Original Article

A Comparative Assessment of Autologous Conditioned Serum and Ozone for Knee Osteoarthritis Treatment: Mid-Term Follow up

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Abstract

Background: Knee osteoarthritis is a common disease associated with knee pain, physical disability, and joint stiffness. The use of non-surgical treatment methods in patients with knee osteoarthritis is important. Autologous conditioned serum (ACS) is a new regenerative therapeutic method that was investigated by a limited number of clinical trials. So far, using ACS in patients with Knee osteoarthritis remains to be controversial among physicians. Thus, the current study was carried out to compare the therapeutic effects of intra-articular ACS and ozone injections in patients with knee osteoarthritis.

Materials and Methods: This prospective, double-blind randomized clinical trial was conducted among 60 patients (30= interleukin-1 receptor antagonist (IL-1Ra) group, 30= ozone group) with knee osteoarthritis, who referred to the Pain Management Clinic of Akhtar Educational Hospital during 2018 to 2019. In the IL-1Ra group, 2 ml of IL-1Ra was injected into the knee joint. The regimen protocol consisted of 4 injections, performed on the first, seventh, fourteenth, and twenty-first days of the treatment and ozone group, 10 ml of ozone (30 μg/ml) + 5 ml of lidocaine 1% were injected into the knee joint. The regimen protocol consisted of 3 injections, performed on the first day of the treatment, one month after the first injection, and two months after the first injection. The severity of pain was assessed by the patients' self-report of pain and using the visual analog scale (VAS), before the treatment and 1, 3 and 6 months after the treatment. The Knee Injury and Osteoarthritis Outcome Score (KOOS) and the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) questionnaires were also measured at before and 6 months following treatment.

Results: The changes in the VAS pain at different time periods showed statistically significant differences in the two groups, (P=0.0001). There was no significant difference between the two groups before the treatment and one month and three months after the initiation of the treatment; however, there was a significant difference between the two groups six months after the initiation of the treatment (P=0.0001). KOOS scores of symptoms, daily activities, and athletic and recreational functions were significantly higher in the IL-1Ra group, and the WOMAC scores of physical function and joint stiffness and the overall scores were significantly higher in the IL-1Ra group, ($p<0.05$).

Conclusion: The intra-articular injection of IL-1Ra is a low-invasive, safe, effective, and long-acting method. In patients with knee osteoarthritis, clinical improvements and responses to the intra-articular IL-1Ra injection are better and longer compared to ozone injection. Therefore, it can be considered as a suitable choice in treating patients with chronic knee pain.

Keywords: Autologous conditioned serum, Ozone, knee osteoarthritis, Outcome

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Introduction

Knee osteoarthritis is a common disease associated with tissue inflammation, physical disability, and cartilage hemostasis imbalance. Almost 25% of people over 50 years of age experience knee pain, joint stiffness, and reduced function caused by knee osteoarthritis[1]. Currently, various surgical treatments are used to treat knee osteoarthritis[2,3]. By performing surgery the cartilage parts, mechanical stimulations, inflammatory cells, and other factors can be removed from the knee joint; however, it cannot result in restoring the joint or repairing knee osteoarthritis. In the last decade, many physicians have used ozone injections in the treatment of knee osteoarthritis. Ozone is a highly oxidative dissolved gas that enhances the nociceptive effects by applying a variety of mechanisms[4]. In many studies, the effect and safety of ozone therapy have been shown in the treatment of knee osteoarthritis and other musculoskeletal diseases[5-11]. Nowadays, injecting ozone is applied to treat knee osteoarthritis in orthopedic centers in Europe[12-14]. However, in recent studies conducted to examine osteoarthritis, the role of biochemical processes in the pathology of the disease and the development of new and regenerative therapies has attracted many researchers' and physicians' attention.

One of the mechanisms of the progression of knee osteoarthritis is the degenerative control pathway of the disease caused due to the pathological increase in inflammation of cytokines and catabolic factors in and around the synovial space. Inflammatory and catabolic proteins, such as interleukin-1 beta, tumor necrosis factor-α, and metalloproteinase matrix, play roles in the cartilage destruction and the progression of osteoarthritis.[15]The approaches that block these changes not only can improve the symptoms of the disease but also can stop or reverse the disease progression.

Anti-inflammatory cytokines in the blood are interleukin-1 receptor antagonist, soluble interleukin-1 receptor type I, soluble tumor necrosis factor receptor-type I, and soluble tumor necrosis factor receptor type II[16].

New therapeutic approaches attempt to produce anti-inflammatory and anabolic proteins at high concentrations by overcoming the high pathological levels of pro-inflammatory and catabolic proteins that cause osteoarthritis.

Autologous conditioned serum (ACS) is a complete autologous blood product used to treat joint osteoarthritis, spinal radiculopathy, tendon and muscle damage[17].

ACS is a non-cellular treatment that has significant biochemical and clinical differences with PRP and other autologous blood substitution treatments[18].

ACS is achieved by venous blood incubations at the physiological temperature (about 37°C) for 6-9 hours in a special syringe. ACS produces products of anti-inflammatory cytokines, such as interleukin-1 receptor antagonist[17], which are important mediators of inflammation and tissue destruction in musculoskeletal diseases[19]. ACS containing this cytokine is extracted from the coagulated blood by centrifugation and is injected into the affected tissue using a sterile filter. In randomized clinical trials, the effect of ACS on the treatment of knee and hip osteoarthritis[20-22], lumbar radicular compression[23], and muscle damage[24] have been shown.

The experimental model of osteoarthritis in the in vivo environment demonstrated that the interleukin-1 receptor antagonist gene (IL-1Ra) significantly improved the clinical parameters of pain, patient activity, maintenance of articular cartilage, and beneficial effects on the histological parameters of the synovial membranes and adjoining articular cartilage[25].

Animal studies have indicated promising results as well. In a placebo-controlled study carried out on a horse suffered from tendinopathy, an ACS injection was performed and a significant reduction was observed in the horse's lameness within 10 days[26].

Clinical trials have revealed that injecting ACS improved pain and joint function and delayed the need for surgery in patients[21,27,28].

ACS is a new regenerative therapeutic method that was investigated by a limited number of clinical trials. Therefore, this study was conducted on the Iranian population due to the high prevalence of knee osteoarthritis in this population, the presence of contradictory views on whether applying ACS can improve clinical outcomes and the high costs of this

treatment.

Methods

This prospective, double-blind randomized clinical trial was carried out on patients with knee osteoarthritis referred to pain Management Clinic of Shohaday-e-Tajrish and Akhtar Hospitals in 2018-2019. Patients who had given their full informed consent, were 40 years old and older, suffered from knee osteoarthritis pain for more than three months, and the radiographic results confirmed the knee osteoarthritis based on the criteria of American College of Rheumatology (ACR)[29] were included. Patients who had not given their consent for taking part in this study, had a history of knee surgery, deformity, lower limb contraction, lower limb neurovascular disease, acute lumbar pathology, injection of steroid drugs in the last two months, inflammatory rheumatoid arthritis, infection, diabetes, pregnancy, and breastfeeding, those who had a BMI>35, were candidates for knee surgery, suffered from knee deviation (varus or valgus more than 5 degrees) confirmed by a three joint view graph, had radicular knee pain, took anticoagulant drugs, suffered from post-traumatic arthritis, had a history of intra-articular injection or ozone therapy in the past 12 months, were sensitive to any of the drugs used in this study, suffered from a systemic or psychiatric disease, had severe osteoarthritis (over stage 3), had an intra-articular hyaluronic acid injection in the past 12 months, suffered from hepatitis, HIV, cytomegalovirus, syphilis, and osteomyelitis or abused substances and alcohol were excluded.

Patients were randomly assigned to treatment groups according to the random numbers table:

I) 2 ml of IL-1RA.

II) 10 ml of ozone (30 μg/ml) + 5 ml of lidocaine 1%.

In the first group, to prepare the IL-1RA, 50 ml of venous blood was taken from the patients using a special syringe (manufactured in Germany by Orthokine) containing glass beads coated with CrSO4. Then, to ensure complete mixing and maximum contact between the beads and blood, the syringe was rotated slowly and was immediately stored in a special incubator at 37°C and transferred to a laboratory in 24 hours. In the laboratory, the blood samples were tested for hepatitis A and B and HIV. If any of the tests were positive, the patients were again tested with new blood samples. If any of the mentioned tests were again positive, the patient would be excluded from the study. In the case of tests being negative, the non-cellular product (IL-1RA) was prepared by the laboratory and was returned to the hospital in 2 ml vials at -20°C in 14-20 days. The regimen protocol consisted of four injections, performed on the first, seventh, fourteenth, and twenty-first days of the treatment.

In the second group, 10 ml of ozone (30 μg/ml) + 5 ml of lidocaine 1% were injected into the knee joint. This group underwent three injections, i.e. on the first day of the treatment, one month after the first injection, and two months after the first injection.

To conduct the procedure, the patient was placed in a supine position and the landmark of the injection area was determined using a knee flexion of about 30 to 45 degrees on the lateral side of the knee. Afterward, the injection site was disinfected with povidone-iodine solution and 2 ml of lidocaine 2% was injected to the skin and articular surface for numbness using a 27-gauge needle. After aspiration and ensuring the correct positioning of the needle by ultrasound guidance (Sono Site, PICO.probe Convex 3-7, Linear 5-12), the intra-articular injection of IL-1RA or ozone was performed using the same needle.

Five items, including pain, symptoms, daily activities, athletic and recreational functions, and knee-related quality of life, were measured by the Knee Injury and Osteoarthritis Outcome Score (KOOS)[29], which is a 4-point Likert-type scale (0-4), and 3 items, including pain, stiffness, and physical function, were measured by the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC)[30], which is a 5-point Likert-type scale (0-5), completed by the patients before the initiation of the treatment and six months after the last injection. The level of pain was evaluated by the VAS (0-10), based on which the patients were required to determine their pain levels by rating their pain levels before the initiation of the treatment, one month after the initiation of the treatment, and three months after the last injection. In the case of complication, the type of complication was also recorded.

It should also be noted that during the study period, the patients did not take steroids, antidepressants and sedative medications.

In the case of pain with a score of more than 3 during the study, they could take acetaminophen (up to 4 grams per day).

The obtained information was then coded and entered into SPSS version 19. In the traditional orthopedics, 100 was considered as no problem and 0 was regarded as the worst state. To comply with this standard, the subscales' scores were calculated by dividing the overall score of each subscale by the maximum possible score of the normalized subscale.

After examining the normal distribution of quantitative data by the Kolmogorov-Smirnov test, the quantitative variables were compared using the t-test, Mann-Whitney test, and repeated measurement ANOVA, and paired t-test and the qualitative variables were examined using the Chi-square test. $P<0.05$ was considered statistically significant.

Results

The results of comparing the demographic information of the patients in the two groups are presented in Table 1.

Comparing the changes in the pain levels examined by the VAS before the initiation of the treatment and one month, three months, and six months after the initiation of the treatment in the two groups showed that there were statistically significant differences between the two groups in terms of the changes in the pain scores ($P = 0.0001$).

The results of comparing the pain levels at different times are presented in Table 2 and show that there was no significant difference between the two groups before the treatment and one month and three months after the initiation of the treatment; however, there was a significant difference between the two groups six months after the initiation of the treatment ($P=0.0001$).

The results of comparing the KOOS scores before the start of the treatment in the two groups are presented in Table 3 and show that there were significant differences between the two groups in terms of the scores of pain, symptoms, daily activities, and athletic and recreational functions.

The results of comparing the KOOS scores obtained six months after the initiation of the treatment by the two groups are presented in Table 4 and indicate that the scores of symptoms, daily activities, and athletic and recreational functions were significantly higher in the IL-1Ra group compared to the other group.

The results of comparing the WOMAC scores obtained before the initiation of the treatment between the two groups are presented in Table 5 and demonstrate no significant differences between the two groups.

The results of comparing the WOMAC scores obtained six months after the initiation of the treatment between the two groups are presented in Table 6. The scores of physical function and joint stiffness and the overall scores were significantly higher in the IL-1Ra group compared to the other group; however, the scores of pain were not significantly different between the two groups.

There were statistically significant differences in terms of changes in the pain levels in each group at different times, i.e., before the injection, and during the six months follow up ($P= 0.0001$).

The changes in each of the KOOS and WOMAC scores in each group were statistically significant before the initiation of the treatment and six months after the last injection ($P= 0.0001$). None of the patients reported any complications related to the procedure.

Table 1: The comparison of the demographic information between the two groups.

		IL-1RA group (n=30)	Ozone group (n=30)	P value
Age (yr.)		56.8±8.6	51.5±5.4	0.006
BMI (kg/m^2)		31.1±3.4	30.5±2.6	0.214
Sex;	Male	14 (46.7%)	9 (30%)	0.184
Female		16 (53.3%)	21 (70%)	

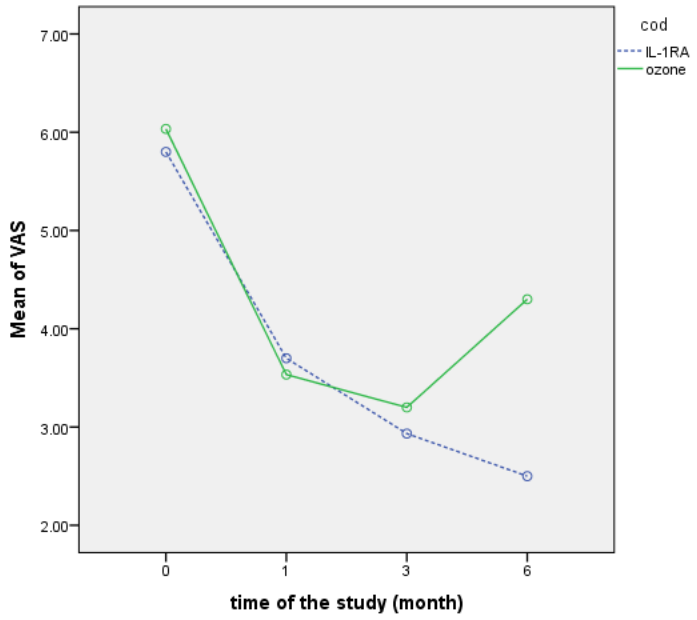

Figure 1. The comparison of the changes in the pain levels between the two groups at different times (P=0.0001).

Table 2: The comparison of the pain levels between the two groups at different times.

	IL-1RA group (n=30)	**Ozone group (n=30)**	**P value**
VAS before injection	5.8±0.6	6.03±0.9	0.215
VAS 1 month after injection	3.7±0.5	3.5±0.6	0.171
VAS 3 months after injection	2.9±0.5	3.2±0.6	0.059
VAS 6 months after injection	2.5±0.6	4.3±0.5	0.0001

Table 3: The comparison of the KOOS scores before the initiation of the treatment between the two groups.

	IL-1RA group (n=30)	**Ozone group (n=30)**	**P value**
Pain	47.2±9.1	40.8±12.9	0.035
symptoms	41.2±9.5	47.7±9.1	0.012
daily activities	52.2±7.6	37.8±9.0	0.0001
athletic and recreational functions.	30.4±11.8	22.3±7.5	0.0001
Quality of Life	27.4±11.8	23.3±11.1	0.124

Discussion

In this study, the effect of intra-articular injection of IL-1Ra was compared with ozone as a control group. In the present study, the changes in the pain VAS at different times and comparing such changes between the two groups showed a significant decrease in the pain VAS in the IL-1Ra group.

There was a significant decrease in the pain VAS in the IL-1Ra group six months after the initiation of the treatment.

Furthermore, six months after the initiation of the treatment, the KOOS pain scores were higher in the

Table 4: The comparison of the KOOS scores obtained six months after the initiation of the treatment between the two groups.

	IL-1RA group (n=30)	**Ozone group (n=30)**	**P value**
Pain	73.7±8.7	70.9±8.4	0.212
symptoms	72.6±13.9	63.1±8.0	0.002
daily activities	73.1±8.6	59.1±9.4	0.0001
athletic and recreational functions.	53.2±13.0	46.7±7.9	0.019
Quality of Life	46.0±10.4	46.9±11.4	0.882

Table 5: The comparison of the WOMAC scores obtained before the initiation of the treatment in the two groups.

	IL-1RA group (n=30)	**Ozone group (n=30)**	**P value**
Pain	45.7±15.8	42.0±12.3	0.313
Stiffness	45.2±9.6	40.3±11.9	0.083
Physical function	45.0±14.8	40.6±8.1	0.155
Overall score	135.9±36.3	122.9±25.6	0.113

Table 6: The comparison of the WOMAC scores obtained six months after the initiations of the treatment between the two groups.

	IL-1RA group (n=30)	**Ozone group (n=30)**	**P value**
Pain	71.4±9.9	68.1±9.1	0.178
Stiffness	61.6±8.8	56.2±9.6	0.028
Physical function	73.3±10.3	67.3±11.4	0.034
Overall score	206.3±24.4	191.5±25.1	0.024

IL-1Ra group and the pain levels showed a higher reduction in this group compared to the other group; however, these changes were not significantly different compared to the ozone group. The KOOS scores of symptoms, daily activities, and athletic and recreational functions obtained by the IL-1Ra group demonstrated a significant improvement. The KOOS scores of knee-related quality of life did not indicate any significant differences between the two groups.

Additionally, the joint stiffness scores and the WOMAC scores of physical activity, and the overall score of WOMAC were higher in the IL-1Ra group compared to the other group and the patients assigned to this group showed a better improvement compared to the ozone group. The comparison of the two groups was statistically significant. The WOMAC pain scores were higher in the IL-1Ra group; however, this difference was not significant between the two groups.

The findings of the current study are consistent with other studies. In a meta-analysis conducted based on scientific evidence, it was revealed that the injection of ozone to patients with knee osteoarthritis improved mild to moderate pain in the short term (1-3 months)[31]. Moreover, RCT studies indicated that the short-term effect of ozone injection on pain relief was better than the placebo[31] and corticosteroids[14]. This is while the short-term effect of ozone injection on the recovery of pain was similar to dextrose[32] and hyaluronic acid[33-36]. The therapeutic effect of ozone had reduced 3-6

months after the injection, and its therapeutic effect was gradually lower than the mentioned injections[31]. This is in line with the results obtained in the present study.

Another study that compared the effects of ozone with hyaluronic acid stated that while the therapeutic effect of ozone injection had significantly reduced after three months, the therapeutic effect of hyaluronic acid had continued six months after the injection. This is while the therapeutic effect of ozone therapy disappeared six months after the injection[34].

It seems that ACS/IL-1Ra has a longer biological beneficial effect on the improvement of clinical symptoms associated with osteoarthritis.

In another study, which compared the effect of hyaluronic acid and autologous conditioned serum, after 104 weeks of follow-up, the symptom and clinical improvements of knee osteoarthritis were significantly higher in the autologous conditioned serum group compared to the hyaluronic acidgroup[37]. In contrast, in some other studies, there were not any significant differences with regard to the improvement in joint function and the reduction of knee osteoarthritis pain in the two groups of ozone and hyaluronic acid during the six months of follow up and none of them was superior to the other one[35].

Furthermore, the WOMAC subscales of joint stiffness and physical function and the KOOS subscales of symptoms, daily activities, and athletic and recreational functions indicated significant improvements in the IL-1Ra group six months after the initiation of the treatment; however, the WOMAC and KOOS subscales of pain were not significantly different between the two groups six months after the start of the treatment. It seems that while ozone still had a significant effect on pain relief six months later, it did not improve the function of the knee joint. This finding is also consistent with other studies[32].

Pre-inflammatory cytokines, such as IL-1β and tumor necrosis factor, are known as mediators of the osteoarthritis process that provide the possibility of achieving therapeutic goals. In a few studies, the blocking effect of these mediators has been studied. Several studies have also shown the role of these mediators in disease progression[38]. Accordingly, because of the role IL-1β in the pathogenesis of osteoarthritis, its antagonistic choice seems logical in treating these patients.

The irreversible inflammatory cytokines may disrupt the cytokine homeostasis, which indicates the need for the treatment in the early stages of the disease.

However, due to the complexity of the pathogenesis of osteoarthritis, a general strategy for treating these patients cannot be considered[39,40].

Some clinical trial studies demonstrated that ACS in the knee osteoarthritis was the last stage of treatment and when no improvements were observed in the patient's used knee arthroplasty.

Serum autologous, which induces the synthesis of anti-inflammatory cytokines, appears to be effective in the symptomatic treatment of knee osteoarthritis. Therefore, the results of applying IL-1Ra produced on the basis of human serum may be different in various people.

Among the limitations of this study, the differences in human serum and molecular profile of the patients that may cause differences in the quality of IL-1Ra, differences in the ozone injection protocol, psychosocial and economic factors, anthropometrics, recommended sports programs, which have a significant impact on the results and the durability of the effects of the treatment, can be mentioned.

It seems that this ACS is associated with beneficial biological effects in patients with knee osteoarthritis.

Conclusion

The intra-articular injection of IL-1Ra is a low-invasive, safe, effective, and long-acting method. In patients with knee osteoarthritis, clinical improvements and responses to the intra-articular IL-1Ra injection are better and longer compared to ozone injection. Therefore, it can be considered as a suitable choice in treating patients with chronic knee pain.

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