Original Article

Effect of intraarticular injection of ozone on inflammatory cytokines in knee osteoarthritis

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Abstract

Background: Knee Osteoarthrosis is one of the most debilitating diseases. Prolotherapy includes intra-articular injection of various drugs to decrease inflammation. Injection of intradiscal O_2-O_3 has reduced pain and disability in patients with low back pain due to prolapsed lumbar disk. To compare the effect of intraarticular injection of Ozone and steroids in improvement of clinical and cellular healing of knee osteoarthritis.

Materials and Methods: in a randomized clinical trial, 70 patients with knee osteoarthrosis were included in the study. In Ozone group, 5 ml (35 µg/ml) of Ozone and in steroid group 5ml (50 mg) Triamcinolone was injected intra-articular. In 1, 2, and 6 months, patients were followed for pain scale, disability index and IL-1β and TNF-α serum levels were measured.

Results: At 1 month after injection pain scale and disability index and IL-1β and TNF-α were decreased in both groups. However, at 2 and 6 month pain scale and disability index compared significantly lower in the Ozone group to steroid group ($p<0.05$). Besides, serum level of IL-1β and TNF-α compared also significantly lower at 2 and 6 months in the Ozone group in the steroid group ($p<0.05$).

Conclusion: Intraarticular Ozone induces significantly longer improvement of pain and disability in knee osteoarthrosis compare to steroid injection. In addition, serum inflammatory cytokines are also lower in Ozone group compared to the steroid group along with clinical improvements.

Keywords: Ozone, Inflammatory, Cytokines, Knee osteoarthritis

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Introduction

Knee Osteoarthritis (OA) of the knee is a degenerative disease manifest as painful joint, articular stiffness and decreased function (1). Osteoarthritis affects the knee and it is currently leading cause of disability in adults (2). The number of adults with arthritis and its associated activity limitation will probably increase little by little; with the resulting great impact on the individual lifestyles, and of course, the health care system (3). The exact mechanism of pain and disability is not well known yet. Various parts have been named as the origin of pain such as articular capsule, ligaments, synovium, bone, lateral part of the meniscus, and involvement of extra-articular ligaments and tendons (4). The role of systemic inflammatory cytokines in the genesis and progression of osteoarthritis has been proposed by a

considerable number of studies. In patients with painful knee, osteoarthritis after an intra-articular injection, alterations in serum cytokines are in good correlation with pain response (5, 6).

Total Knee replacement is the only surgical option but it is expensive. Prolotherapy is a novel technique to alleviate pain in chronic musculoskeletal pain and particularly in knee osteoarthritis (7). Intra-articular injection of various agents could lead to initiate proliferation and regeneration of damaged cartilage tissue. However, controversies over the type of drug injected, duration of action, the extent of pain relief, and retained function still persist. Several researches support the use of intra-articular knee injection as a valuable conservative intervention in the continuum of arthritis management (8). In other similar studies, various agents, including ozone have been tried for intra-articular injection (9-11).

Ozone is more active than oxygen in the universe. Prolotherapy by ozone uses the power of oxygen to regenerate damaged tissues, joints, ligaments, and tendons. This usually causes a partial or complete healing, and ends with pain. Mixture of ozone and oxygen. Injected into cartilaginous tissue could inhibit inflammation and start the regeneration of tissue (12, 13). Ozone affects nucleus pulposus macromolecular structures (proteoglycans and collagens) and has a longer duration of action compared to steroid injection in previous reports (14). The objective of this study is to compare the effect of intra-articular injection of ozone and steroids in improvement of clinical and cellular healing of knee osteoarthritis.

Methods

The study was reviewed and approved by the Shahid Beheshti University of Medical Sciences Ethics Committee. All procedures that performed in this study, were in accordance with the ethical standards of the institution and/or national research committee. Information about the study was given comprehensively both orally and in written form to all patients or their accompanying adult. They gave their informed written consent prior to their inclusion in the study.

In a randomized clinical trial, 70 patients with chronic osteoarthritis were enrolled in this randomized clinical trial. Patients were randomly assigned to a computer based accidental number by which patient group was determined. Physician administering drug and evaluating patients were blind to each other and patient group. All demographic variables, including age, sex, and BMI were measured and recorded on a specified data sheet for each patient.

The inclusion criteria were patients with chronic osteoarthritis aged between 35 to 75 years and knee osteoarthritis. Exclusion criteria were severe underlying disease, any contraindication to steroids and ozone, and indication for surgical arthroplasty.

Intra-articular ozone and steroid injection

Intraarticular injection of 5 ml (35µg/ml) of ozone was injected using regular size syringes. In steroid group 5ml of Triamcinolone (10mg/ml) was injected into the intra-articular space.

Pain and disability measurement

After injection patients were scheduled for follow up visits at 1, 3, and 6 months. At each visits pain scale was measured using numeric rating score (NRS). Disability was measured using an Oswestry Disability Index (ODI) at each visit. Evaluation of pain and disability was performed by a physician blinded to the patient group and were recorded in a specified data sheet.

Blood samples (2ml) were collected at each visit and were stored at -80°C freezer until sent to the laboratory for measurement of IL-1b and TNF-alpha at 37°C.

Il-1b and TNF-α ELISA measurement

Samples were thawed on 4°C on ice bucket and centrifuged at 2000 rpm for 3 minutes. Then 100µl of aqueous phase were transferred on prepared 96 well-plates. For 96 well plates preparation, plates were washed twice the night before and primary antibody for IL-1beta (IL-1β Antibody (H-153), rabbit polyclonal IgG, Santa Cruz, CA, USA) and TNF-alpha (TNF-α antibody (sc-133192), mouse IgG2a, Santa Cruz, CA, USA) were loaded and incubated at 37°C until the next morning. Next morning after proper washing of plates, samples were loaded on the plate. Then secondary antibodies (goat anti-rabbit IgG-HRP, and goat anti-mouse IgG-HRP (sc-2005), Santa Cruz, CA, USA) were added to the samples. IL-1b and TNF-a were measured using an ELISA plate

reader by a standard curve.

Statistical Analysis

Statistical calculations were conducted using SPSS 20 (Chicago, IL, USA). The variables were presented as mean ± standard deviation and were analyzed by the student t-test; parametric texts were used. Other variables were analyzed by Chi-Square, Mann-Whitney U-test or non-parametric tests. P<0.05 was considered as statistically significant. The sample size was estimated using sample size calculator software with 95% confidence interval, p=0.05 and power of 80% and difference between two groups of 30% in primary outcome based on the pilot study.

Results

In this randomized clinical trial, 75 patients with knee osteoarthritis were enrolled and randomly assigned to one of steroid or ozone groups. Five patients in ozone group and 9 patients in the steroid group were lost to follow-up, and 30 patients with ozone and 31 patients in the steroid group finished the study. There were no significant differences in age, sex or BMI, of patients (p>0.05). Mode of osteoarthritis grade at the time of inclusion in the study was 2 in both groups. Mean of osteoarthritis grade were not significantly different in two groups of patients (p=0.33) (Table1). Duration of pain before injection (NRS>4) was 14.5±5.7 months in ozone and 14.9±7.3 months in steroid group which was not significantly different. The mean pain score before injection (NRS score) was 6.8±1.7 in ozone and 6.9±1.5 in steroid group which was not significantly different (p=0.1) (Table 1).

Comparison of pain scale (Numerical rating scale) before and after injection during follow-up time is depicted in figure1. Pain scale was not significantly different at 1 month (p=0.35) after injection, but was different at 2 and 6 month post injection (p=0.011, 0.025; respectively) (Figure 1).

Disability index

Function of joints after injection were measured by the Oswestry disability index (ODI) % (Figure 2). At the beginning of the study before intra-articular injection ODI score was not significantly different between two groups (p=0.44). ODI score was not significantly different between two groups at 1 month after intraarticular injection (p=0.28).

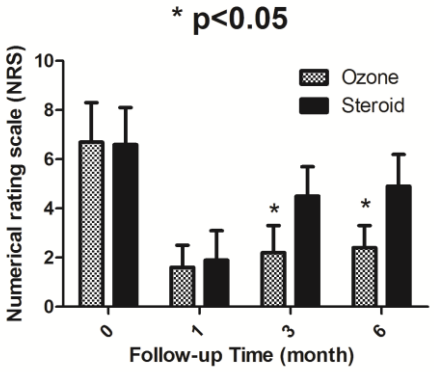

Figure 1. Comparison of pain scale (Numerical rating scale) between patients with knee osteoarthritis after intraarticular injection of ozone and steroid injection (*Mann-Whitney test).

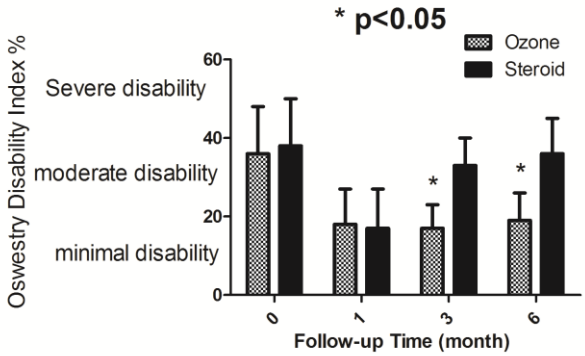

Figure 2. Comparison of functional disability (Oswestry Disability Index) between patients with knee osteoarthritis after intraarticular injection of ozone and steroid injection.

However, ODI score was significantly different at 2 and 6 month post injection (p=0.02, 0.03; respectively).

Inflammatory cytokines

IL-1b and TNF-α were measured and compared in two groups of patients. At baseline samples before intra-articular injection, there were no significant differences in in IL-1b and TNF- α between ozone and steroid groups. At 1 month samples also, there were no significant differences in IL-1b and TNF- α between ozone and steroid groups. However, at 3 and 6 month follow up samples, Il-1b and TNF- α were significantly lower in ozone group compared to steroid groups (Figure 3).

Discussion

In the study, we compared intra-articular

Table 1: Demographic characteristics of patients with intraarticular injection of ozone and steroid.

	Ozone (n=30)	Steroid (n=31)	p-value
Age	56.7±16.9	54.8±19.5	0.19†
Sex (male/female)	11/19	11/20	0.26‡
BMI	22.6±4.3	21.4±3.8	0.35*
Grade of osteoarthritis (Mode/Mean ± SD)	2 /2.2±0.8	2/2.3±0.9	0.33*
Duration of pain before injection (NRS>4) (m)	14.5±5.7	14.9±7.3	0.74‡
Pain score before injection (NRS score)	6.8±1.7	6.9±1.5	0.10‡

Mann-Whitney test ‡ † Wilcoxon-signed rank test *t-test

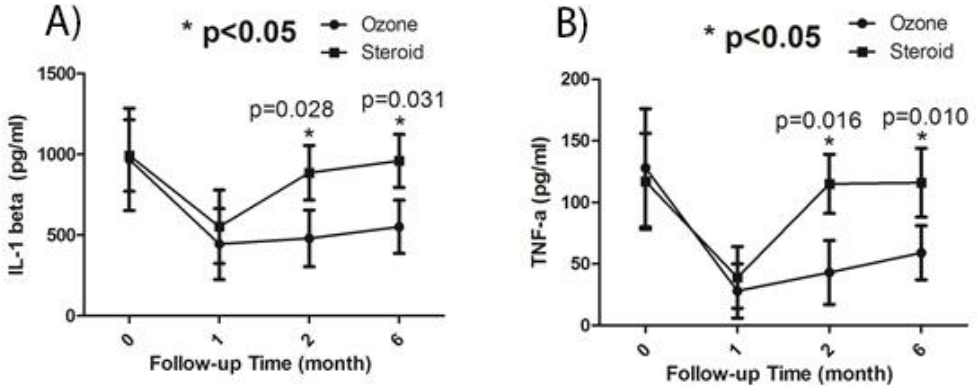

Figure 3. Comparison of serum IL-1 and TNF-α between patients with knee osteoarthritis after intraarticular injection of ozone and steroid injection.

injection of ozone and steroid in decreasing pain and disability and inflammatory response in patients with knee osteoarthritis. Patients who were injected with ozone had less pain and disability after 6 months follow up compare to steroid group.

Ozone effects in decreasing pain were longer compared to steroid. Steroid has been the mainstay of minimally invasive therapies for osteoarthritis. However, there is much debate about the duration of effect in these patients. It is important to note that ozone has invariably showed a longer duration of action in decreasing pain and disability in joint pain. On the other hand, a comprehensive systematic review showed that intra-articular corticosteroid injection results in clinically and statistically significant reduction in osteoarthritis knee pain and the beneficial effect could last for 3 to 4 weeks, but is unlikely to continue beyond that (15).

Specific inflammatory cytokines, IL-1b and TNF-α decreased significantly in ozone group compare to steroid group. Chemical factors include systemic and local inflammatory responses along with mechanical factors could affect cartilage tissue and its destruction (16). The Intracartilaginous ozone injection was first introduced by Muto et al. (17) with positive results in clinical and inflammatory responses. Ozone decreases inflammation and joint injury, providing reduction of pro-inflammatory cytokines, TNF-α and IL-1β transcripts and re-establishment of cellular redox balance (18, 19). Intraarticular glucocorticoid (GC) therapy diminishes synovial cell infiltration, vascularity, expression of pro-inflammatory cytokines (TNF, IL-1beta), and adhesion molecule levels (ICAM-1, VEGF) in patients with chronic arthritis (20). Although steroid has robust anti-inflammatory action against inflammatory cytokines, however, this effect in cartilaginous tissue was shorter compared to ozone in our study coherently with others (21). Actually, their trend reflects a similar pattern in pain and disability, decrease in these patients. These two cytokines showed to be the mainstay of initiation and propagation of chondrocyte destruction. Ozone has probably more stable anti-inflammatory effect, compared to steroid. Besides, ozone, anti-oxidant action could also help to diminish the inflammatory response. Ozone injection has shown to significantly decrease intradiscal inflammation and the

inflammatory response (22).

We did not observe any adverse effects in ozone injected patients. It is an important aspect of our study. On the other hand, intra-articular steroid therapy has not side effects. In a recent paper, researchers showed that the outcome of combination of O_2O_3 and hyaluronic acid demonstrated improved results compared with hyaluronic acid in patients affected by knee OA (23). Meanwhile, a combination of ozone with platelet-rich plasma (PRP) or dextrose could be effective (24, 25).

Conclusion

Patients with chronic knee osteoarthritis could respond better and longer to intra-articular ozone injection compared to intraarticular steroid injection. In addition, serum inflammatory cytokines are also lower in Ozone group compared to the steroid group along with clinical improvements.

Acknowledgment

None.

Conflicts of Interest

The authors declare that they have no conflict of interest.

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