

Nuriye METE¹
Mahmut METE²
İ. Hakkı ÖZTÜRK¹
Leyla ÇOLPAN¹

Plasma Fibronectin and Urine Glycosaminoglycane Levels in Rheumatic Diseases

Received: March 27, 1998

Abstract: In this study, plasma fibronectin and urine glycosaminoglycane levels were determined spectrophotometrically in 20 Rheumatoid arthritis, 10 Ankilosing spondilites, 22 osteoarthritis and 21 healthy control subjects. The mean levels of Fibronectin ($\mu\text{g/ml}$) were 413.3 ± 97 in patients with rheumatoid arthritis, 325.5 ± 93.5 in ankilosing spondilites, 439.1 ± 120.3 in osteoarthritis and 302.2 ± 60.1 in the control group. The ratio of urine glycosaminoglycan excretion to creatinine (g/mol) was 4.52 ± 2.64 in patients with rheumatoid arthritis, 4.18 ± 1.52 in patients with ankilosing spondilites, 2.72 ± 1.19 in osteoarthritis and 2.21 ± 1.02 in the control group. Plasma Fibronectin levels were significantly higher in rheumatoid

arthritis and osteoarthritis when compared to the control group ($p<0.001$). The ratio of urinary glycosaminoglycans excretion to creatinine levels in patients with rheumatoid arthritis and ankilosing spondilites were significantly greater when compared with control group ($p<0.001$).

Having established that plasma fibronectin levels and urine glycosaminoglycane/creatinine ratio were important laboratory findings in rheumatic diseases, and it was decided to employ further research to enlighten mechanisms that are still controversial.

Key Words: Rheumatic disease, fibronectin, glycosaminoglycane.

Department of ¹Biochemistry,
²Microbiology, Faculty of Medicine,
Dicle University, Diyarbakır-Turkey

Introduction

Rheumatoid Arthritis (RA), Ankilosing Spondilites (AS) and Osteoarthritis (OA) are multi-systemic connective tissue diseases. Fibronectin (FN) and Glycosaminoglycans (GAGs) are structural components of this tissue and they have important physiological and biochemical duties (1-10). To dates, there are a number of investigations have been hold for the diagnosis of R.diseases. It is thought that plasma FN and urine GAGs might be in these investigations, while they are the compound of the connective tissue.

So we investigate whether these connective tissue inter-components would help the diagnosis in determining degenerative changes, and to reveal their importance in distinguishing R.diseases and their follow up as well as the correlation between plasma FN level and urinary GAG excretion.

Material and Methods

In this study, plasma FN and urine GAG levels of 21

healthy subjects and 52 cases, 20 of whom were with RA, 10 with AS and 22 with OA, were determined. 17 of the RA cases were female, 3 were male, 1 of the AS cases was female, 9 were male and 18 of the OA cases were female, 4 were male. The mean age of the patients' group was accomplished by evaluating clinical findings, radiologic examinations and ARA (American Rheumatism Association) criteria (11, 12). In addition, laboratory examinations such as liver and renal function tests, C-reactive protein, rheumatoid factor (RF), eritrosit sedimentation rate (ESR), the whole blood and urine. By taking ESR into consideration, the group with RA was divided into sub-groups according to their activities. (L-5 case, M-6 case, H9 case). The all AS cases are in active stage.

The control group was formed from among individuals in whom no degenerative changes seen by arthrography and in whom blood analyses were normal.

Together with the first urinary samples in the

Variables	Control (n=21)	RA (n=20)	AS (n=10)	OA (n=22)	F	P
Age	37.05±8.04	40.60±11.25	25.80±21.50	51.68±7.4	23.2	0.00001
Fibronectin (µg/ml)	302.2±60.1	413.3±97.0	325.5±93.5	439.1±120.3	9.27	0.00001
Urine GAG/ Creatinine (g/mol)	2.21±1.02	4.52±2.64	4.18±1.52	2.72±1.19	8.26	0.0001

Table 1. The statistical values of parameters measured in plasma and urine.

Variables	RA.L (n=5)	RA.M (n=6)	RA.H (n=9)	F	P
Urine GAG/ Creatinine (g/mol)	2.71±0.96	3.87±1.8	5.48±3.02	5.03	<0.001
Plasma FN (µg/ml)	365±60.1	401±98.7	424±106	2.01	>0.05

Table 2. Comparison of urine GAG/Creatinine and plasma FN levels in the cases with RA according to their activity.

Cases with RA were divided into 3 activity groups according to ESR;
 RA.H: 61-125 mm/h
 RA.M: 41-60 mm/h
 RA.L: Under 40 mm/h

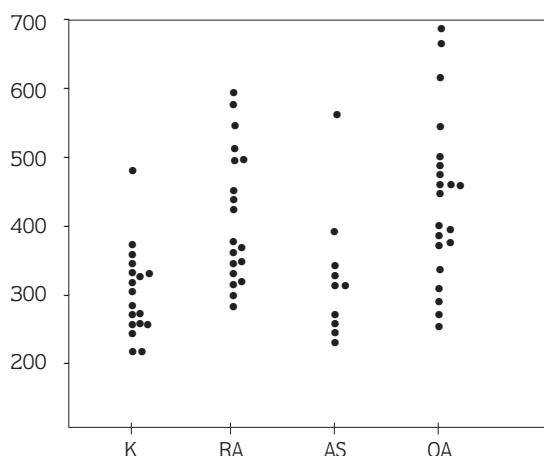


Figure 1. Distribution of plasma FN levels in all groups.

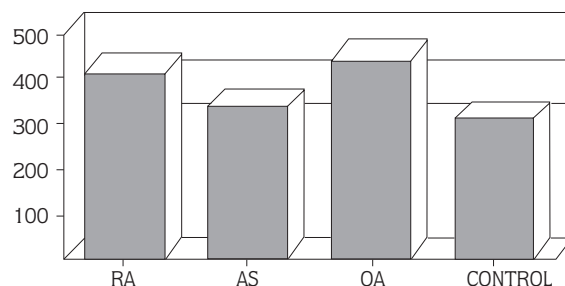


Figure 2. The mean values of plasma FN levels in all cases

morning, the blood samples with venous EDTA were taken simultaneously from the cases.

Plasma FN level was determined by turbidimetric immunoassay method, using Manheim Boehringer Kit (Cat No:401218).

Urine GAG level was determined by spectrophotometric method, based upon the staining of GAGs with cationic dyes (1, 9 dimethyl methylen Blue) (13) and urine creatinine was measured by an autoanalyzer.

Results were evaluated by SPSS computer programme. Analysis of variance was performed for the detection of significance of difference between groups.

Tukey-HSD procedure was used to investigate between which groups the difference was observed. Significance level was taken as 0.05 for Tukey-HSD procedure.

Findings

1- The mean plasma FN level was 413.3±97 µg/ml in patients with RA, 325.5±93.5 µg/ml in patients with AS, 439.1±120.3 µg/ml in those with OA, while it was measured as 302.2±60.1 µg/ml in the control group. The difference between the all groups was significant according to analysis of variance (F=9.27) (p<0.00001). According to Tukey-HSD procedure, the plasma FN level was significantly different between AS and OA, control and RA, control and OA groups (p<0.05) (Table 1) (Fig. 1, 2).

2- In order to diminish the changes that would arise

Variable	Fibronectin		Urine GAG/Creatinine	
RA-AS	413.3±97	325.5±93.5*	4.52±2.64	4.18±1.52
RA-OA	413.3±97	439.1±120.3	4.52±2.64	2.72±1.19***
AS-OA	325.5±93.5	439.1±120.3***	4.18±1.52	2.72±1.19**

Table 3. The statistical comparison of parameters between patient groups.

*P<0.05

**P<0.02

***P<0.01

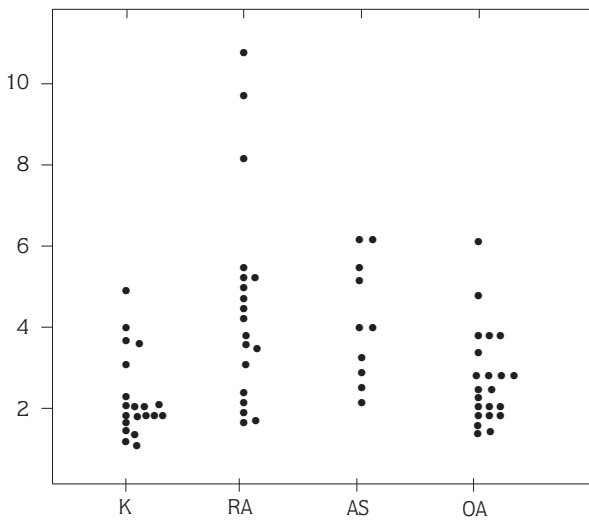


Figure 3. The statistical comparison of parameters between patient groups.

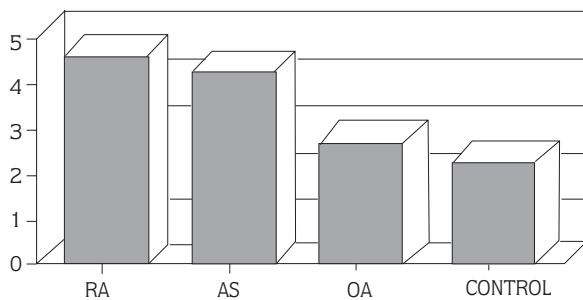


Figure 4. The mean values of urine GAG/creatinine ratios in all cases.

from liquid and excretion, urinary GAG values was evaluated by proportioning them with creatinine, as did other researchers. When the ratio of GAG to creatinine was measured, it was found to be 2.21 ± 1.02 as an average of g/mol in the control group. This ratio was 4.52 ± 2.64 in patients with RA, 4.18 ± 1.52 in those with AS, 2.72 ± 1.19 in patients with OA. The difference between the all groups was significant according to

analysis of variance ($F=8.26$) ($p<0.0001$). According to Tukey-HSD procedure, excretion of GAG in urine was significantly different between RA and OA, control and RA, control and AS groups ($p<0.05$) (Table 1) (Figure 3, 4).

3- Among the groups divided as Low (L, 5 case), Medium (M, 6 case) and High (H, 9 case) according to their activities by taking ESR into account. A significant difference only between urinary GAG excretions of high- and low-activity RA groups was observed (Table 2).

4- In all patient groups, any significant correlation between plasma FN level and GAG excretion through urine was not determined (in RA, AS and OA, $r=0.273$, $r=0.485$ and $r=0.118$, respectively). Between the age and FN levels of patients, marked correlation in patients with AS was observed ($r=0.823$ $p<0.01$).

Discussion

Fibronectin levels:

In this study, plasma FN levels in OA and RA were found to be higher than those in the control group ($p<0.001$). However, any significant difference was not seen among the findings of patients with RA, divided into groups according to their activities. Even though present results were in agreement with findings of others (14-18), there were also studies that were opposite to our findings (19, 20). Another interesting finding was that FN was lower in those with connective tissue disease.

Besides a number of cells, FN is synthesized in chondrocytes (16) and synovial cells. It plays an important role in joint inflammations and pannus extension, since it is an opsonic and chemotactic agent (1, 3). It has been demonstrated that FN is synthesized in neutrofil and in Type-B synovitis in rheumatic diseases (3). It has also been reported that synovial fluid FN fragments increase, and that this does not stimulate synovial proliferation, however (21). When the role of fibronectin fragments in the progress of inflammation was

investigated, it was explained that they are reacted with antibodies arising from streptococcus infections, and that these immune complexes have a role in the RA etiology (22).

In a study in which synovial fluid FN-mRNA level was investigated, the increase in FN was ascribed to the increase in the number of cells synthesizing protein, rather than to the arrangement of genes (10). They have reported that FN-mRNA levels in patients with RA, OA and AS vary, and that they, however, have not any relationship with the diagnosis of the disease and cell infiltration (10). Data obtained in the present study indicated that no differences between patients with AS and control group. This suggestion is confirmed by others finding (16, 17, 18, 21).

Low FN levels may be due to two reasons in cases with AS. One of the two reasons is that the disease involves the vertebral ligaments and synovial space is not wide enough because the peripheral tissue does not participate the process. The other reason is approach to terminal stage of the disease after ligamentary ossification completed (16, 18). However synovial tissue participates the process as polyarticularly in RA. Synovity of all joints is started and then continue with activations joints occurrence of arthrosis in rick from synovial tissue in OA results with progression of secondary synovitis (16, 18, 21).

From these study we suggest that: FN levels increase in patients with RA and OA, while not in those with AS. In order to form complete picture of this event, it will be necessary to look at other aspects such as molecular basis.

GAG excretion with urine

In our study, it was seen that the ratio of urine GAG/creatinine in patients with RA and AS increased significantly in respect to control group. However, there was not any different change in patients with OA. This finding was encountered only in one study (6).

In other studies carried out, increase of GAG in synovial liquid, serum and urine in various rheumatic diseases was reported (6, 23). Urine GAG measurement is a useful index that determines activity (6). Chondrocyte proliferation and damage have been reported as the reason for the increase in urinary GAG excretion. The changes observed in the PG content of tissue constitute the main part of pathophysiology of the disease (11).

Increase in GAG excretion fragmentation occurs during acute phase of reactive arthritis (8). In further stage of the disease, progressive destruction of matrix is prevented by the development of restructive mechanism. The role of GAGs in identifying the degree of cartilage damage in arthritis has been investigated, according to which, when cartilage damage progresses, it has been seen that the hyaluronan-binding site of proteoglicans is more dominant than the site rich in GAGs (8).

In this study, it was seen that GAG excretion through urine was more significant in the active state of RA. The recognition of the RA activity is important in determining the response of the drug to the disease. Another important finding was that there was not any relationship between plasma level and urinary excretion in all patient groups. Releasing and using mechanisms of these compounds which are connective tissue compounds should be investigated more comprehensively.

Data presented in this study suggest that determination of urinary GAG excretion might be an important parameter in inflammatory diseases of arthrosis progressing with matrix destruction, especially in the diagnosis and follow-up of RA and AS.

In addition; the diagnosis of R. patients indicated that plasma FN level is significant together with other parameters, but investigating Its' importance in the etiopathogenesis of the disease.

Results generated in this study also indicated that more comprehensive studies are required related to the role of synovial FN fragments in the progress of the disease.

References

1. Taşcıoğlu C. ve Ark.: Romatoid artritte plazma FN düzeyleri. Ist. Tıp Fak. Mecmuası 55: 169, 1992.
2. Mosesson MW, Amrani DL: The structure and biologic activities of plasma FN. Blood 56 (2): 145, 1980.
3. Shiozova SY, Yoshihara R, Kuroki Y, Fujita T, Shiozowa K, Imura S: Pathogenic importance of fibronectin in the superficial region of articular cartilage as a local factor for the induction of pannus extension on rheumatoid articular cartilage. Ann Rheum Dis 51: 869, 1992.
4. Woessner JF. Jr., Selzer MG: Two latent metalloproteases of human articular cartilage that digest proteoglycan. J. Biol. Chem. 259: 3633-3638, 1984.
5. Endreffy I, Disco F: Glycosaminoglycan excretion in connective tissue diseases: Clin Biochem, 21: 135, 1988.

6. 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, 1986.
7. Carroll G, Mc Coppin S, Bell M, Schwarzer A, Bredahl P: Comparison of keratan sulphate concentrations and the size distribution of proteoglycans in the synovial fluid of patients with osteoarthritis and pyrophosphate arthropathy. *Rheumatol Int*, 11: 63, 1991.
8. Saxne T, Heinegard D: Synovial fluid analysis of two groups of proteoglycan epitops distinguishes early and late cartilage lesions. *Athritis Rheum*, 35: 385, 1992.
9. Şen O, Erdem M: Mesane kanserli hastalarda glikozaminoglikanlar. *Ç.Ü.Tıp Fak. Dergisi*, 17: 35, 1993.
10. Shizawa S, Tokuhisa T: Contribution of synovial mesenchymal cells to the pathogenesis of rheumatoid arthritis. *Semin Arthritis Rheum* 21: 267-73, 1992.
11. Lawrence RC: Rheumatoid arthritis: Classification and epidemiology. In Klippel JH, Dieppe PA (Eds). *Rheumatology*. Mosby. London. 331, 1993.
12. Ball GW: Ankylosing spondylitis. In: Me Carty OJ (Ed): *Arthritis and Allied Conditions*. 1: 1052, 1983.
13. Wladimir Kery, Orłowska M, Stonckova M, Risko M, Zinay D: Urinary glycosaminoglycan excretion in rheumatic diseases. *Clin Chem*, 38: 841, 1992.
14. North ML, Lecocq J, Roth FM, Piffout MC, Asch L: Determination of synovial fibronectin by laser nephelometry. Its diagnostic value in rheumatology. *Ann Biol Clin*, 43: 861, 1985.
15. Ciobanu V, Popescu D, Baluna I, Baroc D, Tarnaw WR, Coralicea M: Plasma fibronectin level in patients with connective tissue diseases. *Med Interne*, 26: 267, 1988.
16. Robinson MW, Walton KW, Scott DG, Scott DL: Diagnostic relevance of fibronectin in cryoprecipitates. *Rheumatol Int*, 10: 81, 1990.
17. Carsons SE, Schwartzman S, Diamond HS, Berkowitz E: Interaction between fibronectin and C1g in rheumatoid synovial fluid and normal plasma. *Clin Exp Immun*, 72: 37, 1988.
18. Dutu A, Vlaicurus V, Bolosiu HD, Parasca I, Christea A: Fibronectin in plasma and synovial fluid of patients with rheumatic disease. *Med Interne*, 24: 61, 1986.
19. Peckhlivanov P, Altunkova I, Toncheva A: Fibronectin and its clinical significance in patient with osteoarthritis psoriatic arthropathy and chronic prostatitis with a joint syndrome. *Vutr-Boles*, 29: 81, 1990.
20. Herbert KE, Mopp PI, Griffiths AM, Revell PA, Scott DL: Synovial fluid fibronectin fragments no evidence for a mitogenic effect on fibroblasts. *Rheum Int*, 10: 199, 1990.
21. Gonzolez GM, Enghild JJ, Pizzo SV: Streptokinase and human fibronectin share a common epitope: Implications for regulation of fibrinolysis and rheumatoid arthritis. *Biochim Biophys Acta*, 1180: 283, 1993.
22. Malik SC, Pearson JP, Dick CW: A comparison of glycosaminoglycans content of human synovial fluid in different disease states. *Biochem Soc Trans*, 12: 874, 1984.
23. Bensouyad A, Hollander AP, Dularay B: Concentrations of glycosaminoglycans in synovial fluid and their relation with immunological and inflammatory mediators in rheumatoid arthritis. *Ann Rheum Dis*, 49: 301, 1990.