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Determining the usefulness of serum hyaluronic acid levels as a predictor of progression of hand osteoarthritis: longitudinal analysis from the Iwaki cohort

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Abstract

Background: Hand osteoarthritis (HOA) causes a significant disfunction in patient's daily life. The predicting factor of hand osteoarthritis has been unknown. We aimed to investigate the usefulness of serum hyaluronic acid (sHA) levels in predicting progression of HOA from a 6-year longitudinal epidemiological study.

Design: In 2008, a total of 417 participants in the Iwaki cohort were followed over 6

years. Hand radiographs were taken at baseline and follow-up and scored according to Kellgren–Lawrence grades and Kallman score for 15 joints. Based on the presence of osteoarthritis, participants were classified into HOA and non-HOA groups. Levels of serum hyaluronic acid (sHA) at baseline were determined by ELISA. Spearman's correlation coefficients between levels of sHA, total number of involved joints, and Kallman score were estimated. Factors related to increasing number of involved joints over a period of six years were analyzed by liner regression analysis.

Results: The prevalence of hand osteoarthritis was 19.9% at baseline and the number of joints involved was 3.6 ± 2.1. Levels of sHA in the HOA group at baseline were significantly higher than non-HOA group ($p<0.0001$), and correlated with the number of involved joints ($r=0.399$, $p<0.0001$) and Kallman score ($r=0.540$, $p<0.0001$). Progression rate was 55.4% and development rate was 19.1% over six years. In HOA group, the number of involved joints increased by 4.9 ± 2.3. Associated factors were age ($p<0.0001$) and higher levels of sHA ($p<0.0001$) at baseline.

Conclusions: Higher levels of sHA correlated with number of involved joints and Kallman score at baseline. In the longitudinal study, Levels of sHA predicted progression of HOA over six years.

Keywords: Serum hyaluronic acid, Biomarkers, Hand osteoarthritis, Kallman score

Introduction

Hand osteoarthritis (HOA) is a common disease in the elderly, and the prevalence as reported radiographically sin population-based studies is 29 to 89% in middle-aged females [1-5]**.** HOA causes chronic pain and disabilities that lead to serious problems in activities of daily living. It also has a significant impact on socio-economic status. Although early detection of higher risk patients is necessary in order to begin a preventive approach, patients could not recognize the severity of their HOA until it progressed and caused serious pain and disabilities. Also, the natural history of this disease and therapeutic strategy for preventing progression has not been established. While there were several potential problems regarding high prevalence and progressive activity of this disease, radiographs could not detect minute changes at an early stage. Hence, an easier quantitative evaluation of disease activity needs to be established.

As the evaluation tool of synovitis, serum biomarkers have attracted attention. Biomarkers are measured from blood and urine, and many substances that specifically reflect the condition of bone, cartilage and synovitis have been reported [6,7]. Biomarkers are suggested as a diagnostic tool and severity predictor of Knee OA, possibly as prognostic predictor [8]. Among them, serum hyaluronic acid (sHA) is strongly related to symptoms and progression of OA since it reflects the state of

synovitis. It is gaining attention as a biomarker for OA severity and a predictor of OA progression. Regarding finger OA, it was revealed that higher sHA levels were corelated with the number of osteoarthritic joints in a population-based cohort study [9], and progression of joint space narrowing from longitudinal observations focusing on the patients [10]. However, there has been no longitudinal evaluation of the relationship between long-term radiographic changes in HOA and levels of sHA in epidemiological studies. Furthermore, it is unclear whether sHA levels could be a predictor of HOA progression.

The aim of this study was to investigate whether sHA levels could reflect the severity and number of involved joint in HOA. Furthermore, we examined the predictive power of sHA levels in determining the progression of HOA in a longitudinal cohort study. We hypothesized that higher levels of sHA at baseline could predict the newly incident number of involved joints over six years

Methods

Subjects were voluntary participants from the Iwaki Health Promotion Project of 2008 and 2014, a community-based program to prevent lifestyle diseases and improve average life expectancy by performing general health checkups and prophylactic

interventions [11,12]. It is an annual program that has been performed in the general population living in the Iwaki area of Hirosaki City located in western Aomori prefecture, Japan, since 2005. This cohort study allows evaluation of many kinds of diseases and disorders from various perspectives and research into the risk factors of locomotive disability. All participants provided written informed consent, and the study was conducted with the approval of the ethics committee of the Hirosaki University School of Medicine.

Subjects

A total of 887 volunteers from approximately 12,000 residents participated in this project in 2008. They were recruited via phone calls from public health nurses and an advertisement in the mass media. Those who had renal failure, liver failure, rheumatoid arthritis, malignant tumors and incomplete questionnaires were excluded from the study. Those who did not undergo radiographic examination were also excluded. A total of 724 participants (273males and 451females) were enrolled at baseline. Among them, 417 participants (145 males, 272 females) were followed up in the Iwaki 2014 cohort. The follow up rate was 57.5%. Height and body weight were measured, and body mass index (BMI) was calculated.

112

113 ***Measurement of sHA levels***

114 Blood samples were taken from all participants early in the morning for biochemical
115 examination at baseline and follow-up. Blood sampling was performed before breakfast
116 because circulating sHA increases following a meal [13]. The levels of sHA were
117 determined using the Hyaluronan Assay Kit (Seikagaku Corporation, Tokyo, Japan) [9].
118 The change in sHA levels over six years was defined as ⊿sHA .

119

120 ***Radiographic diagnosis***

121 Radiographs were taken for joint evaluation: postero-anterior view of bilateral hands
122 and antero-posterior view of weight-bearing bilateral knees. The following regions were
123 evaluated from each joint group by trained orthopedic surgeons (R.U. and H.I.). The
124 second to fifth distal interphalangeal (DIP), proximal interphalangeal (PIP), thumb
125 interphalangeal (IP) and carpometacarpal (CMC), and scapho-trapezial joints for each
126 hand were graded according to the Kellgren-Lawrence classification (KL) [14].
127 Radiographic OA was defined as KL grade ≧2. Participants with at least one involved
128 joint at baseline were assigned to the HOA group while those without radiographic
129 HOA were in the non-HOA group. Furthermore, participants with an increasing number

130 of involved joints over the period of six years, were classified into the Increasing group.
131 Similarly, the presence of knee OA was also evaluated based on the KL scale in both
132 knee radiographs and defined as OA with KL grade 2 or more. Furthermore, the degrees
133 of HOA were also scored according to the Kallman score [15]. Individual hand joints
134 were assessed for the presence of osteophytes (graded 0-3), joint space narrowing (0-3),
135 subchondral sclerosis (0-1), subchondral cysts (0-1), lateral deformity (0-1), and
136 collapse of central joint cortical bone (0-1) with a total of 208 points. To investigate the
137 intra-observer reliability of the scale, 20 randomly selected hand radiographs were
138 scored by the same reader, and two orthopedists (RU and HI) also scored the 20
139 radiographs to assess the inter-observer reliability. The intra- and inter-observer
140 reliability was assessed by the k-statistic, and they were 0.78 and 0.77, respectively.
141
142 ***Statistical analysis***
143 Data input and calculations were performed with SPSS ver. 12.0 J (SPSS Inc.,
144 Chicago, IL, USA). In the baseline data, Chi square testing was performed between
145 HOA and non-HOA groups to compare gender, knee OA and smoking status. The
146 Mann-Whitney U test was performed to compare age, BMI, and sHA levels at baseline.
147 Spearman's correlation coefficients were estimated among sHA levels, number of

involved joints, and Kallman score at baseline. In the longitudinal analysis over six years, the baseline levels of sHA and ⊿sHA were compared using the Mann-Whitney U test between Increasing and non-Increasing groups. Furthermore, logistic regression analysis was performed with a model, in which the presence of increasing number of involved joints was a dependent variable, while baseline levels of sHA or ΔsHA, and relevant factors like age, gender, BMI, smoking, and presence of knee OA were independent variables. A receiver operating characteristic (ROC) analysis was performed to determine whether the levels of sHA at baseline could predict the presence of increasing number of involved joints. We calculated the area under the curve (AUC). The optimal cut-off point was the highest Youden index value (sensitivity + specificity − 1). A p-value below 0.05 was considered to be statistically significant.

Results

Eighty-two of 417 participants (19.7%) were classified into the HOA group **(Table 1)**. The HOA group was older (p<0.0001) and had a higher proportion of females. The prevalence of knee OA was higher (p<0.0001), but no significant difference was observed in BMI **(Table 1)**. The prevalence of HOA at baseline was 16.5% in males and 24.6% in females. Comparing the prevalence of HOA among interphalangeal joints in

166 all cases, the prevalence in the thumb CM joint, the thumb IP joint, and the DIP joints
167 were high **(Fig.1)**. The mean levels of baseline sHA were 56.5 ± 30.1 (ng / ml) in the
168 Non-HOA group and 107±79.3 (ng/ml) in the HOA group, which was significantly
169 higher than the non-HOA group ($p<0.0001$) **(Fig.2A)**. In addition, there was a
170 significant correlation between baseline levels of sHA and the number of involved joints,
171 and the correlation coefficient was 0.399 ($p <0.0001$) **(Fig.2B)**. Similarly, there was a
172 significant correlation between baseline levels of sHA and higher baseline Kallman
173 score, with a correlation coefficient of 0.540 ($p <0.0001$) **(Fig.3)**.
174 Over six years of follow up, 16.8% of males and 24.2 % of females showed an
175 increase in number of HOA joints on X-ray. Eighty-eight (21.1%) participants were
176 classified into the Increasing group and 329 (78.9%) participants were in the
177 non-Increasing group. The mean levels of baseline sHA in the non-Increasing group was
178 59. 3±33.7 ng/ml, and that of the Increasing group was 92.7±78.2 ng/ml ($p<0.0001$)
179 **(Fig.4A)**. In addition, the values of ⊿sHA in the Increasing group were also
180 significantly higher than those of the non-Increasing group **(Fig.4B)**. Logistic regression
181 analysis showed that the levels of baseline sHA were significantly correlated with the
182 increasing number of involved joints **(Table 2)**. From the ROC curve, the levels of
183 baseline sHA had a high predictive ability (AUC = 0.708, $p<0.0001$) for an increase in

number of involved joints, in which the cut-off level was 46.1 ng/ml with an odds ratio of 4.79 **(Fig. 5)**.

Discussion

This is the first population-based longitudinal study to examine the relationship between HOA and levels of sHA. From this epidemiological study, it was revealed that levels of sHA were higher in participants with HOA and correlated with the number of involved joints. Furthermore, longitudinal analysis showed that the increasing number of involved joints over six years was associated with the level of baseline sHA which meant that higher sHA levels could predict the increase in number of involved joints in future. Regarding the relationship between sHA and HOA, similar results were obtained in past cross-sectional studies [10,16], but their validity as a predictor in the longitudinal analysis was not sufficiently investigated.

HA is a glycosaminoglycan found in many joint tissues, and an important component of articular cartilage and synovium [10]. It is a marker for synovitis and joint inflammation and is influenced by a variety of factors such as food intake, activity levels, and presence of disease [18,19]. Therefore, measurement of sHA is performed using blood collected after an overnight fast with less influence of exercise and food.

202 Serum hyaluronic acid levels have been considered a promising biomarker for diagnosis
203 of OA and the disease burden [20-22]. Higher sHA levels have been associated with
204 higher KL grades of knee and hip joints [6,9,17,23,24]. In HOA, the burden of
205 osteophytes [20] joint space narrowing, and the number of involved joints were all
206 related to sHA levels. Although the statistical significance of sHA in the HOA group
207 was not demonstrated in the CARRIAGE family study where the association between
208 sHA and HOA was reported for the first time [25], Filcova reported a significant
209 association with sHA in erosive HOA compared to non-erosive HOA in HOA
210 patients[10]. In normal joints, functional and metabolic activities of hyaluronic acid
211 depend on its high levels and high molecular weight [26]. During inflammation,
212 reactive free radicals from neutrophils in synovial fluid damage and depolymerize HA
213 and that leads to a reduction in its high molecular weight [27-29]. This contributes to
214 reduction in synovial fluid viscosity and to dispersion of HA fragments and disaccharide
215 monomers into the circulation [30-31]. Soluble pro-inflammatory cytokines including
216 interleukin -1 and tumor necrosis factor-α can also be responsible for the production of
217 HA in synovial fluid [32]. Small HA oligosaccharides in the joint combine with high
218 molecular mass HA and interfere with the normal chondrocyte–matrix interactions
219 [33,34]. They also activate production and activity of matrix metalloproteinases and

220 nitric oxide synthesis by articular chondrocytes and inflammatory cells [35,36]. This
221 process is involved in the pathogenesis of OA and it can be inferred that the increased
222 levels of sHA in HOA patients can reflect synovial inflammation and destruction of OA
223 cartilage. Moreover, Chen demonstrated that increased levels of sHA in HOA patients is
224 associated with hand symptoms [25]. However, there is still a lack of sufficient studies
225 analyzing biomarkers in HOA.

226 In this study, there was a significant correlation between levels of sHA and number of
227 involved joints in HOA. Furthermore, sHA levels showed a strong correlation with
228 Kallman score. It has been reported that there is a significant correlation between
229 radiological HOA severity and finger pain [3], and also that serum cartilage oligomeric
230 matrix protein (sCOMP), a type of synovial biomarker, showed association with
231 decreased hand function [16]. In knee OA and hip OA, the association between
232 radiographic severity and sHA has been shown [18,23]. From this study, the relationship
233 between radiographic severity of HOA and levels of sHA were also suggested.

234 In this study, it is suggested that the number of involved joints tends to increase in
235 patients with high levels of sHA, and the risk increases 4.79 times when the cut-off
236 levels of sHA is 46.1 (ng/ml). Filcova reported that a 2-year follow-up study of 88 HOA
237 patients who visited the hospital revealed that Kallman score increased two years later

238 in patients with high levels of sHA [10]. In agreement with this study, we suggest that
239 serum hyaluronic acid levels may be a prognostic factor in HOA. It is considered that
240 the degree of synovitis and cartilage damage may be associated with these correlations.
241 The knee is the largest among weight-bearing joints and has a large volume of cartilage
242 and synovium. Although the individual sizes of finger joints are very small, their
243 number is significant, resulting in large cartilage and synovial volume. Therefore, it
244 seems that association with sHA was also shown in HOA. However, it is important to
245 note that symptoms of HOA do not necessarily coincide with radiographic findings. In
246 daily practice, there are elderly people who live without pain and ADL restrictions, even
247 though their KL grade is high with a significant number of HOA joints, while patients
248 with low KL grades may develop pain and joint swelling and have a great limitation in
249 ADL. Therefore, it is considered necessary to evaluate both symptoms and prognosis
250 when considering the pathology of HOA.
251 This study has several limitations. First, we did not evaluate hand function such as grip
252 strength, handedness, pain and range of motion at the finger joint. Secondly, we did not
253 investigate detailed evaluations of erosion in radiographic images. We assessed joints
254 using anterior–posterior radiographs of the hand. Strictly speaking, it may have been
255 better to use lateral views to assess OA in the hand joints [37]. However in previous

cohort studies, the anterior–posterior view was used to assess OA in all the hand joints [1,2,3,5,38,39]; thus, comparing the prevalence of OA among them might be beneficial. Third, the intake of hyaluronic acid supplements has not been evaluated. Fourth, it is cited that familial OA and OA that co-morbidly affects the whole body other than the knee joint should not be evaluated. Previous studies highlighted the relationships between increased levels of sHA and knee and finger OA but no relationships to other forms of OA [9].

Despite these limitations, our results show that the number of involved joints in the hand gradually increased if the baseline levels of sHA was high. In addition, a significant correlation between the number of involved joints and Kallman score to the levels of sHA was also seen, supporting the previous report that sHA plays an important role in the pathogenesis of HOA. This study is the first report from a long-term longitudinal epidemiological study of the general population concerning the relationship between serum hyaluronic acid levels and HOA.

Conclusion

Serum hyaluronic acid levels correlated significantly with the presence of HOA, the number of joints involved and the Kallman score. In the longitudinal study, sHA was

associated with an increase in number of involved joints after six years, suggesting its usefulness as a predictor of HOA progression.

Abbreviations

HOA	Hand osteoarthritis
sHA	Serum hyaluronic acid
BMI	Body mass index
DIP	Distal interphalangeal
PIP	Proximal interpharlangeal
IP	Interphalangeal
CMC	Carpometacarpal
KL	Kellgren-Lawrence classification
ROC	Receiver operating characteristic
AUC	Area under the curve
sCOMP	Serum cartilage oligomeric matrix protein

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Availability of data and materials

The datasets used and analyzed in the current study are available from the corresponding author on reasonable request.

Author contributions

All authors were involved with the design of the study, interpretation of data, critical

revising of the manuscript and approving the final version for submission. TS, ES and HI were primarily responsible for the data acquisition, and TS primarily did the initial analysis of the data and drafted the manuscript. TS and ES take full responsibility of the integrity of the work from inception to finished article.

Ethics approval and consent to participate

The Ethics Committee of the Hirosaki University Graduate School of Medicine approved the study, and all participants provided written informed consent before participation.

Consent for publication

Consent for publication was not required as no identifying personal information is being published in this manuscript.

Competing interest

The authors declare that they have no competing interests.

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448

449 **Figure titles and legends**

450 Fig. 1. Prevalence (%) of hand osteoarthritis on radiography in each joint in men (M,
451 left) and women (W, right).

452

453 Fig. 2. Increased levels of sHA in HOA groups compared with non-HOA groups (A)

454 and correlation of the levels of sHA with the number of involved joints by Spearman's
455 correlation coefficients (B) at baseline.
456
457 Fig. 3. Correlation of levels of sHA with Kallman score in HOA groups by spearman's
458 correlation coefficients at baseline.
459
460 Fig. 4. Increased levels of sHA (A) and ⊿sHA (B) in Increasing groups compared
461 with non-Increasing groups.
462
463 Fig. 5. The predictability of increasing the number of involved joints by levels of sHA
464 in the receiver operating characteristic curve.
465

Figures

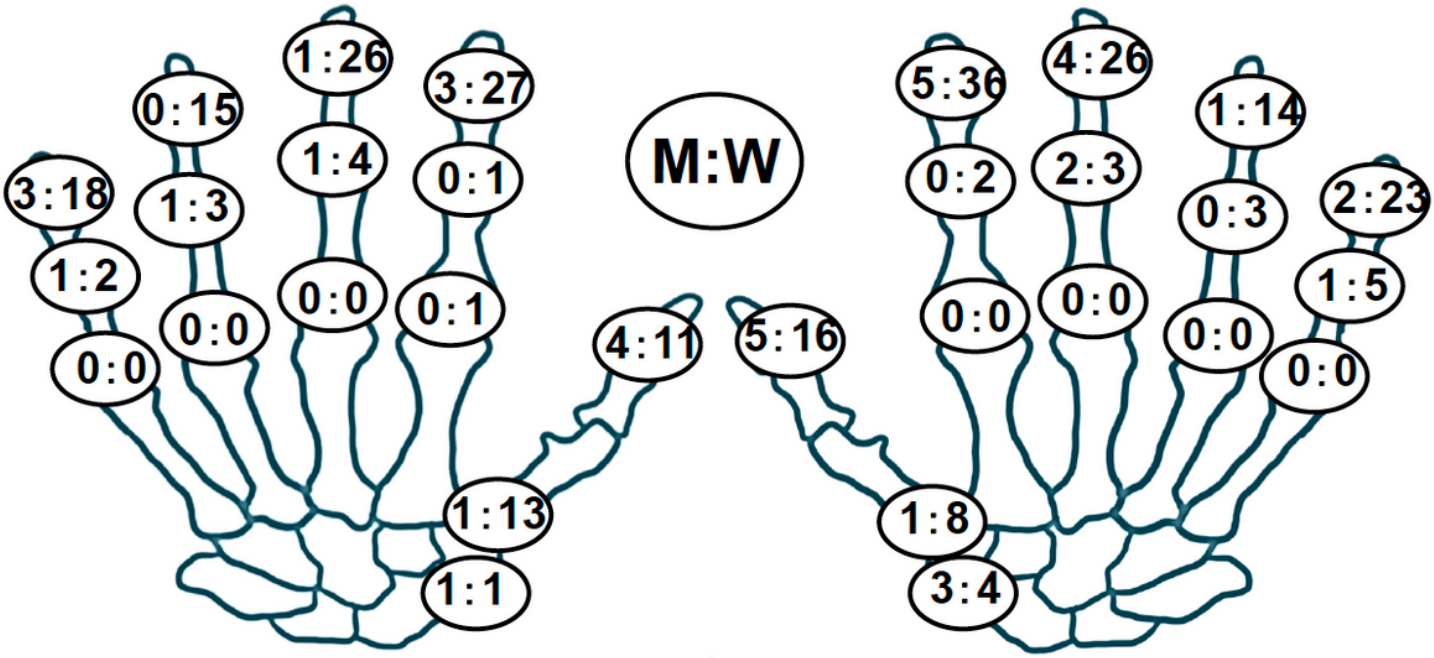

Figure 1

Fig. 1. Prevalence (%) of hand osteoarthritis on radiography in each joint in men (M, left) and w omen (W, right).

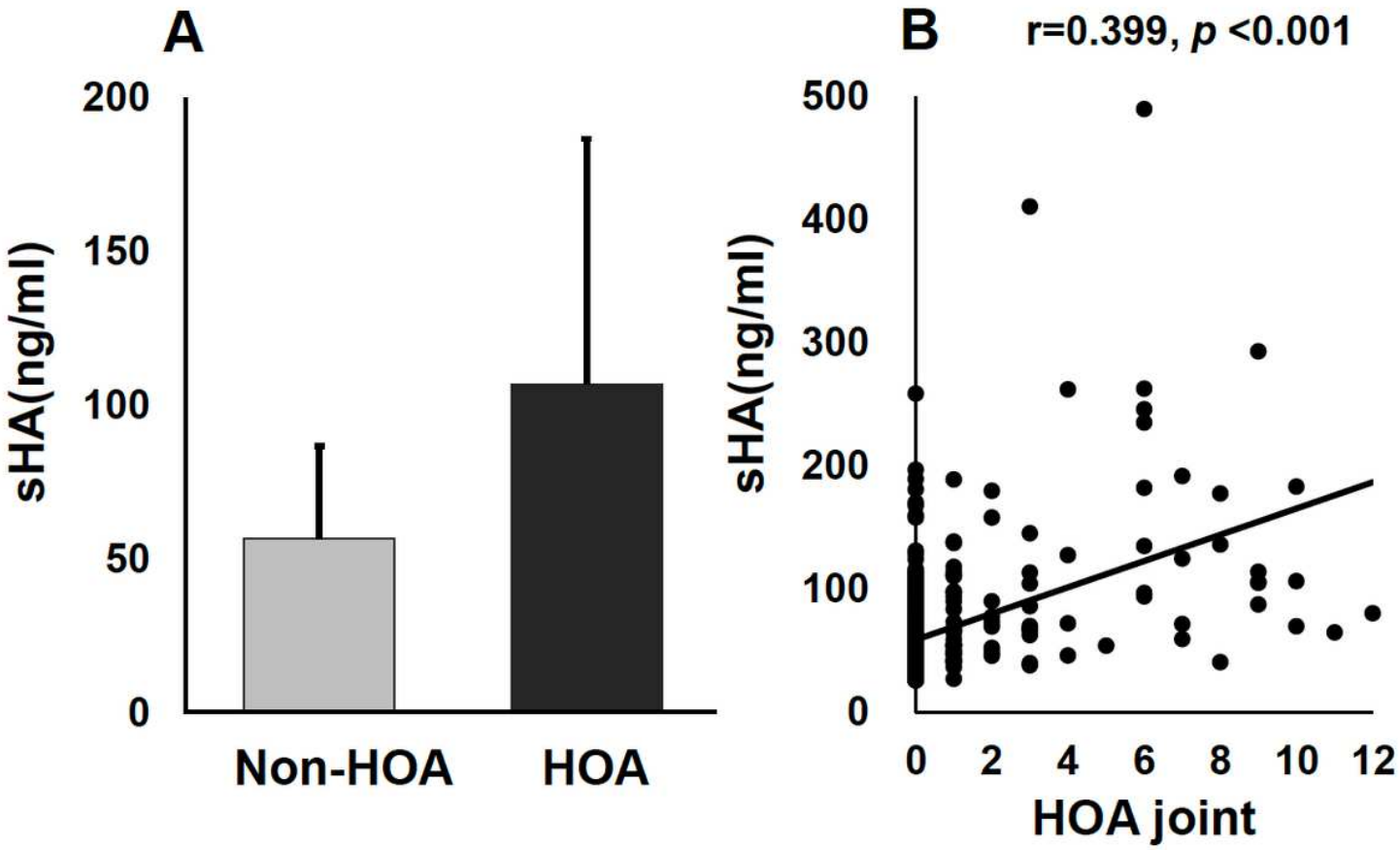

Figure 2

Fig. 2. Increased levels of sHA in HOA groups compared with non-HOA groups (A) and correlation and correlation of the levels of sHA with the number of involved joints by n of the levels of sHA with the number of involved joints by Spearman's correlation coefficients (B) at baseline.

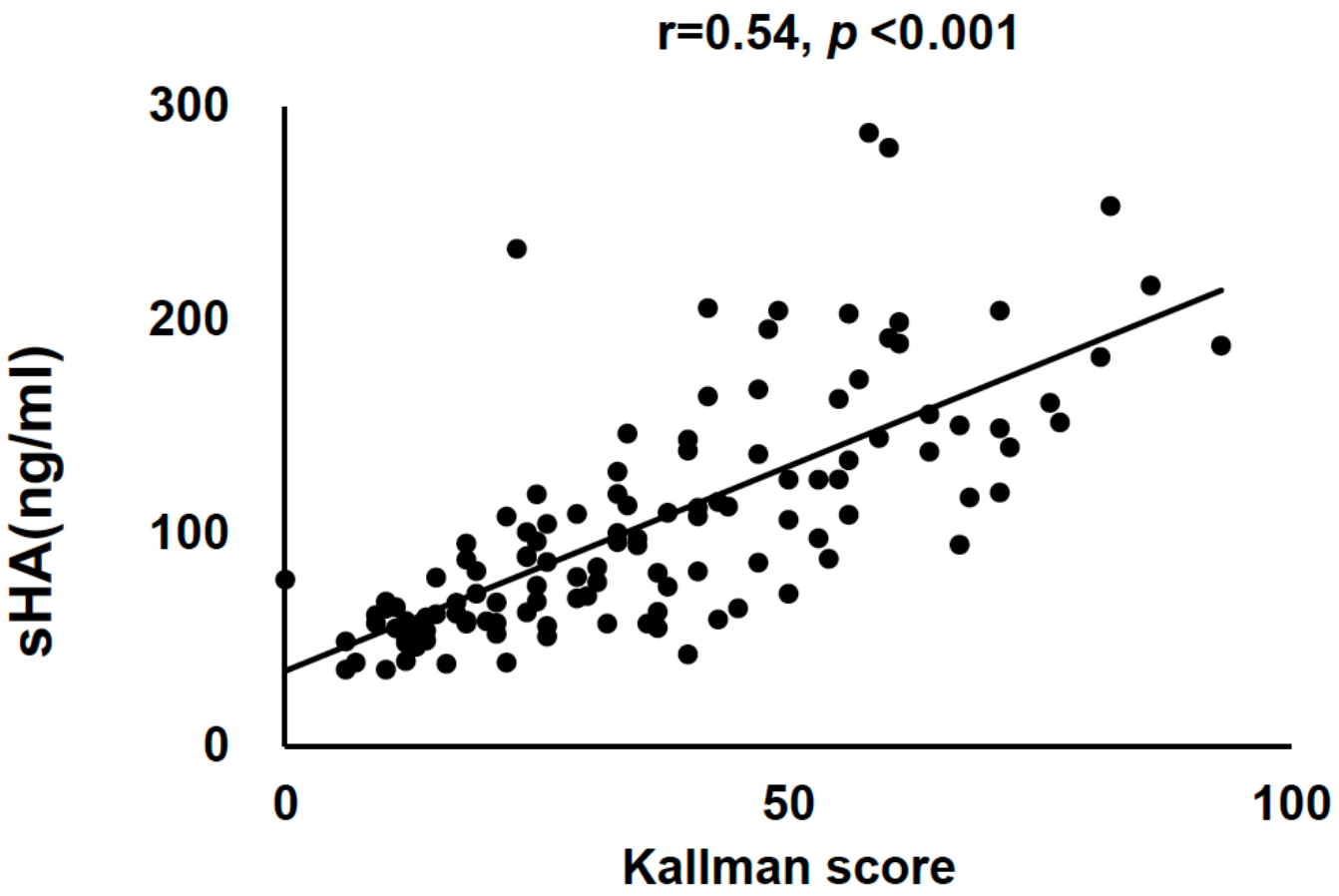

Figure 3

Fig. 3. Correlation of levels of sHA with Kallman score in HOA groups by spearman's correlation coefficients at baseline .

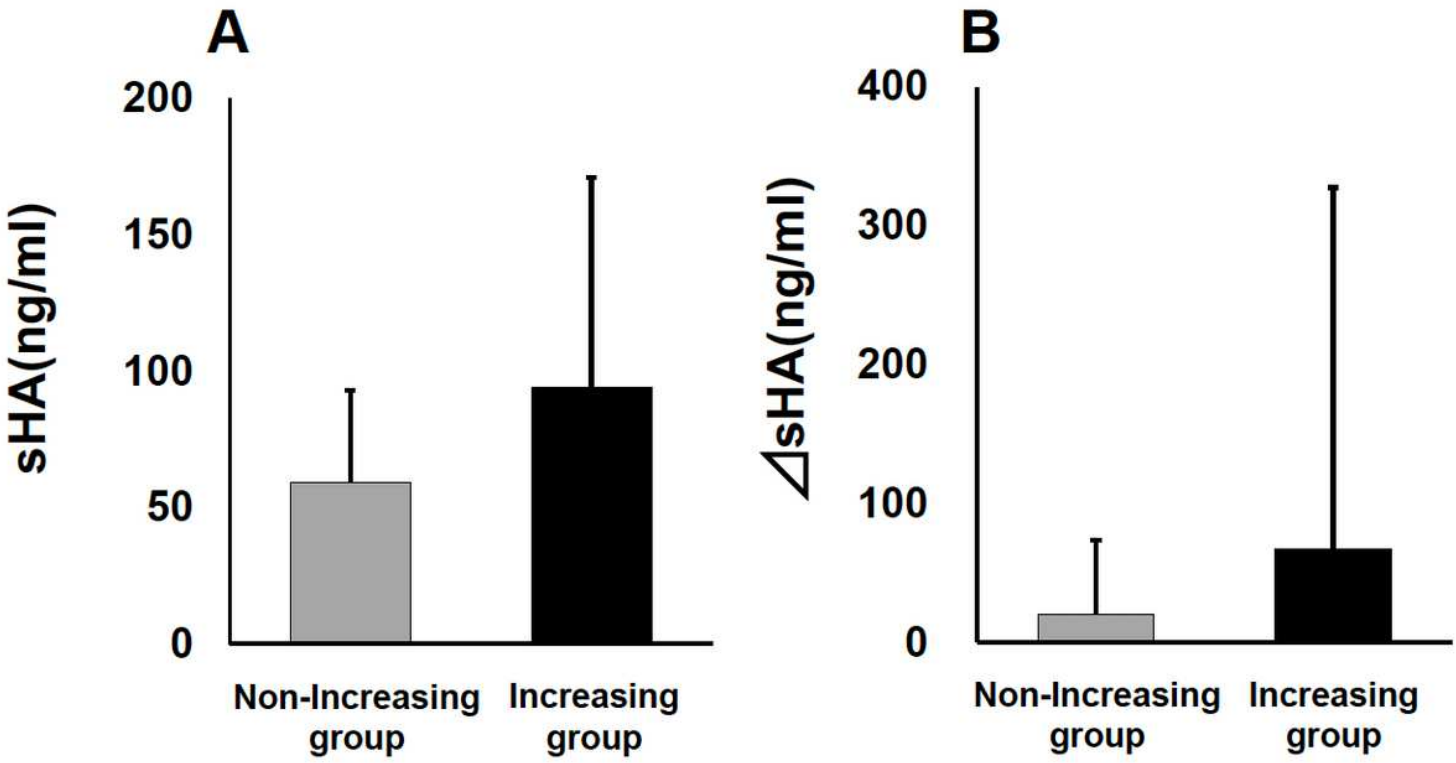

Figure 4

Fig. 4. Increased levels of sHA (A) and sHA (B) in Increasing groups compared with non Increasing groups.

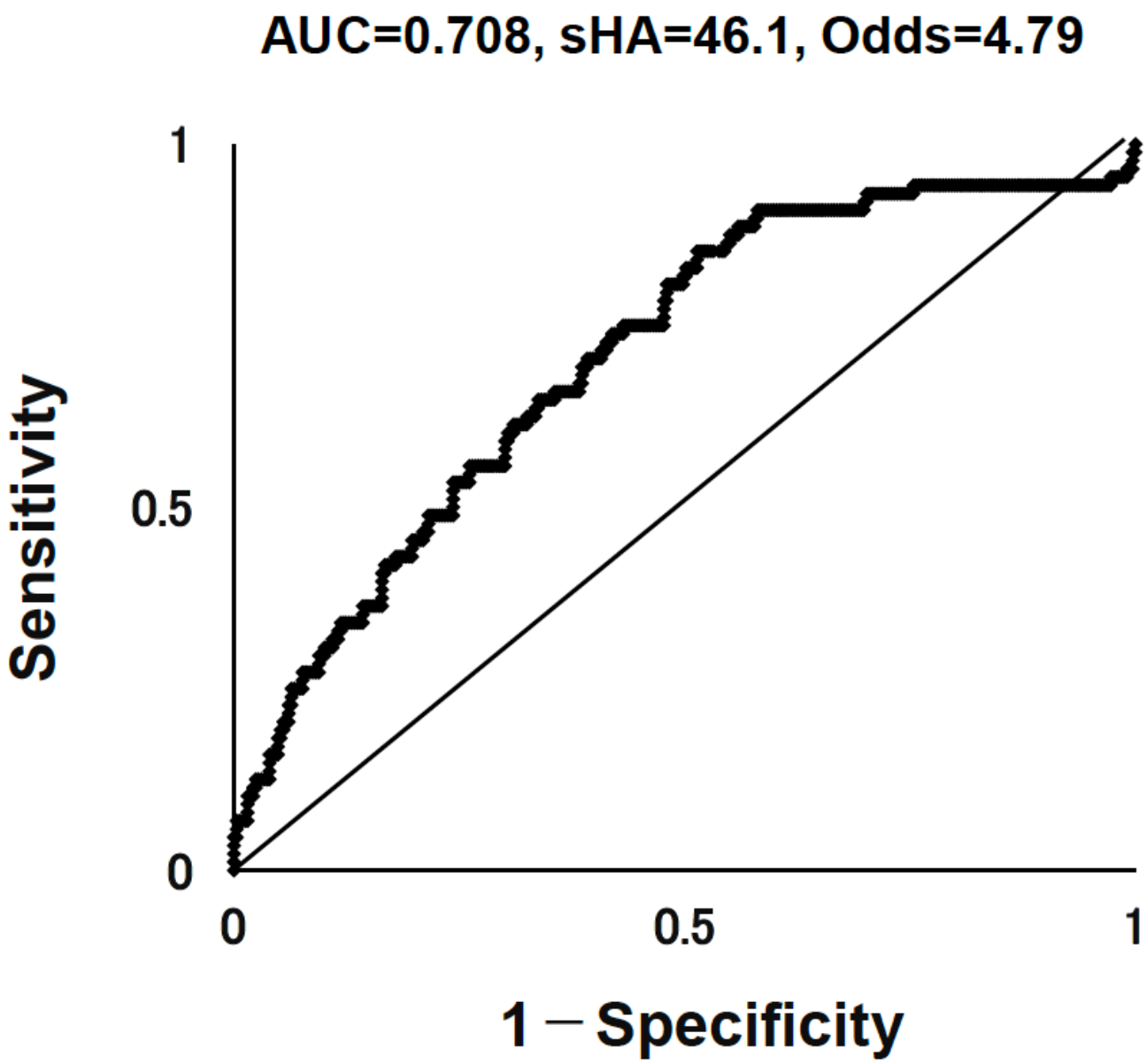

Figure 5

Fig. 5. The predictability of increasing the number of involved joints by levels of sHA in the receiver operating characteristic curve.