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Improvement of self-reported functional scores and thickening of quadriceps and femoral intercondylar cartilage under ultrasonography after single intra-articular injection of a novel cross-linked hyaluronic acid in the treatment of knee osteoarthritis

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Abstract.
BACKGROUND: Most studies used hyaluronic acid (HA) requiring 3–5 intra-articular injections (IAJ) for knee osteoarthritis (KOA).
OBJECTIVE: We evaluated the efficacy of a single IAJ of a novel HA by measuring the thickness of quadriceps and femoral intercondylar cartilage (FIC) under ultrasonography (US) in addition to subjective self-reported measures.
METHODS: Forty-nine patients with KOA (Kellgren-Lawrence grades 2–3) received unilateral IAJ of HYAJOINT Plus to the worse knee and were assessed at baseline and 1, 3 and 6-months after IAJ. Outcome measures were the (1) Visual Analog Scale for pain (VAS), (2) Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC), (3) Lequesne's Index, (4) single-leg-stance duration (5) thigh circumference, and (6) thickness of quadriceps and FIC under US.
RESULTS: Forty-six patients completed the 6-month-follow-up study. All outcome measures improved significantly after HA injection ($p < 0.001$). Both VAS and WOMAC-pain subscale scores improved significantly at 1, 3, and 6 months ($p < 0.01$). The US thickness of the quadriceps and FIC improved significantly at both 3 and 6 months ($p < 0.05$). The Lequesne's index, single-leg-stance and thigh circumference improved significantly at 6 months ($p < 0.01$).
CONCLUSIONS: HYAJOINT Plus is effective both subjectively and objectively for 6 months and is safe as a treatment for KOA.

Keywords: Knee pain, osteoarthritis, hyaluronic acid, ultrasonography

1. Introduction

Osteoarthritis (OA) is the most common musculoskeletal disease around the world. Among populations with OA, 80% of them have limited range of motion of joints, and 25% of them cannot perform major

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activities of daily living [1]. The most common type of OA among older adults is OA of the knee (knee OA) with a prevalence around 30%–40% [2,3]. Knee OA is one of the major conditions that cause disability in older adults and incurs a large economic burden on public health [4].

The most common symptoms of knee OA are pain, functional impairment, and stiffness. Knee OA results in disability and affects quality of life further. Due to the chronicity and incurable nature of knee OA, therapies targeting knee OA should be safe and effective for its long-term management. Current therapies for knee OA include pharmacologic treatment (e.g., analgesics and nonsteroidal anti-inflammatory drugs [NSAIDs]), self-health management (e.g., weight reduction), rehabilitation programs (e.g., therapeutic exercise, modalities, and use of orthotics), intra-articular injection (e.g., corticosteroid, hyaluronic acid [HA], and platelet-rich plasma) and surgery [5,6].

Intra-articular HA injection is a well-established treatment option for knee OA. It is thought that HA is responsible for the restoration of viscoelasticity which deteriorates in OA [7]. Previous studies have shown that HA may provide biological actions including anti-inflammatory, anti-nociceptive, and anabolic effects [8–10]. Moreover, *in vitro* data indicated that HA might regulate the processes of cartilage matrix degradation in OA by binding both CD44 and ICAM-1 receptors, and therefore, slowing chondrocyte apoptosis [11]. According to the guideline for treatment of knee OA published in 2000 by American College of Rheumatology (ACR), HA injections were recommended for patients who could not be effectively managed with nonpharmacologic treatment, simple analgesics, and NSAIDs [12].

Several HA formulations that vary in their origins, concentrations, dosing regimens, molecular weights, and possibly clinical outcomes, are currently available on the market. Most older HA products that were initially introduced are derived from rooster-comb tissue and required 3 to 5 intra-articular injections to achieve clinical efficacy. In contrast, more advanced HA preparations have evolved to provide durable activity and require fewer injections. HYAJOINT Plus is synthesized by a novel cross-linking process (i.e., 1, 4-butanediol diglycidyl ether [BDDE]) to produce an anti-degraded feature. This carefully controlled cross-linking technique enabled the creation of this viscous gel with increased density (2% HA, 20 mg/ml). Furthermore, HYAJOINT Plus is produced by microbial fermentation, thus obviating potential risks of allergies to avian proteins. It is believed to have clinical efficacy with a single injection. The single injection regimen represents an attractive alternative, as it may decrease both patient time expenditure and discomfort associated with the injection procedure [13].

Ultrasonography (US) is widely used by doctors of different specialties. Koca et al. found a significant negative correlation between quadriceps thickness measured by US and using the Kellgren & Lawrence (KL) grading system of knee OA, visual analog scale for pain (VAS), and Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC). Since US is portable, easy to use, and without radiation exposure, evaluation of quadriceps muscle thickness under US could be considered a practical and economical method in both the diagnosis and follow-up of knee OA [14]. Chen et al. evaluated the femoral intercondylar cartilage (FIC) of patients with knee OA under US and graded the severity of knee OA according to thickness and erosion of FIC. They found that this semi-quantitative US grading system might well reflect the clinical symptoms and functions related to knee OA as evaluated by the VAS, WOMAC, and Lequesne's index [15].

Most previous studies evaluated the efficacy of intra-articular HA injection by subjective self-reported measures like VAS, WOMAC, Lequesne's index, short form health survey (SF-36) and so on, with few studies by objective measurements. The aim of this study was to provide a more comprehensive evaluation about the clinical effect of the novel single-dose intra-articular injection of HA (HYAJOINT Plus) by both objective (single-leg-stance duration, thigh circumference, quadriceps and FIC thickness under US of the affected side), and subjective self-reported measures.

2. Materials and methods

2.1. Ethical approval

This study was conducted in accordance with the *Declaration of Helsinki* and was approved by the Institutional Review Board for Human Investigation of Kaohsiung Veterans General Hospital. All subjects gave written informed consent to participate in the study.

2.2. Study design and participants

This investigation was a prospective study with a 6-month follow-up period done in the setting of an outpa-

tient rehabilitation department in a tertiary care medical center between March 2016 and December 2016. Inclusion criteria included both of the following:

1. Both genders, aged 40–85 years, diagnosed as knee OA by ACR criteria [16], with average knee pain of at least 30 mm on a 100-mm VAS for at least 6 months despite conservative treatment such as analgesics, NSAIDs, and/or physical therapy.
2. KL grade 2 or 3 knee OA based on standing anterior-posterior and lateral knee radiographs taken within the previous 6 months [17].

Exclusion criteria included clinically apparent joint effusion or marked valgus/varus deformity, knee instability, disabling OA of either hip or foot, previous orthopedic surgery on the spine or lower limbs, known allergy to avian proteins or HA products, intra-articular injections into the knee in the past 6 months, infections or skin diseases around the target knee, women ascertained or suspected of being pregnant or lactating, or serious medical conditions that would interfere with assessments during the study.

2.3. Outcome measures

We used both subjectively self-reported measures, including VAS, WOMAC, and Lequesne's index and objective measures, including thigh circumference, quadriceps and FIC thickness by US to assess outcomes. Objective measures were done only on the HA-injected side. The detailed information on measures is below:

1. VAS: The patient rated the average severity of knee pain on knee movement over the previous week on a 0–100 mm VAS (0 = no pain to 100 = worst possible pain [18]).
2. WOMAC: A 24-item questionnaire with 3 subscales that measure: pain, stiffness, and physical function. All questions are scored on a scale of 0–4 (0 = none, 1 = mild, 2 = moderate, 3 = severe, 4 = very severe). Maximal score is 96 and lower scores represent better outcomes [19,20].
3. Lequesne's index: An index that assesses the severity of OA knee symptoms during the last week. It includes the measurement of pain, walking distance, and activities of daily living. The total score is 24 and higher scores indicate worse function [21].
4. Single-leg-stance (SLS) duration: An assessment that requires the patient to raise one foot up without touching it to the supported lower extremity with the target knee while maintaining balance as long as possible. The best result of 3 trials was recorded [22].
5. Thigh circumference of the HA-injected site: The patient lies supine and extends both lower limbs fully without wearing pants or skirts. A thin sheet covered their lower limbs to expose only the measured area. We measured the circumference from the center of the line between the anterior superior iliac spine and the upper pole of the patella of the injected site (point A) using measuring tape [14].
6. US: All US examinations were performed on a portable LOGIQ e ultrasound (General Electric Company, U.S.A., 2010), equipped with a 12 MHz linear array transducer by an experienced single operator who was not involved in any further data analysis and was blinded to clinical symptoms, VAS, KL grade of knee OA, WOMAC, and Lequesne's index of patients. All patients received measurement under US in the afternoon.

The patient was laid supine with their legs fully extended for unilateral measurement of the muscle layer thickness of the vastus intermedius muscle (VIM) and rectus femoris muscle (RFM) at point A on the transverse scan [14]. Since the pressure load on the muscle layer might interfere with the measurement of muscle thickness, all sonographic measurements of muscle thickness were done by placing the probe lightly on the skin without applying any pressure. We chose a depth from 4 to 6 cm as the sonographic window to enable visualization of the bony cortex underlying the VIM. The thickest parts of both the VIM and the RFM were measured respectively and summed. Two measurements were taken and the mean was recorded as the quadriceps thickness (Fig. 1).

Patients rested on an examination bed in a supine position, with the examined knee at maximal flexion as tolerated, to expose the weight-bearing FIC as much as possible. We placed the US probe above the upper margin of the patella, perpendicular to the surface of the knee [15]. The probe was dynamically tilted to facilitate better visualization of the hyaline cartilage. The thickest part of the FIC was recorded as the FIC thickness (Fig. 2).

2.4. Study process

The study consisted of a screen visit, a baseline visit-during which single 3 ml intra-articular injec-

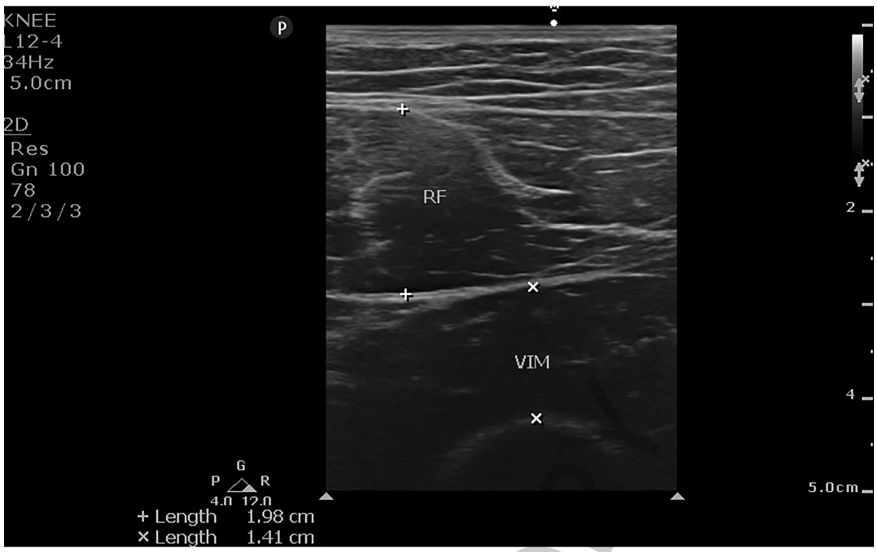

Fig. 1. Measurement of quadriceps thickness of hyaluronic acid injected under ultrasonographic image in short axis. Ultrasonographic probe was placed transversely at the midpoint between anterior superior iliac spine and the upper pole of the patella when patients' legs in full extension. The thickest parts of vastus intermedius and rectus femoris muscles were measured and summed. Two measurements were made and the mean was recorded (RF, rectus femoris muscle; VIM = vastus intermedius muscle).

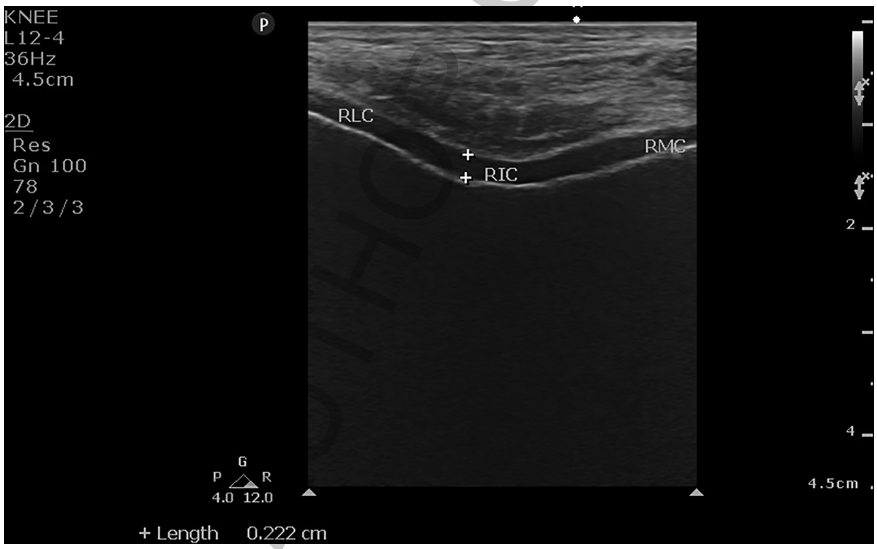

Fig. 2. Measurement of femoral intercondylar cartilage thickness under ultrasonographic image. Ultrasonographic probe was placed above the upper margin of the patella, perpendicular to the surface of the knee with the examined knee in a maximal flexion. The thickest part of femoral intercondylar cartilage (FIC) was record as FIC thickness (RLC, femoral cartilage at right lateral condyle; RIC, femoral cartilage at right intercondyle; RMC, femoral cartilage at right medial condyle).

tion of HYAJOINT Plus (2% microbial fermented HA, 20 mg/ml) was done- and follow-up visits at 1, 3, and 6 months after the injection. If the recruited participants had bilateral knee OA, we only injected the more severe side. The severity was based on K-L grade. If the K-L grade of bilateral knee was the same, we injected the more painful knee based on the patient's statement on VAS. The subjectively self-reported measures in the study were completed by patients themselves or with the help of two well-trained assistants during all follow-ups. Thigh circumference at the injected site was measured and recorded by the same assistant who was blinded to clinical symptoms, VAS, KL grade of knee OA, WOMAC, Lequesne's index

Table 1
Demographic and baseline characteristics of the subjects (total examined knees = 46)

Age (years)	65.07 ± 9.34
Gender	
Male	9 (19.57%)
Female	37 (80.43%)
Height (cm)	156.95 ± 7.67
Body weight (kg)	59.27 ± 11.06
BMI (kg/m^2)	24.01 ± 3.65
OA duration (years)	5.36 ± 5.56
Radiological KL grade	
Grade 2	27 (58.70%)
Grade 3	19 (41.30%)
VAS (mm)	60.12 ± 2.31
WOMAC-Total	36.59 ± 2.61
WOMAC-Pain	7.62 ± 0.49
WOMAC-Stiffness	3.24 ± 1.89
WOMAC-Physical function	23.48 ± 1.91
Lequesne's index	11.04 ± 0.68
SLS duration (sec)	14.95 ± 2.97
Thigh circumference (cm)	48.07 ± 0.87
Quadriceps thickness under US (cm)	2.49 ± 0.10
FIC thickness under US (mm)	22.5 ± 0.12

The values are given as mean and the standard deviation or number of patients, with the percentage in parentheses, BMI = body mass index, OA = Osteoarthritis, KL = Kellgren-Lawrence Scale, VAS = Visual Analog Scale for pain, WOMAC = The Western Ontario and McMaster Universities Osteoarthritis Index, SLS = Single leg stance, US = ultrasonography, FIC = femoral intercondylar cartilage.

and SLS duration of patients during all the visits. Adverse effects of the HA injection were asked and subsequently evaluated during every follow-up.

2.5. Statistical analysis

We used SPSS for Windows version 19.0 (Released 2010. Armonk, NY: IBM Corp) for all analyses. Data were expressed as mean ± standard deviation (SD). A change in outcome measures among baseline, 1, 3, and 6-month post-HYAJOINT Plus injection were assessed using repeated measure one-way analysis of variance (ANOVA) and a Bonferroni post-hoc test. A p value $\leqslant$ 0.05 was considered statistically significant.

3. Results

3.1. Patient characteristics

A total of 49 participants were assessed for eligibility. Among them, 46 patients completed the study and 3 patients withdrew during the study period (1 dropped out during the visit at 3 months and 2 withdrew 6 months after the HA injection, all due to long distances from their homes to the hospital). Patients were predominantly female (80.43%), the mean age was approximately 65 years, the mean body mass index (BMI) was approximately 24, and the mean duration of knee OA was about 5.36 years (Table 1). All 46 subjects had no infections, allergies, or other serious adverse effects. Five subjects developed joint pain without obvious inflammatory signs in the study. One patient developed mild and painful effusion within the injected knee (the effusion was checked by sonography) 3 days after the HA injection. The participant took oral acetaminophen for pain control without aspiration of the joint effusion. The effusion subsided spontaneously 9 days later.

3.2. VAS

The mean VAS decreased by 28.57 mm, 28.98 mm and 28.45 mm from baseline at 1, 3 and 6-months follow-ups. There was a significant reduction on the VAS after the HA injection as compared with baseline using a repeated measures one-way ANOVA ($p <$ 0.001). A post-hoc analysis by a Bonferroni test found significant differences at 1, 3, and 6 months after the HA injection as compared with baseline data (all p values $<$ 0.001). There was no statistical difference of the VAS at 3 months as compared with the one at 1 month, nor were the differences at 6 months as compared with both the one at 1 month and 3 months after the HA injection (Table 2).

3.3. WOMAC

There were significant reductions after the HA injection as compared to baseline in pain, stiffness, functional subscale scores and total scores (all p values $<$ 0.001).

In pain subscale scores, the post-hoc analysis found significant improvements at 1, 3, and 6 months after the HA injection as compared with baseline data (all p values $<$ 0.001). However, there were no statistical differences at 3 and 6 months as compared with the one at 1 month, nor was at 6 months as compared with the one at 3 months after the HA injection.

In stiffness subscale scores, a post-hoc analysis only found significant improvements at 1 and 6 months after the HA injection as compared with baseline data ($p =$ 0.024 and $p < 0.001$, respectively). No other statistically improvements after the HA injection were noted in the other within group comparisons.

Table 2
Self-reported functional scores and single-leg-stance duration at 1, 3 and 6 months after HYAJOINT Plus injection

	1 month after injection	3 months after injection	6 months after injection	F value	p value
VAS	31.55 ± 3.69^{c}	31.14 ± 24.28^{c}	31.67 ± 25.02^{c}	32.03	$< 0.001^{a}$
WOMAC-Pain	4.69 ± 0.69^{c}	3.86 ± 0.42^{c}	3.57 ± 0.40^{c}	21.97	$< 0.001^{a}$
WOMAC-Stiffness	2.21 ± 1.93^{e}	2.31 ± 1.41	$1.69 \pm 1.18^{c,g}$	9.75	$< 0.001^{a}$
WOMAC-Physical function	18.83 ± 2.24	15.91 ± 1.65^{c}	14.12 ± 1.41^{c}	10.41	$< 0.001^{a}$
WOMAC-Total	27.28 ± 2.94^{c}	22.09 ± 2.05^{c}	$17.70 \pm 1.90^{c,f}$	19.10	$< 0.001^{a}$
Lequesne's index	8.88 ± 0.83^{e}	8.46 ± 0.72^{e}	8.10 ± 0.77^{d}	7.68	0.001^{a}
SLS duration (sec)	23.48 ± 3.93	24.07 ± 3.68	26.37 ± 3.83^{d}	5.504	0.004^{b}

The values are given as mean and the standard deviation, VAS = Visual Analog Scale for pain, WOMAC = The Western Ontario and McMaster Universities Osteoarthritis Index, SLS = Single leg stance. [a]Within-group difference using repeated measure one-way ANOVA showed p value $\leqq 0.001$. [b]Within-group difference using repeated measure one-way ANOVA showed p value $\leqq 0.01$. [c]Post-Hoc analysis using Bonferroni test showed p value $\leqq 0.001$ as compared to baseline. [d]Post-Hoc analysis using Bonferroni test showed p value $\leqq 0.01$ as compared to baseline. [e]Post-Hoc analysis using Bonferroni test showed p value < 0.05 as compared to baseline. [f] Post-Hoc analysis using Bonferroni test showed p value < 0.05 as compared to 1 month. [g] Post-Hoc analysis using Bonferroni test showed p value < 0.05 as compared to 3 months.

Table 3
Thigh circumference, quadriceps thickness and femoral intercondylar cartilage thickness under ultrasonography at 1, 3 and 6 months after HYAJOINT Plus injection

	1 month after injection	3 months after injection	6 months after injection	F value	p value
Thigh circumference (cm)	48.47 ± 0.80	49.29 ± 0.86	$50.48 \pm 0.88^{b,e,f}$	11.01	$< 0.001^{a}$
Quadriceps thickness under US (cm)	2.59 ± 0.08	$3.05 \pm 0.10^{b,e}$	$3.10 \pm 0.10^{b,e}$	22.52	$< 0.001^{a}$
FIC thickness under US (mm)	23.1 ± 0.10	25.4 ± 0.10^{b}	25.7 ± 0.11^{b}	7.86	$< 0.001^{a}$

The values are given as mean and the standard deviation, US = ultrasonography, FIC = femoral intercondylar cartilage. [a]Within-group difference using repeated measure one-way ANOVA showed p value $\leqq 0.001$. [b]Post-Hoc analysis using Bonferroni test showed p value $\leqq 0.001$ as compared to baseline. [c]Post-Hoc analysis using Bonferroni test showed p value $\leqq 0.01$ as compared to baseline. [d]Post-Hoc analysis using Bonferroni test showed p value < 0.05 as compared to baseline. [e]Post-Hoc analysis using Bonferroni test showed p value < 0.05 as compared to 1 month. [f]Post-Hoc analysis using Bonferroni test showed p value < 0.05 as compared to 3 months.

In physical function subscale scores, no statistically improvements after the HA injection were noted during the post-hoc analysis except that there were significant improvements at 3 and 6 months after the HA injection as compared with baseline data (both $p < 0.001$).

In WOMAC total scores, post-hoc analysis found significant improvements at 1, 3 and 6 months after the HA injection as compared with baseline data ($p = 0.009$, $p < 0.001$, $p < 0.001$, respectively) and a statistical reduction at 6 months as compared with the one at 1 month after the HA injection. There was no statistical difference at 3 months as compared with the one at 1 month, nor was at 6 months as compared with the one at 3 months after the HA injection (Table 2).

3.4. Lequesne's index

There was a significant reduction after the HA injection as compared to baseline ($p < 0.001$). Post-hoc analysis found significant improvements at 1, 3, and 6 months after the HA injection as compared with baseline data ($p = 0.034$, $p = 0.011$, $p = 0.003$, respectively). No other statistically improvements after the HA injection were noted in the other within group comparisons (Table 2).

3.5. Single-leg-stance duration

There was a significant lengthening of SLS duration after the HA injection ($p = 0.004$). Post-hoc analysis found significant improvement at 6 months after the HA injection as compared with baseline data ($p = 0.008$). No other statistically lengthening of the duration after the HA injection was noted in the other within group comparisons (Table 2).

3.6. Thigh circumference, quadriceps and FIC thickness under US (Table 3)

Significantly thickening of thigh circumference, quadriceps and FIC thickness of the injected site after the HA injection as compared to baseline (all $p < 0.001$) was noted.

As for thigh circumference of the injected site, significant augmentations were noted as compared the data at 6 months to baseline and the one at 1 and 3 months after the HA injection ($p = 0.001$, $p < 0.001$, $p = 0.006$, respectively) during the post-hoc test. No other statistically improvements after the HA injection were noted in the other within group comparisons.

In respect of quadriceps thickness under US of the injected site, there were significant thickening as com-

pared the data at 3 and 6 months with baseline and the one at 1 month after the HA injection (all four p values < 0.001) during the post-hoc test. No other statistically improvements after the HA injection were noted in the other within group comparisons.

As for FIC thickness of the injected site, no statistically improvements after the HA injection were noted during the post-hoc analysis except that there were significant improvements at 3 and 6 months after the HA injection as compared with baseline data ($p = 0.001$ and 0.006, respectively).

4. Discussion

Intra-articular HA injection of knee had been proved to be effective in treating knee OA in several previous studies [23]. However, most initial studies used low-to-moderate molecular weight HA and require 3 to 5 intra-articular injections to achieve clinical efficacy [22]. Multiple times injection increases patient time expenditure and discomfort associated with the injection procedure. Therefore, many of the recent HA products have high molecular weights, which may provide greater bioactivity and longer duration of action, and offer greater potency for tissue repair in OA patients [24]. What's more, it requires only a single injection. Recently, high molecular weight HA was also found to have better joint lubrication in an experimental model [25]. Many of the studies of high molecular weight HA in treating knee OA used Synvisc-One (6 ml, 8 mg/ml) and the relatively high injection volume of HA might cause discomfort and swelling after injection. Due to a novel cross-linking process by BDDE, HYAJOINT Plus provides higher HA density (3 ml, 20 mg/ml), less injection volume with higher HA amount injected (60 mg per injection) than Synvisc-One. To the best of our knowledge, this is the first clinical study to evaluate the efficacy of HYAJOINT Plus for the treatment of knee OA.

Pain reduction is the primary indication for the use of intra-articular HA. In clinical trials of chronic pain treatments, reduction in chronic pain intensity of at least 30% appeared to reflect at least moderate clinically important differences [26]. A previous meta-analysis found a 40%–50% reduction in pain when using HA compared with a placebo [27]. In this study, the mean pain VAS reduced by 28.57 mm (47.5%), 28.98 mm (48.2%), and 28.45 mm (47.3%) at 1-, 3-, and 6-months post-injection, respectively. It appeared that pain relief was documented in our study as in previous studies and the magnitude of pain reductions had clinical significance. As for a minimum clinically important improvement in OA, the accepted threshold for was a mean reduction of 12%–18% by using WOMAC pain subscale score [28]. Our study showed the mean reductions in WOMAC pain subscale score were 38.5%, 49.3%, and 53.1%, respectively, much exceeded the threshold.

The severity of OA could be defined by the Lequesne's index. Scores between 5 and 7, 8 and 10, 11 and 13, were defined as being moderate, severe, and very severe according to Lequesne. He also reported that most patients recruited in OA trials have a score of 9–11 [29], similar to the results of our study. The mean Lequesne's index in our study was 11.04 (very severe handicap) and 8.10 (moderate handicap), before and after 6-month HA injection, respectively. This finding meant a clinical improvement after HA injection at 6 months.

Self-reported measures are subjective and are easily influenced by both different situations and reported ceiling effect [30]. Therefore, we used not only self-reported measures but also established objective tools, including SLS duration, thigh circumference, quadriceps thickness, and FIC thickness at the injected site [14,15], to assess the clinical effect of HYAJOINT Plus.

The mean thickness of the quadriceps muscle (VIM plus RFM) on the injected side of our subjects before the HA injection was 2.49 cm and it was consistent with the results of the 2014 Koca' study (mean thickness of VIM plus RFM was 2.65 cm) [14]. Subjects from both studies, ours and Koca' studies, were of KL grades 2 and 3. However, subjects in the Koca' study (mean age 57.9 years old) were younger than ours (mean age 65.1 years old). A previous study found that muscle activity of the quadriceps in patients with knee OA was significantly lower than that in normal peers [31]. Our data showed that the pain scale, regardless of whether it was VAS or WOMAC pain subscale score, was reduced significantly 1 month after the HA injection but the quadriceps thickness of the injected site did not increase significantly until 3 months after the HA injection. Based on the previous study, we speculated that since pain decreased, patients with knee OA might increase the amount of walking or other activities. This additional activity would use the quadriceps muscle and thus cause neuromuscular adaptation, thereby potentially contributing to an increase in quadriceps thickness. We also found that duration of the SLS lengthened significantly 6 months af-

ter the HA injection, much later than both the improvement of self-reported measurements and the increase in quadriceps thickness. Since the ability and duration of the SLS were highly related to both knee extensors and balance control, we again could verify the above speculation that the HA injection first decreased the pain and then both strengthened muscle structures and improved related functions after neuromuscular adaptation had been established.

Saarakkala et al. evaluated the correlation of a semi-quantitative US grading system (defined by the change of cartilage thickness, the regularity of cartilage interface, and the echogenicity of cartilage) and the degenerative change of articular cartilage using arthroscopic grading. They found that the correlation of the severity of cartilage changes between US and arthroscopy was highest at the femoral intercondylar area [32]. Therefore, we measured the thickness of the FIC rather than that of the medial or the lateral condylar cartilage in our study. Studies also showed that FIC thickness decreased linearly with increases in both age and deterioration of knee OA [33,34]. However, most studies measured cartilage thickness of the knee by computed tomography (CT) or magnetic resonance imaging (MRI) with varied results. A US of the joints offers a non-invasive, portable, fast, and inexpensive imaging method of OA. It is also time-saving if done by a well-experienced physician. Kilic et al. studied the femoral condylar cartilage thickness (FCT) at medial, lateral femoral condylar and intercondylar area of 70 healthy young adults aged between 30 to 32 years old and found that there was a diurnal variation of the FCT [35]. Therefore, all the subjects of our study received US measurement in the afternoon. They also found the mean FIC of healthy young adults was 25.3 mm, which was higher than our data (22.5 mm at baseline) and concurred that FIC thickness might decrease gradually with both aging and disease progression [33,34].

Tuna et al. [38] found that FIC thicknesses were significantly higher than the baseline measurements at the third month but not at the first month after strengthening training, and there was a late-phase thickening of the FIC that paralleled the earlier increase in muscle strength. Therefore, they speculated that the thickening of the FIC might result from regeneration. Our study also found a significant thickening of FIC 3 months after the HA injection as compared with baseline and we assumed that the HA injection might also have some effect on cartilage regeneration.

Five (10.9%) subjects in our study developed mild pain that could be tolerated and subsequently resolved. One (2.2%) patient had effusion of the injected knee joint and resolved spontaneously without aspiration. Adverse effects of the HA injection were similar to the study conducted by Yan et al., which showed the most common adverse effect after the injection of high molecular weight HA was knee pain (12.8%) [36].

Our study had the following limitations. First, there was no control group. Although the improvement in the magnitude of all self-reported measures exceeded many of the minimal clinically important differences, we were unable to compare the efficiency of the HA with the placebo effect. In addition, there might be detection bias since all evaluators were aware that all subjects received an HA injection. Also, unknown confounding factors might have had an effect during the 6-month follow-up period even though we tracked participants and they reassured us that they did not receive any management such as NSAIDs, physical therapy, or other injections during phone call and at each visit. Second, we recruited patients only from a single medical center with KL grades 2 and 3 tibiofemoral OA. Therefore, our results could not be generalized to all knee OA populations with different radiographic severity. Third, several factors might influence the measurement of FIC thickness, e.g., the angle of knee flexion [15], time of measurement [35], and even androgen level [37]. Although all measurements of the FIC were done by the same physician experienced in US regarding maximal flexion of the knee in the afternoon, there might still be some biases that could not be controlled. Studies that are randomized and controlled, have larger sample sizes and longer follow-up periods would be needed to determine both the long-term efficacy of the novel single-dose HA and the reliability of using the objective data by US as a follow-up indicator to assess the therapeutic effect of knee OA in the near future. Ideal candidates for intra-articular HA injection have yet to be defined.

5. Conclusion

Our study shows that an intra-articular injection of the novel single-dose HA, HYAJOINT Plus, is both safe and effective for the treatment of knee OA. Its clinical effects, regardless of whether evaluated by self-reported measures or by objective SLS duration, thigh circumference, and quadriceps and FIC thickness by US, were maintained for at least 6 months. A double-blinded randomized controlled study having a larger sample size that is designed to compare the

efficacy of the novel single-dose injection HA with conventional HAs is required to both determine the cost-effectiveness and provide another new treatment choice for knee OA.

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Conflict of interest

No conflicts of interest were declared in relation to this article.

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