—**Original**—

Serum and Synovial Fluid Levels of Chondroitin Sulfate in Patients with Osteoarthritis of the Knee Joint

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Abstract

We measured serum and synovial fluid levels of chondroitin sulfate (CS) in patients with osteoarthritis (OA) of the knee joint to clarify whether CS levels differ in various stages of OA, and whether measurement of CS levels is useful in evaluating the pathology of OA. The study population was 117 OA patients and 23 healthy young volunteers. Synovial fluid was obtained from 69 of 117 OA patients. The mean serum level of C4S in all of the OA patients was significantly higher than that in the controls (p<0.05). However, there was little difference between respective OA stages. A small amount of serum C6S (0.2 to 0.6 nmol/ml) was detected in 31.6% of the OA patients and in 26.1% of the control subjects. The mean serum C0S level in all OA patients was significantly higher than that in the controls (p<0.01). However no significant difference was found between respective OA stages. As for synovial fluid CS, the C4S level in the early stage of OA tended to be higher than that in the advanced stage. C6S levels showed a markedly decreasing trend with advancing OA stage. C0S was not detected in synovial fluid. The present study demonstrated that serum levels of C4S and C0S in OA patients are elevated compared with normal subjects, and that synovial fluid levels of C4S and C6S may provide useful in assessing the pathology of OA. (J Nippon Med Sch 2001; 68: 165—170)

Key words: blood, synovial fluid, chondroitin sulfate, osteoarthritis, knee joint

Introduction

Glycosaminoglycans (GAGs) consist mainly of chondroitin sulfate (CS) and hyaluronic acid (HA) with other minor components[1]. GAGs are present in various connective tissues[2,3], and are also found in synovial fluid and serum[4-7]. They are not only essential matrix components, but also have some physiological functions[8]. In recent years, it has been demonstrated that measurements of HA levels in serum and synovial fluids are useful in the diagnosis of rheumatoid arthritis (RA) and of osteoarthritis (OA)[9-13]. Much attention has been focused on the role of CS as a marker of joint diseases, and changes in CS levels have been reported in various joint diseases[14-20]. However, to our knowledge, there have been no detaile, studies of CS levels in OA patients at various stages of the disease.

The aim of this study was to measure CS levels of serum and synovial fluid in patients with OA of the knee joint at various stages, and to clarify whether CS levels differ at various stages of OA, and whether measurement of CS levels is useful in evaluating the pathology of OA.

Table 1 Study population

	Number of patients			Age (yrs)	Disease period (month)
	Total	Male	Female		
Stage I	26	7	19	53.7 ± 7.6	8.9 ± 14.0
Stage II	50	10	40	66.3 ± 9.0	22.4 ± 20.6
Stage III	32	5	27	72.8 ± 9.6	68.9 ± 52.4
Stage IV	9	3	6	79.8 ± 9.1	122.6 ± 71.1
OA total	117	25	92	66.3 ± 11.8	39.8 ± 49.1
Control	23	15	8	26.3 ± 4.6	—

Table 2 Chondroitin sulfate levels in serum and synovial fluid

	Serum (nmol/ml)				Synovial fluid (nmol/ml)			
	No.	C4S	C6S	C0S	No.	C4S	C6S	C0S
Stage I	26	23.2 ± 5.7	trace	21.6 ± 3.6	9	20.0 ± 7.5	111.2 ± 61.3	N.D.
Stage II	50	24.6 ± 6.5	trace	22.6 ± 3.9	31	16.7 ± 5.8	76.0 ± 41.2	N.D.
Stage III	32	23.1 ± 5.3	trace	22.7 ± 3.9	21	14.6 ± 3.9	50.3 ± 17.7	N.D.
Stage IV	9	21.2 ± 6.4	trace	22.6 ± 2.5	8	16.8 ± 4.9	44.8 ± 17.1	N.D.
OA total	117	23.6 ± 6.0	trace	22.4 ± 3.7	69	16.5 ± 5.6	69.2 ± 41.8	N.D.
Control	23	20.8 ± 7.0	trace	20.0 ± 4.4	—	—	—	—

N.D.: not detectable

Materials and Methods

(1) Study population

We measured serum CS levels in 117 patients with osteoarthritis of the knee joint (25 men, 92 women) and 23 healthy young volunteers as controls (15 men, 8 women) without a history of knee symptoms and injuries (**Table 1**). Synovial fluids were obtained from 69 of 117 OA patients. The mean volume of synovial fluid was 10.4 ± 9.1 ml (range: 2.0 to 45.0 ml). The mean age of the patients and the control subjects was 66.3 years (range: 40 to 90 years) and 26.3 years (range: 18 to 35 years), respectively. Of the 117 patients, 26 were radiologically evaluated as stage I, 50 as stage II, 32 as stage III and 9 as stage IV by the Kellgren and Lawrence scale (knee version)[21]. The mean time interval from the onset of OA to examination was 39.8 months (range: 1 to 240 months).

(2) Biochemicals

Blood samples were centrifuged to obtain the serum at 10,000g for 15 minutes at 15℃. Synovial fluid samples were also centrifuged at 10,000g to remove cells and joint debris for 15 minutes at 4℃. Serum and synovial fluid samples were kept frozen at −80℃ until biochemical assay. The unsaturated disaccharides derived from chondroitin 4-sulfate (C4S), chondroitin 6-sulfate (C6S) and unsulfated chondroitin sulfate (chondroitin: C0S) were measured by high performance liquid chromatography (HPLC)[22]. The detection limit for CS concentration was 0.2 nmol/ml by this method. Statistical analysis was performed by analysis of variance and chi square test, and p = 0.05 was accepted as the minimum of significance.

Results

The measurements of serum and synovial fluid CS levels in the OA patients and control subjects are summarized in **Table 2**. C4S, C0S and small amounts of C6S could be detected in serum by the HPLC method used in this study. When the serum C4S levels in the OA patients and the controls were compared, the mean serum C4S level in all of the OA patients was significantly higher than that in the control (p < 0.05) (**Fig. 1**). However, there was little difference between the respective OA stages (**Fig. 2**). A small amount of serum C6S (0.2 to 0.6 nmol/ml) was detected in 31.6% of the OA patients and in 26.1% of
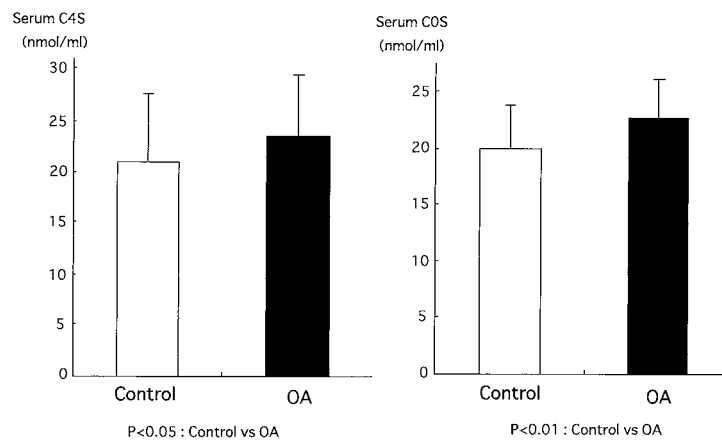
Fig. 1 Serum C4S and C0S levels in the OA patients and controls. The mean serum C4S and C0S levels in the OA patients were significantly higher than in the controls.
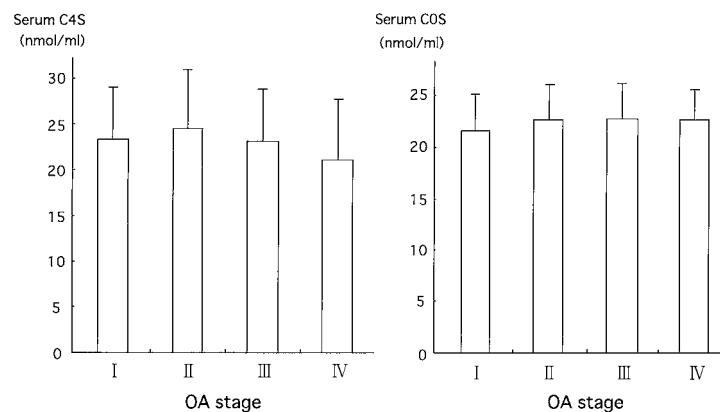

Fig. 2 Serum C4S and C0S levels at respective OA stages. No significant difference was found in serum C4S and C0S levels between respective OA stages.

the control subjects. The mean serum C0S level in all of the OA patients was significantly higher than that in the control ($p < 0.01$) (**Fig. 1**). However, no significant difference was found between respective OA stages (**Fig. 2**). As for synovial fluid CS, the mean C4S level in stage I tended to be higher than that in stage II or above (**Fig. 3**). The mean C6S level in stage I was also significantly higher than in stage II, III and IV. Moreover, C6S levels showed a markedly decreasing trend with advancing OA stage (**Fig. 4**). C0S was not detected in synovial fluid.

Discussion

In joint tissues, GAGs are continuously synthesized and degraded. They are released first into the synovial fluid, and then via the lymph vessels into the bloodstream[2].In studies of joint clearance, a consider-

able amount of labeled radioactive GAG injected into the joint cavity was released into the blood within a few hours[23]. The determination of GAG levels in the synovial fluid and serum may therefore allow an assessment of joint tissue metabolism. In regard to the GAG levels in OA, Alwan et al[24] noted that horses with OA showed high GAG levels in synovial fluid as well as in serum.

In recent years, CS derived from GAG chains has been specifically and quantitatively determined by the HPLC method[7,22]. C4S is distributed widely not only in articular cartilage, but also in synovium, ligament and meniscus[25]. Because C4S levels in synovial fluid are markedly elevated in RA and traumatic arthritis[14,15], C4S levels in synovial fluid have been thought to relate to the inflammatory process of joint tissues. Nishida et al[26] found in a study of GAG localization that high levels of C4S were located in in-

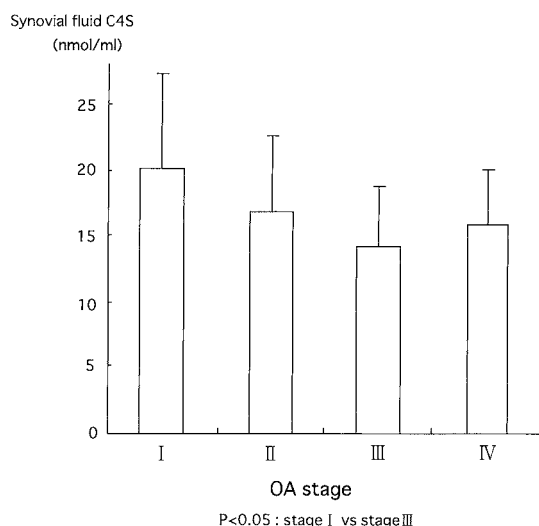Synovial fluid C4S
(nmol/ml)

OA stage

P<0.05 : stage I vs stage III

Fig. 3 Synovial fluid C4S levels at respective OA
stages. Synovial fluid C4S levels in stage I
tended to be higher than in stage II or above.

Synovial fluid C6S
(nmol/ml)

OA stage

P<0.05 : stage I vs stage II, stage II vs stage III, stage II vs stage IV
P<0.001 : stage I vs stage III, stage I vs stage IV

Fig. 4 Synovial fluid C6S levels at respective OA
stages. Synovial fluid C6S levels showed a
markedly decreasing trend with advancing
OA stage.

flamed synovial tissue. Thus, an elevation in synovial
fluid C4S level may reflect joint inflammation. In the
present study, synovial fluid C4S levels tended to be
elevated in the early stage of OA. This suggests that
C4S synthesis and degeneration in joint tissues may
increase due to inflammation and early OA change. It
also suggests that joint tissue inflammation such as
synovitis and cartilage degeneration by cytokines
may play a part in the pathology of OA in the early
stage. With regard to early OA and inflammation, Pa-
nula et al[27] reported in experimental OA that an in-
crease in synovial fluid levels of tissue inhibitor of
metalloproteinases (TIMP-1), stromelysin (MMP-3),
and the activity of phospholipase A_2 enzyme (PLA_2)
suggests the activation of inflammation-related proc-
esses in synovium and cartilage in the early stage of
OA. Smith et al[28] also stated that production of proin-
flammatory cytokines in synovial membranes are a
feature of early OA.

As for serum CS, Greenwald et al[29] reported that se-
rum CS increased in papain-induced arthritis in rab-
bits. Murata et al[30] stated that serum C4S elevated in
inflammatory diseases and in the early stage of wou-
nd healing. However, there is little information on se-
rum C4S levels in joint diseases. In this study, serum
C4S levels in all OA patients were significantly higher
than in the controls, but there was little difference be-
tween respective OA stages.

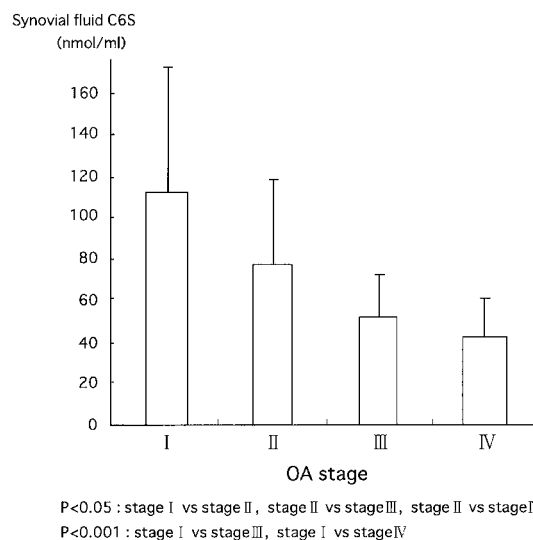Articular cartilage is rich in C6S. Shinmei et al[14]

stated that synovial fluid C6S derives mostly from ar-
ticular cartilage because of its large tissue volume in
the joint. In this study, C6S levels in synovial fluid de-
creased with advancing OA stage. We considered
that changes in synovial fluid C6S levels in OA may
reflect cartilage turnover and the cartilage mass re-
maining in the joint. Recently, Bayliss et al[31] reported
some interesting findings concerning zonal distribu-
tion of CS isomers in normal cartilage. They stated
that C6S concentration increases from the mid-zones
of the tissue toward the articular surface, and that the
highest concentration of C6S is located in the upper
quartile of the tissue. Therefore, when the articular
surface wears to some degree with advancing OA
stage, the amount of C6S released into the joint de-
clines precipitously, resulting in marked decreases in
C6S concentration. An additional explanation could be
an altered CS metabolism in the cartilage. Synthesis of
C6S in articular cartilage has been shown to decrease
with the progression of OA[32], The present study dem-
onstrates that changes in C6S levels in synovial fluid
reflect cartilage turnover and the cartilage mass re-
maining in the OA joint. This suggests that the meas-
urement of C6S levels may be useful in assessing the
pathology of OA. However the clinical significance of
measuring serum C6S is uncertain because serum C6S
levels are impossible to analyze quantitatively.

The major site of metabolism for circulating CS is

the liver, where CS may lose its sulfate residues as inorganic sulfate. Unsulfated CS (C0S) and inorganic sulfate are excreted in the urine. Part of CS is taken up by the polymorphonuclear leukocytes, where it is degraded[2,33]. In general, therefore, serum C0S has been thought to be an intermediary metabolite of CS. In fact, most C0S exists in the blood and urine, and no detectable amounts of C0S are found in joint tissues or synovial fluid. Generally, increased amounts of metabolite indicate increased tissue turnover. Poole et al[34] reported in a study of epitope 846 as a maker of CS synthesis that CS turnover in serum increased 56% in RA patients and 19% in OA patients. Thus elevated serum C0S levels in OA patients may indicate an increased turnover in joint tissues. However, Hata et al[35] reported that C0S may not be an intermediary metabolite of CS, but rather a material with physiological functions.

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