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Variation of serum hyaluronan with activity in individuals with knee osteoarthritis[1]

Lisa G. Criscione M.D.†[2], Alan L. Elliott M.D., M.P.H.‡[2], Thomas Stabler B.Sc.†, Joanne M. Jordan M.D., M.P.H.‡, Carl F. Pieper D.P.H.§ and Virginia B. Kraus M.D., Ph.D.†*

† *Department of Medicine, Duke University Medical Center, Durham, NC, USA*

‡ *Thurston Arthritis Research Center, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA*

§ *Department of Biometry and Bioinformatics, Duke University Medical Center, Durham, NC, USA*

Summary

Purpose: Serum hyaluronan (HA) was evaluated for diurnal variation in participants with osteoarthritis (OA) of the knee.

Methods: Twenty participants with radiographic OA of at least one knee were admitted overnight to the General Clinical Research Center for serial serum sampling. Serum was obtained between 6:00 p.m. and 8:00 p.m. on Day 1 (T3) after a day of normal activity. During the night of bed rest, participants remained supine for a minimum of 5 h between the hours of 3:00 a.m. and 8:00 a.m. Blood was drawn prior to arising from bed (T0), and 1 h (T1) and 4 h (T2) after arising and performing usual morning activities, including eating breakfast. During the morning, participants were encouraged to remain physically active and were not permitted to sit for more than 30 min at a time. Serum HA was measured by enzyme-linked immunosorbent assay. Results were analyzed using non-parametric Freidman's test with Dunn's *post-hoc* Multiple Comparison test.

Results: Serum levels of HA increased significantly from T0 to T1 ($P < 0.01$). There were no other significant changes in serum HA levels observed between any of the other time points.

Conclusions: Although a rise in serum HA with activity and eating has been demonstrated previously in individuals with rheumatoid arthritis, this is the first study to demonstrate a similar rise in individuals with OA. These results suggest that serum sampling for HA in OA clinical trials should be performed more than 1 h after arising in the morning and at least 1 h after breaking an overnight fast.

Key words: Hyaluronan, Osteoarthritis, Diurnal variation, Biomarker, Physical activity.

Introduction

Serum hyaluronan (HA) is a marker of osteoarthritis (OA) status[1,2]. HA is a high molecular weight glycosaminoglycan, composed of alternating subunits of glucosamine and glucuronic acid. The strong association of serum HA with OA is somewhat surprising given the widespread distribution of HA as a constituent of loose connective tissue throughout the body. HA is however present in high concentrations in joint tissues including synovium, cartilage, and synovial fluid.

The majority of HA (85%) is cleared from joints by way of lymphatics with the remainder cleared by the liver via the general circulation[3,4]. The daily variation in serum HA, and optimal sampling procedure, has not previously been evaluated in the setting of OA. In rheumatoid arthritis (RA), serum HA levels have been shown to increase in association with activity[5,6], and as a function of synovial mass[7]. Healthy controls have been shown to have low baseline concentrations of serum HA which increase up to two times baseline levels with activity[5,6,8] (Table I). However, no elevation in serum HA has been seen with activity in a reference group of hospitalized patients with other non-arthritic diseases which included glaucoma, orbital tumor, mycosis fungoides, and polymorphous light eruption[8]. We were interested in determining whether diurnal and activity related variation in serum HA was evident in OA, a joint disease typically characterized by less overt and less frequent episodes of synovitis than RA.

Methods

PARTICIPANTS

Participants with radiographic knee OA of at least one knee were recruited through the Rheumatology outpatient clinic. Participants were excluded on the basis of arthropathy due to RA, systemic lupus erythematosus, psoriasis, gout, or hemochromatosis. Based upon self-report, no participant in our study had liver disease, a cause of elevated serum HA levels. All procedures were approved by the Institutional Review Board of Duke University Medical Center. Participants were admitted to the General Clinical

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[2]The first two authors contributed equally to this work.

*Address correspondence and reprint requests to: Virginia Byers Kraus, M.D., Ph.D., Box 3416, Duke University Medical Center, Durham, NC 27710, USA. Tel: 1-919-681-6652; Fax: 1-919-684-8907; E-mail: vbk@acpub.duke.edu

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Table I
Summary of studies evaluating daily variation in serum HA levels in individuals with arthritis

Study	Mean serum HA after bed rest (ng/ml)	Mean serum HA after morning activities (ng/ml)	Magnitude increase in HA with activity*	ELISA method
Engström-Laurent *et al.*[5]	Controls: 26 RA: 124	Controls: 54 RA: 402	2.08 3.24	Affinity method
Lindqvist *et al.*[8]	Controls: 65 RA: 196	Controls: 93 RA: 1183	1.43 6.04	Competitive radioimmunoassay
Manicourt *et al.*[6]	Controls: 32 RA: 69	Controls: 62 RA: 173	1.94 2.51	Sandwich ELISA
Present study	OA: 69	OA: 136	1.98	Competitive ELISA

*$P < 0.01$ for change with activity for each group in each study.

Research Center (GCRC) of Duke University Medical Center in the late afternoon for an overnight stay. Participants were allowed to take all of their usual medications, including non-steroidal anti-inflammatory drugs and dietary supplements such as vitamins and glucosamine and/or chondroitin sulfate.

OA ASSESSMENT

On the day of admission, weight-bearing, 30° semiflexed posteroanterior radiographs of both knees were taken with the SynaFlex™ x-ray positioning frame (Synarc, San Francisco, CA)[9]. Knee radiographs were scored for Kellgren–Lawrence (K–L) grade[10] by the consensus of two trained observers. Knee OA was quantified as the sum of the K–L grades for both knees, and totaled ≥ 2 for each participant. Four participants had undergone unilateral total knee replacement and the replaced knee was assigned a K–L score of 0 for purposes of analysis. Hip OA was defined clinically by physician assessment based upon ACR criteria: hip pain with either (1) internal hip rotation $<15°$ degrees and hip flexion $\leq 115°$, or (2) internal hip rotation $\geq 15°$, pain associated with internal rotation, morning stiffness of the hip of ≤ 60 min, and age over 50 years[11]. The number of affected hips was recorded as 0, 1, or 2. The total number of knees with radiographic OA and hips with clinical OA was summed as 1, 2, 3, or 4 joints.

CLINICAL VARIABLES

Morning stiffness of the knees was quantified on a 0–3 scale as follows: 0 (none), 1 (up to 30 min), 2 (31–60 min), or 3 (more than 60 min). All participants completed a western Ontario and McMaster Universities (WOMAC) OA index[12] on the evening of admission. Weight and height were recorded for computation of body mass index (BMI, kg/m^2).

BLOOD SAMPLING

Blood was first collected between 6:00 p.m. and 8:00 p.m. on the evening of admission to the GCRC, after a normal day of activity and after eating. As this was the latest sample of the day, this sample was designated T3. Individuals retired to sleep at their usual hour, with instructions to remain supine, at a minimum between 3:00 a.m. and 8:00 a.m. If necessary, a bedpan or urinal was provided during this interval. At 8:00 a.m., participants were awakened, and phlebotomy was performed while the participant remained supine (T0). Immediately thereafter, participants were served breakfast, and asked to perform their usual daily routine. Phlebotomy was again performed 1 h after arising (T1), and 4 h after arising (T2). Participants were encouraged to remain physically active throughout the morning, and were not permitted to sit for more than 30 min at a time. Sera were separated and immediately frozen to $-20°$C within 2 h of collection, then transferred to $-86°$C for long-term storage.

HA ELISA ASSAY

Samples were analyzed for HA in duplicate, blinded, and in random order using an indirect competitive enzyme-linked immunosorbent assay (ELISA) as previously described[1]. The inter- and intra-assay variability for this assay were 13.6% and 6.3%, respectively.

STATISTICAL ANALYSIS

Statistical computations were performed using GraphPad Prism® (GraphPad Software, Inc., San Diego, CA) and JMP® (SAS, Cary, NC) software. Results were analyzed using non-parametric Freidman's test with Dunn's *post-hoc* Multiple Comparison Test (with Bonferroni correction). To evaluate associations of HA with variables and risk factors related to OA status (total WOMAC score, minutes of morning stiffness, K–L sum score of the knees, total numbers of OA joints, age and BMI), we performed multiple linear regression. The change in HA levels with activity in this cohort were compared to the levels reported in the literature for RA patients and controls. A P value of <0.05 was considered significant.

Results

A total of 20 participants (65% female, 80% Caucasian, and 20% African American) with radiographic knee OA underwent serial serum sampling within a 16-h period. Participants ranged in age from 59 to 91 years (mean 70 years). A total of five (25%) participants had unilateral knee OA, the remainder had bilateral disease. A total of seven (35%) participants also had hip OA by clinical exam (100% bilateral).

Serum HA levels varied up to a factor of 8 among participants at any particular time point. However, upon arising in the morning and performing typical daily activities for 1 h, the serum HA levels rose in 17 of the 20 participants (Fig. 1). The change in HA was significant from T0, before arising, to T1, after 1 h of morning activities ($P < 0.01$) (Fig. 2). The tendency of serum HA levels to decrease after

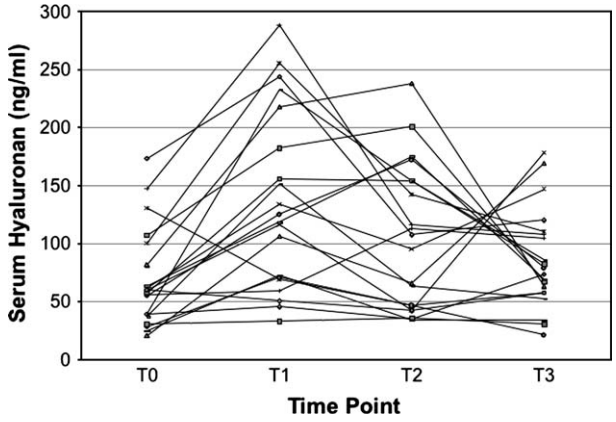

Fig. 1. Changes in serum HA with activity in individual participants ($N = 20$). T0 – 8:00 a.m. sample, prior to arising from bed; T1 – 9:00 a.m. sample, 1 h after arising from bed and performing morning activities; T2 – 12:00 noon sample, 4 h after arising from bed; T3 – 8:00 p.m. sample, after approximately 12 h of daily activities.

the first hour of morning activity was not statistically significant. Mean serum levels of HA were 69 ± 43 ng/ml (mean ± SD) at T0, 136 ± 76 ng/ml at T1, 104 ± 63 ng/ml at T2, and 86 ± 44 ng/ml at T3. The mean serum HA levels in these OA participants were 2.5–5.4 times greater than the mean level in a control population ($N = 298$, mean HA level of 25 ng/ml) that we have analyzed previously using the same HA assay method[1]. The absolute levels of serum HA are difficult to compare among other studies utilizing different assay methods; nevertheless, it is possible to compare the magnitude of the increase in HA in response to activity across these various studies. The magnitude of change from T0 to T1 (1.98 times) in this cohort, was less than the activity related increase reported for RA patients (2.51–6.04 times), and comparable to or greater than the increase reported for controls (1.43–2.08 times) (Table I).

In a multiple linear regression model, the only significant associations between the OA variables and HA in this small sample were observed for the HA T3 value. Significant associations were observed between HA T3 and duration of morning stiffness of the knees (parameter estimate $b = 33.5$, $P = 0.02$), the total WOMAC score ($b = -1.41$, $P = 0.03$), and weakly with K–L grade sum score ($b = 9.1$, $P = 0.09$) but not with age. After the addition of BMI to the model, the association of the HA T3 value with K–L grade sum score was not significant ($P = 0.17$), but the associations with duration of morning stiffness of the knees and total WOMAC score were unchanged.

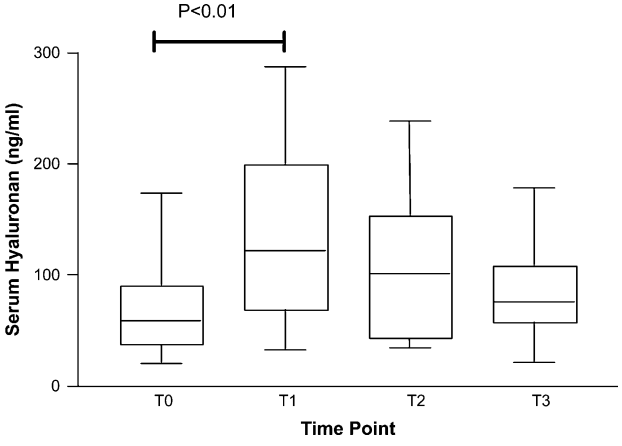

Fig. 2. Serum HA levels over time. The box plots depict the median, 25th and 75th percentile values and upper and lower limits of serum HA levels (expressed in ng/ml).

Discussion

To our knowledge, this is the first study to evaluate the daily serum HA variation in a cohort of individuals with radiographic knee OA. In this OA cohort, serum HA levels rose significantly with morning activities including eating breakfast. This pattern is similar to the change in serum HA observed for patients with RA[5,8,13]. In RA patients, the magnitude of the transient increase in serum HA upon joint mobilization was associated with the amount of synovitis and duration of morning stiffness[5]. These findings have been ascribed to accumulation of water with the hydrophilic HA during joint immobilization in proportion to the amount of inflamed joint tissue. It is speculated that this mechanism results in hindered joint movement and the symptoms of joint stiffness, which are alleviated as the joint is mobilized and HA is transported via the lymphatics to the circulation. We therefore believe the variations in HA with activity in OA patients to be mechanistically similar, namely that HA accumulates in lymph nodes, synovium, and subsynovial tissues of OA joints during rest, as has been postulated for RA[7], and is transported via the lymphatics to the circulation during physical activity.

Several past studies have provided insight into the variability in serum HA due to assumption of an upright posture[14], activity[15], and eating[16], all of which lead to increases in serum HA. Increases in serum HA with activity were more pronounced in individuals with inflammatory arthritis than normal controls, as demonstrated by Engstrom-Laurent and Hallgren *et al.*[5], Manicourt *et al.*[6] and Lindqvist *et al.*[8]. By changing the time of the first meal of the day, Rossler *et al.*[16] were able to change the time of day of the serum HA peak in five healthy males. When standard portions were provided to resting individuals, HA increased 1 h after the first meal of the day, and did not increase with further meals. When meals were supplied in small aliquots every 30 min rather than as one large meal, peak serum HA levels were attenuated, though the only significant change still occurred 1 h after the first feeding. These studies together suggest that serum HA levels are influenced by both the first food intake and the first episode of mobilization of the day, independent of the actual time of day. In this regard, the variation in HA is not strictly diurnal but rather activity related. Based upon these results, and insights derived from past studies, we suggest serum sampling for HA more than 1 h after arising and becoming active in the morning and at least 1 h after breaking an overnight fast.

The HA macromolecule exists as a stiff random coil in solution[17]. Conformational changes in HA, induced by oxidation or by digestion into smaller fragments, can alter the apparent HA concentration as measured with an HA-protein binding assay of the type used in this study[17,18], presumably as a result of unmasking new HA-protein binding sites. It has been shown that a minimum of 10 HA-derived oligosaccharide units are needed to bind to an HA-binding protein, and the ability to bind diminishes above a size of 100 HA-derived oligosaccharide units[17]. In RA patients, the transient peak in response to morning activity consists of

relatively low molecular weight HA[13], a size probably readily detected by a HA-protein binding assay. Thus the apparent concentrations of HA, and in particular, the transient rise in the morning after 1 h of activity, may reflect both the quantity of HA and the size and conformation of HA.

A limitation of our study was the small sample size that limited our ability to find relationships of statistical significance between HA levels and OA variables. However, the repeated measures design increased the study power to detect diurnal changes in HA levels. In summary, our study demonstrates variation in serum HA in an OA population in response to activities of daily living in the morning. These results suggest that serum HA levels drawn more than 1 h after arising can be considered representative of a stable level of this OA biomarker in individuals with knee and hip OA.

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