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## Evaluation of Glycosaminoglycans Levels in Normal Joint Fluid of the Knee

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### Abstract

Glycosaminoglycans (GAGs) level in joint fluid have been investigated in various joint diseases as a joint maker. However, there are very few studies of normal joint fluid providing a baseline for a better understanding of altered GAGs level in pathological joint fluid. We investigated GAGs level in knee joint fluid for 25 healthy young volunteers with a mean age of 27.5 years (range: 18 to 36 years). Biochemical evaluations included the concentration of hyaluronic acid (HA), chondroitin 4-sulfate (C4S) and chondroitin 6-sulfate (C6S), and the C6S/C4S ratio. The unsaturated disaccharides derived from HA by Morgan-Elson methods, and those from CS were measured with high performance liquid chromatography. The mean HA concentration was  $3.4 \pm 0.6$  mg/ml. The mean concentration of C4S and C6S, and C6S/C4S ratio were  $19.0 \pm 4.9$  nmol/ml,  $125.5 \pm 44.4$  nmol/ml and  $6.5 \pm 1.1$  respectively. (J Nippon Med Sch 2000; 67: 92—95)

**Key words:** hyaluronic acid, chondroitin sulfate, normal joint fluid, biochemical analysis

### Introduction

Glycosaminoglycans (GAGs) in joint fluid consist of hyaluronic acid (HA), chondroitin sulfate (CS) and keratan sulfate (KS) isomers. These GAGs have an important role in the joint lubrication and cartilage nutrition. Recently their concentrations have been thought to reflect joint tissue metabolism in normal and diseased states. GAGs levels have been investigated in various joint diseases<sup>1,3</sup>, and considerable interest has been developing as a joint marker for rheumatoid arthritis (RA) and osteoarthritis (OA).

However, there have been some debates<sup>4,5</sup> about whether GAGs levels in joint fluid increase or decrease in RA and OA, compared with those in normal subjects, because there are few studies of normal joint fluid. Our review of current published studies on nor-

mal joint fluid reveals the following limitations: studies are small, few description regarding to subject selection, inadequate study population and discrepancy between the results of HA and CS concentration. To our knowledge, is no detailed study evaluating normal GAGs levels, especially CS levels in joint fluid. The aim of this study is to clarify normal GAGs levels of joint fluid.

### Materials and Methods

We examined joint fluid from healthy young volunteers with no history of knee symptoms and injuries. Moreover athlete participating high level sports was excluded from the present study. Sufficient volumes of joint fluid for assay (0.2 ml) were obtained from 25 of 59 volunteers. The mean age of these 25 volunteers was 27.5 years (range: 18 to 36 years).

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Table 1 The results of glycosaminoglycans levels of synovial fluid in 25 healthy young volunteers

Case	Age	Sex	Volume	HA	C4S	C6S	C6S/C4S
			ml	mg/ml	nmol/ml	nmol/ml	
1	26	M	0.5	3.3	15.2	75.8	5.0
2	31	F	0.5	4.4	16.3	100.7	6.2
3	24	M	0.5	3.5	15.7	86.7	5.5
4	36	M	0.5	4.2	19.1	102.2	5.4
5	18	F	0.8	3.9	21.7	157.6	7.3
6	35	M	1.0	2.6	16.9	134.4	7.7
7	35	M	0.3	3.6	25.2	139.7	5.5
8	26	F	0.5	3.4	18.1	80.3	4.4
9	34	M	0.4	3.1	12.0	86.0	7.2
10	35	F	0.8	3.9	14.6	96.4	6.6
11	26	F	0.6	3.6	14.1	92.0	6.5
12	21	F	1.0	3.2	12.7	83.8	6.6
13	26	M	0.3	3.4	18.4	134.3	7.3
14	20	M	0.6	3.3	16.6	104.0	6.3
15	22	M	1.0	3.8	20.9	153.1	7.3
16	29	M	0.9	3.0	16.7	108.6	6.5
17	22	M	1.3	3.5	29.7	214.4	7.2
18	25	M	0.3	2.5	26.0	181.4	7.0
19	28	M	0.3	2.7	22.9	219.1	9.6
20	26	M	1.1	2.0	15.8	102.6	6.5
21	28	F	0.3	3.8	16.4	99.4	6.1
22	23	M	0.4	4.4	27.7	218.3	7.9
23	29	F	0.3	3.4	17.6	107.6	6.1
24	32	M	0.6	3.9	27.8	161.5	5.8
25	31	F	0.2	3.2	16.9	97.0	5.7

M : male, F : female, HA : hyalunonic acid, C4S : chondroitin 4-sulfate, C6S : chondroitin 6-sulfate, C6S/C4S : a ratio of C6S to C4S

Joint fluid samples were collected in sterile plastic tubes and centrifuged at  $10,000 \times g$  for 15 minutes at  $4^{\circ}\text{C}$  to remove cells and joint debris. The supernatants were stored at  $-80^{\circ}\text{C}$  until biochemical assay. The unsaturated disaccharides derived from hyaluronic acid (HA) by Morgan-Elson methods<sup>6</sup>, and those from CS were measured by high performance liquid chromatography (HPLC)<sup>7</sup>. Biochemical evaluations included the concentration of HA, chondroitin 4-sulfate (C4S), and chondroitin 6-sulfate (C6S), and C6S/C4S ratio.

### Results

The results of GAGs levels of joint fluid in 25 healthy volunteers were shown in **Table 1**. The mean volume of joint fluid was  $0.6 \pm 0.3 \text{ ml}$  (range: 0.2 to 1.3 ml). The mean HA concentration was  $3.4 \pm 0.6 \text{ mg/ml}$  (range: 2.0 to 4.4 mg/ml). The mean concentration of C4S and C6S were  $19.0 \pm 4.9 \text{ nmol/ml}$  (range: 12.0 to

$29.7 \text{ nmol/ml}$ ) and  $125.5 \pm 44.4 \text{ nmol/ml}$  (range: 75.8 to 219.1 nmol/ml) respectively. The mean C6S/C4S ratio was  $6.5 \pm 1.1$  (range: 4.4 to 9.6).

### Discussion

The determination of GAGs in human joint fluid has been difficult due to diversity of structures and limited amount of unsaturated disaccharides. Recently the development of HPLC allows more specific and sensitive determination of HA and CS isomers derived from GAG chains<sup>8,9</sup>.

HA is a main component of synovium and joint fluid. The viscoelastic property of joint fluid is mainly due to HA with a high capacity for binding water. Detection and quantitation of HA is of interest in many fields, especially in clinical chemistry. Recent studies demonstrated that HA has anti-inflammatory functions including inhibition of prostaglandin E2 synthesis<sup>10,11</sup>,

suppression of leucocyte migration and proliferation<sup>12</sup>, and protection against cytotoxicity caused by reactive oxygen species<sup>13</sup>.

In normal condition, HA concentration in joint fluid is maintained at a higher level, comparing with blood level. In inflammatory diseases<sup>1-3</sup> such as RA, gout and traumatic arthritis, HA concentration has been reported to be lower than normal. Balazs et al.<sup>14</sup> stated that a considerable variation was found in HA concentration of joint fluid collected from various joints. They reported that HA concentration in joint fluid of the knee was 2.5~3.8 mg/ml in normal subjects aged 18 to 35 years. Our results for HA concentration agree well with their study.

CS contain a repeating disaccharides of N-acetyl-galactosamine and glucuronate. In aggrecan<sup>15</sup>, most galactosamine residues carry a single sulphate group in 4 or 6 position, and a small portion are non-sulphated. Articular cartilage are rich in CS<sup>16</sup>, especially in C6S. Bayliss et al.<sup>17</sup> reported that C6S makes up 72~95% of CS in normal articular cartilage. C4S is distributed widely not only in articular cartilage, but also in synovium and ligament. Shinmei et al.<sup>18</sup> stated that C6S in joint fluid derives mostly from articular cartilage because of its large tissues volume in the joint, and that C6S concentration and C6S/C4S ratio may reflect cartilage metabolism.

Asari et al.<sup>19</sup> reported in a study of 6 young volunteers that the mean concentrations of C4S and C6S, C6S/C4S ratio were  $11.8 \pm 1.98$  nmol/ml,  $58.0 \pm 19.5$  nmol/ml and 4.9 respectively. However no subject selection and detailed back ground data of study population were described in their study. Namiki<sup>20</sup> noted in a study of 12 young athletes that the mean concentrations of C4S and C6S, C6S/C4S ratio and HA concentration were  $14.8 \pm$  nmol/ml,  $63.3 \pm 19.9$  nmol/ml,  $5.5 \pm 3.5$  and  $2.7 \pm 0.7$  mg/ml respectively. These authors stated that questions remain about whether athletes are adequate for study population, and found that the results of HA concentration was lower compared with a study of Balazs et al. In comparison with their results, our results showed higher values for all three parameters. The reason for the discrepancy between these results is unclear, but may be mainly differences in subject selection or study population.

Change in CS concentration of joint fluid have been

reported in various joint diseases. In recent studies, C4S and C6S concentrations and C6S/C4S ratio in RA<sup>21,22</sup> were reported to be 26.4~29.7 nmol/ml, 28.9~43.2 nmol/ml and 1.1~1.9 respectively, and those in OA<sup>23-25</sup> 15.4~20.4 nmol/ml, 56.8~81.1 nmol/ml and 3.8~4.0. Judging from normal level obtained in the present study, in RA, C4S concentration considerably increased and C6S concentration and C6S/C4S ratio markedly decreased. In contrast, in OA, C4S concentration is similar to normal and C6S concentration C6S/C4S ratio are considerably decreased. Shinmei et al.<sup>26</sup> noted that the proliferated synovium may accelerate the production and release of C4S into joint fluid, causing increased C4S concentration in RA, and loss of articular cartilage or an altered cartilage metabolism in RA and OA relate to decreased C6S concentration.

The present study demonstrates that GAGs level in normal joint fluid. Measurements of GAGs levels in joint fluid may provide useful information in monitoring joint tissue metabolism, and in assessing severity and the prognosis of joint diseases.

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