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Percutaneous transplantation of allogenic bone marrow-derived mesenchymal stem cells for the management of paraplegia secondary to Hansen type I intervertebral disc herniation in a Beagle dog

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Abstract

Background: Intervertebral disc herniation (IVDH) is one of the common causes of spinal cord injury (SCI) in dogs. It is commonly treated by performing surgical decompression that involves the removal of the extruded disc material. However, the recovery rates after surgical interventions are variable and many times unsatisfactory. This report aims to document a case of paraplegia associated with IVDH in a Beagle dog and its therapeutic management using allogenic bone marrow-derived mesenchymal stem cells (aBM-MSCs). **Case description:** The dog was presented with paraplegia that was initiated three weeks back. Based on the findings of computed tomography (CT), the condition was diagnosed as Hansen type I IVDH at T_{12}-T_{13} intervertebral space. **Findings/treatment and outcome:** Neurological examination was performed to grade the neurological deficit. The isolation, culture, and characterization of aBM-MSCs were done as per the standard protocol. The prepared cell suspension of aBM-MSCs was percutaneously transplanted to the spinal cord parenchyma at the site of injury. A total of four doses of 1×10^6 cells were given at an interval of 15 days along with methylcobalamin and gabapentin orally. Improvement was evaluated based on the neurological examination and grading. Considerable improvement was noticed after the first dose of aBM-MSCs. The animal started complete weight bearing on its pelvic limbs after two doses. **Conclusion:** Percutaneous transplantation of aBM-MSCs might have played an important role in reversing the neurological deficits secondary to IVDH in this dog. Further studies are required preferably in a larger population to confirm the efficacy of aBM-MSCs therapy in ameliorating neural deficits associated with IVDH.

Key words: Dog, Intervertebral disc, Mesenchymal stem cells, Paraplegia, Spinal cord injuries

Introduction

Type I intervertebral disk disease (IVDD) is a result of intervertebral disk degeneration that is severe enough to cause extrusion of the central nucleus pulposus into the vertebral canal. This can result in compressive injury to the spinal cord and the continued compression may cause severe impairment in neurological function (Gordon-Evans *et al*., 2019). Degeneration of disc can result in two types of disc herniation, extrusion, and protrusion. The extrusion of the intervertebral disc associated with chondroid degeneration is called Hansen type I lesion. It is commonly observed in chondrodystrophic dog breeds (Smolders *et al*., 2013). Intervertebral disc herniations (IVDH) occur most commonly in the thoracolumbar region, causing upper motor neuron (UMN) deficit in the pelvic limbs (Ruddle *et al*., 2006). The dogs that are affected by thoracolumbar IVDD may have classical clinical signs ranging from back pain to paralysis.

Diagnosis of IVDH in dogs can be done using diagnostic modalities like myelography, computed tomography (CT), and magnetic resonance imaging (MRI). Among these, high-field MRI is considered to be the ideal modality for the diagnosis of dogs with disc disease. Plain CT can be used to identify the extrusion of mineralized disc material, a common condition in chondrodystrophic dog breeds (Robertson and Thrall, 2011). Surgical management of IVDH involves decompression by performing hemilaminectomy (Mateo *et al*., 2019).

The transplantation of bone marrow-derived mesenchymal stem cells (BM-MSCs) can be considered as a promising, safe, and reliable treatment strategy for the management of chronic spinal cord injury (SCI) in canines (Sarmento *et al*., 2014; Sharun *et al*., 2020). Percutaneous transplantation of neurogenically-induced BM-MSCs has been used for the treatment of paraplegia in dogs secondary to IVDD after performing surgical decompression (Besalti *et al*., 2015). Dogs with chronic

SCI secondary to thoracolumbar IVDH have been successfully treated by fetal bone marrow stem cells injected intramedullary immediately following hemilaminectomy (intra-operative transplantation) (Sarmento *et al*., 2014). Further, transplantation of adipose-derived MSCs into the injured spinal cord parenchyma following decompression surgery was associated with better clinical outcome compared to decompression surgery alone (Kim *et al*., 2016). Percutaneous transplantation has also been attempted using autologous enriched olfactory ensheathing cells in dogs with chronic IVDD that had not received decompressive surgery (Granger *et al*., 2012).

The aim of this clinical communication is to put on record the diagnosis and treatment of Hansen type I IVDH without surgical decompression using percutaneous transplantation of allogenic BM-MSCs (aBM-MSCs) in a Beagle dog.

Case description

A four-year-old, male Beagle dog was presented with spontaneously developed paraplegia three weeks back. The patient was previously treated and was found to be refractory to medical management using steroids and other supportive therapy. Upon examination, the patient was found to be active and alert. The animal was dragging its pelvic limbs while walking indicating paraplegia. Neurological examination revealed the absence of superficial pain perception whereas deep pain was conserved. Postural reactions like conscious proprioception, wheel barrowing, hopping, tactile, and visual placing were absent in the pelvic limbs. All the spinal reflexes (patellar reflex, flexor reflex, and perianal reflex) were found to be normal and the animal had voluntary control over urination. The panniculus (cutaneous trunci) reflex was found to be absent caudal to T_{12} vertebrae. The dog was classified under the grade 3 (paraplegia with intact deep pain perception) category of neurological signs as suggested by Wheeler and Sharp (2005). Based on the neurological examination, neurolocalization of the lesion was done to the T_3-L_3 segment of the spinal cord. Further investigations were recommended to confirm the findings of the neurological examination.

Diagnostic imaging

Radiographic examination identified reduced disc space between the vertebral body of T_{12} and T_{13} thoracic vertebrae (Fig. 1). Computed tomography was performed to localize the site of the lesion. Computed tomography scans identified herniated disc material within the vertebral canal of the T_{12} and T_{13} thoracic vertebrae. Mineralized disk material from the T_{12}-T_{13} intervertebral disk space was found to be herniating into the vertebral canal, resulting in focal spinal cord compression (Figs. 2a-b and 3a-b). Hence, the case was diagnosed as paraplegia secondary to Hansen type I IVDH at T_{12}-T_{13} intervertebral space.

Treatment

All procedures performed are in accordance with the ethical standards of the institution at which the studies were conducted. The owner declined the request for surgical decompression due to its invasive nature. Hence, we suggested stem cell therapy using BM-MSCs as an attempt to reverse the neurological deficit associated with Hansen type I IVDH. Bone marrow was collected from two healthy dogs presented to the clinic for elective surgeries with the consent of pet owners. The animals were anesthetized before the collection of bone marrow (iliac crest) as per the protocol described by Sharun *et al*. (2020). The protocols used for the isolation and culture of aBM-MSCs were similar to those performed by Bhat *et al*. (2019). Mesenchymal stem cells at 3rd passage were characterised as per the International Society for Cellular Therapy criteria and following the method of Ansari *et al*. (2013). Briefly, the growth characteristics of MSCs were observed for their plastic attachment and fibroblastic growth, trilineage differentiation potential (osteogenic, adipogenic, and chondrogenic), expression of certain stem cell surface markers (CD73, CD90,

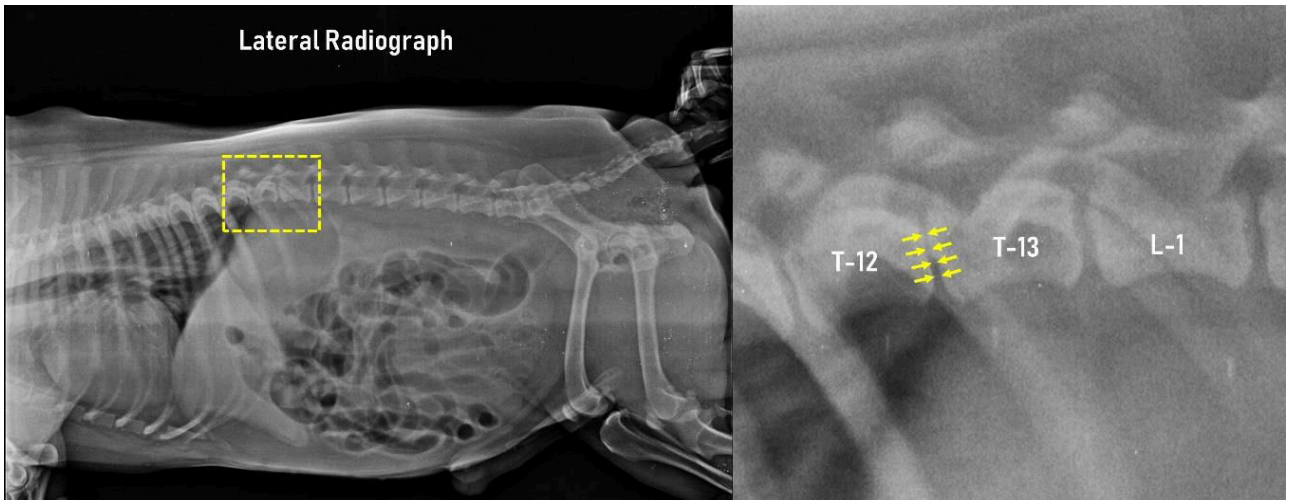

Fig. 1: Lateral radiograph of thoraco-lumbar spine. Reduced intervertebral space between T_{12} and T_{13} thoracic vertebrae (yellow arrows)

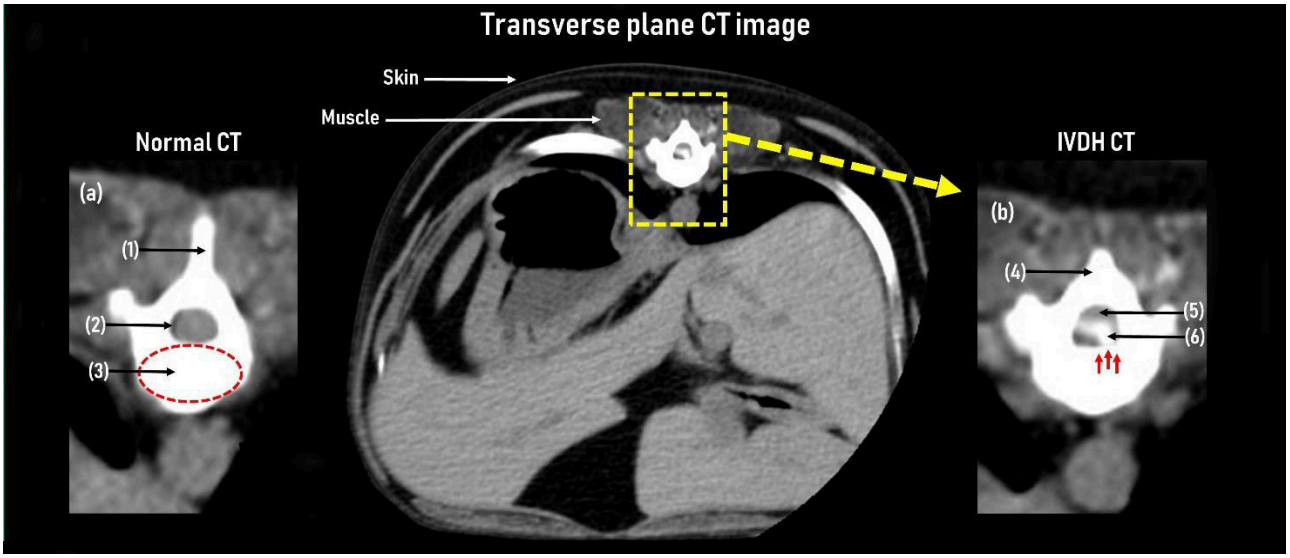

Fig. 2: Transverse plane CT image showing the presence of extruded disk material in the vertebral canal of T_{12} vertebrae (2.5 mm slice thickness, 120 kV, and 89 mA). (**a**) Normal CT image for comparison. (1) Spinous process, (2) Vertebral canal, (3) Vertebral body, (**b**) Mineralized disc material extending into the spinal cord canal. (4) Vertebral lamina, (5) Spinal cord, and (6) Extruded material pressing on the spinal cord. CT: Computed tomography, and IVDH: Intervertebral disc herniation

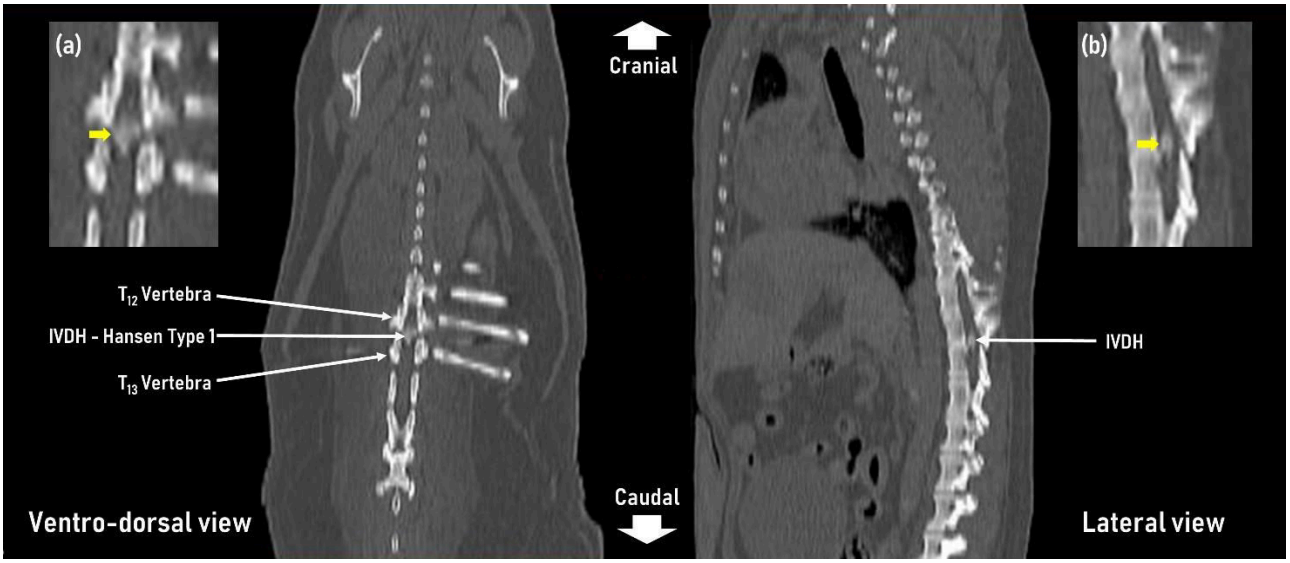

Fig. 3: Reconstructed CT images. (**a**) Dorsally reconstructed CT image of the same dog allowing accurate lesion localization at T_{12-13} intervertebral space (arrow), and (**b**) Sagittal reconstructed CT image showing dislocated intervertebral disc material seen as a hyper-attenuating mass in the vertebral canal (arrow). IVDH: Intervertebral disc herniation

CD105, and CD34) by reverse transcription-polymerase chain reaction (RT-PCR) and immunocytochemistry. The MSCs were also evaluated for expression of pluripotency markers like Nanog, Oct4, Sox2 using RT-PCR assay. Well characterized allogenic canine BM-MSCs at 3rd passage was ready for use in our laboratory.

For injecting the stem cells, the dog was anesthetized with xylazine (1 mg/kg body weight (BW)) followed by ketamine (5 mg/kg BW) intramuscularly. One ml of aBM-MSCs suspension containing 1×10^6 cells/ml was percutaneously transplanted to the spinal cord parenchyma at the site of injury. The animals were positioned in sternal recumbency and a 26-gauge spinal needle was inserted into the T_{12}-T_{13} interface. It was then directed through the interarcuate space till it penetrated the spinal cord parenchyma and a sudden jerk produced by the lightly anaesthetized animal. After the tip entered the parenchyma, half of the stem cells were injected slowly into the spinal cord. The remained half was injected while withdrawing the needle from the spinal cord parenchyma to deposit the cells at multiple layers. A total of four doses were given at an interval of 15 days along with supportive therapy including methylcobalamin (500 mcg/dog q12 h) and gabapentin (10 mg/kg BW q12 h) orally throughout the study period. Improvement was evaluated based on the neurological examination and grading. Recovery was scored using the scale (on a scale of 0 to 14) suggested by Olby *et al.* (2001).

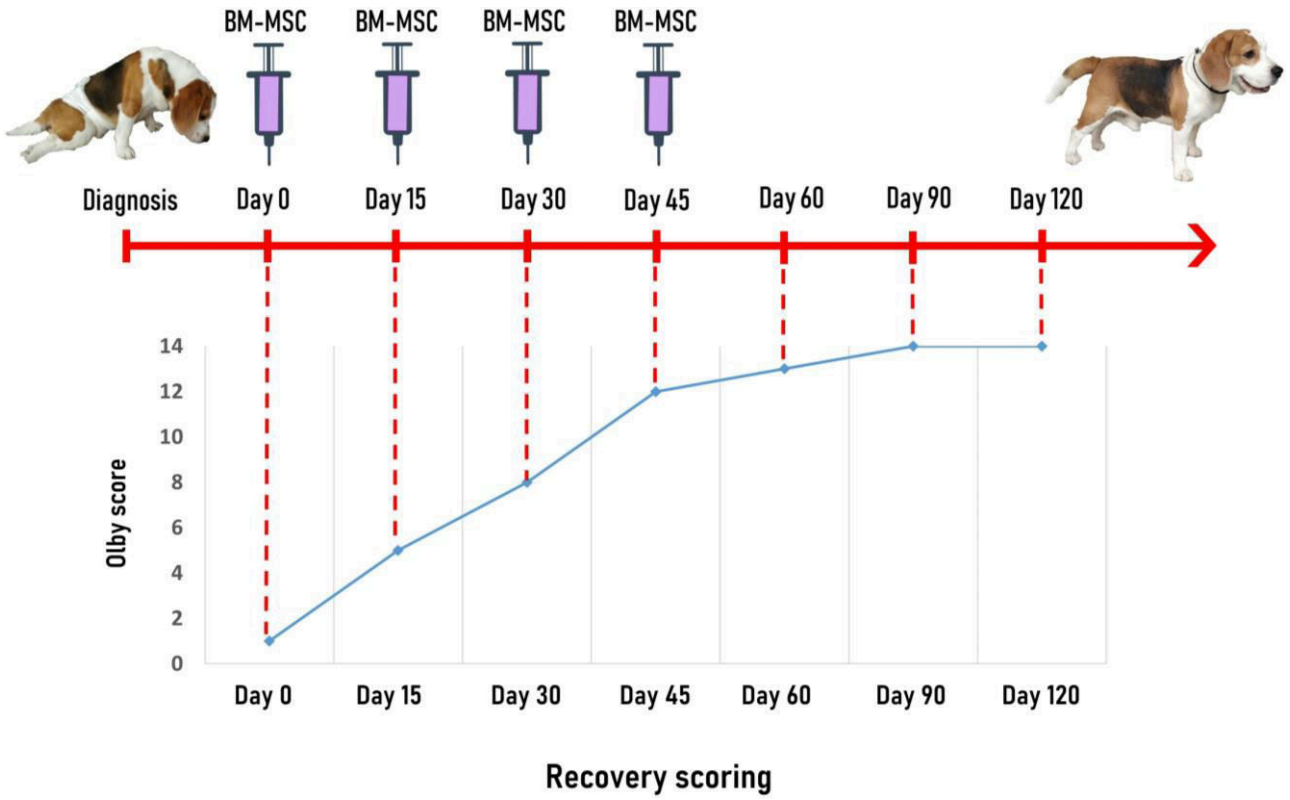

Fig. 4: Graph plotting the progressive improvement of neurological function of the dog. Considerable improvement in Olby score was observed immediately after the first BM-MSCs injection. Complete weight bearing was observed after two doses. BM-MSCs: Bone marrow-derived mesenchymal stem cells

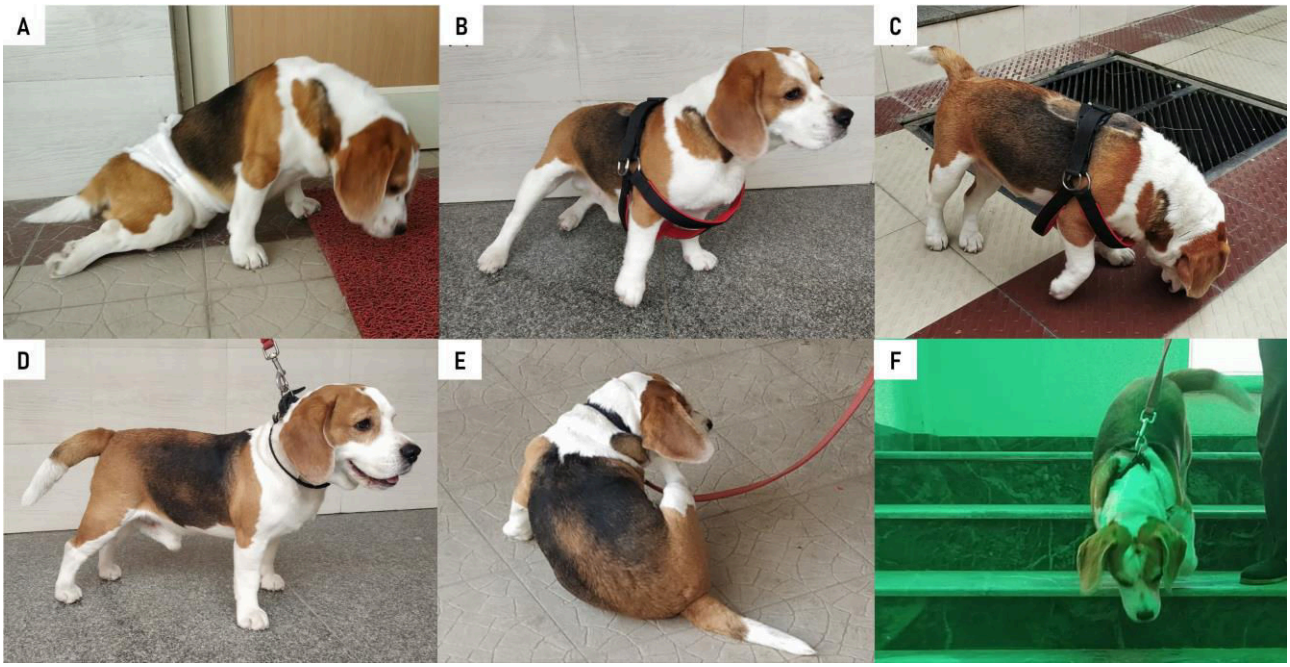

Fig. 5: Progressive improvement in the neurological function. (**A**) Day 0, paraplegia with dragging of hind limbs, (**B**) Day 30, initiated weight bearing on the hind limbs, (**C**) Day 45, weight bearing on hind limbs that has normal strength, but had ataxia, (**D**) Day 60, almost normal gait with mild ataxia during walking, (**E**) Day 90, complete recovery with return of normal behaviour (scratching of ear with hind limbs), and (**F**) Day 90, dog started moving up and down the stairs with ease

Result

Substantial improvement was noticed after the first dose of aBM-MSCs. The animal started complete weight bearing on its hind limbs after two doses. Improvement was also observed in the neurological score which

indicated improvement in sensory function. Progressive improvement in neurological function was evident from the increased recovery score (Figs. 4 and 5A-F). Excellent improvement was observed in pain perception (nociception), leading to the return of superficial pain perception. Gradual improvement in postural reactions like conscious proprioception, visual/tactile placing, and wheel barrowing were also recorded.

Discussion

Computed tomography images of type I disk extrusion are characterized by the presence of hyper-attenuating disk material in the epidural space. The density of the extruded disk material is directly dependent upon the degree of mineralization. The disk material can either migrate horizontally along the vertebral canal floor and circumferentially around the spinal cord or can migrate dorsolaterally into the intervertebral foramina. The extruded disc can displace and compress the spinal cord depending on the volume and distribution of disk material (Wisner and Zwingenberger, 2015). The calcified disc, before extrusion can be visualized in the disc space. Whereas the extruded calcified disc material will be visible at the intervertebral foramen above the disc. Once it is extruded, the amount of calcified disc material remaining in the disc space is less and hence cannot be detected using radiography (Stigen *et al*., 2019). In the present case, radiographs could not identify the presence of any calcified disc material in the T_{12}-T_{13} interface but a remarkable reduction in the disc space was observed. Narrowing of disc space cannot be considered as a sign of disc herniation since the disc can herniate laterally, dorsally as well as ventrally. Extruded disc material may not be visible radiographically as radiography had less sensitivity compared to CT in detecting calcified disc material in the vertebral canal. Nevertheless, radiograph may exhibit higher sensitivity in detecting remained calcified material in the disc space (Stigen *et al*., 2019). It is also very difficult to identify calcified disc in thoracic vertebrae since the opacification of intervertebral foramina occurs naturally due to the ribs superimposition.

Treatment options for IVDH involve the use of corticosteroids, acupuncture, decompressive surgical techniques, cage rest, or a combination of the aforementioned options. Surgical decompression is the established treatment for non-ambulatory dogs which involves laminectomy followed by the removal of disc material from the spinal canal (Ruddle *et al*., 2006). Surgical management of disc extrusions has a higher success rate in dogs with intact pain perception compared to dogs with no conscious pain sensation. But the neurological recovery following surgical management in IVDH may be time consuming, expensive, and frustrating to the owners (Olby *et al*., 2003; Ruddle *et al*., 2006). Percutaneous cellular therapy using autologous enriched olfactory ensheathing cells has been reported to have therapeutic potential in chronic SCI (Granger *et al*., 2012). Here, we chose aBM-MSCs for the percutaneous implantation without performing any decompressive surgery.

Compared to dogs that had traumatic SCI, the prognosis of motor function recovery is significantly higher in dogs suffering from SCI secondary to IVDH. This can be attributed to the fact that vertebral fracture or luxation are associated with spinal cord lacerations in addition to contusive/compressive injuries (Olby *et al*., 2003). In the present case, Hansen type I IVDH at T_{12}-T_{13} was associated with the grade 3 injury characterized by paraplegia with intact deep pain perception. The mild nature of the injury might have contributed to the complete neurological recovery without the need for any surgical decompression. Stem cell therapy might have played an important role in the sudden recovery in neurological function. MSCs isolated from different sources not only can differentiate multiple cell lineages, but also have several trophic, anti-inflammatory, and regenerative effects (Figueroa *et al*., 2012). The transplantation of MSCs is associated with clinical signs improvement due to their ability to modify the inflammatory response that promotes the survival of endogenous nerve cells. Mesenchymal stem cells also modulate various regulatory signal molecules, leading to reduce glial scar formation (Kim *et al*., 2016). They are also reported to restore the myelination of injured axons. MSCs possess immunosuppressive properties and also secrete certain growth/trophic factors that promote regenerative processes (Meirelles *et al*., 2009). The present case, refractory to the routine treatment, responded to stem cell therapy very quickly showing the efficacy of stem cell therapy in IVDH cases, with intact deep pain sensation.

The prognosis of IVDH cases is greatly dependant on the presence or absence of deep pain perception (Olby *et al*., 2003; Ruddle *et al*., 2006). The absence of deep pain perception following SCI is indicated as the functional spinal cord transection (Olby *et al*., 2003). Studies have shown that those patients lacking deep pain perception were 1.7 times less likely to become ambulatory following the treatment (Ruddle *et al*., 2006). The reason for the good prognosis, in this case, can be attributed to the conservation of deep pain perception, even though superficial pain perception was absent.

In conclusion, percutaneous transplantation of aBM-MSCs might have helped the dog to regain its neurological function completely without performing surgical decompression. Percutaneous delivery of stem cells was preferred to avoid the need for any surgical intervention. Hence, this non-invasive technique may be further evaluated for its efficacy in managing paraplegia secondary to Hansen type I IVDH. Further evaluation is required in a larger cohort of dogs to determine the efficacy and success rates associated with this procedure.

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Conflict of interest

None of the authors has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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