

Comparison of two different ozone injection sites for knee osteoarthritis, Tibio-femoral joint versus Supra-patellar recess : an open randomized clinical trial

Abstract

Background: The aim of this study was to compare the efficacy of ozone injection at Tibio-femoral joint with Supra-patellar recess on knee osteoarthritis (OA).

Methods: In this randomized, controlled clinical trial, 99 patients with symptomatic knee OA were randomized into two groups. 47 patients selected to receive 7-8 ml ozone (20 µg/ml) through Tibio-femoral joint injection, and 49 patients received 10 ml ozone (20µg/ml) through supra-patellar recess injection by using in-plane ultrasound-guided. The primary outcome was the change from baseline in the visual analogue scale (VAS)(0-100 mm) pain score during 3 months after injection. Secondary outcome measures included the Western Ontario and McMaster universities OA Index (WOMAC), Lequesne index, time “Up and Go” (TUG) test, single-limb stance (SLS) tests, patient satisfaction, and adverse effects. All of these measurements were evaluated at the base time, 48 hours, 2 weeks, 1 month, 2 months, and 3 months after injection.

Results: Both groups had significantly improvement in the primary and secondary outcome measures. VAS pain score except at the 3-month follow-up (16.8±13.3 versus 18.1±16.6, 95% CI, -7.33 to 4.73, p =0.6), WOMAC pain score, WOMAC stiffness score at all evaluated times, and WOMAC total score at 48 hours after injection (95% CI, -19.4 to -4.9) were significantly better among Tibio-femoral ozone injection compare to Supra-patellar recess injection (95% CI, -20.68 to -6.51, p<0.001). In both groups satisfaction, TUG and SLS times were improved, but no significant difference was seen between groups (p>0.05). At the 3-month follow-up, WOMAC pain and total scores for only Tibio-femoral joint injection group as well as WOMAC function, TUG and SLS times for both groups were gradually coming back to the baseline.

Conclusion: Ozone injection in both groups was associated with pain relief, functional improvement, and quality of life in patient with knee OA. Pain and stiffness of joint were improved better in Supra-patellar recess ozone injection.

Key words: ozone, osteoarthritis, sonography, injection

Introduction

One of the most common forms of arthritis in the world is osteoarthritis (OA) which affects over 20 million individuals in the US [1]. In general practice, OA is one of the most common complaints and reported as the most common form of arthritis which affected more than 20 million individuals in the United States [2]. It is also known as a leading cause of lower extremity disability among older adults [3]. Unfortunately, the exact etiology of OA still remains unknown.

The pathophysiology of OA is characterized by degeneration of articular cartilage, osteophytes formation, subchondral remodeling, and joint swelling [2,3]. OA presents by deep articular pain and progressive loss of function leading to disability and significant burden on the healthcare network [4]. The treatment of OA is based on pharmacological and non-pharmacological interventions, including oral non-steroidal anti-inflammatory drugs (NSAIDs), and intra-articular (IA) injection of several products such as corticosteroids, viscosupplements, blood-derived substances, as well as physical exercise [4]. At present, there is no approved OA treatments to reduce structural progression or need for total knee replacement [5,6].

Ozone (O₃) is a variety of oxygen, applied for therapeutic aims in humans, especially in chronic diseases with little efficacy of allopathic medicine, such as rheumatic disease and OA [7-9]. The mechanism of action of ozone are: anti-inflammatory, analgesic and acts as an antioxidant effects by improvement of cellular metabolism, lowering prostaglandin synthesis, modifying the redox system function properly (by induction the synthesis of oxidant enzymes such as superoxide dismutase, glutathione peroxidase, and catalase), leading to reduction in cellular oxidative stress, as well as improvement of tissue oxygen supply via hemoreologic action, vasodilation, and angiogenesis [8,9,10]. There are few published studies concerning the use of intra-articular ozone in the treatment of knee OA and most of them are clinical series reports [7-12]. Complication of knee injections have been related to pain or swelling at the site of injection [13], granulomatous inflammation of the synovium [13], saphenous neuropathy [14], aseptic acute arthritis [15,16], embolia cutismedicamentosa (Nicolau Syndrom) [17], and albicans arthritis [18].

There have been an increasing number of image-guided procedures, including ultrasound, lately offering safer, comfortable, with fewer complications or adverse events and more efficient approaches to patients when compared with surgery or non-guided (blinded) procedures [19]. The method of in-plane ultrasound-guided knee injection through a lateral supra-patellar approach has been shown to be a safer when compared with other methods [20-22]. On the other hand, supra-patellar recess is an easy access point for injection and a little far away from intra-articular space. Thus, we propose the hypothesis that the complications of injection in this point of knee

may be associated with fewer complications following knee injection, especially when performed by ultrasound-guided knee injection.

As far as we know, there are no studies to date to assess the supra-patellar recess ozone injection with Tibio-fibular ozone injection by using ultrasound-guided. The aim of this study was to compare the efficacy of ozone injection in supra-patellar recess with the Tibio-femoral joint injection in the setting of pain reduction, functional improvement of knee, and fewer adverse events.

Material and Methods

Study design

This study was a prospective, an open randomized clinical trial study based on three months of follow-up exerted between September 2017 and December 2017. The patients were enrolled in clinic of pain of Akhtar university hospital, Tehran, Iran. All patients read and signed a written informed consent prior to participating in the study. The institutional review board for human investigation has approved this survey and has been conducted based on the principles of the Declaration of Helsinki. This study was registered at clinical trial registry of Iran with IRCT# 20131124015515N5.

Inclusion criteria. Patients who participated in this study were males and females aged between 60 and 85 years old, suffering from osteoarthritis of knee according to the criteria defined by American College of Rheumatology[23]. They also have confirmatory knee X-ray diagnosis (Kellgren Lawrence grades II-III) and pain in the affected knee [24].

Exclusion criteria. Patients aged less than 60 years over 85 years, those who had neurologic or mental deficit based on Mini Mental State Examination, Kellgren Lawrence grades I and IV, recent knee trauma or suspicion of another joint affection, uncontrolled systemic disease, thrombocytopenia, bleeding tendencies, use of anti-coagulants or anti-aggregates, recent myocardial infarction or stroke, and those who had been left the study during 3 months of following-up. Those patients who could not return for visit at any of these times, excluded from the study.

Groups. All patients were divided randomly into two groups: one group received ozone in Tibio-femoral (TF) joint and another group received ozone in Supra-patellar recess (SR).

Patients from TF group received 7-8 ml of one intra-articular injection of ozone 20 µg/ml and patients from SR group received 10 ml of one intra-articular injection of ozone 20 µg/ml[7]. As ozone has short half-life (nearly 45 minutes at 20 °C)[8,9], it was freshly generated in the Akhtar hospital clinic(Tehran,Iran), using an Ozone & Life O&L 3.0 RM generator (Sao Jose Campos-Brazil) connected to a pure oxygen source and applied for the patient, promptly. The oxygen was used by ozone generators through high voltage tubes with outputs ranging from 4000-14000 and a mixture of ozone-oxygen with concentration ranges extending to 5% [25].

Sample Size Determination : The decision to select a proper sample size was based on the two primary end points. To achieve this goal, a sample size of 40 evaluable patients was selected with 80% statistical power and 30% between groups efficacy. The Chi Squared test was used with the alpha level of 0.025 and beta level of 0.20 in hypothesis testing. A total of 80 evaluable patients were selected for this study. Approximately 96% evaluable patients was aimed or randomization purposes with the possibility of 20% dropout rate. The clinical trial was aimed at comparing a new treatment to the existing one, requiring the utilization of more than half of the selected sample at the cost of losing the statistical efficacy for the trial [27].

Allocation concealment: Proper allocation concealment keeps trial investigators and participants unaware of upcoming allocations so that each patient has an equal chance of being assigned to a given group. To achieve this goal, opaque envelopes containing the group each patient would belong to were sequentially numbered and were given to a nurse not been involved in the trial. After a patient consented to the trail study, he or she selected one of the opaque envelopes and was given the allocated ozone. The opaque envelopes were opened by another nurse in a sequential manner after the patient's evaluation.

Patient inclusion in the study. A baseline visit was performed for each participant attended in the study and the history, physical examination, evaluation of knee radiography and others tests were performed in this session. The questionnaires and tests used in our study consist of Visual Analogue Scale (VAS) [28], Time up and go test (TUG Test) [29], Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) [30]. Those compatible with the inclusion criteria were instructed to go on their medical treatment just the same as their physicians' orientation. They signed the printed informed consent paper prior to the enrollment in this trial.

Randomization. participants were sequentially assigned to receive Ozone in Tibio-femoral joint or supra-patellar recess according to a pre-established computer-generated global randomization. That list was prepared by Dr.Hashemi, on September 2016, using software ETCETERA, version 2.46, and constituted 96 numbers with the corresponding treatments (Fig1).

Except for the physician who performs the injection, the main researcher and those who evaluated the results did not know which group the patients were allocated to. The patients were also not aware of two different approaches of injection.

Treatment.

The study consisted of a baseline visit during which the intra-articular injection was exerted and the second visit at 48 hours, followed by 2 weeks, 1 month, 2 months, and 3 months post-injection. Patients who used non-steroidal anti-inflammatory drugs (NSAIDs), one week period allowed them to stop NSAIDs consumption for seven days. Demographic data and baseline assessments were collected prior to group randomization.

All syringes containing ozone for Tibio-femoral injection was placed in a vertical position with their beaks upwards prior to the needle and blood collection bottle cover placement. Then a needle was placed on the beak of the syringe containing ozone by each nurse and a blood collection bottle cover was put over the needle by the same nurse. Subsequently, the main researcher received the syringes to be used for the treatment.

Ozone. Our source for the ozone for the medical use was an ozone generator (Ozone & Life model O & L 3.0 RM, of Brazilian fabrication). Oxygen passes through a high voltage tube in this generator and then ozone is generated by oxygen dividing into molecules while passing

through this tube. The exit port of the generator was connected to a 10 cc syringe that collected the produced gas using the following parameters:

O₂ flux=1L/min and Feeder adjusted in position 8

An ozone with concentration of 20 µg/ml was prepared according to the aforementioned protocol [7]. The room was kept ventilated while the ozone was being generated, to facilitate the process of dispersion of the gas that could escape to the environment.

Technique. The more painful and less functional knee was chosen to be injected. The pain of joint was measured by numerical rating pain scale (NRPS) and those with severe pain (score ≥ 7) were selected as well as knee injury and osteoarthritis outcome score (KOOS) was used for selection the patients with less functional knee. Each patient was positioned sitting over a stretcher. The same knee was prepared in a sterile fashion and with an antiseptis procedure with gauzes soaked in 70° GL alcohol, using round centrifugal movements, a few times, from puncture site to its periphery. The needle was placed at puncture site at the tibio-femoral articular interline, 1.5 cm below the apex of patella and same distance medially to the patellar tendon. To prevent the puncture of the of the Hoffa`s fat pad, we always kept the direction of needle anteroposteriorly [10]. We performed a second puncture when there was an error in the knee puncture at first attempt or the needle contacted the femoral condyle. Local anesthesia was obtained by using a 1 ml syringe and 30 × 7 mm needle containing 0.5 cc of 2% lidocaine solution at puncture site [31]. In all cases, the syringe was aspirated prior to injection to prevent

entering into joint effusion that might be presented to confirm that the needle was not inside the joint. Subsequently, the syringe with ozone was connected to the needle used for local anesthesia of the puncture site and the ozone was injected slowly in continuous fashion. Then syringe and needle were both withdrawn from the joint. The injected place was packed and then dress for at least 30 minutes.

All procedures were performed in an outpatient clinic, **Akhtar hospital, Tehran, Iran..** Injections were performed by a physician, who has experience of more than 600 cases per year in knee injections or aspiration **and no anesthetic medication was used for injection.** This physician did not involve into the study. Patient were in a supine position, the knee was flexed approximately 60° and was prepared in a sterile fashion, and 1 ml of 2% lidocaine hydrochloride with 1: 80,000 epinephrine was infiltrated into the skin and subcutaneous tissue at the lateral soft spot of the knee joint just inferior to the lower pole of patella with 27gauge needle for patient comfort. The accuracy of the injection was assessed by an ultrasound. All patients had radiographs of the knees that included an anteroposterior standing view, a lateral standing view, and a patellar skyline view. The Kellgren and Lawrence grading scale was used to classify the severity of OA by 2 authors. The graders were blinded to treatment group at the time of radiographic evaluation. The primary outcome were knee pain and functional improvement at 48 hours, 2 weeks, 1 month, 2 months, and 3 months follow-up. Secondary outcome measures included the WOMAC , Lequesne index, time up and Go (TUG) test, single-limb stance (SLS) test, patient satisfaction, and adverse events at different evaluated times.

Evaluation tools. Visual Analogue Scale (VAS): The most frequently used tools for pain measurement in the general population is VAS since it is identified as the most sensitive, reproducible, and simplest pain scale. It is a 10-centimeter line with newscasters at both ends. The words “without pain” and “unbearable pain” are at each end side. The patient is asked to mark a point showing their pain. To count the measure a 0-100mm ruler is used [28].

Lequesne Index: Lequesne index includes 10 detailed questions related to patients with knee osteoarthritis. pain or discomfort, extreme distance walked, and daily life doings indicated with 5, 1 and 4 respectively). The score differs between 0 to 24 points, and the worse the pain and function [31].

Timed Up and Go (TUG test): TUG test, consists of a stand up from a chair/9height of seat = 45 cm and of arms = 65 cm), 3 meters return walk and sitting down, while the time spent performing the test is booked. The preposition of the test is to assess stability when sitting, shifting from a sitting position to a standing position, stability when walking and turning and walking when walking without using compensatory strategies. The test takes 10 seconds or less in independent individuals without balance alterations and 20 seconds or less in independent for basic transfers, Those need 30 seconds or more to complete the test are dependent in many daily life activities and moving, facing a greater risk of falling [29].

WOMAC (Western Ontario and McMaster Universities) Index: WOMAC index assesses pain, stiffness, and physical function in daily life activities using 24 queries (for example, climbing down stairs). The degree of difficulty in pain and stiffness is indicated by individuals from 0 (none) to 5 (very strong) during the previous 72 hours. The 24 items value between 0 and 96; the higher the value, the worse the symptoms of the patients [30].

Statistical analysis: The efficacy analysis was conducted in the pre-protocol (pp) patient population for randomized patients who met the inclusion/exclusion criteria and received treatment using the baseline of 48 hours, 2 weeks, one month, 2 months, and three months of

visits. No protocol deviations were observed. Major protocol deviations were classified as fully meeting the selection criteria, voluntary trail exist, and non-compliance with the required study treatment. For 2 patients who dropped out the study, Intention to treat analysis (ITT) was not conducted.

Data analysis: Acquired data was fed to Excel for the purposes of score variables calculation, such as Lequesne Algofunctional Index, WOMAC, and Geriatric Pain Measure. Further, the data was analyzed using SPSS 20.0 (SPSS Inc.Chicago) statistical software to determine average (mean),median, standard deviation between groups using Student'S t-test to look at the ratio between and within the patients' groups. Qualitative characteristics were described according to the groups was verified with tests such as Chi-squared, Fisher's exact test, likelihood ratio test, and non-parametric Man-Whitney's test. Summary-measures Scales were utilized to define scales according to the groups and evaluation moments. Generalized estimating equations with Autoregressive Correlation Matrices of order 1 between the moments, with normal marginal distribution and identity or logarithmic link function was also used to analyze comparison between the groups and moments. Since the models showed statistical significance at 95% confidence level, Bonferroni's multiple comparison test was used to establish between groups means pairwise comparison to determine which means are significantly different. Results were illustrated using medium profile graphics, with the respective standard errors and according to the groups, and the tests used a significance level of 5%.

Results

A total of 118 participants were selected to conduct this survey. Of them 11 patients did not meet the eligibility criteria at the screening visit and 8 other patients decided not to enroll or not to return for follow-up visits during the study period. Therefore, 99 patients were in access for analysis during 3 months follow-up assessment. Of them, 48 patients allocated in Tibio-femoral injection and 51 patients in Supra-patellar recess injection. One patient in Tibio-femoral and two patients in Supra-patellar recess group were non-compliance. Finally, 47 patients for Tibio-femoral and 49 patients for Supra-patellar recess ozone injection selected to conduct this study. There was no significant differences between Tibio-femoral and Supra-patellar recess injection groups regarding demographic or baseline data ($p > 0.05$) (Table1). In both groups significantly improvement in the VAS pain, WOMAC score, and Lequesne index score at the baseline, 48 hours, 2 weeks, 1 month, 2 months, and 3 months was seen ($p < 0.001$). Supra-patellar recess injection was significantly more effective than Tibio-femoral joint injection to alleviate the pain based on the VAS pain scores in 48 hours, 2 weeks, 1 month, and 2 months after injection ($p < 0.05$; CI 95% -20.68- -6.51, 9.76-26.43, -20.18- -4.81, -14.47- -2.72; respectively). WOMAC pain and stiffness scores were significantly decreased among Supra-patellar recess group

compared to Tibio-femoral group at any time interval evaluated after the baseline ($p < 0.05$; 95% CI -3.16- -0.03 at 48 h follow-up visit). Regarding WOMAC function score and WOMAC total score, no difference between two groups was found ($p = 0.5$ and 0.3 ; 95% CI, -6.47- 3.47, -9.02- 2.82, at 3 months follow-up, respectively) (Table-II). It is of note that, within-group comparison of TUG times did not demonstrate significant change in either group during the study period ($p > 0.05$). Meanwhile, the SLS time improved significantly in both groups ($p > 0.4$) (Table-IV).

In the Tibio-femoral joint injection group, the acetaminophen consumption decreased from a mean (and standard deviation) of 16.7 ± 4.8 tablets per week at baseline to 6.2 ± 3.1 , 5.8 ± 3.6 , 6.3 ± 2.8 , 7.1 ± 2.1 , and 9.3 ± 2.5 tablets per week at the 48 hours, 2 weeks, 1 month, 2 months, and 3 months follow-up evaluation compared with a decrease from 15.1 ± 3.6 tablets per week at baseline to 5.8 ± 3.3 , 5.3 ± 2.8 , 5.8 ± 2.2 , 6.6 ± 3.7 , and 9.9 ± 2.9 tablets per week at the follow-up visit among patients received Supra-patellar recess injection. There were no significant difference between-groups in the setting of patient satisfaction (Table IV). The satisfaction was highest among 3 months in both groups (Table IV).

The frequency of pain and complications were comparable between the two groups (Table V). Most complications were not severe and approximately lasted 1 to 3 days and alleviated by simple analgesics easily or resolved spontaneously. Joint pain was the most common complain

among patient after injection in both groups and infection was not repeated from any patient (Table V). Joint effusion was seen in 1 patient in Tibio-femoral joint injection group and 2 patients in Supra-patellar recess injection group. Within 1 week after injection, they resolved spontaneously, without the need of arthrocentesis for pain relief.

Safety and tolerability

The application of ozone for Intra-articular injection has been demonstrated to be safe. The complications are rare and comprise acute and transitory pain, effusion, swelling and stiffness in the knee, lasted 1-3 days, and improved well or getting better by simple analgesics. In this study, adverse events were based on a questionnaire. No allergic reactions, sepsis, or serious adverse events occurred during the study. Adverse events did not cause to study discontinuation in either group.

Discussion

This randomized, clinical trial study presents the results of 1.5 years survey conducted in patients with knee osteoarthritis receiving intra-articular ozone through either Tibio-femoral joint or Supra-patellar recess injection. This study shows that ozone injection of either through Tibio-femoral joint or supra-patellar recess is safe and effective for 3 months. Pain was alleviated, function improved, and satisfaction was increased by using ozone in both groups. Adverse reverse were rare and transient. VAS pain score, WOMAC pain and stiffness scores were more improve among those with supra-patellar recess injection compare to traditional Tibio-femoral joint injection.

Several studies in the world indicate the ability of ozone to control or improve different diseases [37]. Most evidences indicating the efficacy of medical use of O₃ is based on results of observational studies and case reports in which it has been used in the symptomatic treatment of human [7-12]. Release of endorphin inhibits transmission of the noxious signal to the cortex and thalamus, activation of the descending anti-nociceptive system, hypo-stimulation linked to the oxidative degeneration of c-nociceptors, simultaneous psychogenic stimulation of the central analgesic system activated by the gas injection, somehow due to placebo effect, and the localized oxygenation and analgesia lead to vasodilation and muscle relaxation, thus a reactivation of the muscle metabolism, by favoring oxidation of lactate, neutralization of acidosis, enhanced synthesis of ATP, calcium ion reuptake and edema reabsorption are all the proposed mechanism of ozone for symptomatic treatment of pain and functional improvement(38).

Lopes de Jesus CC, et al conducted a randomized, double-blinded controlled study, in which patients who suffer from knee osteoarthritis divided into two groups of ozone and placebo and compared the effects of intra-articular ozone on pain reduction and joint functional improvement between these two groups [37]. They understood a significant improvement of joint function and reduction in pain intensity and when compare with placebo group after 8 weeks of treatment. In their study the number of patients in the ozone group was significantly more than placebo group. In addition to that, a significant difference in basal data regarding marital status and schooling level which in turn may interfere in the observed results. Although they reported very rare adverse events, the long time of 4.5 years period of this study may miss to report the accurate adverse events. The results achieved by Giombini et al, who used oxygen-ozone for 23 patients with knee osteoarthritis were also corroborated by Lopes de Jesus's study [39]. In both of aforementioned studies the injection of ozone was performed in Tibio--femoral joint and demonstrated the effectiveness of ozone in reduction the pain and improvement of knee joint function. In our study, we perform the injection in Tibio-femoral joint the same as their study, but compared it with supra-patellar recess point injection in order to assess the improvement the

disabilities result from OA as well as we assist ultrasound guided probe to enhance the accuracy of injection.

Chagas-Neto FA et al showed that in-plane ultrasound-guided injection of the knee in semi-flexion approaching the lateral supra-patellar recess is a safe and useful technique administers intra-articular contrast solution, as an alternative method without radiation exposure [20]. Bum Park Y et al showed that accuracy of ultrasound-guided supra-patellar bursal injection was more than blind approach [40]. In the study conducted by Curtis, HM and Park Y demonstrated that supra-patellar approach was safer than other approach for intra-articular injection [41]. Such technique permits to control the needle throughout the entire procedure from the puncture site to

the articular cavity, avoiding vital structures such as tendons and cartilage [20]. In our study, we performed this technique to increase the accuracy in order to compare two methods of approaching.

One interesting finding in the current study was that the improvement in the mean VAS pain , WOMAC (pain,stiffness,function) scores and Lequesce index score from baseline to 2 months was increasing, but after that it has been moved back to the baseline within 3 months, not as severe as that the baseline. In our study, we found that supra-patellar recess injection improved VAS pain scores better than group with Tibio-femoral joint injection at 48 hours, 2 weeks, 1 month, 2 months, and with no difference at 3 months. It was also showed that WOMAC pain and stiffness score was significantly improved among supra-patellar recess injection group compare to Tibio-femoral injection group. In addition to that, WOMAC total score for 48 hours was better improved among supra-patellar recess injection. There was no significant difference for satisfaction, adverse effects, TUG and SLS time between two groups. This study was the first study in which ozone injection was applied in two different sites of knee joint using ultrasound-guided. Supra-patellar recess has a little more distance from the joint, therefore we increased the the dosage of ozone compare to Tibio-femoral joint injection. The injection in this point may be associated with little adverse events and more efficient related to the intra-articular injection.

We have found several limitations for our study. First, this study was conducted at a single center, and special patients with Kellegren-Lawrence grade-2 or 3 Tibio-femoral osteoarthritis were recruited. The results cannot be generalized to all other recruited patients with osteoarthritis. The outcomes cannot be generalized to all patients with different degree of radiographic. Second, because of the different site of injection, the researcher who performed injection in both groups could not be blinded. However, that researcher was not involved in the results evaluation. Third, in our survey, we did not consider a placebo group. On the other hand, injection into the joint could have a strong placebo effect, which may alleviate pain by approximately 30% over following weeks [42]. Therefore, our results may have overestimated the real effects of both sites of injection. Fourth, the volume and dosage of ozone are very important to show the effectiveness and changing the outcomes. The possibility of a dose-

dependent response that could increase efficacy should be studied in the future. On the other hand, the lack of imaging exam control to assess the impact of treatment on its evolution was the fifth limitation of the current study. The last limitation could be brought up, was the time for treatment and follow-up. Thus, longer treatment and follow-up periods would confirm the results or elicit more or less favorable results over time. Moreover, results of current study are warrant and encouraging us to conduct further studies in patients with knee OA to evaluate the effects of ozone for a longer period of time.

Conclusion

VAS pain points, WOMAC pain score, and WOMAC stiffness points, at 48 hours, 2 weeks, 1 month, and 2 months after injection was better among ozone injection in supra-patellar recess compared with Tibio-femoral joint ozone injection as well as at 3 months after injection WOMAC pain points and WOMAC stiffness points were better for supra-patellar recess injection. There was no difference between groups regarding TUG time, SLS time, patient satisfaction and adverse events. Further studies are needed to enhance our understanding of the other unknown mechanisms exist behind ozone injection in supra-patellar recess for symptomatic treatment of the knee osteoarthritis. If the effectiveness of supra-patellar recess injection to reduce pain and improve function is demonstrated in the upcoming studies, it may provide a new insight into whether this point of injection inhibits one of several known mechanism of pain in osteoarthritis or another cause will be proposed for our findings.

Ethical Approval

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

Consent

All patients read and signed a written informed consent prior to participating in the study. The institutional review board for human investigation has approved this survey and has been conducted based on the principles of the Declaration of Helsinki.

Acknowledgement

This study was funded by Shahid Beheshti University of Medical Sciences, Tehran, Iran. The authors would like to sincerely express their gratitude to the president of Akhtar hospital and Dr.Hashemi for preparing the necessary facilities to conduct this study.

Conflict of interest

There was no conflict of interest in this study to declare.

References:

- 1.Lawrence RC, Felson DT,Helmick CG,Arnold LM,Choi H,et.al.Estimates of the prevalence of arthritis and other rheumatic conditions in the united States.Part II.Arthritis Rheum.2008;58:26-35.
- 2.Hsaio CJ,Cherry DK,Beatty PC,Rechtsteiner EA.National Ambulatory Medical Care Survey:2007 summary.Natl Health Stat Report.2010:1-32.

3. Guccione AA, Felson DT, Anderson JJ, Anthony JM, Zhang Y, et al. The effects of specific medical conditions on the functional limitations of elders in the Framingham study. *Am J Public Health*. 1994;84:351-8.
4. Ayhan E, Kesmezacar H, Akgun I (2014). Intra-articular injections (corticosteroid, hyaluronic acid, platelet rich plasma) for the knee osteoarthritis. *World J Orthop* 5:351-361.
5. Losina E, Daigle ME, Reichmann WM, Suter LG, Hunter DJ, et al. Disease-modifying drugs for knee osteoarthritis: can they be cost-effective? *Osteoarthritis Cartilage*. 2013;21(5):655-667.
6. Fibel KH, Hillstorm HJ, Halpern BC. State-of-the Art management of knee osteoarthritis. *World J Clin Cases*. 2015;3(2):89-101.
7. Huanqui C, Cruz M, Miranda A, Poblete P, Mamani R. Eficacia y seguridad del ozono intra-articular en artrosis de rodilla refractaria al tratamiento. *Rev. Peru Reum*. Vol 12, n° 1, 2006. P. 19-24.
8. Benvenuti P. Oxygen-ozone treatment of the knee, shoulder and hip-a personal experience. *Rivista Italiana di Ossigeno-Ozonoterapia* 2006;5:135-144.
9. Calunga JL, Menendez S, Leon R, Chang S, Guanche D, et al. Application of ozone therapy in patients with knee osteoarthritis. *Ozone: Science & Engineering*. 2012;34:469-475.
10. Bocci V. Scientific and Medical Aspect of Ozone therapy. State of the art. *Archives of Medical Research*. 2006;37(4):425-435.
11. Wong R, Gomez M, Menendez S, Ceballos A. Medical application of ozone therapy on arthrosis [abstract]. IN: 1er Congreso Iberoamericano de Aplicaciones del Ozono, 1990 October 31-November 3, Ciudad de la Habana, Cuba.
12. Conde B, Casas M, Delgado M, Ramos F. Ozone therapy in the treatment of osteoarthritis [abstract]. In: 2do Simposio Interacional de Aplicaciones del Ozono; 1997 March 24-28, Ciudad de la Habana, Cuba.
13. Chen AL, Desai P, Adler EM, Di Cesare PE. Granulomatous inflammation after Hyalan G-F 20 viscosupplementation of the knee: a report of six cases. *Journal of Bone & Joint Surgery-American Volume*. 2002;84-A(7):1142-7.
14. Lizuka M, Yao R, Wainapel S. Saphenous nerve injury following medial knee joint injection: a case report. *Archives of Physical Medicine & Rehabilitation*. 2005;86:2062-2065.

15. Bernardeau C, Bucki B, Liote F. acute arthritis after intra-articular hyaluronate injection: onset of effusion without crystal. *Annals of the Rheumatic Diseases*.
16. Roos J, Epaulard O, Juvin R, Chen C, Pavese P, et al. Acute pseudoseptic arthritis after intra-articular sodium hyaluronan. *Joint, Bone, Spine: Revue du Rhumatisme*. 2004;71:352-354.
17. Beissert S, Presser D, Rutter A, Metze D, Luger TA, et al. Embolia cutis medicamentosa (Nicolau syndrome) after intra-articular injection. *Hautarzt*. 1999;50:21421-6.
18. Christensson B, Ryd L, Dahlberg L, Lohmander S. *Candida albicans* arthritis in a nonimmunocompromised patient. Complication of placebo intra-articular injections. *Acta Orthopaedica Scandinavica*. 1993;64:695-698.
19. Di Cesare PE, Haudenschild DR, Samuels J, Abramson SB. Pathogenesis of Osteoarthritis. In: Firestein GS, Budd RC, Gariel SE, McInnes IB, O'Dell JR. *Kelly's Textbook of rheumatology*. 9th Edition. Philadelphia: Elsevier Saunders; 2013. V 2, p.1617-1633.
20. Chagas-Neto FA, Taneja AK, Gregio-Junior E, Nogueira-Barbosa MH. *Ultrasound Q*. 2017.
21. Otjen J, Parmell SE, Menashe S, Thapa MM. Ultrasound-guided joint injections for MR arthrography in pediatric patients: how we do it. *Pediatr Radiol*. 2015;45(3):308-316.
22. Ng AW, Hung EH, Griffith JF, Tong CS, Cho CC. comparison of ultrasound versus fluoroscopic guided rotator cuff interval approach for MR arthrography. *Clin Imaging*. 2013;42(1):99-105.
23. Altman R, Asch E, Bloch D, Bole g, borenstein D, et al. Development of criteria for the classification and reporting of osteoarthritis: classification of osteoarthritis of the knee. *Arthritis Rheum* 1986;29:1039-1049.
24. Kellgren JH, Lawrence JS. Radiological assessment of osteoarthrosis. *Ann Rheum Dis*. 1957;16(4):494-501.
25. Al-Jaziri AA, Mahmoodi Sm. Painkilling effect of ozone-oxygen injection on spine and joint osteoarthritis. *Saudi Med. J* 2008;29(4):53-557.
26. Nagib H. Metodologia de estudos em ciencias da Saude-como planejar, analisar e apresentar um trabalho científico. Sao Paulo: Rocca, 2004:185-201.
27. Pocock SJ. *Clinical trials: a practical approach*. Chinchester: John Wiley; 1983:87-88.
28. Price DD. The of visual analogue scales as ratioscalemeasures for chronic and experimental pain 1983, 17:45-56.

29. Podsiadlo D, Richardson S. The Timed “up & Go”: a test of basic functional mobility for frail elderly persons. *Journal of American Geriatrics Society* 1991;39:142-148.
30. Bellamy N, Buchanan WW, Goldsmith CH, Campbell J, Sitt LW. Validation study of WOMAC: a health status instrument for measuring clinically important patient relevant outcomes to antirheumatic drug therapy in patients with osteoarthritis of the hip or knee. *The Journal of rheumatology*. 1998;15(12):1833-1840.
31. Neustadt DH. Intra-articular injections for osteoarthritis of the knee. *Cleveland Clinic Journal of Medicine*. 2006;73(10):897-911.
32. Faucher M, Poiraudéau S, Lefreuve-Colau MM, Rannou F, Fermanian J, Revel M. Assessment of the test-retest reliability and construct validity of a modified Lequesne index in knee osteoarthritis. *Joint Bone Spine*. 2003 70(6):521-525.
33. Gupta SK. Intention-to-treat concept: a review. *Perspect Clin Reside*. 2011;2(3):109-112.
34. Kirkwood BR, Sterne JAC. *Essential medical statistics*. 2nd edition. Massachusetts, USA; Blackwell Science; 2006:502.
35. McCullagh P, Nelder JA. *Generalized linear models*. 2nd edition. New York, USA: Chapman and Hall; 1989:511.
36. Neter J, Kutner MH, Nachtsheim CJ, Wasserman W. *Applied linear statistical models*. 4th edition. Illinois, USA: Richard D. Irwing; 1996:1408.
37. Lopes de Jesus CC, Dos Santos FC, de Jesus LMOB, Monteiro I, Sant`Ana MSSC et al. Comparison between intra-articular ozone and placebo in the treatment of knee osteoarthritis; A randomized, double-blinded, placebo-controlled study. *PLoS One*. 2017.
38. Giombini A, Menotti F, Di Cesare A, Giovannangeli F, Rizzo M, Moffa S et al. Comparison between intra-articular injection of hyaluronic acid, oxygen ozone, and the combination of both in the treatment of knee osteoarthrosis. *J Biol Regul Homeost Agents*. 2016 April-Jun;30(2):621-5.
39. Borrelli E. Mechanism of action of oxygen ozone therapy in the treatment of disc herniation and low back pain. *Acta Neurochir Suppl*. 2011.
40. Park Y, Lee SC, Nam HS, Lee J, Nam SH. Comparison of sonographically guided intra-articular injections at 3 different sites of the knee. *J Ultrasound Med*. 2011;30(12):1669-1676.

41.Curtiss HM,Finnoff JT,Peck E,Hollman J,Muir J,et al. Accuracy of ultrasound-guided and palpation-guided knee injections by an experienced and less-experienced injector using a suprolateral approach: a cadaveric study. PM&R.2011;3(6):507-515.

42.Kirwan J. Is there a place for intra-articular hyaluronate in osteoarthritis of the knee? Knee.2001 Jun;8(2):93-101.

Table I .Demographic and Baseline Characteristics of Intension to treat population

Parameters	Tibio-femoral Joint Injection(N=47)	Supra-patellar Recess Injection(N= 49)	P Value
Sex*			
Female	32 (68%)	33(67%)	0.93
Male	15 (31%)	16(32%)	
Age¥(yr)	62.3±8.7	61.6±6.3	0.65
Body mass index ¥(kg/m2)	25.6±4.6	26.1±3.3	0.54
Radiographic Kellgren-Lawrence grade*			
I	10(%21)	11(22%)	0.98
II	13(27%)	12(24%)	
III	17(36%)	18(36%)	
IV	7(%14)	8(16%)	
Osteoarthritis site*			
Left	23(48%)	20(60%)	0.42
Right	24(52%)	29(40%)	
Osteoarthritis duration¥ (yr)	5.8±4.1	5.2±3.9	0.46
≤5 yr*	25(53%)	27(55%)	0.52
>5 to ≤10yr*	19(40%)	16(32%)	
>10 yr*	3(6%)	6(12%)	
VAS score¥ (mm)	59.4±16.1	56.9±17.4	0.46
WOMAC score¥ (points)			
Pain (0-20)	10.5±3.1	9.8±3.6	0.30
Stiffness (0-8)	3.9±1.8	3.8±1.6	0.77
Function(0-68)	36.4±14.5	36.1±13.5	0.92
Total (0-96)	50.8±17.7	49.7±16.7	0.75
Lequesne index¥(points)			

TUG time¥(sec)	12.9 ±7.7	12.6±12.8	0.89
SLS time¥(sec)	18.2±20.8	17.7±19.3	0.90

*The values are given as the number of patients with the percentage in parentheses. ¥ The values are given as the mean and the standard deviation.

TableII . Comparison of VAS,WOMAC, AND Lequesne Index Scores Between Groups

	Tibiofemoral Joint Injection 47	Suprapatellar Injection 49	95% Confidence Interval	P Value
VAS Pain (Points)				
Baseline	59.4±16.1	56.9±17.4	-7.21-6.21	0.88
48 h	34.9±19.5	21.3±15.6	-20.68- -6.51	P<0.001
2wks	30.4±14.9	18.1±15.7	9.76-26.43	P<0.001
1 mo	30.2±18.8	17.7±19.6	-20.18- -4.81	0.002
2mo	25.5±12.8	16.9±16.4	-14.47- -2.72	0.005
3mo	18.1±16.6	16.8±13.3	-7.33-4.73	0.67
WOMAC pain score (points)				
Baseline	10.5±3.1	9.8±3.6	-0.44-2.24	0.19
48h	7.6±3.6	6.0±4.2	-3.16- -0.03	0.04
2wks	6.8±3.9	4.8±3.8	-3.54- -0.45	0.01
1mo	6.4±3.7	4.5±4.3	-3.50- -0.29	0.02
2mo	6.2±3.4	4.3±3.9	-3.36- -0.43	0.01
3mo	5.5±2.8	4.4±2.1	-2.09- -0.10	0.03
WOMAC stiffness score (points)				
Baseline	3.9±1.8	3.8±1.6	-0.58- 0.78	0.77
48h	3.1±1.6	2.5±1.2	-1.16- -0.03	0.04
2wks	2.5±1.4	1.8±1.3	-1.24- -0.15	0.01
1mo	2.3±1.8	1.5±1.1	-1.39- -0.20	0.01
2mo	2.2±1.8	1.3±1.5	-1.56- -0.23	P<0.001
3mo	1.8±1.2	1.3±1.2	-0.98- -0.01	0.04
WOMAC function score (points)				
Baseline	36.4±14.5	36.1±13.5	-5.30- 5.90	0.91
48h	29.2±13.8	32.1±10.9	-7.86- 2.06	0.25

2 wks	26.3±14.1	29.2±13.3	-8.38- 2.58	0.30
1mo	25.5±12.8	27.3±11.6	-6.68- 3.08	0.47
2mo	23.3±10.5	25.9±12.8	-7.29- 2.09	0.28
3mo	24.8±11.7	26.3±13.1	-6.47- 3.47	0.55
WOMAC total score (points)				
Baseline	50.8±18.1	50.4±18.2	-6.86- 7.66	0.76
48h	37.7±17.5	49.7±17.7	-19.4- -4.95	0.01
2wks	32.9±15.4	38.5±16.8	-12.05- 0.85	0.09
1mo	31.5±13.8	36±16.1	-10.50- 1.51	0.14
2mo	28.9±12.8	34.3±14.4	-10.85- 0.06	0.06
3mo	30.5±14.7	33.6±14.9	-9.02- 2.82	0.31
Lequesne Index(points)				
Baseline	11.7±3.9	10.8±4.1	-0.70- 2.50	0.27
48h	8.8±5.1	9.4±5.7	-2.76- 1.56	0.58
2 wks	7.7±3.9	8.6±4.0	-2.48- 0.68	0.26
1mo	7.4±3.4	8.5±4.3	-2.65- 0.45	0.17
2mo	7.1±4.2	8.3±4.5	-2.94- 0.54	0.18
3mo	7.0±2.9	8.1±3.3	-2.34- 0.14	0.08

*The values are given as the mean and standard deviation.**Between groups difference determining using independent-samples 1-way ANCOVA.¥ A significant difference ($p < 0.05$).£Within-group difference determined using repeated-measures 1-way ANOVA.

III Comparison of TUG and SLS Times Between Groups

	Tibiofemoral Joint Injection*	Suprapatellar Recess Injection*	P Value¥
TUG time(sec)			
Baseline	12.8±7.7	12.3±13.1	0.82
48h	11.2±5.3	11.8±3.6	0.51
2 wks	10.2±4.8	11.1±7.8	0.49
1 mo	10.0±5.1	10.6±5.4	0.57
2mo	10.0±4.8	10.3±4.6	0.75
3mo	11.5±5.6	11.8±6.5	0.81
SLS time (sec)			
Baseline	18.6±20.5	17.3±18.7	0.75
48h	22.4±20.2	21.7±20.0	0.86
2wks	25.5±19.7	23.8±21.1	0.68
1mo	26.6±20.8	24.4±22.2	0.62
2mo	27.7±21.8	25.5±23.9	0.64
3mo	25.8±22.4	24.7±21.9	0.81

*The values are given as the mean and standard deviation.¥Between-group difference determined using independent-samples 1-way ANCOVA or Johnson-Neyman analyses.¥Within-group difference determined using repeated-measures 1-way ANOVA.£ A significant difference (p<0.05).

TableIV. Comparison of Patient Satisfaction Between Groups

	Tibio-femoral Joint Injection*	Supra-patellar Recess Injection*	95% Confidence Interval	P.value
VAS satisfaction score				
48h	69.3±23.4	68.8±22.9	-8.76- 9.76	0.91
2 wks	72.1±22.0	70.8±20.7	-8.24- 8.84	0.94
1 mo	74.5±23.7	71.8±23.3	-6.70- 12.10	0.57
2mo	76.7±19.5	73.3±20.5	-4.61- 11.41	0.41
3mo	75.8±18.8	70.7±18.4	-2.34- 12.54	0.18

*The values are given as the mean and standard deviation .Patients were asked to rate their satisfaction with treatment, as compared with their preinjection condition, using a 100-mm VAS (0= completely dissatisfied and 100=completely satisfied).

Table V. Adverse Events

	Tibiofemoral Joint Injection*(N=47)	Suprapatellar Recess Injection*(N=49)	P Value
Joint pain	6(12%)	2(4%)	0.12
Joint Swelling	5(10%)	1(2%)	0.09
Jointstiffness	4(8%)	0(0%)	0.16
Joint effusion	2(4%)	1(2%)	0.48
Limb weakness	1(2%)	2(2%)	0.58
Injection site paresthesia	1(2%)	0(0%)	0.30
Infection¥	0(0%)	0(0%)	Not compatible
Back pain¥	1(2%)	0(0%)	0.3

*The values are given as the number of patients with the percentage in parentheses.

Patients are counted once for each unique adverse event and may have had > 1 unique adverse event.

¥ Judged to be unrelated to the study treatment.

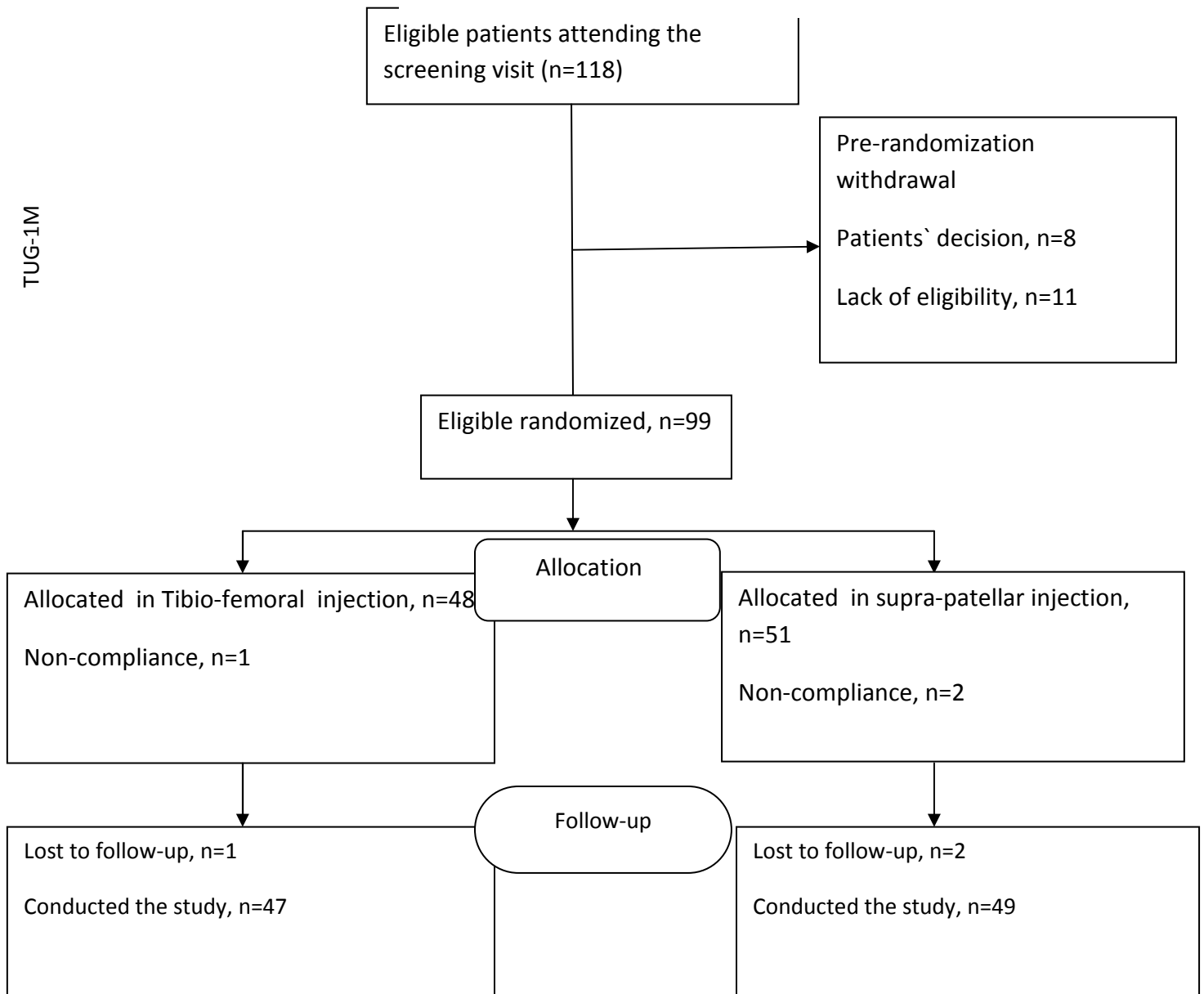


Fig.1 Flow of participants through the trial