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Original Article

Ozone or hyaluronic acid in the intra-articular treatment of knee osteoarthritis?

Diz osteoartritinin eklem içi tedavisinde ozon mu hyaluronik asit mi?

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ABSTRACT

Background: Both intra-articular ozone and hyaluronic acid injections are commonly used for treatment of knee osteoarthritis (KOA). It was aimed to evaluate and compare the effectiveness of intra-articular ozone and hyaluronic acid (HA) injections on pain and functional limitations of participants with KOA.
Methods: One hundred and eight consecutive patients (eighty-four women, twenty-four men) aged 40-75 years visited to outpatient clinic with knee pain for longer than 3 months. HA injections were performed as a single dose, and ozone injections were administered once a week as three doses in total. The Visual Analog Scale (VAS) and the Western Ontario and McMaster Universities Arthritis Index (WOMAC) were performed before treatment, one and three months after treatment.
Results: Seventy-six participants were included in the study. The randomization was done as Hyaluronic Acid (n=39) and Ozone (n=37) groups. No significant difference was found in terms of WOMAC-total, WOMAC-pain, and VAS (p>0.05) at all stages among groups. WOMAC-stiffness score was found significantly different between first month and third month follow-ups (p=0.011). Also, there was a significant change in WOMAC-function scores between before treatment and first month follow-ups (p=0.008), and before treatment and third month follow-ups (p=0.002) in inter-group analysis.
Conclusion: Both ozone and HA injections were effective treatment methods for KOA. However, intraarticular HA injection had a longer-lasting effect on pain and function than ozone injection.

Keywords: Hyaluronic acid, injection, intraarticular, osteoarthritis, ozone therapy

ÖZ

Amaç: Diz osteoartritinin (DOA) tedavisinde hem eklem içi ozon hem de hyaluronik asit enjeksiyonları yaygın olarak kullanılmaktadır. Bu çalışmada, DOA'lı katılımcıların ağrı ve fonksiyonel kısıtlılıkları üzerine eklem içi ozon ve hyaluronik asit (HA) enjeksiyonlarının etkinliğinin değerlendirilmesi ve karşılaştırılması amaçlandı.
Yöntem: Üç aydan uzun süredir diz ağrısı şikayeti ile polikliniğe başvuran, yaşları 40-75 olan ardışık 108 hastanın (seksen dört kadın, yirmi dört erkek) 32'si dahil etme kriterlerine uymadığı için dışarda bırakılarak 76 hasta çalışmaya dahil edildi. HA enjeksiyonları tek doz, ozon enjeksiyonları ise haftada bir olmak üzere toplam üç doz olarak uygulandı. Katılımcılar, Vizüel Analog Skala (VAS) ve Western Ontario ve McMaster university Arthritis Indeksi (WOMAC) kullanılarak tedaviden önce, tedaviden bir ve üç ay sonra olmak üzere değerlendirildi.
Bulgular: Yetmiş altı katılımcı çalışmaya dahil edildi. Katılımcılar, Hyaluronik Asit (n=39) ve Ozon (n=37) grupları olarak iki gruba ayrıldı. Gruplar arasında tüm evrelerde WOMAC-toplam, WOMAC-ağrı ve VAS (p>0.05) skorları açısından anlamlı fark saptanmadı. WOMAC-sertlik skoru birinci ay ve üçüncü ay takipleri arasında anlamlı olarak farklı bulundu(p=0.011). Ayrıca gruplar arası analizde tedavi öncesi ve birinci ay takipleri (p=0,008) ve tedavi öncesi ve üçüncü ay takipleri (p=0,002) arasında WOMAC fonksiyon skorlarında anlamlı değişiklik saptandı.
Sonuç: Hem ozon hem de HA enjeksiyonları DOA için etkili tedavi yöntemleridir. Ancak eklem içi HA enjeksiyonunun ağrı ve fonksiyon üzerine etkisi ozon enjeksiyonuna kıyasla daha uzun süreli olarak saptanmıştır.

Anahtar kelimeler: Ağrı, eklem içi, enjeksiyon, hyaluronik asit, osteoartrit, ozon tedavisi

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INTRODUCTION

Osteoarthritis (OA) is a common disease affecting the joints in adults. Recent studies suggest that OA is an inflammatory disease involving mechanical degeneration of articular cartilage, and functional and structural alteration of the synovium, subchondral bone, meniscus, and periarticular ligaments (1). The prevalence of knee osteoarthritis (KOA) increases with age. With the prolongation of the average life expectancy, the frequency of KOA has also increased in society (2).

Viscosupplementation, which is one of the conservative treatment methods in the KOA, is the injection of hyaluronic acid (HA) into the affected joints to restore physiological viscoelasticity in the synovial fluid. HA is an integral part of healthy cartilage and synovial fluid. Composed of repeating disaccharide units formed by N-acetyl-d glucosamine and d-glucuronic acid (3). The function of the HA is to provide joint lubrication and shock absorption during movements (2). HA has an anabolic effect on cartilage by reducing cytokine-induced enzyme production and decreasing inflammation. Additionally, it provides a direct analgesic effect on the joint by masking the nociceptors (4).

Ozone (O3) is one of the strongest antioxidant agents (5). The molecule of O3 consists of three unstable oxygen atoms (5,6). Oxygen-ozone (O2O3) mixture was produced by using a medical ozone generator, and the maximum ozone concentration in it can be 5% (5). Low doses of O2O3 have an anti-inflammatory effect by suppressing inflammatory cytokines and mediators (TNF-alfa, IL-6, IL-8, etc.), and regulating prostaglandin and bradykinin mechanisms (7-9). Also, O2O3 therapy has a key role in nociception and modulation by increasing serotonin and endogenous opioids (10). Ozone has a paradoxical antioxidant effect. Controlled O3 administration stimulates the antioxidant endogenous system, provides adaptation to oxidative stress, and thus, prevents tissue damage (11).

This study aimed to compare the effectiveness of ozone and HA injections on pain and functional limitations of the participants with KOA.

MATERIALS AND METHODS

Settings and Participants

This was a retrospective study that was conducted between January 2021 and September 2021. One hundred and eight consecutive patients (eighty-four women, twenty-four men) aged 40-75 years who visited Kanuni Sultan Suleyman Training and Research Hospital, Department of Physical Medicine and Rehabilitation outpatient clinic with knee pain for > 3 months were recruited for the study. The criteria of American College of Rheumatology were based on the diagnosis of KOA, and the classification of KOA was applied according to the Kellgren-Lawrence(KL) grading system (12,13). Participants aged 40-75 years, diagnosed with moderate KOA (KL grade 2-3), treated with HA or Ozone injection, and had normal blood test results were included in the study. Severe KOA, malalignment of the knee, rheumatological diseases, abnormal blood results, a history of previous knee surgery, corticosteroid injection in the previous 12 months, and history of malignancy or infection in the knee, hypersensitivity to hyaluronate, glucose 6-phosphate dehydrogenase enzyme deficiency, and using ACE inhibitors were accepted as exclusion criteria.

Ethical consideration

The present study was approved by the local ethical board (KAEK/2021.11.304) in accordance with the Declaration of Helsinki. The study was designed retrospectively, so the participants did not sign an informed consent form.

Interventions

All injections were prepared and administered by an experienced clinician who specialized in intraarticular injection and ozone therapy. The injection area was sterilized with 10% povidone-iodine, with round centrifugal movements from the center to the periphery. The intra-articular

injections were performed on the participants in the supine position while the knee flexed at 30 degrees, through the lateral mid-patellar approach.

HA injections were performed as a single dose, and ozone injections were administered once a week as 3 doses in total (14). The same synthetic HA product (Viscoial®, prefilled syringe containing 60 mg/4 ml of HA) was applied to the HA groups with molecular weight between 1500-1600 KDalton. In the ozone group, 10 cc of O2O3 with 10 microgram/ml concentration was used for the first session, and 15 microgram/ml was used for the second and third sessions (15). The Salutem Plus® ozone generator (Has medical equipment, Turkey) was used for the preparation of O2O3 mixture with a 5% concentration.

All participants were recommended not to stand for a long time in the 24 hours after injection, an exercise program including isometric exercises for quadriceps femoris, range of motion of the knee, and patellar mobilization was taught practically to all participants by an experienced therapist. All participants were asked to perform the exercises 3 times a week, with 10 repetitions in a set for 3 months.

Outcomes

The Visual Analog Scale (VAS) is performed for scoring the pain intensity. This scale is a 10-centimeter line between 'no pain' and 'severe pain' (16).

Western Ontario and McMaster Universities Arthritis Index (WOMAC) is applied for evaluating pain, joint stiffness, and functional limitations. The questionnaire consists of twenty-four items: five of them for pain, two for stiffness, and seventeen for functional limitation (17).

The scales were performed before treatment, one and three months after treatment by another investigator who was blinded to the injected product for each participant.

Data Analysis

For sample size calculation, the G*Power program (G*Power version 3.1.9, Germany) was used and it was calculated according to the change in pain intensity. In order to achieve the test power of 80% at the significance level of 0.025 with an effect size of 0.70 according to the VAS, it was calculated that at least 33 participants per group would be required as described by Lopes de Jesus et al (18).

The IBM SPSS (Statistical Package for the Social Sciences) Statistics for Mac, version 21.0 (Armonk, NY, USA) was used for the analysis. Distributions of the variables were investigated using a histogram and the Shapiro-Wilk test. Descriptive statistics were expressed as frequency (percentage) for categorical variables and mean (standard deviation) for continuous variables. The Friedman Test was used to show the difference between follow-up values in both groups for intra-group analysis. For post-hoc evaluation, Bonferonni Corrected-Wilcoxon's Signed Ranks Test was used for intra-group comparisons of repeated measures in two groups. Kruskal Wallis test was performed for inter-group comparisons. For post-hoc analysis, Bonferonni Corrected-Mann Whitney U test was used.

P value of less than 0.05 was considered statistically significant. In addition, p value of less than 0.0167 was accepted as significant for Bonferonni correction.

RESULTS

One hundred and eight participants aged 40-75 years who were diagnosed with moderate KOA (KL grade 2-3) and treated with ozone or HA injection were evaluated for recruiting. Seventy-six participants who met the inclusion criteria were included in the study. A total of thirty-two participants have excluded: Twelve of them had systemic diseases, six had rheumatological diseases, six of them had a history of knee

surgery, and eight participants had malalignment of the knee (genu varum and valgum more than 20 degrees). The participants were randomized into the Hyaluronic Acid Group (HAG; n=39) and Ozone Group (OG; n=37). All participants attended all treatment sessions in the final analysis. Clinical and demographic characteristics are seen in Table 1.

Table 1. Demographic and clinical characteristics of the participants.

Variable	HA Group (n=39)	Ozone Group (n=37)	p value
Age (year) Mean (SD)	55.9 (7.3)	55.2 (9.2)	0.144
Sex (male/female)	33/6	31/6	0.921
BMI Mean (SD)	33.2 (5.1)	33.3 (4.7)	0.506
KL grade n(%)			0.966
Grade 2	16 (41.0%)	15 (40.5%)	
Grade 3	23 (59.0%)	22 (59.5%)	
Laterality n(%)			0.966
Right knee	23 (59.0%)	22 (59.5%)	
Left knee	16 (41.0%)	15 (40.5%)	
VAS Mean (SD)	7.5 (0.9)	7.4 (1.1)	0.210
WOMAC Mean (SD)			
Total	50.5 (12.5)	48.1 (14.3)	0.420
Pain	12.3 (2.8)	10.8 (3.6)	0.265
Stiffness	4.1 (2.1)	2.0 (1.4)	0.063
Function	34.1 (8.6)	35.3 (11.3)	0.123

HA: hyaluronic acid, BMI: body-mass index, KL: Kellgren Lawrence, VAS: visual analog scale, WOMAC: Western Ontario and McMaster Universities Arthritis Index, SD: standard deviation, p<0.05 is considered as significant.

Table 2. Within-group analysis of the hyaluronic acid and ozone groups.

	HA group (n=39)	p^a	p^b	Ozone group (n=37)	p^a	p^b
VAS (mean±SD)						
Pre-treatment	7.5±0.9	<0.001*	First-PreT p<0.001*	7.4±1.1	<0.001*	First-PreT p<0.001*
First month	4.1±1.5		Thr-First p=0.935	4.0±2.1		Thr-First p=0.065
Third month	4.1±1.7		Thr-PreT p<0.001*	4.5±2.4		Thr-PreT p<0.001*
WOMAC (mean±SD)						
Pre-treatment	50.5±12.5	<0.001*	First-PreT p<0.001*	48.1±14.4	<0.001*	First-PreT p<0.001*
First month	36.5±13.7		Thr-First p=0.132	24.7±12.9		Thr-First p=0.002*
Third month	37.1±14.2		Thr-PreT p<0.001*	28.2±12.5		Thr-PreT p<0.001*
WOMAC pain (mean±SD)						
Pre-treatment	12.3±2.8	<0.001*	First-PreT p<0.001*	10.8±3.6	<0.001*	First-PreT p<0.001*
First month	8.2±3.2		Thr-First p=0.525	5.5±3.0		Thr-First p=0.682
Third month	8.5±3.1		Thr-PreT p<0.001*	5.8±3.1		Thr-PreT p<0.001*
WOMAC stifness (mean±SD)						
Pre-treatment	4.1±2.1	<0.001*	First-PreT p<0.001*	2.0±1.4	<0.001*	First-PreT p<0.001*
First month	3.0±1.8		Thr-First p=0.156	0.8±1.05		Thr-First p=0.003*
Third month	2.9±1.8		Thr-PreT p<0.001*	1.0±1.1		Thr-PreT p=0.001*
WOMAC function (mean±SD)						
Pre-treatment	34.1±8.6	<0.001*	First-PreT p<0.001*	35.3±11.3	<0.001*	First-PreT p<0.001*
First month	26.3±9.9		Thr-First p=0.090	19.8±9.7		Thr-First p=0.676
Third month	27.0±10.3		Thr-PreT p<0.001*	19.8±9.0		Thr-PreT p<0.001*

*HA: hyaluronic acid, BMI: body-mass index, KL: Kellgren Lawrence, VAS: visual analog scale, WOMAC: Western Ontario and McMaster Universities Arthritis Index, SD: standard deviation, PreT: Pre-treatment, Thr: Third-month, p^a <0.05 is considered as significant (Friedman Test); p^b (<0.0167 is considered as significant for post-hoc analysis for the Friedman Test), *: significant values.*

Intra-group analysis

Based on the post-hoc evaluation results, VAS and WOMAC (pain, stiffness, function, and total) scores were decreased significantly at the first and third-month follow-ups as compared to before treatment results in both groups. However, no significant change was seen in terms of VAS, WOMAC-pain, and WOMAC-function scores between the first month and third month follow-up sessions in both groups. According to the comparison between the first-month and third-month follow-up measurements, WOMAC-total and WOMAC-stiffness were significantly increased in OG, whereas there was no significant change in these scores in HAG (Table 2).

Table 3. Pairwise comparison between the treatment sessions in hyaluronic acid and ozone groups.

		Mean difference	95% CI for difference		p
			Lower bound	Upper bound	
VAS	HA				
	First-PreT	-3.43	-3.92	-2.97	
	Thr- First	0.0	-0.28	-0.28	First-PreT: 0.817
	Thr-PreT	-3.43	-3.97	-2.92	
					Thr-First: 0.124
	Ozone				
	First-PreT	-3.40	-4.02	-2.73	Thr-PreT: 0.225
	Thr-First	0.51	-0.03	1.05	
	Thr-PreT	-2.89	-3.57	-2.19	
WOMAC Total	HA				
	First -PreT	-14.0	-16.11	-11.84	
	Thr-First	0,59	-0.23	1.45	First-PreT: 0.017
	Thr-PreT	-13.41	-15.79	-11.14	
					Thr-First: 0.083
	Ozone				
	First-PreT	-23.45	-28.77	-18.44	Thr-PreT: 0.269
	Thr-First	3.52	1.60	5.88	
	Thr-PreT	-19.93	-25.61	-14.66	
WOMAC pain	HA				
	First -PreT	-4.08	-4.73	-3.36	
	Thr-First	0.27	-0.11	0.64	First-PreT: 0.144
	Thr-PreT	-3.82	-4.46	-3.15	
					Thr-First: 0.987
	Ozone				
	First-PreT	-5.29	-6.73	-4.00	Thr-PreT: 0.806
	Thr- First	0.31	-0.33	0.96	
	Thr-PreT	-4.98	-6.64	-3.52	
WOMAC stifness	HA				
	First -PreT	-1.09	-1.41	-0.78	
	Thr-First	-0.05	-0.21	0.11	First-PreT: 0.502
	Thr-PreT	-1.15	-1.50	-0.83	
					Thr-First: 0.011*
	Ozone				
	First-PreT	-1.18	-1.63	-0.73	Thr-PreT: 0.085
	Thr-First	0.25	0.11	0.39	
	Thr-PreT	-0.93	-1.41	-0.48	
WOMAC function	HA				
	First -PreT	-7.69	-9.27	6.25	
	Thr-First	-0.66	-0.08	1.44	First-PreT: 0.008*
	Thr-PreT	-7.03	-8.87	-5.37	
					Thr-First: 0.426
	Ozone				
	First-PreT	-15.51	-19.26	-11.68	Thr-PreT: 0.002*
	Thr-First	-0.28	-0.99	0.93	
	Thr-PreT	-15.54	-19.37	-11.68	

*HA: hyaluronic acid, VAS: visual analog scale, WOMAC: Western Ontario and McMaster Universities Arthritis Index, PreT: Pre-treatment, Thr: Third-month, p <0.0167 is considered as significant for Bonferonni Corrected-Mann Whitney U test; *: significant values.*

Inter-group analysis

According to the comparison of the HAG and OG, no statistically significant difference was seen in terms of VAS, WOMAC-total, and WOMAC-pain ($p>0.05$) at all stages. WOMAC-stiffness score was found significantly different between the first and third-month follow-ups ($p=0.011$). Additionally, a significant change was found in WOMAC-function scores before treatment and first-month follow-up ($p = 0.008$), and before treatment and third-month follow-up sessions ($p=0.002$) in inter-group analysis (Table 3).

DISCUSSION

This study purposed to evaluate and compare the efficacy of intraarticular ozone and HA injections on pain and functional limitations in participants with KOA. Based on the results, both ozone and HA injections decreased pain intensity and joint stiffness, improved functional limitations of the participants at mid-term stages (3-month follow-up). However, joint stiffness was found to increase in ozone injection between first and third-month follow-ups. In addition, the functional limitation was improved with ozone injection more than with HA injection.

Several studies reported that the pain relief with ozone was more rapid, but HA provided a longer-term reduction in pain intensity (19,20). Lopes de Jesus et al.[18] evaluated the efficacy of intra-articular ozone therapy and placebo injections and they found that ozone was more effective than placebo in pain reduction after 8 weeks of treatment. Similarly, in the current study, it was found that both HA and ozone injections decreased pain intensity in participants at the 3-month follow-up, and there was no difference between the two products on pain relief.

In one study, it was determined the effects of Platelet-rich plasma (PRP), HA, and ozone injections on joint stiffness in participants with KOA, and reported that all three injections have a similar effect on joint stiffness (19). In the current study, both HA and ozone injections decreased the joint stiffness, but after the first-month evaluation, the stiffness started to increase in the ozone group. This may be related to the regulatory and reducing effect of HA on cartilage damage.

Raeissadat et al.[21] reported that both ozone and HA injections improved the function of the participants with KOA, but the function domain of the WOMAC score was better after HA injection than ozone. Contrarily, in the current study, the function domain in the ozone group decreased more than the HA group. Duymus et al.[19] showed that the effects of ozone injection on pain and function disappeared completely in the 6th-month evaluation. This effect may not have been observed in the current study due to the 3-month follow-up period.

The best protocol of the ozone concentration for intra-articular injections still remains unclear. Based on the knowledge of the Madrid Declaration the safe range is specified as 5 to 20 µg/mL (15). In some studies, the injection dose of ozone therapy was used 20 µg/mL or more (18,19,21). On the other hand, there were also studies using 15 µg/mL of ozone concentration (8,22). In one review article, it was evaluated the superiority of the ozone concentrations and reported that it was needed more studies with long follow-up periods for analyzing optimal dose and frequency of ozone in pain relief (23). In the current study, 10 µg/mL x 10 mL of O2O3 was used in the first session, and 15 µg/mL x 10 mL was administered in the second and third sessions. The reason why a lower dose was preferred in the first session was a burning sensation due to the oxidation of ozone (15).

HA injection is an effective treatment for symptomatic KOA. However, there are limited studies on evaluating different formulations of HA products. In one retrospective study, three different intra-articular HA injection regimens (single injection, weekly for 3 weeks, weekly for

5 weeks) were evaluated and it was found that there was no significant difference between all three treatments on pain relief (14). In the present study, a single HA injection was administered to participants with KOA.

Being a retrospective study, a relatively short follow-up period, and subjective evaluation of participants can be considered limitations. There are some strengths of the study. Limited numbers of studies have evaluated the effect of ozone injection for KOA and compared the efficacy with HA. Additionally, the study was described as single-blind.

As a conclusion, both ozone and HA injections were effective treatment methods for patients with KOA. However, intraarticular HA injection had a longer-lasting effect on pain and function compared to ozone injection.

Ethics Committee Approval: The study protocol was approved by the University of Health Sciences, Istanbul Kanuni Sultan Suleyman Training and Research Hospital Ethics Committee (KAEK/2021.11.304/26.11.2021).

Conflict of Interest: The authors have declared that they have no conflict of interest.

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