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Mesenchymal Stem Cell Therapy in a Case of Bilateral Chronic Osteoarthritis Occurring in Articulatio Genu in a Dog of Cane Corso Breed

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ABSTRACT

Background: In this study, mesenchymal stem cell therapy and the findings were discussed in a bilateral chronic osteoarthritis case in a Cane Corso breed dog.

Methods: A patient was brpought to the clinic with the complaint of lameness, malaise, fear, anxiety and reluctance in running, stair climbing and descending movements. In the orthopedic examination, Art. Genu was found to have pain in flexion and extension movements and on radiological examination, joint space was reduced and bilateral chronic osteoarthritis was found. The patient was sedated and mesenchymal stem cells were injected into both knee joints. These procedures were repeated twice with an interval of three weeks.

Result: The joint space of both knees widened and the joint fluid increased after the treatment. In addition, there was a relief in flexion and extension movements on the both knees. The patient's lameness decreased and he did not experience uneasiness when going up and down the stairs and there was a significant improvement. It was determined that mesenchymal stem cell therapy gave a positive response in the current osteoarthritis case. It has been concluded that this method can be applied as an alternative to steroids, NSAID (Nonsteroid anti-inflammatory drugs) and GAG (Glycosaminoglycan) applications in similar cases.

Key words: Arthritis, Cane corso, Mesenchymal stem cell, NSAID, Steroids.

INTRODUCTION

The general name given to the tissues in loose connective tissue structure that takes place in the embryo development and later in the life of the fetus, starting from the differentiation of the epiblast, is called "mesenchyme". Mesenchymal stem cells differentiate in tissues such as fat, cartilage, muscle, tendon and bone and ensures the repair of damage in these tissues (Can, 2014; Phinney and Prockop, 2007). In a study on mice after the discovery of hematopoietic stem cells, the bone marrow stroma was transplanted into another tissue. It was proved in this study in 1966 that this transplanted tissue can transform into fat, cartilage and bone tissue (Friedenstein *et al*., 1966). Depending on the gene expression changes of stem cells, their morphology, behavioral characteristics and functions also change. In this way, they show the ability to make cells similar to themselves in the tissue they are transplanted (Gardner, 2002; Krieger and Simons, 2015). Mesenchymal stem cells have surface markers. With the detection of these markers, mesenchymal stem cells can be recognized. Being positive for CD 73, CD90, CD105 molecules is accepted as the basic marker (Can, 2014).

Even though the main source for mesenchymal stem cells is bone marrow, apart from this, mesenchymal stem cells are isolated and separated from many tissues such as bone, dental pulp, liver, cord blood, placenta, amniotic fluid, synovial fluid and even peripheral blood, thanks to their adhesion properties (Ding *et al*., 2011).

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With the increase in studies on mesenchymal stem cells, it has been reported that it is used in cases other than orthopedics such as medulla spinalis, myocardium, kidney, as well as its use in damage to orthopedic tissues (Lakshmipathy and Verfaillie, 2005; Patel *et al*., 2013). Again, there are studies showing that it is used successfully in diabetes, heart, liver and kidney diseases and some autoimmune diseases (Undale *et al*., 2011; Otto and Rao, 2004). Mesenchymal stem cells were first used in the field of orthopedics in 1951. Especially successful results in fracture repair and metabolic bone diseases have been discussed (Undale *et al*., 2011). Delayed union or nonunion of fractures in orthopedics accounts for 5-10% of all fractures. For this reason, new methods were needed especially in the treatment of such cases. After understanding the

osteogenic potential of mesenchymal stem cells *in vitro*, there are studies showing positive effects on bone regeneration with their osteoblastic activities *in vivo* (Javazon *et al*., 2004; Kılıç *et al*., 2007). In a study, it was reported that in the use of mesenchymal stem cells in cartilage damage caused by traumatic or degenerative reasons in the knee joint, both chondrogenic and osteogenic differentiation of mesenchymal stem cells and full cartilage formation and mesenchymal stem cells have a positive effect on bone and cartilage repair in macroscopic, histological and immunofluorescent studies (Tatebe *et al*., 2005). Treatment with mesenchymal stem cell applications has become an important option in diseases that cause cartilage damage such as rheumatoid arthritis, senile osteoarthritis, osteochondritis dissecans (Sonomoto *et al*., 2014). Among the knee joint components, the menisci take an important place. Menisci are important especially in the load distribution of the body and stabilization of the knee joint (Kurzewil and Friedman, 2002). The operative treatment of meniscal tears is repair or resection according to the peripheral or central zone (Yoon and Park, 2014; Jeong and Lee, 2012).

This study was carried out to test the effectiveness of mesenchymal stem cell therapy in a bilateral chronic osteoarthritis case occurring in articulatio genu in a Cane Corso breed dog.

MATERIALS AND METHODS

Long-term malaise and lameness occurred in a 7-year-old, uncastrated, 40 kg male Cane Corso dog. He was brought to the Academy Animal Hospital, Bursa, Türkiye with the complaints that he experienced anxiety and fear while running and going up and down the stairs in 2022. The patient was taken to another veterinary clinic due to these complaints and that Glycosaminoglycan (GAG) and Nonsteroidal Anti-Inflammatory (NSAID) drugs were used for about six months, but they could not get a positive response in 2021.

In the orthopedic examination of the patient; Art. Coxae movements were found to be normal. Both patellae were in their normal position. Normal response was obtained from the cruciate ligament tests applied to both knees. There was a painful limitation in flexion and extension movements in genu. The patient's hip and knees were imaged in the ventro-dorsal position with a high-resolution 3D volumetric computed tomography device (Vigamo, Hasvet, Turkey). On the CT scan, narrowing of the joint spaces of both knees and chronic osteoarthritis were observed (Fig 1).

Despite the fact that GAG and NSAID were administered to the patient approximately six months no positive response was obtained and considering the age of the patient, it was decided to perform intraarticular mesenchymal stem cell therapy. The patient was sedated by 2.5 cc intravenous injection of xylazine (Rompun, Bayer, Germany). Both knees were shaved and prepared for the process according to the rules of asepsis antisepsis. Sterile 3 million units of mesenchymal stem cells were injected into the left knee and 2 million units of mesenchymal stem cells were injected into the right knee. The medium used was Dulbecco's Modified Eagle Medium (DMEM) with Sodium Pyruvate and L-Glutamine Free (Biological Industry, Israel). There are 1 g/l D-Glucose (low glucose) and 1.1 g/l sodium pyruvate in the DMEM used. Canine adipose tissue was used as a source of mesenchymal stem cells. This tissue was stored in liquid nitrogen at-78 degrees K. It was thawed before use. After thawing, it was kept under the cold chain and injected within a maximum of 3 hours. This application was repeated twice with an interval of three weeks (Fig 2). After the last injection applied to the patient, radiological images were taken again and both Art genu were checked.

RESULTS AND DISCUSSION

Due to chronic osteoarthritis in both knees of the patient, pain while running, difficulty in going up and down stairs, uneasiness and fear almost disappeared after mesenchymal stem cell therapy applied with an interval of two weeks. The patient's relief was observed both by the patient's owner and during clinical examinations. In addition, in radiological examination findings; It was observed that the joint space of both knees was enlarged. Increased joint space can be considered as a radiological finding of decreased inflammatory reactions in the region (Fig 3).

In a study, it was reported that in the use of mesenchymal stem cells in cartilage damage caused by traumatic or degenerative reasons in the knee joint, both chondrogenic and osteogenic differentiation of mesenchymal stem cells and full cartilage formation and mesenchymal stem cells have a positive effect on bone and cartilage repair in macroscopic, histological and immunofluorescent studies (Tatebe *et al*., 2005). In the present study, it was found that local injections of mesenchymal stem cells applied to both knee joints were effective. Optimal results were obtained, especially in the second week. Positive feedback from the patient's owner was most ideal during this period. The reason for this is that the stem cells settle in the area at least 2-3 weeks after the injection, transform into the original chondrocyte cell and initiate the release of natural hyaluronic acid fluid. We attribute this to the fact that the peak effect was achieved after the second application. In general, a second dose is sufficient in such cases of osteoarthritis. In the next process, it will be beneficial to repeat the injection once a year for preventive purposes.

Current treatment for knee osteoarthritis includes conservative and operative treatments (Price *et al*., 2018; Wilson *et al*., 2019). Intra-articular injection of hyaluronic acid and administration of oral anti-inflammatory drugs are recommended for the treatment of osteoarthritis (Zhang *et al*., 2010; Bannuru *et al*., 2019). Mobility Plus® tablets may be recommended for the treatment of rheumatoid arthritis and

osteoarthritis in dogs (Ashwath *et al*., 2021). The methanol extract of Gossypium herbaceum was found to have an antiarthritic effect. A higher test dose of methanol extract of Gossypium herbaceum (MEGH) has proven to be significantly effective in the treatment of rheumatoid arthritis (Reddy and Raju, 2018). α-Bisabolol treatment ameliorates CIA-induced inflammation by inhibiting proteases and inflammatory mediators, suggesting that á-bisabolol might be a potential candidate against arthritis inflammations (Gao *et al*., 2022).

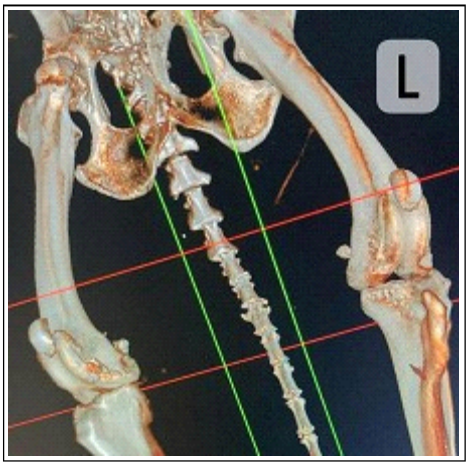


Fig 1: CT image of the patient.

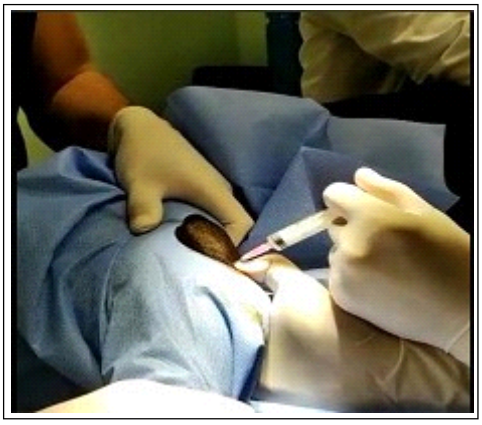


Fig 2: Mesenchymal stem cell injection.

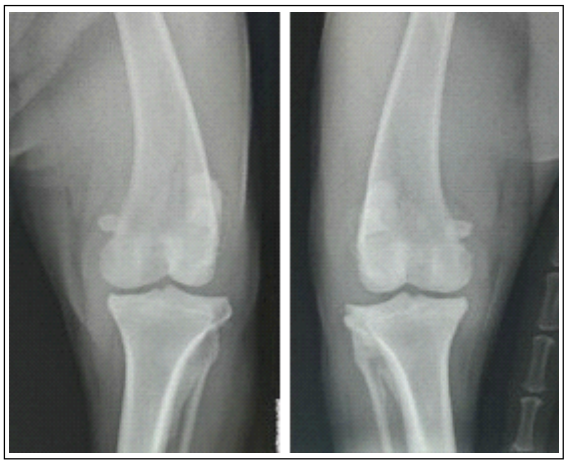


Fig 3: Control radiograms (left/right) of the patient taken after the last injection.

Mesenchymal stem cell applications are relatively costly compared to other conventional applications. Synthetic preparations containing hyaluronic acid should also be applied 3-4 times with an interval of one week. In addition, it is repeated every year for protective purposes. However, synthetic preparations containing hyaluronic acid only increase the joint fluid. Mesenchymal stem cell application provides chondrocyte cell formation, creates repair in damaged tissue and supports the formation of natural joint fluid.

NSAIDs quickly relieve pain but have very serious side effects (da Costa *et al*., 2017). Although the administration of intra-articular steroid drugs to patients with osteoarthritis reduces pain, it deforms chondrocytes. Intra-articular cortisone applications are not recommended in recent years. Oral administration of NSAIDs to such patients may also reduce pain, but may cause serious damage to the gastrointestinal tract.

CONCLUSION

Stem cell therapy has started to be used in human medicine especially in the last ten years. This treatment method has been applied in veterinary medicine as of 2017. Stem cells are the basic cells that form the structure of all tissues and organs in the body and thus can turn into all cells in the organism. Stem cells applied locally or systemically play a major role in repairing damaged tissues or organs.

Stem cell therapy is applied in many cases such as some ocular diseases, wound healing, fractures that do not heal and autoimmune allergies. In this study, it was understood that a 7-year-old male Cane Corso breed dog had chronic osteoarthritis in both knee joints that did not respond to GAG and NSAID drugs and responded positively to mesenchymal stem cell therapy. Mesenchymal stem cell injection can be as an alternative to steroids, NSAID (Nonsteroid anti-inflammatory) drugs and GAG (Glycosaminoglycan) applications in similar cases.

Conflict of interest: None.

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