Histological features of knee osteoarthritis treated with triamcinolone acetonide and hyaluronic acid

PATTARANATCHA CHARNWICHAI[1,2], RACHANEEKORN TAMMACHOTE[2], NATTAPOL TAMMACHOTE[3], THIAMJIT CHAICHANA[4] and NAKARIN KITKUMTHORN[5]

[1]Program in Biotechnology, Faculty of Science, Chulalongkorn University; [2]Department of Botany, Faculty of Science, Chulalongkorn University, Pathumwan 10330; [3]Department of Orthopedics, Faculty of Medicine, Thammasat University Hospital, Khlong Nueng, Pathumthani 10120; [4]Department of Pathology, Faculty of Medicine, Chulalongkorn University, Pathumwan, Bangkok 10330; [5]Department of Oral Biology, Faculty of Dentistry, Mahidol University, Bangkok 10400, Thailand

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Abstract. Osteoarthritis (OA) is one of the most common degenerative joint diseases leading to disability in the end stage. Although intra-articular triamcinolone acetonide (TA) is one of the OA treatments that have been widely used, the side effects of such corticosteroids are still controversial. Intra-articular hyaluronic acid (HA) injection is another therapeutic option for patients with OA who do not want to use corticosteroids because of their side effects. However, the difference between the histological features associated with TA and HA in the treatment of OA remains unclear. Thus, the present study aimed to compare the histological effects of TA and HA on the cartilage of patients with knee OA. In the current study, 31 patients diagnosed with grade 3-4 knee OA on the Kellgren-Lawrence radiographic grading scale were separated into three groups: TA (n=12); HA (n=7) and untreated group (n=12). Histological examination of the whole articular cartilages of the patients was performed with hematoxylin and eosin and Alcian staining, as well as using a TUNEL assay. Clinical data such as cartilage thickness, structural and component deterioration, proteoglycan levels, apoptosis and empty lacunae were compared between the three groups. The results showed a high level of deterioration in both TA and HA groups but not in the untreated group, although the thickness of cartilage in the HA group was lower compared with that in the TA and untreated groups. The proteoglycan levels in the TA group were lower compared with those in the HA group. Moreover, the number of empty lacunae in the HA group was higher compared with that in the TA group, while no difference in apoptosis was found between TA and HA groups. A significant difference was not found in the histological staining between TA and HA groups. On the other hand, a significant difference was found in cartilage deterioration between the medial and lateral sides in these groups. TA and HA groups showed comparable histological results. TA injection is cheaper and easier but has more adverse effects for patients with knee OA than HA injection. Therefore, orthopaedists should select TA or HA based on the economic and specific needs of patients.

Correspondence to: Dr Nakarin Kitkumthorn, Department of Oral Biology, Faculty of Dentistry, Mahidol University, 6 Yothi Road, Ratchathewi, Bangkok 10400, Thailand
E-mail: nakarinkit@gmail.com

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Introduction

Osteoarthritis (OA) is the most common chronic degenerative joint disease and its prevalence has grown by 113.25% worldwide from 247.51 million cases in 1990 to 527.81 million cases in 2019. It characterized by degenerative changes of cartilage, bone and synovial tissue, and leading to disability, in particular knee OA (1,2). Symptoms of OA include stiffness, crepitus and swelling and joint pain is the hallmark of OA which tends to worsen with physical activity (3). OA is defined by imbalance between mechanical strain on cartilage and resistance (4) OA is a multifactorial disease, including factors such as age, sex, obesity, genetic predisposition, occupational knee-bending and joint trauma, but the actual cause of OA remains unclear (5). Thus, the primary purpose of OA treatment is to reduce pain and stiffness, slow disease progression and improve joint function (6). There are several treatment options available to patients with OA, such as physical modalities and exercise, pharmacological treatment, intra-articular (IA) injection of corticosteroids and surgery, depending on the symptoms and severity of the disease (7).

IA corticosteroids are administered into the joint space and are used as an alternative therapeutic option for the treatment of OA when oral medications are not effective to relieve symptoms (8). IA injection of corticosteroids has been widely used for >50 years to treat patients with knee OA for pain relief and control of local inflammation (9-11). It acts directly on nuclear steroid receptors to alter synthesis of cytokines and enzymes and to inhibit phospholipase A2, resulting in a decrease in proinflammatory derivatives of arachidonic acid (12).

Moreover, corticosteroids inhibit synthesis of prostaglandin and decrease the activity of collagenase and production of interleukin-1 and tumour necrosis factor α, which can degrade the cartilage (8). Nevertheless, use of corticosteroids is still controversial due to their side effects, particularly when used as a long-term treatment or used repeatedly (10).

The effects of corticosteroids on the articular cartilage have been reported. Several studies have showed that both short- and long-term IA injection of corticosteroids or its combination with anaesthetics have no detrimental effect and do not cause chondrocyte death in *Macaca irus* monkeys and patients with OA (10,13,14). On the other hand, adverse effects of corticosteroids on articular chondrocytes have been reported. A decrease in chondrocyte density has been reported in male Sprague-Dawley rats following 6 months of corticosteroid injection (15). Chondrocyte apoptosis induced by corticosteroids has been reported in both mice transplanted with human articular cartilage and in human chondrocyte cultures (15,16). Moreover, the rate of apoptosis is increased both in *in vitro* chondrocyte cultures and in osteochondral *ex vivo* specimens when treated with combined corticosteroids and local anaesthetics (17). Corticosteroids are suggested to increase oxidative stress and alter expression of cyclin-dependent kinase inhibitor 1A, growth differentiation factor 15 and protein c-Fos genes, which are involved in cell death and chondrotoxicity (18). Moreover, corticosteroids may inhibit proteoglycan metabolism by decreasing aggrecan expression and proteoglycan concentration (19). Concerning the effects in patients, a transient decrease of meniscal thickness and joint space width is observed in the knee medial compartment of patients with knee OA who receive corticosteroid injections for 1 year (20). Triamcinolone acetonide (TA), methylprednisolone acetate, prednisolone acetate and betamethasone are used to treat patients with OA and exhibit similar general efficacies (21). These corticosteroids provide good results for pain relief, especially TA which is shown to be more effective than other corticosteroids (22). On the other hand, viability of both human and canine OA chondrocytes is significantly reduced upon being treated with TA in a concentration-dependent manner (18,23). Moreover, TA significantly increases MMP-3 mRNA expression in chondrocytes, a gene involved in the degradation of cartilage (24).

In addition to corticosteroids, hyaluronic acid (HA) is commonly administered via IA injection to treat knee OA. HA is a linear non-sulphated glycosaminoglycan that is composed of repeating D-glucuronic acid and N-acetylglucosamine units (25,26). It is found in multiple types of animal tissue, especially in the extracellular and pericellular matrix of soft connective tissue (25,26). HA provides viscoelasticity and lubrication to synovial fluid and has an anti-inflammatory effect (25). Moreover, exogenous HA increases proteoglycan and chondrocyte HA synthesis, decreases activity and production of MMPs and proinflammatory mediators and acts as an immune regulator (26,27). There are several studies on the effect of HA injection: Certain studies have showed no significant differences following HA injections and placebo in patients with knee OA (28,29), while others have reported that IA HA injections increase the short-term improvements in patients with early to moderate-onset knee OA with a modest effect that peaks at ~6-18 weeks after injection (30,31). Furthermore, a comparison between the effects of corticosteroids and HA reported no differences when comparing the Western Ontario and McMaster Universities Arthritis Index (WOMAC) scores of patients treated with either corticosteroids or HA at 6-month follow-up (32). However, at 6 months after injection, HA is reported to be more effective than corticosteroids, especially for movement restriction relief (33), while corticosteroids are reported to be superior in reducing pain (33,34). Although HA has become widely adopted to treat patients with OA, whether it decreases pain remains unclear.

To the best of our knowledge, although IA injections of corticosteroid and HA are widely used in patients with knee OA, the effects of these drugs have been compared only by a limited number of studies (7-13). Moreover, the comparison of the IA knee injection effects of TA and HA has not been studied. Therefore, the present study aimed to assess the effects of IA corticosteroids or HA on the articular cartilage of patients with knee OA. The structures and components of articular cartilage and proteoglycan levels, as well as levels of apoptosis and empty lacunae in chondrocytes, were analysed.

Materials and methods

Sample collection. In the present study, fresh knee articular cartilages were collected from patients with knee OA who were diagnosed with grade 3-4 on the Kellgren-Lawrence radiographic grading scale (35) at the Department of Orthopaedic Surgery, Faculty of Medicine, Thammasat University Hospital (August 2019-March 2021). Demographic data is presented in Table I. Patients were separated into three groups depending on their clinical history. Patients who had received IA injection of 40 mg Kanolone-F (TA extended-release injectable suspension; L.B.S. Laboratory Limited) within 6 months before total knee arthroplasty (TKA) were included in the TA group (n=12). The HA group (n=7) included patients who received IA injection of Synvisc® (Hylan G-F 20; Sanofi S.A.) in the 6 months before TKA. Lastly, patients who had not received IA injections with either TA and/or HA within 6 months before TKA were included in the untreated group (n=12). After TKA, eight knee articular cartilage samples from each patient, including posterior lateral, posterior medial, anterior lateral, anterior medial, tibia lateral, tibia medial, distal lateral and distal medial, were collected and immediately soaked in 10% neutral buffered formalin (Bio Optica Milano SpA) for 24 h at room temperature. Other clinical data including age, sex, affected knee side, weight and height were collected from all patients. All procedures were approved by the Ethics Committee for Research in Human Subjects, Faculty of Medicine, Thammasat University (approval no. MTU-EC-OT-4-233/62; Pathumthani, Thailand) and conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all patients.

Sample preparation. Formalin-fixed articular cartilage samples were cut into 2x1x1 cm^3 and were decalcified using 10% EDTA with a commercial ultrasonic machine (Cavitator® ultrasonic cleaner; Mettler Electronics Corp.; power, 85 watts; frequency, 67 kHz) for 8 h at room temperature (36). Then, the decalcified articular cartilage samples were embedded in paraffin wax using a tissue embedding machine (Leica Biosystems) according to a standard histological protocol (37).

Table I. Characteristics of participants in TA, HA, and untreated groups.

Characteristic	TA (n=12)	HA (n=7)	Untreated (n=12)	P-value
Male:female (%)	3:9 (25.00:75.00)	2:5 (28.57:71.43)	6:6 (50.00:50.00)	0.4012
Median age, years (range)	66 (49-87)	76 (63-83)	73 (59-82)	0.2360
Left:right knee (%)	4:8 (33.33:66.67)	4:3 (57.14:42.86)	5:7 (41.67:58.33)	0.5975
Median BMI, kg/m^2 (range)	25.50 (18.70-28.90)	27.00 (20.70-29.40)	26.00 (20.90-36.40)	0.3933
Normal:overweight:obese (%)	5:7:0 (41.67:58.33:0)	2:5:0 (28.57:71.43:0)	3:6:3 (25.00:50.00:25.00)	0.2265

TA, triamcinolone acetonide; HA, hyaluronic acid; BMI, body mass index.

Haematoxylin and eosin (H&E) and Alcian blue staining. A total of 16 slides of 3-μm paraffin-embedded sections for each patient were prepared. Alcian blue staining (Bio-Optica Milano SpA); Alcian blue 0.1 g in 100 ml of 3% acetic acid was performed for 30 min while H&E staining (C.V. Laboratories Co., Ltd.) was carried out according to the manufacturer's procedure (1 g haematoxylin in 10 ml of ethanol). Both staining steps were conducted at room temperature. H&E staining was utilized to study the overall structure and components of articular cartilage and chondrocytes using OA Research Society International 0-6 histologic features grading (13,38). The paraffin-embedded sections were mounted and scanned using Aperio CS2 Scanscope (Leica Microsystems, Inc.) at 40X magnification. All slides were graded by three operators (NK, TC, and PC). The overall structure and components were graded as follows: Grade 0, intact surface and cartilage morphology; grade 1, intact surface; grade 2, surface discontinuity; grade 3, vertical fissures; grade 4, erosion; grade 5, denudation and grade 6, deformation (8). The grading score was calculated based on the grade (0, grade 0-2; 1, grade 3-4 and 2, grade 5-6). A grading score <1 was interpreted as low deformation while a grading score ≥1 was interpreted as high deformation.

To study the proteoglycan levels in the articular cartilage, 8 paraffin-embedded section from each patient was stained with Alcian blue according to a standard histological protocol (13). The paraffin-embedded sections were mounted and scanned, as aforementioned. The proteoglycan levels were graded as 0-10 staining intensity (0; no stain, 1; extremely weak, 2; very weak, 3; weak, 4; below average, 5; average, 6; above average, 7; intense, 8; very intense, 9; extremely intense, 10; dark).

TUNEL assay. 8 paraffin-embedded sections from each patient were stained with TUNEL reagent to study the apoptosis of chondrocytes and empty lacunae. ApopTag® Plus Peroxidase *in situ* Apoptosis Detection kit (MilliporeSigma; cat. no. S7101) was used according to a modified protocol. Briefly, the sections were treated with proteinase K for 10 min at room temperature following deparaffinization for nucleic acid retrieval. For peroxidase blocking, the sections were treated with hydrogen peroxide for 5 min at room temperature. Subsequently, the sections were treated with equilibration buffer and terminal deoxynucleotidyl transferase enzyme for 2 h in a hybridizer at 37˚C. The sections were washed with stop solution for 10 min and treated with anti-digoxigenin peroxidase for 30 min at room temperature. For colour development, the sections were treated with 3,3'-diaminobenzidine for 10 min at room temperature. Both apoptosis and empty lacunae were graded as follows: 0, none; 1-24, low; 25-75, moderate and 76-100%, high. Empty lacunae grading score was classified as follows: 0, none/low; 1, moderate and high, 2. A grading score <1 was interpreted as low levels of empty lacunae while a grading score ≥1 was interpreted as high levels of empty lacunae.

Statistical analysis. Clinical data of all patients, including age, sex, affected knee side, weight and height, were recorded in Microsoft Excel software (version 2021; Microsoft Corporation) and statistical analysis was performed using GraphPad Prism (version no 9; GraphPad Software, Inc.). Data are presented as the mean ± SD. The entire experiments were independently duplicate. All data were tested for parametric distribution by Shapiro-Wilk test. Data such as sex, affected knee side, overall structure, apoptosis, and empty lacunae were analysed using χ^2 test whereas age, BMI, thickness of cartilage and proteoglycan levels were analysed using Kruskal-Wallis's test followed by Dunn's multiple comparison for comparisons between the three groups. Bonferroni's correction was used for all statistical adjustment. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Clinical data of participants. The present study involved 31 patients with knee OA who were separated into three groups: TA (38.71); HA (22.59) and untreated (38.71%). Patients comprised 11 males and 20 females, aged 49-87 years with a BMI range of 18.7-36.4 kg/m^2. A total of 13 patients were diagnosed with knee OA on the left side and 18 with knee OA on the right side. No significant differences between TA, HA and untreated groups were found in sex, age, knee side, BMI and BMI category (Table I).

Histological observation. All the results exhibited non-parametric distribution (Table SI). Almost all the articular cartilage samples deterioration (Fig. 1). However, high levels of deterioration were observed in both TA and HA groups (16.67 and 28.57% of patients, respectively) but not in the untreated group (Figs. 1A-C and 2A). The TA group showed the highest thickness of articular cartilage, while the HA group showed the lowest thickness. No significant differences were found between the three groups in both the overall

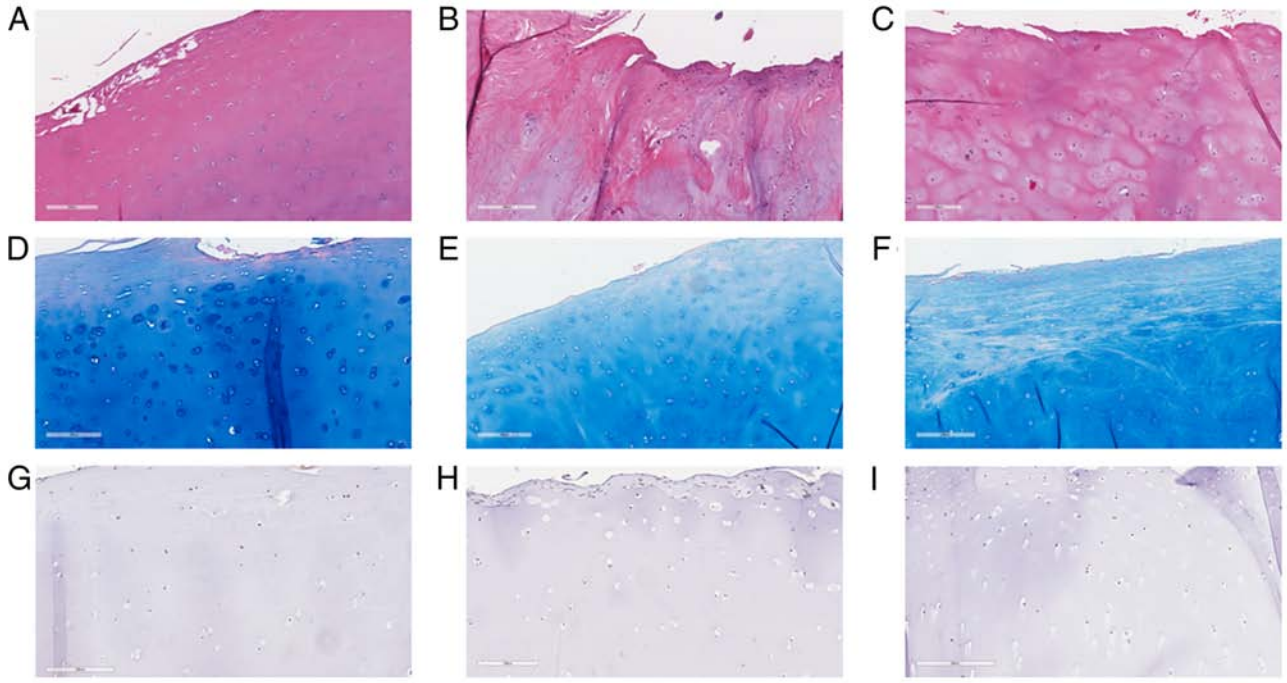

Figure 1. Representative histological features of articular cartilage. (A) Untreated, (B) HA and (C) TA samples stained with hematoxylin and eosin. (D) Untreated, (E) HA and (F) TA samples stained with Alcian blue. (G) Untreated, (H) HA and (I) TA samples stained with TUNEL assay. TA, triamcinolone acetonide; HA, hyaluronic acid. Scale bar is 200 μm length.

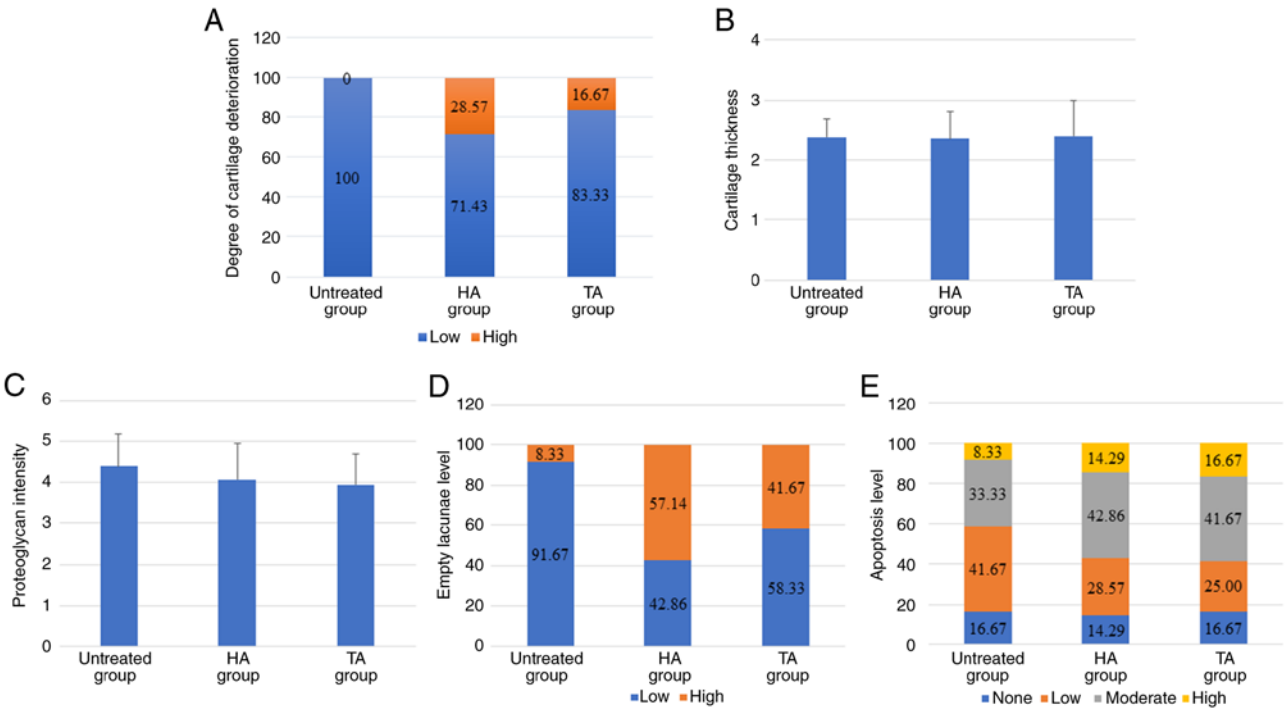

Figure 2. Histological features of articular cartilage in untreated (n=12), HA (n=7), and TA (n=12) groups. (A) Cartilage deterioration. (B) Mean cartilage thickness. (C) Mean proteoglycan intensity score. Levels of (D) empty lacunae and (E) apoptosis. TA, triamcinolone acetonide; HA, hyaluronic acid. Denoted, the scale bar is 200 μm length.

structure and thickness of articular cartilage (Fig. 2B). The proteoglycan levels were analysed using Alcian blue staining (Fig. 1D-F). The proteoglycan levels in the TA and HA groups were decreased compared with those in the untreated group, while those in the TA group were decreased compared with those in the HA group, although no significant difference was found between the three groups (Fig. 2C). Apoptosis and empty lacunae in articular cartilage were studied by TUNEL assay (Fig. 1G-I). Between the three groups, there were no statistically significant differences in empty lacunae and

Table II. Histological features.

A, Triamcinolone acetonide

Histological feature	DM (n=9)	TM (n=6)	PM (n=8)	AM (n=7)	M (n=30)	DL (n=6)	TL (n=6)	PL (n=9)	AL (n=7)	L (n=28)	P-value[a]	P-value[b]
Median cartilage thickness, mm (range)	2.255 (1.048-4.632)	2.550 (1.240-3.850)	2.299 (1.927-3.853)	2.676 (0.542-4.007)	2.255 (0.542-4.632)	1.825 (1.030-2.619)	2.693 (1.777-4.531)	2.554 (1.567-3.550)	3.024 (1.602-3.885)	2.367 (1.030-4.531)	0.4535	0.9343
Low:moderate:high deterioration	3:4:2	3:3:0	3:5:0	0:5:2	9:17:4	5:0:1	4:2:0	6:3:0	6:1:0	21:6:1	0.0551	0.0009
Proteogylcan intensity score	3.778	4.375	3.813	3.286	3.806	3.958	4.042	4.222	4.036	4.025	0.8143	0.5925
Low:moderate:high levels of empty lacunae	5:3:1	1:5:0	4:4:0	2:4:1	12:16:2	1:5:0	3:2:1	1:4:4	2:4:1	7:15:6	0.2398	0.1920
Apoptosis (%)	22.220	16.670	12.500	85.710	32.260	50.000	16.670	33.330	57.140	40.000	0.0017	0.2386

B, Hyaluronic acid

Histological feature	DM (n=7)	TM (n=6)	PM (n=6)	AM (n=3)	M (n=22)	DL (n=6)	TL (n=2)	PL (n=6)	AL (n=3)	L (n=17)	P-value[a]	P-value[b]
Median cartilage thickness, mm (range)	2.046 (1.292-4.981)	2.578 (1.621-3.779)	2.184 (1.431-3.182)	3.144 (2.090-3.375)	2.288 (1.233-4.981)	2.457 (1.702-3.300)	2.168 (1.455-2.880)	2.627 (1.862-4.476)	2.882 (2.185-3.496)	2.589 (1.455-4.476)	0.6456	0.4617
Low:moderate:high deterioration	1:4:2	3:2:1	0:6:0	0:3:0	4:15:3	5:1:0	0:2:0	3:3:0	3:0:0	11:6:0	0.0230	0.0081
Proteogylcan intensity score	3.964	4.125	3.875	3.917	3.977	5.083	4.375	3.583	3.917	4.265	0.8111	0.4242
Low:moderate:high levels of empty lacunae	3:4:0	0:5:1	2:4:0	1:1:1	6:14:2	1:4:1	1:0:1	2:2:2	1:1:1	5:7:5	0.6505	0.2101
Apoptosis (%)	42.860	33.330	16.670	33.330	31.820	33.330	50.000	66.670	0	41.180	0.0017	0.3175

C, Untreated

Histological feature	DM (n=11)	TM (n=9)	PM (n=11)	AM (n=4)	M (n=35)	DL (n=9)	TL (n=12)	PL (n=10)	AL (n=11)	L (n=42)	P-value[a]	P-value[b]
Median cartilage thickness, mm (range)	2.137 (1.380-4.902)	2.283 (0.469-3.312)	2.640 (0.778-4.218)	2.686 (1.916-3.425)	2.283 (0.469-4.902)	2.344 (1.936-2.655)	2.389 (1.274-3.845)	2.604 (2.198-4.061)	3.308 (1.829-4.436)	2.5075 (1.274-4.436)	0.1023	0.0843
Low:moderate:high deterioration	7:4:0	5:4:0	5:6:0	2:2:0	19:16:0	9:0:0	11:1:0	7:3:0	10:1:0	37:5:0	0.0448	0.0041
Proteogylcan intensity score	4.674	4.907	4.098	4.146	4.493	4.889	4.111	3.858	4.576	4.339	0.7214	0.4991
Low:moderate:high levels of empty lacunae	9:1:1	8:1:0	6:2:3	2:1:1	25:5:5	6:3:0	7:5:0	4:6:0	9:2:0	26:16:0	0.0495	0.0060
Apoptosis (%)	25.910	34.890	12.730	40.000	48.900	42.890	50.000	33.330	70.910	41.180	0.0017	0.5575

[a]DM, TM, PM, AM, DL, TL, PL, AL); [b]M vs. L. M, medial; L, lateral; D, distal; T, tibia; P, posterior; A, anterior.

apoptosis. The levels of empty lacunae in the HA group were increased compared with that in the TA group while the level of apoptosis showed no significant difference in these groups (Fig. 2D and E).

Histological observations between medial and lateral sides in 3 groups. In both TA and HA groups, no significant differences between medial and lateral sides were observed in terms of thickness and proteoglycan, empty lacunae, and apoptosis levels. The deterioration of the medial side was significantly increased compared with that of the lateral side in both TA and HA groups (Table II). No significant difference between medial and lateral sides was observed in thickness, proteoglycan, and apoptosis levels in the untreated group. The deterioration and empty lacunae levels of the medial side were higher than those of the lateral side in the untreated group (Table II).

Table III. Comparison of TA and HA for knee osteoarthritis treatment (3,5-13).

Factor	TA vs. HA
Availability	TA>HA
Cost	TA<HA
Side effects	TA>HA
Histology	
Deterioration	TA=HA
Cartilage thickness	TA=HA
Proteoglycan intensity	TA=HA
Empty lacunae	TA=HA
Apoptosis	TA=HA

TA, triamcinolone acetonide; HA, hyaluronic acid.

Discussion

Knee OA is a degenerative joint disease characterized by cartilage degeneration leading to disability in the final stage. The primary goal in treating patients with knee OA is to relieve pain (10). IA injection is used to treat patients with knee OA when oral drugs are not effective (39). IA corticosteroid injections are typically used to treat patients with knee OA to increase joint mobility, decrease joint inflammation and reduce acute pain and swelling, although several adverse side effects that have been reported (40). Several studies have reported the side effects of corticosteroids on chondrocytes: Corticosteroids induce chondrocyte apoptosis and decrease chondrocyte density at 6 months after administration (15-17).

Because of the adverse effects of corticosteroids, IA HA injections are used to treat patients with knee OA. High molecular weight HA is a component of synovial fluid that provides joint lubrication and hyalin cartilage nutrients (41). Several animal models and clinical trials have showed that IA HA injections are a more effective and safer symptom-modifying therapy for decreasing pain of patients with knee OA compared with steroids in short- and mid-term (41,42). In addition, IA HA injection significantly decrease chondrocyte apoptosis rate immediately after administration in a rabbit model (43). However, to the best of our knowledge, the are no studies comparing the effects of corticosteroids and HA IA knee injections in patients with knee OA. The present study aimed to compare the overall structure and components of articular cartilage and proteoglycan, apoptosis, and empty lacunae levels in the articular cartilage of patients with knee OA.

The present study used human articular cartilage from patients with knee OA who underwent TKA as these cartilage samples can accurately represent the pathological conditions of patients with OA. Patients in the TA group received IA injection of Kanolone-F within 6 months prior to TKA, while patients in the HA group received IA injection of Synvisc® within 6 months prior to TKA. Patients in the untreated group did not receive TA or HA within 6 months before TKA. TA is a classical corticosteroid that is used to decrease pain in patients with OA and is more effective than other corticosteroids for pain relief (22,44). Moreover, TA injections activate anti-inflammatory macrophages by inducing $CD163^+$ and Folate receptor β expression. Because of macrophage activation, osteophyte formation is prevented and IL-10 production is reduced (22). By contrast, TA increases oxidative stress and alters expression of cell death signals, leading to chondrotoxicity (18). Moreover, TA induces chondrocyte apoptosis both in cartilage layers and in cultured chondrocytes (16). HA is a linear polysaccharide composed of disaccharide repeats of N-acetyl-glucosamine and glucuronic acid [(1→3)-β-D-GlcNAc-(1→4)-β-D-GlcA-] and is found in the extracellular matrix (26). HA serves as a lubricant that can reduce friction between cartilage, especially in its high molecular weight form (45). Patients with knee OA injected with 6 ml Hylan G-F 20 do not show greater pain relief compared with a placebo group over a period of 26 weeks using WOMAC scores (46). By contrast, certain studies found that Hylan G-F 20 exerts better symptom improvement in the early stage of knee OA compared with placebo and sodium hyaluronate using Visual Analogue Scale and WOMAC scores (47,48). Moreover, TA shows increased pain relief and knee functional improvement compared with Hylan G-F 20 in the first and second week, respectively; however, at 6 months follow-up, TA and Hylan G-F 20 show similar results in pain relief, function and range of motion (49). However, to the best of our knowledge, side effects in patients, in particular apoptosis rate, between corticosteroids and HA remains unclear.

Results from the present study revealed no significant differences in all histological results including deterioration, cartilage thickness and proteoglycan, empty lacunae and apoptosis levels between TA and HA groups (Table III). To the best of our knowledge, previous studies (7-13) have focused mostly on TA and HA efficacy; however, the present study aimed to investigate histological features of articular cartilage treated with TA or HA injection. The present results indicated that TA and HA were comparable in histological features. Although TA induces apoptosis in chondrocyte culture and apoptosis is observed in the superficial and middle layer of severe combined immunodeficiency mouse cartilage (16), the present results indicated that there was no difference in apoptosis levels following treatment with TA or HA. Stove *et al* (19) showed that the corticosteroid dexamethasone decreases proteoglycan concentration *in vitro*. On this basis, the present study compared the proteoglycan levels and showed that these

were comparable between TA, HA and untreated groups. Moreover, the cost of HA is higher than TA, which presents a disadvantage (Table III) (3,38). Conversely, the side effects are the biggest disadvantage of TA. TA exerts rapid pain relief compared with HA (34,50). Cost-effectiveness is a key concern. The present results can assist doctors in choosing the suitable treatment for patients with OA. Moreover, the present results showed that the deterioration on the medial side was significantly higher than on the lateral side in the untreated, HA and TA groups. Consistent with this, knee OA most often presents in the medial compartment of the joint with a prevalence 5-10 times higher than in the lateral compartment because during walking, 60% of the load goes through the medial side of the knee (51-53).

In conclusion, the present histological results showed that the TA and HA groups were comparable in terms of deterioration, cartilage thickness and levels of proteoglycan, empty lacunae, and apoptosis. The present results may inform decisions about whether to use TA or HA in the treatment of knee OA, but patient financial and health status should be considered.

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Availability of data and materials

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

Authors' contributions

PC and NK conceived the study, designed experiments, analyzed and interpreted data, and wrote the manuscript. PC, RT, TC, and NT interpreted data. PC and NK confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethic Committee for Research in Human Subjects, Faculty of Medicine, Thammasat University (approval no. MTU-EC-OT-4-233/62; Pathumthani, Thailand) and conducted in accordance with the Declaration of Helsinki. All participants provided written informed consent.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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