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Histological Evaluation of Intra-Articular Injections of Medical Ozone on the Repair of Experimentally Induced Articular Defects in Dogs

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Damage to articular cartilage can eventually lead to osteoarthritis, a debilitating, degenerative joint disease. The limited natural healing ability of cartilage and the limitations of currently available therapies make treatment of cartilage defects a challenging. In recent years, interest has increased in the effects of medical ozone, which can be used safely inside the joint, and the ease of preparation has increased its use in vet clinics.

Aim: Aim of research, we investigated the utilization of medical ozone to treatment cartilage damage, the dogs were euthanized on day 56 after surgery. Histological assessments of the repair tissue were assessed for the treated and control defects at various times.

Methodology: Methodology ten healthy adult mongrel dogs were used in the current study, all animals underwent surgery to create a defect in the middle of the trochlear groove of the left

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femoral bone. The dogs were divided into two equal groups, Group I (control group) was left without treatment. While group II (O_3 group) was treated by the injection of medical ozone.
Results: The result of the ozone group showed superior results in the cartilage development and reduction of the inflammatory process than control group, The cartilage appears to occupy in ozone group more than control group. the signs of mineralization occur in the peripheral portion, particularly in the edges near the trochlear groove in the ozone group.
Conclusion: Conclusion the process of injecting medical ozone into the joint affected by osteoarthritis has proven to be significantly effective compared to dogs that were left untreated. Therefore, we can go towards recommending the use of medical ozone as an effective and safe non-surgical treatment, continued for at least two months, in the treatment of severe osteoporosis.

Keywords: Articular cartilage; articular cartilage defect; medical ozone.

1. INTRODUCTION

The articular cartilage tissue is avascular, a neural and without lymphatic vessels and it is composed of a single cell type [1]. The chondrocyte and for this reason, it has a narrow range of follow-injury-based self-repairing [2]. Osteoarthritis (OA) is the most joints disease that shows joint pain, loss of function and impaired mobility [3].

Regardless of the actual prevalence of osteoarthritis, its progressive and debilitating nature makes it a significant welfare issue for dogs [4]. osteoarthritis is still a lack of treatment options that provide sustained pain relief and improved quality of life without the risk of significant side effects [5]. Ozone is made up of three oxygen atoms with a cyclic structure. ozone may be found in the stratosphere of nature [6]. However, it may also be created artificially by exposing diatomic oxygen (O2) to a high-voltage electrical discharge [7].

Ozone therapy is often used to treat osteoarthritis. ozone is empirical, with limited studies providing histological and biochemical evidence of its effectiveness [8]. Ozone is now another suitable option for intra-articular injection in treating stifle osteoarthritis [9]. Ozone has been shown to reduce pain and inflammation associated with stifle osteoarthritis [10,11,12]. Upon interacting with bodily fluids, by inducing the release of various factors from endothelial cells and restoring an equilibrium of the cells redox balance, O_3 serves as a bioregulation component [13].

Aim of Study: Evaluate the efficacy of medical Ozone on healing of articular cartilage degeneration in dogs.

2. MATERIALS AND METHODS

2.1 Experimental Animals Design

Ten healthy mongrel dogs, aged between 1 and 2 years, with an average weight of 20 kg, were utilised. The animals were housed in individual enclosures and administered a dosage of 0.2 mg/kg of Ivermectin (Ivomec, Holland). then, the dogs were administered anaesthesia using a combination of xylazine-hydrochloride 2% at a dosage of 5 mg/kg body weight intramuscularly (I/M) and ketamine-hydrochloride 10% at a dosage of 10 mg/kg body weight I/M [14]. The dogs were positioned in lateral recumbency on the surgical table, and the left hind limb was prepared aseptically for surgery. The dogs were created defect in the middle of the trochlear groove of the left femoral bone. This defect had a diameter of 8 mm and a depth of 4 mm. The defect was induced using a manual drill. A longitudinal incision was made from the proximal end of the patella to the site of attachment of the patellar ligament to the tibial tuberosity. Bleeding was controlled using haemostatic forceps. The stifle joint was flexed, causing the displacement of the capsule and adjacent tissues towards the centre in order to access the distal portion of the femoral articular surface. Subsequently, the surgical site was washing with a solution of normal saline (0.9% NaCl) in order to eliminate any debris present. The surgical incision was closed in the standard manner using polyglactin 910 size (2-0) to bring the joint capsule, lateral fascia, and subcutaneous tissue together. Each layer was closed separately using a continuous suture pattern. The skin was then closed using an interrupted suture pattern with nylon size (2-0) suture material. The dogs were separated into two groups of identical size, with each group including 5 dogs. Group I, the control group, were dogs left without treatment. The ozone group, the group II, received intra-articular

injection of medical ozone at a concentration of 37.3 mg/ml [15]. The dogs were euthanized 56 days after the procedure.

2.2 Ozone Production

Ozone was freshly obtained from an ozone generator (Model NO: ATO-MD 520) (Fig. 1). After creating defect, the dogs were prepared for ozone injection, after which the joint is prepared aseptically. then, the ozone generator is connected to the oxygen bottle and the oxygen regulator is set to ½ L/min. then, the ozone generator is set to a concentration of 37.3 mg/ml. Leave 30 seconds and then connect the 10 ml syringe to the gas outlet [16]. The ozone is then injected into the stifle joint after ensuring the correct location by withdrawing a sample of synovial fluid (Fig. 2).

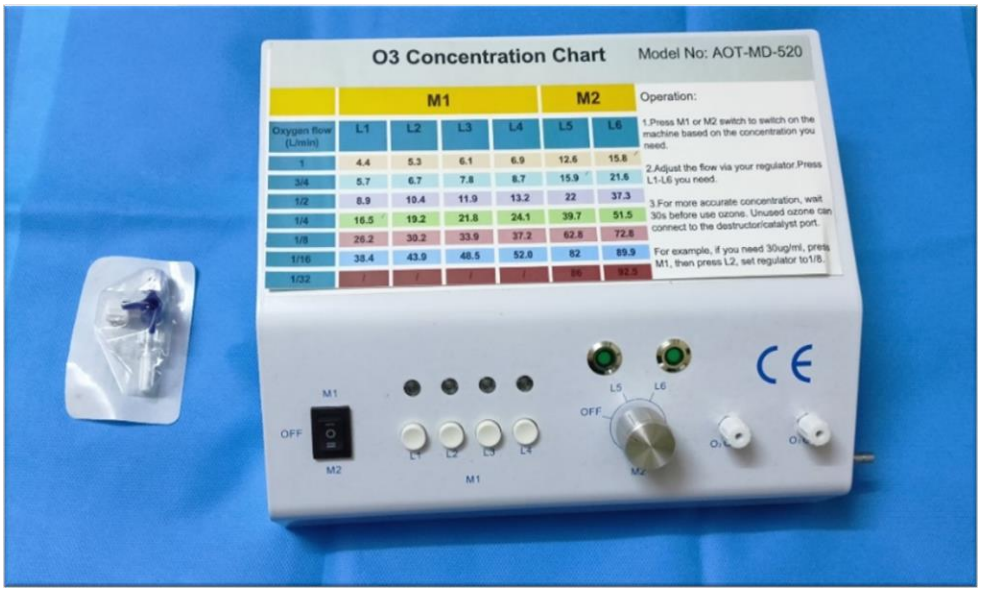

Fig. 1. Shows ozone generator

Fig. 2. show ozone injected into stifle joint

Table 1. shows the scoring of cartilage healing [18]

Sl. No.	parameter	Ratio	Score	Notes
1	Inflammation	No inflammation	0	Parameter ration is an
		≥ 25	1	average of 10 fields per
		$26-50$	2	animal for all animals in
		$51-75$	3	the group
		Above 75%	4	
2	Angiogenesis	No angiogenesis	0	
		≥ 25	1	
		$26-50$	2	
		$51-75$	3	
		Above 75%	4	
3	Fibrous tissue	No fibrous tissue	0	
		≥ 25	1	
		$26-50$	2	
		51.75	3	
		Above 75%	4	
4	Regenerated cartilage	No cartilage regeneration	0	
		≥ 25	1	
		$26-50$	2	
		$51-75$	3	
		Above 75%	4	

2.3 Histopathological Evaluation

Immediately after euthanized, the stifle joint was opened, and chondral samples of site of operation were harvested. Samples were soaked with PBS throughout the test period. They have been covered with 10% neutral buffed formalin. After that, the specimens were de calcinated, cut, and stuffed with paraffin. They cut and stained the sagittal sections with haematoxylin and eosin from there. A pathologist evaluated sections microscopically [17].

2.4 Scoring of Cartilage Healing

Healing of the cartilage was scored according to (ICRS) scoring system [18], which includes the criteria shown in the Table 1.

3. RESULTS

Control group showed that the site of operation was filled with collagen fibres (score 4), and newly generated blood vessels were evident and numerous (score 3). However, this group does not feature cartilage regeneration (score 0), as showed in Figs. 3 and 4. Ozone group showed marke cartilage regeneration in the operation site (score 3); the regenerated cartilage is not well differentiated. also, there is still fibrous tissue between the regenerated cartilage and the original one in the area surrounding the operation site; the fibrous tissue in this group shows a score (1), and the newly generated blood vessels are showing moderate development (score of 1), (Figs. 5 and 6). show in Table 2.

Table 2. shows the scoring of the changes that associate the cartilage healing process

Group	Parameter	Value	Score
1	Inflammation	10 ± 0.1	1
	Angiogenesis	54 ± 0.31	3
	Fibrous tissue	88 ± 0.12	4
	Cartilage regeneration	0	0
3	Inflammation	0	0
	Angiogenesis	19 ± 0.00	1
	Fibrous tissue	23 ± 0.09	1
	Cartilage regeneration	72 ± 0.20	3

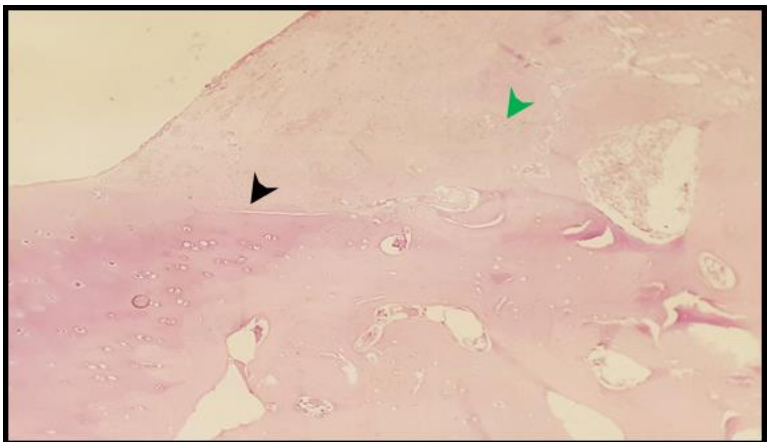

Fig. 3. Shown damage of articular cartilage surface (control group) well demarcated line between the original cartilage and the operation site (black arrow) chondrocyte differentiation in the operation site (green arrow) H&E 10X.

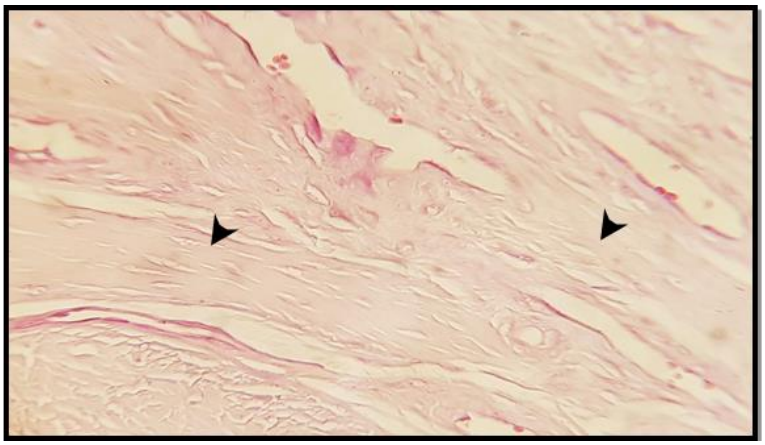

Fig. 4. Shown damage of articular cartilage surface (control group) intensive collagen ingrowth in the site of operation (black arrow). H&E 40X

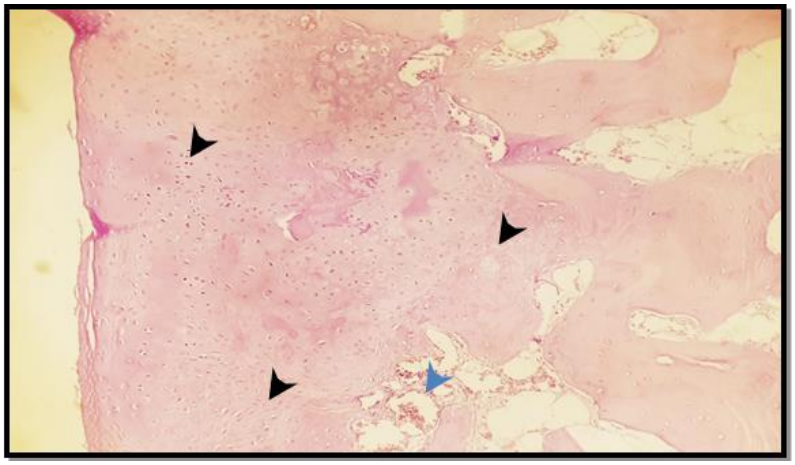

Fig. 5. Shown damage of articular cartilage surface (ozone group) marked cartilage regeneration in the grafting site (green arrow) and newly generated blood vessels around the new cartilage (blue arrow). H&E 4X

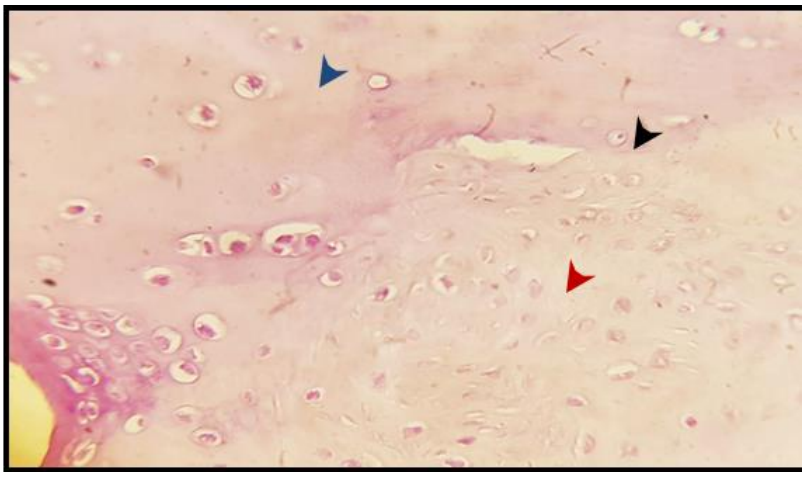

Fig. 6. Shown damage of articular cartilage surface (ozone group) a marked area between regenerating cartilage in the grafting site (black arrow) and the original cartilage (blue arrow), regenerating cartilage (red arrow). H&E 40X

4. DISCUSSION

These ozone groups showed marked cartilage development, which appears to be more fixed in the defect site, surrounded by an area of intensive collagen deposition; the newly generated cartilage was more than in the control group, superior Results in cartilage development and reduction of the inflammatory process in the defect site according to Yang et al.,[19]. shown this result because ozone (O_3) acts as a bioregulator by releasing factors from endothelial cells and normalising the cellular redox balance when it comes into contact with a biological fluid. Ozone is capable of altering the levels of cytokines such as interleukin 8, TNFa, transforming factor beta1 and platelet-derived growth factor PDGF, These Results were confirmed by de Sire et al. [20]. 'The authors have shown that it is capable of repairing degenerated articular cartilage in animals with stifle osteoarthritis to a certain extent and increases their capacity for exercise [21]. Injection of ozone to treat cartilage defect is intra-articular, dissolving it in the entire joint cavity and affecting the treatment [22]. Furthermore, ozone reacts with polyunsaturated fatty acids, antioxidants, reduced glutathione, and albumin These results matched those of Mehana et al. [23].

5. CONCLUSION

Demonstrated that the medical ozone can be used to significantly improved cartilage repair, cartilage development and reduction of the inflammatory process in the cartilage defect.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Authors hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

ETHICAL APPROVAL

The Ethics Committee of the College of Veterinary Medicine, University of Basrah, approved all operations conducted in this investigation.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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