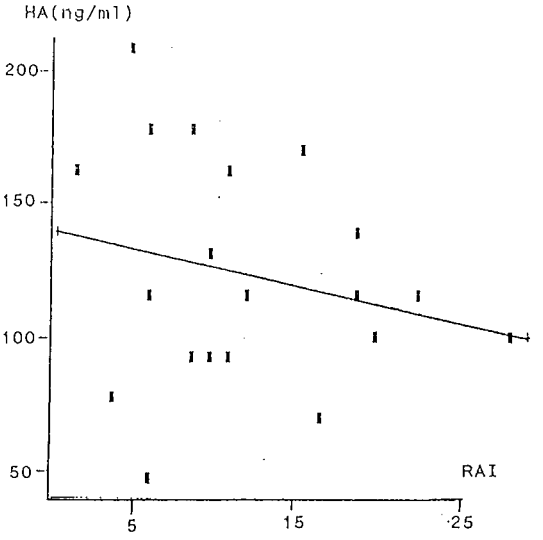

Fig 2 b : Regression analysis between urine hyaluronate level and Ritchie articular index in patients with osteoarthritis (r=-0.20, p=0.432).

In OA group, urine HA levels correlated with objective-subjective function levels of daily activities and grip strength of both hands (p=0.009) but there was not a significant correlation between urine HA levels and sedimentation rates (p=0.464).

DISCUSSION

It has been well defined that serum HA levels increase with synovial inflammatory activity. However, the results of several reports about the practical value of HA measurements are controversial. Goldberg et al have documented that the serum levels of hyaluronate are increased in RA and OA

	rha	rdd	rms	rgsr	rgsl	resr	rcrp	rlat	rrai	rss	rofc
rdd	0.066										
rms	-0.015	-0..323									
rgsr	0.083	-0.476	0.074								
rgsl	0.133	-0.382	0.096	0.966							
resr	0.188	0.165	0.180	-0.513*	-0.497						
rcrp	0.197	-0.074	-0.158	-0.037	-0.151	0.252					
rlat	0.125	-0.450	0.106	-0.007	-0.186	0.150	0.431				
rrai	-0.130	0.558*	-0.026	-0.505*	-0.528*	0.555*	0.245	-0.044			
rss	0.133	0.557*	-0.317	-0.607*	-0.603*	0.411	0.113	-0.128	0.631		
rofc	0.183	0.558*	-0.414	-0.485	-0.426	0.076	-0.044	-0.418	0.352	0.770*	
rsfc	0.224	0.340	-0.039	-0.356	-0.354	0.263	-0.036	-0.279	0.439	0.769*	0.674

dd : disease duration
ms : morning stiffness
grs : grip strength (right)
gsl : grip strength (left)
esr : erithrocyte sedimentation rate
crp : C-reactive protein
lat : latex
rai : Ritchie articular index
ss : Steinbrocker score
ofc : objective functional capacity
sfc : subjective functional capacity
* : $p<0.01$

Table - 5 : Correlation (r) Between Urine HA levels and other parameters in RA patients.

when compared with healthy controls (Goldberg et al. 1991). They found the mean plasma level of HA sevenfold higher in the RA group and twofold higher in the OA group with respect to the controls. The observed high serum concentrations of HA in RA patients when compared with OA patients should depend on the much more marked proliferation of synoviocyte-rich synovium in RA than in OA. Interleukin-1 is an inflammatory mediator, which is known to stimulate HA synthesis by synoviocytes. The higher concentrations of Interleukin-1 in the synovial fluid of patients with RA than in that of patients with OA has been reported previously (Fontana et al. 1982; Nouri et al. 1984). Our results confirmed the earlier observations but we did not find HA elevation in both patient groups as high as the levels reported. This may be related mainly to the suppressive effect of prescribed anti-inflammatory drugs and steroids. This effect on hyaluronate synthesis has been demonstrated previously in fibroblast tissue culture (Saarni and Hopsu-Havu, 1978). Many of our OA and RA patients were receiving NSAIDs and some RA patients were receiving steroids and DMARDs (disease modifying antirheumatism drugs) as well. The heterogeneity of the patient populations in different studies might also alter the results. It was established that male RA patients have higher levels of HA when compared with females (Paimela et al. 1991). The higher values in males is believed to be the result of the larger amount of synovial mass in men. In both of our patient groups, 80% of the subjects were female. The elevation of serum HA levels due to physical activity in RA patients and healthy subjects is also well documented (Engström-Laurent and Hallgren, 1987). But in this study, 24 hour urine samples were collected from thehospitalized patients performing restricted and uniform daily activities.

Severe, more destructive and progressive forms of RA may also elevate HA levels more consistently. Paimela et al concluded that in patients with progressive joint erosions, HA concentrations were elevated remarkably (Paimela et al. 1991). They also suggested that HA may have a prognostic value in early RA to predict subsequent joint damage. In our study, there was only one RA patient with a Steinbrocker radiologic score of 9, indicating an early less severe disease. It appears difficult to discuss

the prognostic value of HA in this study because a prospective follow-up study is needed to correlate radiologic deterioration and changing HA levels in time.

Although there still are conflicting reports in the literature about the correlation of the clinical and biochemical markers of inflammation, we found a correlation of these parameters when compared with each other, but these parameters did not correlate with urine HA excretion levels in RA patients.

Engström-Laurent and Hallgren did not also find any correlation between levels of plasma HA and ESR in RA (Engström-Laurent and Hallgren, 1985). Goldberg et al reported that levels of plasma HA did not correlate with levels of plasma elastase or ESR in their RA and OA patients but they found a correlation of HA with Articular Index and functional capacity score in both patient groups (Goldberg et al. 1991). They suggested that the increase in the level of synovial HA is independent from the systemic inflammatory response but is a sensitive marker of synovial hyperactivity. In another study, Levesque et al also found that HA levels did not correlate with clinical or biochemical markers of inflammation (Levesque et al. 1991).

The data obtained in our study suggest that HA does not behave as an acute phase reactant. The higher HA levels in RA and OA patients reflect an ongoing local synovial inflammatory process. Additional prospective studies are needed to discuss the practical value of measuring HA levels in assessing both the pathogenic changes in joints and prognosis in RA and OA.

Correspondence to : Dr.Jale TAN
Gazi Üniversitesi Tıp Fakültesi
Fiziksel Tıp ve Rehabilitasyon
Anabilim Dalı
Beşevler
06510 ANKARA - TÜRKİYE
Phone : 4 - 212 65 65 /486

REFERENCES

1. Balazs EA, Watson D, Duff IF, Roseman S : Hyaluronic acid in synovial fluid. I. Molecular parameters of hyaluronic acid in normal and arthritic human fluids. Arthritis Rheum 10 : 357-376, 1967
2. Balazs EA : The physical properties of synovial fluid and the special role of hyaluronic acid, in : Disorders of the knee, ed. A. Helft (JB Lippincott, Philadelphia). 1974
3. Chuck AJ, Murphy J, Weiss JB, Grennan DM : Comparison of urinary glycosaminoglycan excretion in rheumatoid arthritis, osteoarthritis, myocardial infarction and controls. Ann Rheum Dis 45 : 162-166, 1986
4. Dahl IMS, Laurent TC : Concentration of hyaluronate in serum of untreated cancer patients with special reference to patients with mesothelioma. Cancer 62 : 326-330, 1988
5. Engström-Laurent A, Lööf L, Nyberg A, Schröder T : Increased serum levels of hyaluronate in liver disease. Hepatology 5 : 638-642, 1985
6. Engström-Laurent A, Hallgren R : Circulating hyaluronate in rheumatoid arthritis : relationship to inflammatory activity and the effect of corticosteroid therapy. Ann Rheum Dis 44 : 83-88, 1985
7. Engström-Laurent A, Hallgren R : Circulating hyaluronic acid levels vary with physical activity in healthy subjects and in rheumatoid arthritis patients : relationship to synovitis mass and morning stiffness. Arthritis Rheum 30 : 1333-1338, 1987
8. Fontana A, Hengartner H, Weber E, Fehr K, Grob PJ, Cohen G : Interleukin-1 activity in the synovial fluid of patients with rheumatoid arthritis. Rheumatol Int 2 : 49-53, 1982
9. Fraser JRE, Laurent TC, Pertoft H, Baxter E : Plasma clearence, tissue distribution and metabolism of hyaluronic acid injected intravenously in the rabbit. Biochem J 200 : 415-424, 1981
10. Fraser JRE, Laurent TC, Engström-Laurent A, Laurent UBG : Elimination of hyaluronic acid from the blood stream in the human. Clin Exp Pharmacol Physiol 11 : 17-25, 1984
11. Goldberg RL, Huff JP, Lenz ME, Glickman P, Katz R, Thonar EJMA : Elevated plasma levels of hyaluronate in patients with osteoarthritis and rheumatoid arthritis. Arthritis Rheum 34 : 799-807, 1991
12. Klein F, Valkenburg HA, Cats A : On standardization of the latex fixation test. Bull Rheum Dis 26 : 866-868, 1976
13. Levesque H, Delpech B, Le Loet X, Deshayes P : Serum hyaluronate in rheumatoid arthritis : study by affinoimmunoenzymatic assay. Br J Rheumatol 27 : 445-449, 1991
14. Lindqvist U, Engström-Laurent A, Laurent U, Nyberg A, Bjorklund U, Erikson H, Pettersson R, Tengblad A : The diurnal variation of serum hyaluronate in health and disease. Scand J Clin Invest 48 : 765-770, 1988
15. Lundin A, Engström-Laurent A, Hallgren R, Michaelsson G : Circulating hyaluronate in psoriasis. Br J Dermatol 112 : 663-671, 1985
16. McCarthy M : Historical perspective on C-reactive protein. Ann NY Acad Sci 389 : 1-17, 1982
17. Moskowitz RW : Clinical and laboratory findings in osteoarthritis, in : Arthritis and allied conditions, ed. McCarty DJ (Lea and Febiger, Philadelphia). 1985, pp. 1408-1432
18. Nouri AME, Panayi GS, Goodman SM : Cytokines and chronic inflammmation of rheumatic disease. I. The presence of interleukin-1 in synovial fluid. Clin Exp Immunol 55 : 295-302, 1984

19. Paimela L, Heiskanen A, Kurki P, Helve T, Leirisalo-Repo M : Serum hyaluronate level as a predictor of radiologic progression in early rheumatoid arthritis. Arthritis Rheum 34 : 815-821, 1991
20. Ritchie DM, Boyle JA, McInnes JM, Jasani MK, Dalakas TG, Grieveson P, Bucharan WW : Clinical studies with an articular index for assessment of joint tenderness in patients with rheumatoid arthritis. QJ Med 37 : 393-406, 1968
21. Ropes MV, Bennett GA, Cobbs S, Jacox R, Jessar RA : 1958 revision of diagnostic criteria for rheumatoid arthritis. Bull Rheum Dis 9 : 175-176, 1958
22. Saami H, Hopsu-Havu VK : The decrease of hyaluronate synthesis by anti-inflammatory steroids in vitro. Br J Dermatol 98 : 445-449, 1978
23. Steinbrocker O, Traeger CH, Batterman RC : Therapeutic criteria in rheumatoid arthritis. JAMA 140 : 659-662, 1949
24. Westergren A : Studies of the suspension stability of the blood in pulmonary tuberculosis. Acta Med Scand 54 : 247-282, 1920
25. Whiteman P : The quantitative measurement of alcian blue - glycosaminoglycan complexes. Biochem J 131 : 243-350, 1973
26. Wu AHB, Parker OS, Ford L : Hyperviscosity caused by hyaluronic acid in serum in a case of Wilms' tumor. Clin Chem 30 : 914-916, 1984