ORIGINAL ARTICLE

Investigation the efficacy of intra-articular prolotherapy with erythropoietin and dextrose and intra-articular pulsed radiofrequency on pain level reduction and range of motion improvement in primary osteoarthritis of knee

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Background: Osteoarthritis is one of the most common diseases and the knee is the most commonly affected joint. Intra-articular prolotherapy is being utilized in acute and chronic pain management setting. This study was designed to compare the efficacy of three methods of intra-articular knee joint therapies with erythropoietin, dextrose, and pulsed radiofrequency. **Materials and Methods:** After approval by the Ethics Committee and explaining the therapeutic method to volunteers, 70 patients who were suffering from primary knee osteoarthrosis went through one of the treatment methods (erythropoietin, dextrose, and pulsed radiofrequency). The study was double-blind randomized clinical trial performed from December 2012 to July 2013. Patients' pain level was assessed through the visual analog pain scale (VAS), and range of motion (ROM) was measured by goniometric method. Furthermore, patients' satisfaction was assessed before and after different treatment methods in weeks 2, 4, and 12. For analysis, Chi-square, one-way ANOVA, and repeated measured ANOVA were utilized. **Results:** The demographic results among the three groups did not indicate any statistical difference. The mean VAS in erythropoietin group in the 2nd, 4th, and 12th weeks was 3.15 ± 1.08, 3.15 ± 1.08, and 3.5 ± 1.23, respectively ($P \leq 0.005$). Knee joint ROM in the erythropoietin group in the 2nd, 4th, and 12th weeks was 124 ± 1.50, 124 ± 1.4, and 123 ± 1.53 respectively ($P \leq 0.005$). Satisfaction score in the 12th week in erythropoietin group was extremely satisfied 15%, satisfied 55%, and moderately satisfied 30%, ($P = 0.005$). No specific side-effects were observed. **Conclusion:** Intra-articular prolotherapy with erythropoietin was more effective in terms of pain level reduction and ROM improvement compared with dextrose and pulsed radiofrequency.

Key words: Erythropoietin, knee osteoarthritis, prolotherapy, pulsed radiofrequency, visual analogue pain scale

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INTRODUCTION

Osteoarthritis is one of the most common diseases inflicting human beings, and the knee is the most common joint that is affected by osteoarthritis.[1,2] This disease is painful and is resistant to various pharmacologic or nonpharmacologic treatments. Patients have to take high doses of analgesics, especially nonsteroidal antiinflammatory drugs, which may cause deleterious side-effects if used for long periods.[3] Doctors have always been seeking more efficient treatment methods causing the least side-effects. One of the recent methods for treating acute and chronic joint pain is the utilization of intra-articular prolotherapy.[1,4-6] In this method, through insertion of growth stimulants, the inflammatory cascade is activated, which leads to inflammatory factors release, cellular growth, and acceleration of cartilage growth.

The most commonly used stimulant used for intra-articular prolotherapy is dextrose.[5,6] Now-a-days, using different blood components such as platelets and whole blood has been taken into consideration as well. Erythropoietin, a growth stimulant and red blood cells proliferator released from kidney, has stimulant effects on bone marrow cells' growth.[7-10] Myriad of studies are being done on the effects of erythropoietin on the nonhematopoietic parts such as musculoskeletal system.[11-17] However, there is little experience with its intra-articular functions, but it's usage has shown enhancement in osteochondral healing and cartilage repair in an animal study due to the paucity of previous

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study on human setting for erythropoietin intra-articular injection and because in formerly done animal studies 400 IU/kg erythropoietin have been used.[17-20] This dose (4000 IU) was chosen because it was the smallest used dosage as intravenous (IV) injection. IRCT (IRCT2013092210336N4) and Ethical Committee license (IUMS-13840) achieved for this proposal.

Moreover, radiofrequency has been practiced in recent years to treat numerous painful conditions including neurolysis of the facets, intradiscal application for discogenic pain, trigeminal neuralgia and knee pain. Pulsed radiofrequency has been reported to be effective in treating painful conditions such as sacroiliac joint, shoulder, meralgia paresthetica, occipital neuralgia, and intra-articular prescription.[21-25] This study was conducted to investigate the efficacy of three methods in knee osteoarthritis: Intra-articular prolotherapy with erythropoietin, intra-articular prolotherapy with dextrose, and intra-articular pulsed radiofrequency, and to evaluate their therapeutic effects and to seek a novel and practical solution to more efficient pain management with minimum side-effects. In fact, performing intra-articular injection is technically more convenient than radiofrequency. Furthermore, in terms of cost-effectiveness, these injection techniques are cheaper techniques than the radiofrequency. Hence, in case of being equal or better effectiveness with intra-articular injection, this method could be more rationally recommended than the radiofrequency method.

MATERIALS AND METHODS

After obtained approval by the Ethics Committee of Iran University of Medical Sciences this study was carried out as a double-blinded, randomized clinical trial on patients diagnosed with primary knee osteoarthritis from December 2012 to July 2013. Considering $\alpha = 0.05$, $\beta = 20\%$ and the calculation power 80%; sample size calculated to be 70 patients. The sample size formula was:

$$2N = \frac{4\,(Z\alpha + Z\beta)^2\,\sigma^2}{\delta^2}$$

They were selected from patients who had visited Rasoul Akram Pain Clinic, undergone primary examinations to reject other causes and checked for inclusion and exclusion criteria. The eligibility criteria were: Osteoarthritis according to the American College of Rheumatology's (formerly, American Rheumatology Association)[2] criteria, age 40-70, clinical Class I-III and radiologic Stage 1-3 based on Kellgren–Lawrence criteria.[2] The noneligible criteria were: Drugs or alcohol addiction, hemophilia, knee surgery, rheumatoid arthritis, or other rheumatologic diseases. After explaining the therapeutic method, its benefits and possible side-effects, the measured tools and signing the written consent by them, the patients entered the study. First, the patients were examined for pain level based on visual analog pain scale (VAS) (from 0 to 10 score for pain)[25] and the knee joint range of motion (ROM)[26] values determined through goniometric method were recorded in the pertinent forms. Afterwards, the patient was randomly scheduled by a second colleague for one of the aforementioned methods in the operating room using robust (pseudo-) random number generation software. According to the study's therapeutic method, they underwent prolotherapy or pulsed radiofrequency as delineated below.

After fasting for 6 h the patients were transferred to the pain ward. The venous line was implemented for all patients. Next, the patients were transferred to pain operating room lying supine. Standard monitoring including electrocardiogram, pulse oximetry, and blood pressure monitoring were performed for all patients through an automatic device every 15 min. After scrubbing and covering the area with sterile covers under aseptic conditions, local anesthesia and fluoroscopically guidance, the needle 22G and 10 cm length in Groups 1 and 2, through anteroposterior method from the superolateral part of the patella with an angle of about 45°, was entered into the knee articular area and was confirmed by dye injection. The erythropoietin group (Group 1) received intra-articular injection of 5 cc of ropivacaine 0.5% (naropin, AstraZeneca, Germany) together with 4000 international units of erythropoietin (PD poetin, Pooyesh Darou, Iran). As the matter of fact no previous study was done on human setting and in previous animal study 400 IU/kg erythropoietin was used in investigations. This dose (4000 IU) was chosen because it was the smallest used dosage as IV injection. Moreover, 25% dextrose is the usual dosage for prolotherapy. The dextrose group (Group 2) received fluoroscopically guided intra-articular injection of 5 cc 0.5% ropivacaine together with 5 cc dextrose 25% (Samen Darou, Mashhad, Iran). In the pulsed radiofrequency group (Group 3), under aseptic conditions and local anesthesia with fluoroscopic guidance, through anteroposterior method from the superolateral part of the patella with an angle of about 45°, RF needle G 22, 100 mm long and 10 mm active tip (OWL, Diros Tech, Ontario, Canada) entered the articular area. From the anteroposterior fluoroscopic view the needle tip was embedded at the center of patella. Then, the probe (Diros Tech, Ontario, Canada) was entered and the patients underwent pulsed radiofrequency (20 ms, 2 Hz, 45 V, 15 min, 42°C, 2 cycles). Following the operation, the patients were transferred to the recovery room for 1 h to be monitored for any possible side effects, in case there were not any, the patient was discharged. During the 2nd, 4th, and 12th following weeks they were examined by a second colleague who was not aware of the patients' group, for level of pain according to the VAS (0 for no pain at all, 10 for worst pain imaginable),

and knee joint's ROM was examined through goniometry (Exacta Goniometer, North Coast Medical, USA) the normal knee ROM is −5-140°, and the result were recorded. The acceptable response to treatment was a reduction of at least two units from the expressed pain rate. Besides, age, gender, and level of satisfaction ([1] extremely satisfied,[2] satisfied, [3] moderately satisfied, and [4] not at all satisfied) and the side-effects were recorded and then statistically analyzed. For analysis, Chi-square, one-way ANOVA, and repeated measured ANOVA were utilized, and $P < 0.05$ was considered as statistically significant.

RESULTS

Seventy patients aged 40-70, diagnosed with primary osteoarthritis of knee with clinical Classes of I-III were randomly placed in the three groups under study. The demographic results among the three groups did not show any statistically significant differences [Table 1]. The mean VAS of pain in Group 1 at the beginning of the study was 6.65 ± 0.98, in Group 3 was 7.08 ± 1.41, and in the Group 2 was 7.11 ± 1.03 [Table 2]. This level in Group 1 in the 2nd, 4th, and the 12th week was 3.15 ± 1.08, 3.15 ± 1.08, and 3.5 ± 1.23, respectively; this difference was statistically significant as compared to the other two groups ($P \leq 0.005$). In addition, the mean VAS of pain after the intervention in Group 3 was lower as compared to Group 2; this difference in the 2nd week was statistically significant [Table 2]. ROM of knee in Group 1 in the 2nd, 4th, and 12th week after the intervention was 124 ± 1.50, 124 ± 1.4, and 123 ± 1.53; this difference was statistically significant as compared to the other two groups ($P \leq 0.005$). Furthermore, the level of improvement of knee ROM was higher in Group 3 as compared with Group 2; this difference in the 2nd and 4th week after the study was statistically significant [Table 3]. Regarding the patients' satisfaction in the 12th week, in Group 1 the patients rated their level of satisfaction as, extremely satisfied (15%), satisfied (55%), and moderately satisfied (30%), which was significantly different as compared to the other two groups (Chi-square $P = 0.005$). Furthermore, the level of satisfaction in Group 3 was: Satisfied (29%), moderately satisfied (50%), and not at all satisfied (21%) and in the Group 2, satisfied (30%), moderately satisfied (35%) and not at all satisfied (35%) in the 12th week. The difference between these two groups was not statistically significant [Table 4]. No particular side-effect related to the above-mentioned interventions was observed.

DISCUSSION

Based on our findings, having a prolotherapy with erythropoietin session for primary osteoarthritis patients is more efficient in pain management or improving ROM than having a prolotherapy with dextrose, or intra-articular pulsed radiofrequency session. Normal cells require growth factors for proliferation without which human

Table 1: Patient's demographic findings

Variable	All patients	Erythropoietin group	Pulsed radiofrequency group	Dextrose group	*P* value
Number of patients	70	20	24	26	0.78
Average age (mean±SD)	59.90±8.08	61.15±7.47	56.95±8.31	60.57±7.47	0.45
Male (number)	30	9	11	10	0.23
Female (number)	40	11	13	16	0.33

#$P \leq 0.05$ = Statistically significant; SD = Standard deviation

Table 2: Mean of VAS in different groups

Mean VAS of pain	Erythropoietin group**	Pulsed radiofrequency group***	Dextrose group&	*P* value
Before the intervention	6.65±0.98	7.08±1.41	7.11±1.03	0.349
2nd week	3.15±1.08	3.25±2.00	4.50±1.36	0.005#
4th week	3.15±0.87	3.87±1.70	4.65±1.38	0.002#
12th week	3.50±1.23	5.50±1.93	5.53±1.60	0.002#
P value	0.005	0.05	0.072	

**4000 IU erythropoietin intra-articular; ***Pulsed radiofrequency intra - articular; &Dextrose 25% intra-articular; #$P \leq 0.05$ = Statistically significant; VAS = Visual analogue pain scale

Table 3: ROM's mean scores

Knee joint's ROM's mean scores (mean ± SD)	Erythropoietin group*	Pulsed radiofrequency group**	Dextrose group&	*P* value
Before the intervention	98.08±1.60	95±1.97	101±1.36	0.339
2nd week	124±1.50	105±2.06	106±1.43	0.005#
4th week	124±1.4	110±2.11	110±1.26	0.004#
12th week	123±1.53	113±2.16	113±2.16	0.039#
P value	0.01	0.05	0.112	

*4000 IU erythropoietin intra-articular; **Pulsed radiofrequency intra-articular; &Dextrose 25% intra-articular; #$P \leq 0.05$ = Statistically significant; ROM = Range of motion; SD = Standard deviation

Table 4: Patient's satisfaction in pulsed radiofrequency and prolotherapy groups in the 12th week

Group	Extremely satisfied (%)	Satisfied (%)	Moderately satisfied (%)	Not at all satisfied (%)	*P* value	Total number
Erythropoietin number (%)*	3 (15)	11 (55)	6 (30)	–	0.04	20
Dextrose number (%)**	–	8 (30)	9 (35)	9 (35)	0.123	26
Pulsed radiofrequency number (%)***	–	7 (29)	12 (50)	5 (21)	0.05	24
P value		0.15	0.05	0.05		

Chi-square $P = 0.005$; *4000 IU erythropoietin intra-articular; **Dextrose 25% intra-articular; ***Pulsed radiofrequency intra-articular; $P \leq 0.05$ = Statistically significant

body's evolution will stop.[1,7] Studies have indicated that growth factors such as transforming growth factor β-1, erythrocyte growth factor, and the platelet factors released from the fibroblasts cause enhancement of cell proliferation.[7-9] The effect of the inflammatory factors on cartilage restoration has been exhibited in human and animal studies.[8,9] Inflammation leads to secondary growth factors' production; and injecting inflammatory factors, without causing any damage, leads to the outset of proliferation phase. Glucose, sodium borate, and some other injection materials work with the same mechanism.[4-6,27]

Erythropoietin is primarily known as a hematopoietic hormone. However, following the discovery of erythropoietin receptors outside the hematopoietic system, numerous studies have been carried out on its effect on the nonhematopoietic body parts.[11,12] In several studies, the effects of erythropoietin on the musculoskeletal system were investigated.[10,16] It has been suggested that this hormone can affect osseous tissue both directly and indirectly. However, there is no consensus on erythropoietin's effects on musculoskeletal tissue restoration yet.[16,17,20,28] One of the most important effects of erythropoietin is its protective effect on the endothelial cells of the small brain vessels, which assumes an important role in protecting the central nervous system.[18] However, compared with the hematopoietic effects, for starting the erythropoietin cellular protective effects, prescription of high doses of medication is required. In a study conducted by Holstein *et al*. on bone restoration speed, two groups (one with prescribing erythropoietin, and the other without prescribing erythropoietin) were investigated.[28] During the study, 500 IU/kg/day of intraperitoneal injection of erythropoiethin was prescribed. After 2 weeks, the amount of appearance of erythropoietin receptors in chondrocytes of the osseous defects restoration area demonstrated a marked increase. Prescribing erythropoietin in this study resulted in a substantial rise in the speed of the osseous defects restoration. Nevertheless, the beneficial effects of erythropoietin in the 5th week were not tractable. To investigate the effects of erythropoietin on osseous defects restoration Mihmanli *et al*. carried out a study on a number of adult male rabbits with fractured mandible bone.[29] Restoration and repair in the group receiving erythropoietin occurred faster and with a higher quality; moreover, the rate of the osteoblast cells and angiogenesis in the area of composition of the new bone was higher in this group. Erythropoietin had also resulted in considerable reduction of the osteoclast cells' activity, heightening osteoblasts' activity, and developing vessels as compared with the control group. Diverse studies have demonstrated the effects of erythropoietin on bone repair; however, the regulatory mechanisms of this process are not recognized yet. Furthermore, in a variety of studies, the cellular protective effects of erythropoietin on the cardiovascular system and retina have been shown. In separate studies done by Calvillo *et al*.,[30] Moon *et al*.,[31] and Cai *et al*.[32] in 2003, the potential ability of erythropoietin for treating myocardial infarction was shown. Yet, no study has been conducted on the intra-articular prescription of erythropoietin on human. Even no animal studies have been reported on the effect of erythropoietin on joints.

Moreover, in several studies pulsed radiofrequency has been reported as a suitable mode with minor side-effects for treating many painful conditions. Pulsed radiofrequency is a method in which a particular voltage is applied next to the nerve, the waving duration is about 20 ms and the resting time is 480 ms, which is applied at 2 Hz frequency, and the long resting time between the waves lets the stimulated area to cool, in a way that the area's temperature is kept under 45° centigrade, a temperature that is below the tissue destruction threshold. Pulsed radiofrequency effect mechanism is not completely identified; investigations have brought up a nervous moderator effect for it. However, Sluijter *et al*. put forward two hypotheses:

1. Inhibition of C fibers leads to inhibition of neurotransmission of pain and
2. Influence on the immune system causes pain reduction. In this action mode, the created electric field influencing the immune cells causes the reduction of inflammatory factors such as interleukin β-1, tissue necrosis α factor, and interleukin-6. It also has a general and widespread effect through affecting cell connections.[33] Despite the reports of many studies conducted on radiofrequency in the conventional mode, articles on the subject of pulsed radiofrequency are scarce. In a study by Shah and Racz conducted on shoulder joints, they reported performing pulsed radiofrequency on supraspinatus nerve effective in one case.[34] Sluijter *et al*. have reported positive long- and short-term effects on six cases of

different joints pain (cervical facet, thigh, shoulder, sacroiliac joint, atlantoaxial joint, and wrist joint).[33] Some articles have reported the positive effects of this mode for short-terms on knee osteoarthritis[23] and some have shown positive results for 6 months on saphenous nerve for managing knee pain. Halim *et al*. promulgated that pulsed radiofrequency in C1-C2 for cervicogenic headache, reduced pain level more than 50% for 1-year.[35] Pulsed radio frequency on articular branches of femoral nerve and obturator has been shown to be a suitable method in the knee joint pain management.[36] Taverner *et al*. have reported satisfactory short-term effects for controlling knee pain.[37] Based on the results of this study, the effects of performing one session of pulsed radiofrequency on minimizing patients' pain in a short time has been very impressive (reduction of 40-50% of pain level up to the 4th week); however, the long-term effects of this method are still under question; these findings are in the same line with Taverner's results.[38-42] In our investigations, prescribing dextrose has resulted in lower pain level and amelioration of joint's ROM. In a study carried out by Reeves and Hassanein six sessions of injecting intra-articular dextrose 10% every 2 months on knee osteoarthritis cases, reduced 63% of articular inflammation, 44% of pain level, improved joint's ROM 14°, and ameliorated knee balance by 85%.[5] Another study conducted by the same group following six sessions of prolotherapy for osteoarthritis of finger joints, with a 1-year follow-up, showed a lack of disease progression, 53% of pain reduction, and amelioration of joints' ROM up to 8°.[6] Although pain reduction and improvement of joint's ROM were lower in our study as compared to the other two groups, our findings are in line with the above-mentioned findings. In this study, 70 primary knee osteoarthritis patients were randomly divided into three groups (erythropoietin group with 20 patients, pulsed radiofrequency group with 24 patients, and dextrose group with 26 patients). All three groups experienced pain minimization with different levels, although this effect was much more considerable in the group receiving erythropoietin. The undertaken investigations in this study indicate that intra-articular prescription of erythropoietin leads to the better improvement of the knee joint ROM and patients' higher satisfaction. In conclusion, our findings showed that intra-articular prescription of erythropoietin in the joint is more effective for knee osteoarthritis patients' pain management than prolotherapy with dextrose or joint intra-articular pulsed radiofrequency. Moreover, one session of performing pulsed radiofrequency in the knee joint resulted in lower pain level and improved knee joint ROM. This effect was more pronounced in the short-term (the first 4 weeks); as time passed by it became less effective as also demonstrated by Taverner *et al*. No particular side-effect was observed among the patients.

There were some limitations to this study, including:

1. The follow-up time was limited to 12 weeks, which did not allow judgment for long-term effects. It is necessary to conduct studies with more than 3 months follow-up.
2. Regarding the lack of literature on intra-articular prescription of erythropoietin, the amount of prescribed medications was not specified. However, in the mentioned animal studies on osseous fracture, amounts up to 500 IU/kg were used as well. Although much lower than this amount has been suggested, it is likely to obtain better results by increasing the prescribed amount in future studies. Besides, this study was the first to investigate the intra-articular prescription of erythropoietin in the knee joint, taking beneficial and various effects of erythropoietin in the nonhematopoietic parts into account, more studies with more factors and increased prescribed medications are required to prove its possible salutary effects on the articular cartilage tissue.

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AUTHOR'S CONTRIBUTION

- PR; study design, conduct of the study, data interpretation, obtaining equipements.
- FI; conduct of study, administration technical and scientific revision of the article,
- SHRF; literature search and clinical analysis.
- AAN; data interpretation and clinical analysis and manuscript preparation.
- SRE; data interpretation and critical revision of the article.
- MZ; study design, data collection.

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