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Original Research

Double-blinded prospective randomized clinical trial in knee joint osteoarthritis treatment: safety assessment and performance of trehalose hyaluronic acid versus standard infiltrative therapy based on medium-weight sodium hyaluronate

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ABSTRACT

Introduction: Newly formulated trehalose-hyaluronic acid (T-HA) has proven to be more stable in vitro to the effects of hyaluronidase enzyme.
Objectives: To compare clinical outcomes of T-HA with standard non–trehalose (NT-HA) hyaluronic acid when administered as infiltrative therapy in patients with symptomatic osteoarthritis of the knee.
Methods: A prospective controlled trial with parallel arms was performed. Sixty patients with persistent symptomatic knee osteoarthritis were randomized to T-HA or non–trehalose-hyaluronic acid groups. Each patient received 3 doses of either of the products separated by 15 days, with a follow-up at 3 (T_1) and 6 (T_2) months. The study was blinded for participants, caregivers, and outcome assessors. Treatment performance was measured with the International Knee Documentation Committee (IKDC), Knee Injury and Osteoarthritis Outcome Score (KOOS), and Visual Analogue Scale (VAS) for pain outcome scores and was compared with basal scores and between groups.
Results: Each group consisted of 30 patients; the mean age was 56.4 ± 15.6 years. At 3 months, IKDC, KOOS, and VAS improved for both groups ($P < .05$). At 6 months, group T-HA continued to improve IKDC, KOOS, and VAS ($P < .05$), while group NT-HA scores decreased ($P < .05$). IKDC increased to 66.98 (60.92-78.79) for T-HA, while it decreased to 59.77 (35.34-73.03) for NT-HA.
Conclusions: Both T-HA and NT-THA are safe and effective for treating early osteoarthritis symptoms. T-HA provides a longer duration of symptom relief than NT-HA does. Further studies are needed to determine the total lasting effects of T-HA.

Introduction

Hyaluronic acid is an effective treatment for early symptomatic knee osteoarthritis (OA). It decreases symptoms by lubrication and shock absorption. Chemically it has anti–inflammatory effects by binding to the CD44 receptor, thus inhibiting pro-inflammatory mediators through toll-like receptors 2 (TLR2) and TLR4; this finding has demonstrated that its chondroprotective effects.[1-2]

Trehalose, a non–reducing disaccharide, has long been used in the biopharmaceutical industry to stabilize proteins; it is formed by 2 glucose molecules linked by an α, α–1,1 bond. These bonds confer resistance on trehalose to acid hydrolysis, stabilizing the

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Table 1
Inclusion-exclusion criteria.

Patient screening criteria
Inclusion criteria
• Age between 18 and 80 y
• Ability to provide informed consent
• Persistent pain >3 mo that failed non–invasive treatments.
• OA diagnosed by radiographic imaging
• Grade I-III radiographic OA as defined by the K-L classification
Exclusion criteria
• Knee instability
• Patella maltracking
• Systemic disorders such as diabetes, rheumatoid arthritis,
• Hematological diseases (coagulopathies), severe cardiovascular diseases, infections, or immunodeficiencies.
• Use of anticoagulant medications
• History of known anemia
• Recent intra-articular injection treatment (within 30 d)
• Pregnancy or possible pregnancy

substance at high temperatures and in acidic environments (pH 2 and 100 °C for 24 hours) thus, providing high resistance to oxidation and early degradation.[3]

A recent in-vitro study showed that hyaluronic acid combined with trehalose had enhanced resistance to hyaluronidase enzyme degradation compared to standard therapy (2). In this study, a hyaluronate composed of 80% medium weight plus 20% low-weight hyaluronic acid was compared to one with the same structure plus 1% trehalose. The 2 samples were tested in a reactive solution with hyaluronidase for its activity evaluation. The sample containing trehalose showed no significant amount of hydrolyzed hyaluronic acid in the reaction mix after 60 min, while the control demonstrated 54.1% of hydrolyzed HA, implying a lower susceptibility, and accessibility of the product to the hyaluronidase than the sample without trehalose had.[4,5]

Our primary endpoint evaluates whether infiltrative trehalose therapy provides longer-lasting effects than standard non–trehalose therapy does in treating persistent symptomatic knee osteoarthritis (OA). Second; we assess trehalose hyaluronate's safety and performance based on an increment of KOOS, IKDC, and VAS clinically from the basal scores.

As trehalose hyaluronic acid (T-HA) has improved resistance to hyaluronidase in vitro compared to non–trehalose (NT-HA), we hypothesize that in-vivo T-HA has longer-lasting clinical results than NT-HA has when applied as an injectable formula for OA symptomatic knees.

Methods

This was a prospective double-blinded, randomized control trial with parallel arms and an allocation ratio of 1:1 of patients with persistent symptomatic OA of the knee receiving either T-HA or NT-HA. The Consolidated Standards of Reporting Trials (CONSORT) Statement was followed.[6]

The study was performed according to the ethical standards outlined in the 2013 revision of the 1975 Declaration of Helsinki, approved, and monitored by our institutional review board. Additionally, trehalose hyaluronate is under the directive 93/42/EEC; it is in line with medical device documents (MEDDEV), and approved by FDA for its use. Trial ID: ISRCTN18428696.

Participants

The patients were recruited at a single clinical institution by the chairman of the orthopedic department. The main inclusion criteria consisted of patients aged 18 to 80 years, with symptomatic knee OA, grade I to III according to K-L classification, without pain relief after at least 3 months of non–invasive treatment OA was diagnosed by X-rays and classified according to the Kellgren and Lawrence classification.[7] Every patient had to sign the informed consent before enrolment; the potential benefits and risks of hyaluronic acid injection were explained and understood by all. The main exclusion criteria were any recent intra-articular injection therapy, knee instability, significant axial deviation, systemic disorders such as rheumatoid arthritis, coagulopathies, or infections. Full detail is illustrated in Table 1.

Intervention

Patients that meet the inclusion criteria were centrally randomized by research personnel at the same working center into either group A or B. The type of randomization was simple by an electronic randomizer used (Research Randomizer Uriabank, G.C, & Plus.S, 2013 computer software Version 4.0), which provided a random number table to group A or group B, generating an unpredictable random 1:1 allocation sequence.

The manufacturer provided the products labeled as A or B (corresponding to group A or B, respectively). Both products had the same syringe, color (transparent), texture, and quantity (2 mL). Thus, the study was blinded for patients, clinicians, researchers, and the manufacturer who handled the product. The nature of the product was revealed only when the study was finished.

After basal clinical scores were recorded, an intraarticular injection with either of the products was always performed by the same orthopedic physician in a sterile environment. The knee was held in extension, and a suprapatellar approach was used. Precisely, 2 mL of hyaluronate were injected with a 20 gauge by 38 mm needle after which ice was applied for 5 minutes. Then patients were instructed to avoid intense exercise for 48 hours and to apply ice for 15 minutes 3 times a day.

Each patient received 3 doses of the same product (A or B) separated by 15 days. Afterward, they were invited for follow-up at 3, and 6 months. Follow up for clinical evaluation was by the same senior author. Both groups received the same rehabilitation protocol for 4 weeks, starting 1 week after the first injection to improve strength, and range of motion.

Throughout the follow-up period, it was suggested that patients consistently follow isometric knee exercises.[8]

The use of medication by the patients was not recorded.

Outcomes

To address the primary endpoint, patients were clinically evaluated by a senior author using VAS scores[9] (0-10, with 0 meaning no pain and 10 meaning extreme pain); IKDC[10] (0-100, with 100 denoting no functional limitation or pain with high-level activity) and KOOS[11] (percentage score obtained from the evaluation of 5 separately scored subscales: pain, other symptoms, functions of daily living, function in sport and recreation, and knee-related quality of life. It ranges from 0 to 100, 0 representing severe problems, and 100 representing no problems). Scoring systems were recorded through questionnaires filled by the same patients before the first injection,

(T_0), at 3 months (T_1) and 6 months (T_2). Patients were also assessed for any adverse reaction such as effusion, flare, or pain during the clinical evaluation. After clinical evaluation and score recording, an independent researcher archived the data into a database.

The infiltration products used were NT-HA composed of 80% medium-weight sodium hyaluronate (1200-1500 kDa) and 20% low-weight hyaluronic acid (200-400 kDa); T-HA had the same components plus the addition of 1% trehalose. These HAs are from a non–animal source, obtained by bacterial fermentation (*Streptococcus Equi*). Both came in the dosage presentation of 2 mL, 50 mg/2 mL hyaluronate.

Sample size

A-priori power analysis was based on sample size calculation from prior studies. A total sample of 60 patients, 30 for each group, was estimated to be adequate to detect a 15-point difference in KOOS between group A and B using a Wilcoxon-Mann Whitney 1-sided test assuming a standard deviation of 20, an alpha of 0.025, and a power of 0.80. The sample of 30 subjects for each group also has a power of 0.92 to detect a 15-point difference in KOOS among periods (T_0 vs T_1, T_0 vs T_2, and T_1 vs T_2) within the same group, assuming a standard deviation of 20, an overall alpha of 0.025, and a Wilcoxon signed ranks 1-sided test.

Statistical methods

Normal distribution was determined graphically and by the Shapiro-Wilk test. Summary statistics were reported as absolute frequency and percentage change for categorical or continuous variables, such as the median and interquartile range (IQR), as they were non–normally distributed. Groups A and B were compared with the χ^2 test for categorical variables and the Wilcoxon-Mann Whitney test for continuous variables.

A Wilcoxon signed ranks test with Bonferroni adjustment for multiple time comparison was performed to assess whether scores differed among periods (T_0 vs T_1, T_0 vs T_2, and T_1 vs T_2). Statistical significance was considered for P values less than .05 in all tests. A per-protocol analysis was used for result interpretation.

All analyses were performed with R version 3.6.1 and Stata/S.E. version 14.0 (Stata Corporation, College Station).

Results

The mean age of the 60 study patients was 56.3 ± 15.9 years. Of the 155 patients assessed for eligibility, 75 were included in the study. A total of 38 were randomized into group A and 37 into group B (Flow diagram is reported in Fig. 1). Patients were recruited from June 2019 to April 2021 until the objective sample of 60 patients was reached: 30 per group. Patients who were unwilling to complete full treatment or were lost to follow-up were not included.

No significant difference ($P > .05$) among the 2 study groups regarding age, gender, affected size, smoking, Kellgren-Lawrence, or BMI regarding the demographic data was found. Detailed results are reported in Table 2.

There was a statistically significant improvement in all scores for T-HA in KOOS, IKDC, and VAS at 3 and 6 months ($P < .05$) compared to basal scores. From the third to the sixth month, improvements in IKDC, KOOS, and VAS continued, but this improvement was not statistically significant.

Regarding NT-HA, IKDC, KOOS, and VAS improved clinically at 3 months compared to the basal score. This improvement was clinically significant for the IKDC score ($P < .05$). However, scores returned to the previous basal results from the third to the sixth month; this finding was statistically significant for IKDC scores, and KOOS ($P < .05$).

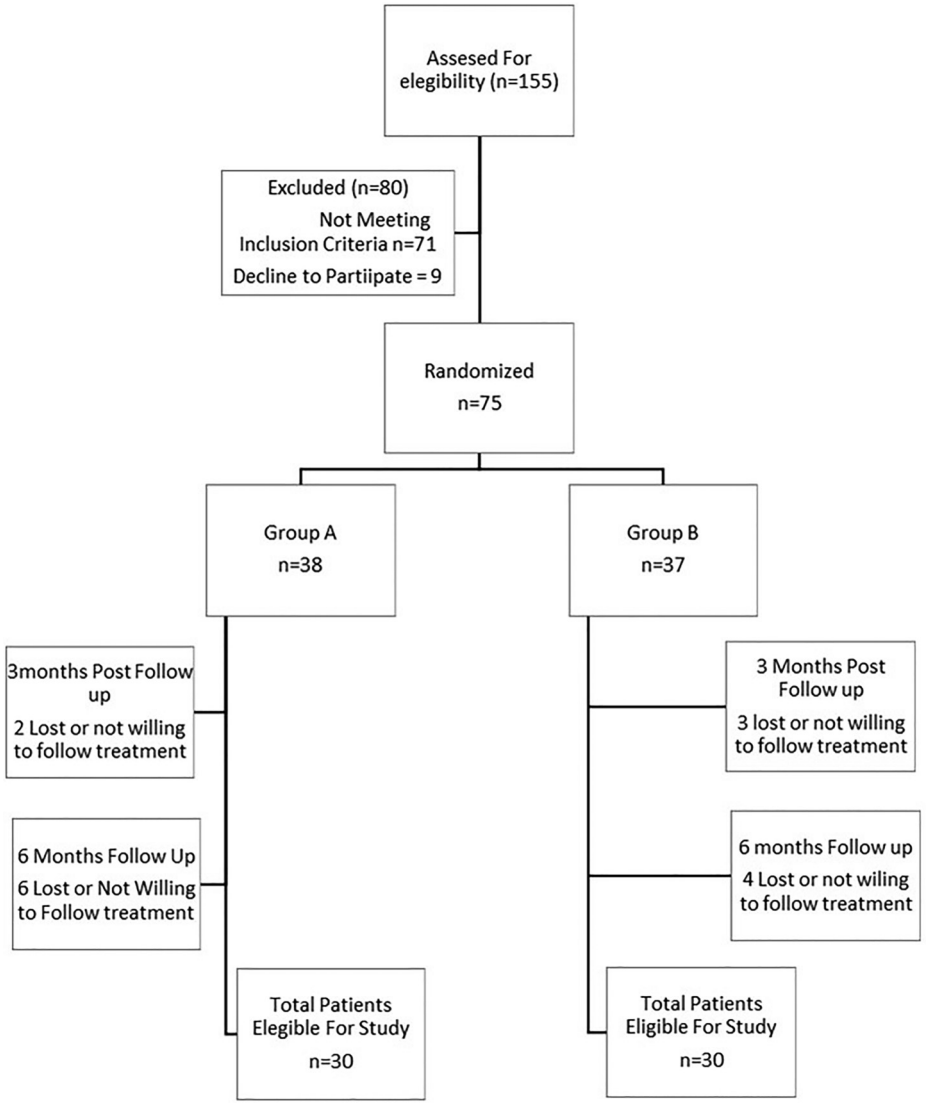

Fig. 1. Flow diagram used in the design of the trial.

When comparing both groups at 3 months, T-HA showed no significant clinical results than NT-HA did for KOOS and VAS ($P > .05$). At 6 months, T-HA reported significant clinical outcomes for IKDC and KOOS compared to NT-HA ($P < .05$). Detailed results are reported in Table 2 (Figs. 2-4).

One patient in group A and 2 in group B reported mild effusion as an adverse effect. This event occurred in the first days after the initial injection and was alleviated by ice and low-strength analgesics and resolved completely.

Discussion

To our knowledge, this is the first prospective randomized controlled trial to evaluate and compare the effects of a trehalose hyaluronate formulation used as an infiltrative therapy for knee OA.

Our clinical results corroborate those in the literature regarding the effectiveness of hyaluronic acid as a treatment for symptomatic knee OA, as both compounds of hyaluronic acid demonstrated a statistically significant improvement in pain and function from the basal time point.[12,13]

Novel trehalose hyaluronic acid was demonstrated to significantly improve clinical outcomes at 3 (T_0-T_1) and 6 months (T_0-T_2) compared to basal scores, and the high scores persisted at 6 months. On the other hand, control hyaluronate behaved similarly to standard therapy, as it showed clinical improvement at 3 months but with no persistent effect at 6 months. This correlates with literature that describes that the peak effectiveness of intra-articular HA is reached between 1 and 2 months, and at 6 months, only some residual effects would remain.[14,15]

Table 2
Demographic data.

	Overall N = 60	Group A N = 30	Group B N = 30	P value
	n (%)	n (%)	n (%)	
Sex				
Female	27 (45.0)	11 (36.7)	16 (53.3)	.299
Male	33 (55.0)	19 (63.3)	14 (46.7)	
Side				
Bilateral	36 (60.0)	16 (53.3)	20 (66.7)	.409
Right	17 (28.3)	9 (30.0)	8 (26.7)	
Left	7 (11.7)	5 (16.7)	2 (6.7)	
Kellgren-Lawrence Grade				
Grade 1	5(8.3)	2(6.6)	3(10)	N.A.
Grade 2	23(38.3)	11(36.6)	12(40)	
Grade 3	32(53.3)	17(56.6)	15(50)	
	Median (IQR)	Median (IQR)	Median (IQR)	
Age	56.00 (49.00, 69.00)	55.00 (41.75, 61.25)	63.00 (49.00, 69.00)	.149
Height	1.70 (1.65, 1.78)	1.70 (1.66, 1.78)	1.70 (1.63, 1.75)	.490
Weight	73.50 (67.00, 77.00)	72.00 (63.75, 80.00)	74.00 (67.00, 76.75)	.737
BMI	24.60 (22.50, 25.71)	24.50 (22.35, 25.27)	25.17 (23.32, 26.00)	.379

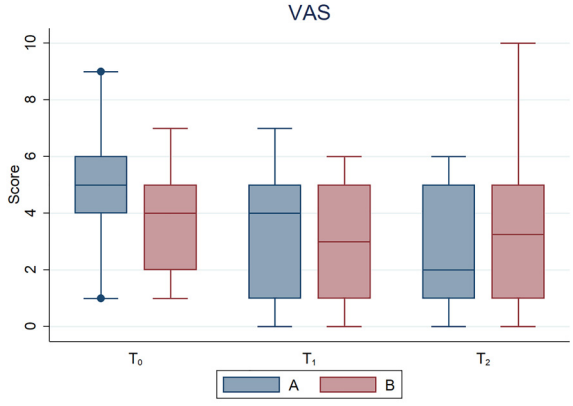

Fig. 2. VAS score box-plot.

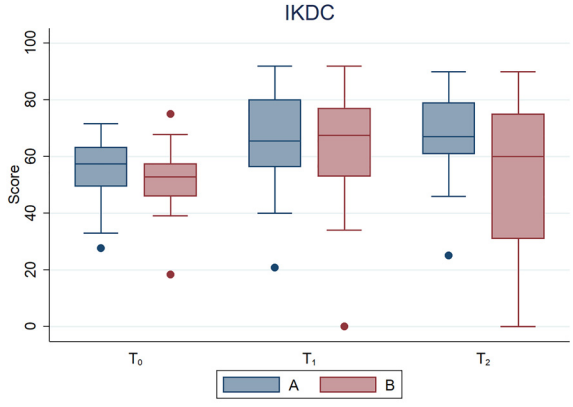

Fig. 3. IKDC score box-plot.

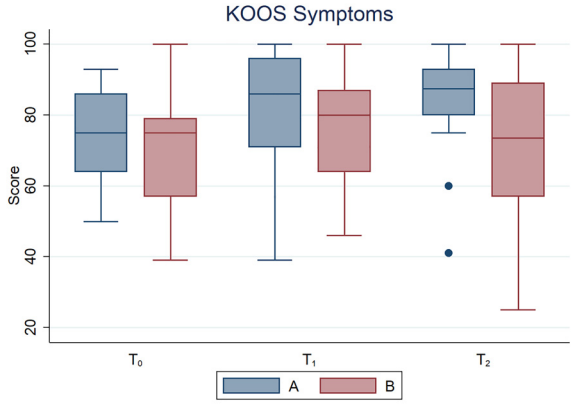

Fig. 4. KOOS symptoms box-plot.

Comparing both hyaluronates at 3 months, they had similar clinical outcomes. However, the difference was statistically significant at 6 months because the novel compound maintained the effects, while the NT-HA markedly returned to basal scores. This finding supports our primary hypothesis showing that trehalose hyaluronate provides longer-lasting effects than NT-HA does, thus proving it to be more effective.

The background of trehalose is not new; it has been widely used in many forms in pharmaceuticals and has proven to be safe.[4,5] The compound consists of 2 glucose molecules linked by an $\alpha,\alpha-1,1$ bond. This bond confers some of its remarkable properties, such as the ability to remain stable to heat, and acid (pH 2 at 100 ˚C for 24 h). Furthermore, due to its low hygroscopicity (no moisture absorption at 90% RH and 25 ˚C for 10 days), it remains stable, and free-flowing in high humidity environments such as the knee joint.[16]

Protein-based compounds such as hyaluronic acid are easily degraded by strong acids, bases, inorganic salts, and organic solvents. The active end groups of these proteins, generally associated with hydrogen-bonded water, can bind to other molecules, leading to their denaturation, and loss of function. Trehalose helps preserve the structure and function of proteins by hydrogen bonding to the polar residues in hyaluronic acid, serving as a water substitute. In this setting, by removing H_2O, chemical reactions do not occur. This happens with trehalose hyaluronate, which does not react with the hyaluronidase enzyme, and thus extends its effects, as previously demonstrated in vitro.

This fact is highly relevant in bio-orthopedics because further inflammatory damage could be prevented by stabilizing the chemical reactions in the knee joint. Additionally, the trehalose molecule has previously demonstrated its anti–inflammatory effects. It has been used in organ preservation to increase organ transplantation timing and as a reducer of postoperative adhesions for fibrosis prevention.[17,18] Thus, this anti–inflammatory property could be another reason T-HA clinical outcomes continued to improve over time, making it an interesting focus for future research.

Our control therapy was performed with medium- plus low-weight hyaluronic acid. The background behind this choice is that the mixing of the 2 allows a high concentration of HA (2.5%), maintaining a viscosity suitable enough for supplementation, easy to handle, and with an optimum extrusion from the syringe but without the adverse inflammatory effects of low or high-weight HA.[19]

High-weight hyaluronic acid (HW-HA) has also been evaluated in previous studies to achieve longer-lasting effects. However, the disadvantage of high-viscosity hyaluronate is that the product is more difficult to administer, and primarily that during the inflammatory phases of OA it has been related to decreased functional outcomes. Overall, the current evidence does not support the superiority of one kind of HA preparation over another, perhaps except for a slightly lower efficacy for low-weight HA preparations versus intermediate and high-weight hyaluronic acid,[20] and increased safety risk for HW-HA; in some studies, even doubling the frequency of post-injection effusion, and inflammation.[21] This fact also correlates with our findings, as with our control, we did not find significant adverse reactions proving it to be safe to administer.

Limitations

Our study is not exempt from limitations; the short clinical follow-up of 6 months should be taken into consideration. Another limitation is that patients with loss of follow-up or without completed therapy were not included (8 patients for group A and 7 for group B), leaving in doubt why they failed to continue. Moreover, our primary outcome was clinical scores, but they are an indirect sign of persistency of hyaluronate in the knee joint. Furthermore, the relatively small number of patients did not allow for subpopulation analysis, particularly, regarding pre-treatment knee OA.

Another limitation is the lack of recording of the rescue medicine by the patients during the follow-up period. Perhaps for further research, it would be interesting to correlate clinical findings with intraarticular inflammatory markers over time, and with possible hyaluronate present in the articular joint to objectify the results.

Table 3
Clinical comparison between T-HA and NT-HA groups.

	Group		Comparison between A and B *P* value	Comparison between time points		
	T-HA Group A *N* = 30 Median (IQR)	NT-HA Grop B *N* = 30 Median (IQR)			T-HA Group A Adj *P* value	NT-HA Group B Adj *P* value
IKDC						
T_0	57.47 (49.43, 62.64)	52.87 (45.98, 57.47)	.111	T_0-T_1	.005*	.012*
T_1	65.16 (56.90, 79.02)	67.48 (53.00, 75.75)	.610	T_1-T_2	1	.006*
T_2	66.98 (60.92, 78.79)	59.77 (35.34, 73.03)	.045	T_0-T_2	<.001*	1
KOOS						
T_0	75.00 (65.00, 85.25)	75.00 (58.75, 79.00)	.744	T_0-T_1	.010*	.921
T_1	86.00 (71.00, 96.00)	80.00 (64.00, 87.00)	.189	T_1-T_2	1	.015*
T_2	87.50 (80.00, 92.75)	73.50 (57.00, 88.25)	.030	T_0-T_2	.001*	1
PAIN						
T_0	72.00 (58.75, 81.00)	66.50 (58.00, 82.50)	.923	T_0-T_1	<.001*	.005*
T_1	83.50 (76.00, 96.25)	83.00 (73.50, 92.00)	.953	T_1-T_2	1	.212
T_2	87.50 (75.00, 93.50)	81.00 (69.00, 91.25)	.441	T_0-T_2	.001*	.031*
ADL						
T_0	85.00 (65.00, 92.50)	73.00 (65.00, 86.25)	.329	T_0-T_1	.044*	.012*
T_1	93.50 (78.00, 97.00)	83.00 (69.00, 93.00)	.083	T_0-T_2	1	.525
T_2	90.00 (83.25, 98.75)	82.00 (65.00, 93.00)	.006*	T_1-T_2	.006*	1
SPORT						
T_0	50.00 (36.25, 65.00)	50.00 (30.00, 55.00)	.345	T_0-T_1	<.001*	.007*
T_1	70.00 (50.50, 86.50)	65.00 (51.25, 80.00)	.336	T_0-T_2	.017*	.100
T_2	78.50 (60.00, 90.00)	60.00 (50.00, 77.75)	.003*	T_1-T_2	<.001*	.003*
QOL						
T_0	50.00 (38.00, 56.00)	45.50 (31.00, 56.00)	.576	T_0-T_1	.188	.002*
T_1	60.00 (38.00, 70.00)	63.00 (50.00, 69.00)	.801	T_0-T_2	.265	.164
T_2	59.50 (45.50, 80.00)	53.00 (38.00, 69.00)	.159	T_1-T_2	.021*	.044*
VAS						
T_0	4.88 (3.81, 6.00)	4.00 (2.00, 4.69)	.020*	T_0-T_1	.006*	.170
T_1	4.00 (1.10, 5.00)	3.20 (1.00, 4.75)	.385	T_0-T_2	.009*	1
T_2	2.00 (1.00, 5.00)	3.15 (1.00, 5.00)	.506	T_1-T_2	<.001*	.571

* Statistical Significant Value ($P < 0.05$)

Conclusions

Both T-HA and NT-THA are safe and effective for treating early OA symptoms. However, T-HA provides a longer duration of symptom relief than NT-HA Further studies are needed to determine the total lasting effects of T-HA Table 3.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jcjp.2022.100043.

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