Cell J
Cell Journal(Yakhteh)
Homepage: https://www.celljournal.org/
Original Article
Vol 25, No 5, May 2023, Pages: 338-346

Comparison of Ultrasound-Guided Percutaneous and Open Surgery Approaches in The Animal Model of Tumor Necrosis Factor-Alpha-Induced Disc Degeneration

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Abstract

Objective: Animal models provide a deeper understanding about various complications and better demonstrate the effect of therapeutic approaches. One of the issues in the low back pain (LBP) model is the invasiveness of the procedure and it does not mimic actual disease conditions in humans. The purpose of the present study was to compare the ultrasound-guided (US-guided) percutaneous approach with the open-surgery method in the tumor necrosis factor-alpha (TNF-α)-induced disc degeneration model for the first time to showcase the advantages of this recently developed, minimally invasive method.

Materials and Methods: In this experimental study, eight male rabbits were divided into two groups (open-surgery and US-guided). Relevant discs were punctured by two approaches and TNF-α was injected into them. Magnetic resonance imaging (MRI) was performed to assess the disc height index (DHI) at all stages. Also morphological changes (annulus fibrosus, nucleus pulposus) were evaluated by assessing Pfirrmann grade and histological evaluation (Hematoxylin & Eosin).

Results: The findings indicated targeted discs became degenerated after six weeks. DHI in both groups was significantly reduced (P<0.0001), however the difference was not significant between the two groups. In the open-surgery group, osteophyte formation was seen at six and eighteen weeks after the puncture. Pfirrmann grading revealed significant differences between injured and adjacent uninjured discs (P<0.0001). The US-guided method indicated significantly fewer signs of degeneration after six (P=0.0110) and eighteen (P=0.0328) weeks. Histological scoring showed significantly lower degeneration in the US-guided group (P=0.0039).

Conclusion: The US-guided method developed a milder grade condition and such a model better mimics the chronic characteristics of LBP and the procedure is more ethically accepted. Therefore, the US-guided method could be a merit approach for future research in this domain as a safe, practical and low-cost method.

Keywords: Animal Model, Disc Degeneration, Open Surgery, Ultrasound-Guided Percutaneous

Citation: **Tayebi B, Molazem M, Babaahmadi M, Ebrahimi E, Hajinasrollah M, Mostafaei F, Makvand Gholipour N, Baharvand H, Baghaban Eslaminejad MR, Hassani SN, Hajizadeh-Saffar E. Comparison of ultrasound-guided percutaneous and open surgery approaches in the animal model of tumor necrosis factor-alpha-induced disc degeneration. 2023; 25(5): 338-346. doi: 10.22074/CELLJ.2023.1972610.1168**

Received: 20/November/2022, Revised: 07/February/2023, Accepted: 05/April/2023
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Introduction

The number of patients affected by low back pain (LBP) has been increasing for years, which is known as the number one cause of disability in the world (1). Intervertebral disc degeneration (IDD) is recognized as a common cause of LBP, which is characterized by changes in the structure and following dysfunction of the disc (2, 3).

Since this condition has a high economic burden on the health systems of countries, finding a way to address it has become a therapeutic priority (4). Conventional therapies emphasize on reducing pain symptoms; however, none of these interventions stop the progression of degeneration or restore the physiological function of the disc (5, 6). It is known that the effectiveness of any new intervention is closely related to the correct simulation of the underlying disease, so establishing an optimal animal model is essential. However, because the exact pathophysiology of disc destruction is unknown, creating an animal model similar to the original disease has remained challenging (3).

Despite the various methods available for developing an animal model of IDD, such as using mechanical force (3), structural disorders, or genetic predisposition (7), none of these methods can fully simulate the fundamental nature of the disease in humans (7, 8). Among these methods, rupturing the disc with a specific size and depth of the needle can induce a slow and spontaneous process (8), just like the procedure that occurs in most patients, so it can be used as a suitable model for mimicking the IDD. Different studies reported punctures through the posterior (9, 10) or anterior (10, 11) direction. The advantage of these approaches is the ease of establishment, but due to injury to the posterior spinal structure, irritating the spinal cord and nerve roots using these approaches is challenging (1). Recent studies have identified inflammatory mediators and signaling pathways as essential factors in the onset and progression of disc degeneration (1, 12, 13). An annular puncture with simultaneous injection of tumor necrosis factor-alpha (TNF-α), as the critical proinflammatory cytokine, can induce degeneration of intervertebral discs (IVD) by inhibiting the extracellular matrix synthesis of the IVD and create a pain mechanism similar to that occurring in patients (12, 14-16). Different studies have demonstrated the role of TNF-α and interleukin-1beta (IL-1β) in the progression of disc degeneration and the association of these cytokines with painful behavior (14, 17, 18). Additionally, surgical samples derived from symptomatic back pain patients in comparison to tissues from asymptomatic discs have demonstrated a higher degree of TNF-α producing cells (19).

Since Russell and Burch (20) raised the 3R rules (replacement, refinement, reduction) in 1959 to observe ethical issues of animal studies, surgical procedures have been replaced by minimally invasive techniques. Also, in defining animal models, one of the main aims is to reduce the pain in animals under the circumstances in which their use cannot be replaced.

Using the ultrasound-guidance (US-guided) method can be a suitable method for developing a non-invasive and efficient model for the degeneration and regeneration of structural IVDs studies because of its availability and low cost (21). Minimally invasive techniques reduce the risk and complexities of invasive surgeries, including bleeding, infections, and long recovery period. Also, with the help of non-invasive techniques without cutting the animal's body, the mortality risks associated with surgery will be reduced as well.

Therefore, the purpose of the current study was to compare TNF-α-induced disc degeneration using the US-guided percutaneous injection method as a minimally invasive procedure with the open surgery method for the first time. Moreover, by evaluating changes at 12 weeks after the disease was established, it was made possible to set a baseline to evaluate the effectiveness of treatments in similar studies. Proper *in vivo* simulation of degenerative disc disease is a research priority that can provide deep insight in the study of disc degeneration or regeneration.

Material and Methods

Study design

In this experimental study, eight male New Zealand White (NZW) rabbits (provided by Razi Vaccine and Serum Research Institute, Karaj, Iran) with approximately 2.5 kg weight were adapted in the laboratory seven days before application. Throughout the study, each animal was housed in a single standard cage in a controlled room at a temperature of 15-24°C with a 12-hour light/dark cycle and relative humidity of 30-70%. Food and water were freely available to all animals. Animals were divided into open surgery (as an invasive approach) and US-guided (as a minimally invasive approach) groups. For both groups, the L4-L5 and L6-L7 discs were punctured with an 18-G needle, and 20ng TNF-α (210-TA-005, R&D, USA) was injected into the discs at the same time.

Open surgery approach

In the open surgery group, the rabbits were anesthetized by intramuscular injection of 10 mg/kg Xylazine 2% (Alfasan, Holland) and 50 mg/kg Ketamine 10% (Alfasan, Holland), the abdomen was shaved and sterilized with 10% betadine swabs (povidone-iodine) and 70% alcohol. Surgery was performed under sterile conditions and general anesthesia [Isoflurane in oxygen (2%, Piramal, India)]. The procedure was performed in a way that the abdominal area was split, and the lumbar part of the spine was exposed (Fig.1A). The aortic bifurcation (22) (Fig.1B) is considered a sign of the location of the L7 vertebra. The IVD L4-L5 and L6-L7 were punctured by an 18-G needle to a depth of five mm (using handmade tools) (Fig.1C) for five seconds and simultaneously TNF-α [20 ng per 100 µl phosphate buffered saline (PBS)+0.1% bovine serum albumin (BSA)] was injected into the relevant discs. The abdomen muscles were then sutured with Vicryl suture 3-0 and the skin with nylon suture 0-2. The animal was examined to ensure that there were no postoperative complications, including hemorrhage, wound infection, ulceration, spinal cord injury, and rupture of the abdominal arteries. To control infection and pain, Enrofloxacin 10% (Aburaihan Pharma, Iran, 5 mg/kg) and tramadol (Alborz Darou, Iran, 15 mg/kg) were injected subcutaneously for three days, respectively.

Ultrasound-guided approach

In the US-guided (non-invasive) group, rabbits were anesthetized as described previously. The left side of the rabbit's flank was shaved and sterilized with 10% betadine swabs (Povidone-iodine) and 70% alcohol. US-guided annular percutaneous needle puncture was used to induce this model and assess this method's feasibility and safety. First, US was conducted through the retroperitoneal approach to identify the iliac spine and the location of intervertebral discs. Then, the target disc was confirmed based on the location of the aortic bifurcation (Fig.2A) (22). Once the target disc was confirmed, an 18-G needle was slowly penetrated percutaneously toward the disc under US-guidance. The needle was then confirmed to be positioned in the disc at a depth of five mm (Fig.2B) for five seconds, and simultaneously TNF-α (20 ng per 100 µl PBS+0.1% BSA) was injected into the relevant discs (Fig.2C). In this way, L4-L5 and L6-L7 segments were injured with the 18-G needle to induce IDD. Also, L2-L3 segments were used as adjacent uninjured discs in all animals. To control infection and pain, Enrofloxacin 5% (5 mg/kg) and Tramadol (15 mg/kg) were injected into all rabbits for three days respectively. There is no need to suture in this method. After puncture, computed tomography (CT) scan (SOMATOM Spirit, Siemens Healthcare, Germany) was conducted to confirm the injection of TNF-α (Fig.2D).

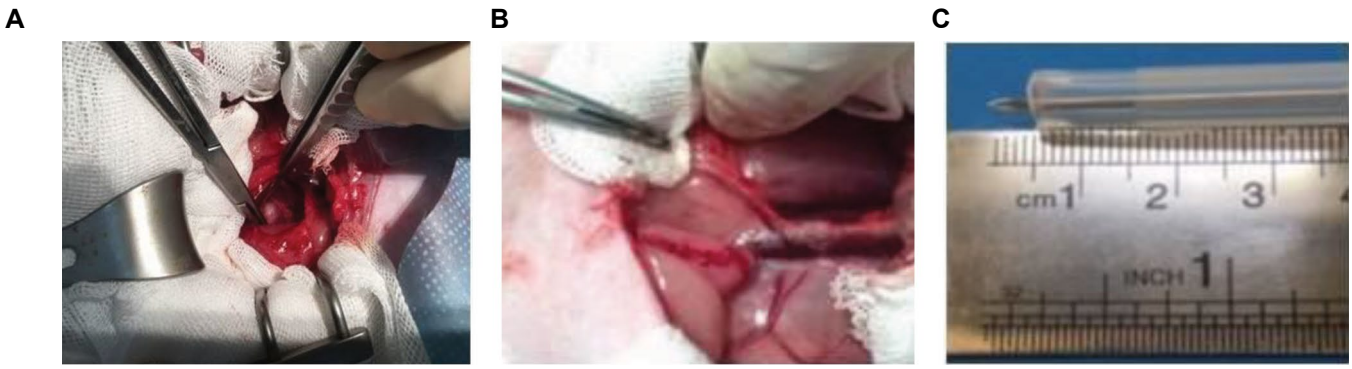

Fig.1: Open surgery approach from the anterior direction. **A.** Intraoperative picture demonstrating an anterior approach to expose the lumbar part of the spine. After exposing the designated discs, they were punctured by an 18-G needle to a depth of five mm and at once 20 ng tumor necrosis factor-alpha (TNF-α) was injected. **B.** Aortic bifurcation as a landmark space for the L6-L7 intervertebral disc. **C.** A handmade tool to control the depth of the needle puncture (5 mm depth); the cap of needle was cut to address this issue.

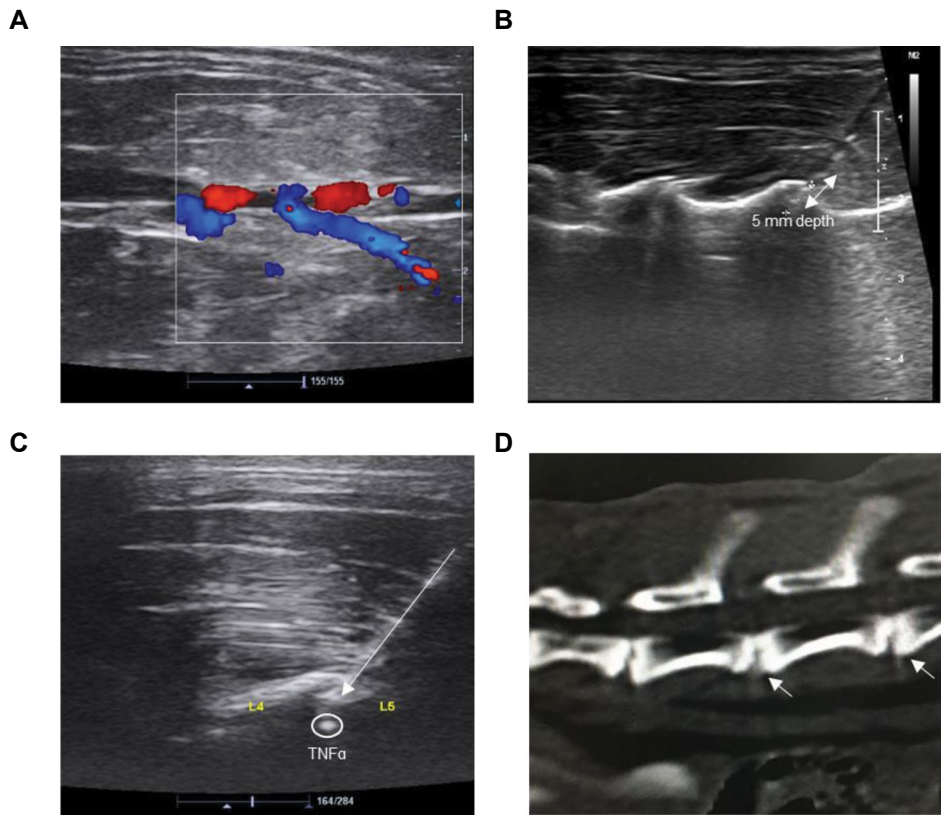

Fig.2: Detailed procedures of the ultrasound-guided (US-guided) percutaneous puncture method. **A.** Aortic bifurcation as a landmark space for the L6-L7 intervertebral disc. **B.** The needle tip was stabbed into the annulus fibrosus for about 5 mm according to US guidance. **C.** Needle route and injected tumor necrosis factor-alpha (TNF-α). **D.** CT scan verified the injection of TNF-α; for this purpose, a radiocontrast agent was added to TNF-α and CT scan was performed after the injection. L; Lumbar and CT; Computerized tomography.

Magnetic resonance imaging analysis

The rabbits were placed in a supine position on a quadrature surface coil. Lumbar spine imaging was performed using a standard clinical 3.0-T MR unit (SIEMENS MAGNETOM Prisma; Siemens Healthcare, Germany). Scanning was done three times, before the annular puncture and at six and eighteen weeks post annular puncture. Sagittal T2-weighted images, were obtained using a spin-echo sequence (pulse repetition time/echo time=3150/93 ms, echo train length=14, field of view=160×100 mm, pixel bandwidth=289 kHz, slice thickness=2 mm, phase encoding direction=head to feet).

As the measurement of disc height index (DHI) by x-ray depends on the depth of sedation and muscle relaxation, in this study the Chai et al. (23) method was utilized for measuring DHI by magnetic resonance imaging (MRI.) DH was defined as the shortest dimension drawn between the medians of adjacent endplates. DHI was measured by two independent radiologists, and averaged values were obtained. The relative disc height (RDH) compared to the control disc was used for DHI calculation. RDH and DHI were calculated as follows:

RDH=disc height after annular puncture×(height of control disc before annular puncture/height of control disc after annular puncture)

DHI=(disc height before annular puncture-RDH after annular puncture)/disc height before annular puncture.

The rabbits were followed up by MRI at baseline, six and eighteen weeks after the puncture. After eighteen weeks, rabbits were anesthetized and then sacrificed with an IV injection of 3% Sodium pentobarbital.

Pfirrmann grading of discs

T2-weighted images on the sagittal plane of MRI were used for morphological evaluation of IVD degeneration according to the Pfirrmann grading system at six and eighteen weeks after the operation. Pfirrmann grading as a qualitative measurement was used to classify discs between five grades of degeneration (24).

Two independent observers checked out sagittal T2-weighted MR images of each intervertebral disc, and the Pfirrmann grade was determined based on defined criteria followed in Table 1.

Histological evaluation

After eighteen weeks, all animals were euthanized, and all motion segments, including L2-L3, L3-L4, L4-L5, L5-L6, and L6-L7, were harvested. By using a rotary tool angle grinder (Ronix 3402), each disc was cut with adjacent vertebras (vertebral body–IVD–vertebral body) and fixed in 10% neutral buffered formalin (NBF, PH=7.26) for 48 hours. Then discs were decalcified using ethylenediaminetetraacetic acid (EDTA) for more than 45 days. Decalcified discs were serially dehydrated in ethanol and embedded in paraffin. The 5 µm thick sections were prepared and, after staining with Hematoxylin-eosin (H&E) for structural evaluation, and Masson's trichrome (MT) (HT15-1KT, Sigma-Aldrich, USA) for collagen fiber, were evaluated by two independent pathologists, using light microscopy (Olympus BX51, Olympus, Japan). In each disc, a separate score was assigned for each of the following 4 regions: 1 (normal) to 3 (worst); Annulus fibrosus (AF), the border between AF and nucleus pulposus (NP), cellularity of NP and matrix of NP (8).

Statistical analysis

Statistical analysis for open surgery and US-guided groups were done by IBM SPSS version 25 (IBM Corp., Armonk, NY, USA) and Prism 8 (GraphPad Software., La Jolla California USA) software using $P<0.05$ to identify statistically significant differences. Differences between groups were evaluated with the two-way ANOVA for DHI and Pfirrmann grade. The graphs were plotted with GraphPad Prism 8 software.

Ethics approval and animal care

All aspects of the procedures were approved by the Ethics Committee of the Royan Institute (IR.ACECR.ROYAN.REC.1397.155) and were performed under the standard guidelines of the NIH Guide for the Care and Use of Laboratory Animals (8th edition).

Table 1: Grading system of intervertebral disc degeneration

Grade	Definition
I	Disc has a uniform high signal in the nucleus on T2-Weighted MRI.
II	Central horizontal line of low signal intensity.
III	High intensity in the central part of the nucleus with lower intensity in the peripheral regions of the nucleus.
IV	Low signal intensity centrally and blurring of the distinction between nucleus and annulus.
V	Homogeneous low signal with no distinction between nucleus and annulus.

MRI; Magnetic resonance imaging.

Results

Open surgery and the US-guided approach were both safe and feasible

There were no severe side effects related to the modeling procedures, and there were no severe bleeding or infections at the operation or needle insertion sites. However, in the open surgery group, anorexia, binge drinking, and weakness were observed after the operation, which resolved within 48 hours without intervention. However, in the US-guided approach, all animals were active. During the follow-up period, the rabbits were well and gained weight. Degeneration of designated discs were seen in all animals of each group after six weeks.

The US-guided approach demonstrated fewer signs of degeneration after six weeks

T2-Weighted MRI image showed reduced fluid signal within the discs, the disc appeared blacker than usual, compared with the adjacent uninjured disc in all experimental groups after six and eighteen weeks post-puncture. This reduced fluid signal was milder in the US-guided group (Fig.3A, B). In the open surgery group after six weeks, the discs were bulging and reduced in height. MRI demonstrated a progressive endplate osteophyte formation which is the bone spur's outgrowth on the spine's bones or near the joints; from six weeks after a puncture in two cases (four discs) of the open surgery group. There was a dorsal protrusion of the AF. Also at the vertebral endplate of the IVD, a focal fluid signal was observed which could be caused by edema in the vertebral soft tissue. A ventral fluid signal was observed, which might be from the remnants of the destroyed disc or soft tissue edema. In this group after eighteen weeks, the described ventral soft tissue edema and the protrusion were regenerated. However, a further decrease in the DHI was observed and osteophyte was also observed in the discs. Compared to the open surgery group, in the US-guided approach, none of the side effects were observed. There was no sign of protrusion or soft tissue edema, and the NP was more homogenic and regular.

DHI decreased in each group after degeneration. The DHI of both groups has been normalized to the DHI of adjacent uninjured discs of each rabbit. It indicated that injury which was induced by both methods could significantly reduce the DHI compared to its own adjacent uninjured discs ($P<0.001$), but no significant differences were seen between the two groups (Fig.4A).

In the baseline imaging study, all vertebral discs in qualitative MRI studies were categorized as Pfirrmann grade 1. Analysis for mean Pfirrmann grades for adjacent uninjured discs, discs treated with open surgery, and US-guided methods were 1, 4.1, and 3.2, respectively, after six weeks (Fig.4B). Pfirrmann grading showed a significant difference between injured and adjacent uninjured discs ($P<0.0001$). Based on Pfirrmann score, the US-guided method showed significantly fewer degenerative signs compared to the open surgery method after six and eighteen weeks ($P=0.0110$, 0.0328 respectively).

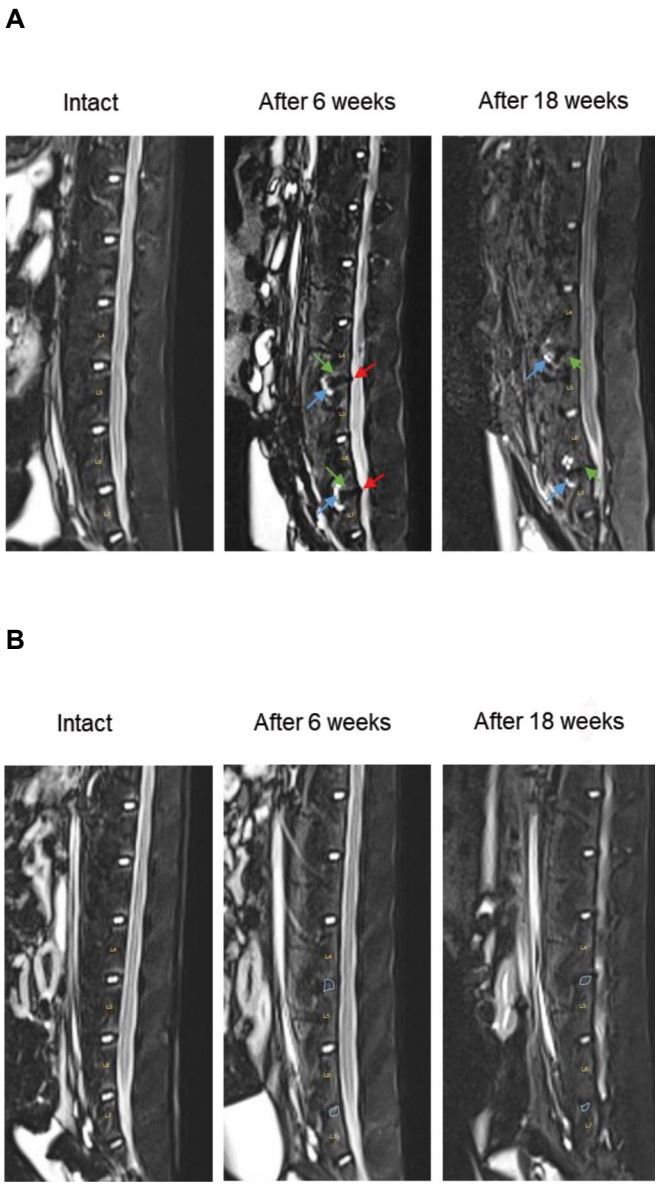

Fig.3: Degeneration of the designated discs was seen after six and eighteen weeks in two experimental groups on sagittal conventional T2-weighted STIR MRI sequences. **A.** In the open surgery group, degeneration was more severe, herniation of the disc (red arrows), vertebral endplate edema (green arrows) and a ventral fluid signal (blue arrows) were seen. **B.** Milder degeneration was seen in the US-guided group. Despite the decrease in signal intensity, the border of the disc was well-defined and there was no sign of soft tissue edema at both time points. MRI; Magnetic resonance imaging and US; Ultrasound.

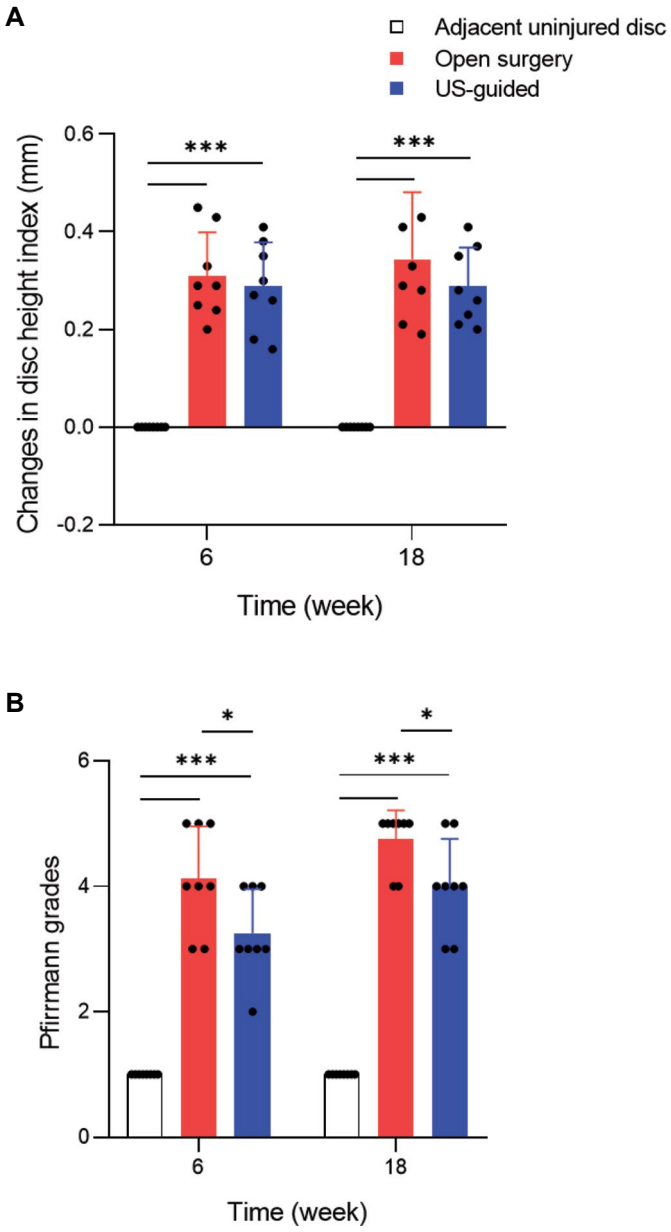

Fig.4: Changes in DHI and Pfirrmann grades at six and eighteen weeks after needle puncture and injection versus adjacent uninjured disc (each group was compared with its own adjacent uninjured disc). **A.** A significant decrease in the DHI was observed (P<0.01) after 6 and 18 weeks compared to the intact control. **B.** A significant decrease was observed between the open surgery, US-guided group, and adjacent uninjured discs. Pfirrmann grades compared between experimental groups, where the US-guided group demonstrated significantly lower scores (thus less degenerative changes) than the open surgery group. Biological replicate=4 rabbits; 2 discs of each animal. *; P<0.05, ***; P<0.001, DHI; Disc height index, and US; Ultrasound.

Histological evaluation showed significant changes between two approaches after eighteen weeks

In addition to changes in disc height and disc puncture, simultaneous TNF-α injection induced histological changes after 18 weeks. Hematoxylin and eosin (H&E) and Masson Trichrome (MT) staining of uninjured discs showed numerous large, vacuolated cells within normal extracellular matrix (Fig.5A, B) with a distinct border between AF and NP in contrast to injured discs. Comparison of histological sections between the adjacent uninjured (Fig.5A, B) and injured discs in the open surgery (Fig.5C, D) and US-guided (Fig.5E, F) groups clearly showed a loss of normal disc height, disorganization of the AF lamellae and loss of the gel-like appearance of the NP in both injured groups. There were about 50-80% NP cell loss and more than 80% loss of cells in the NP compartment, in the US-guided and open surgery approaches, respectively. The matrix of NP was denser in the open surgery group than the US-guided group. The NP compartment was filled with fibrous lamella with loss of border between AF and NP in the open surgery group, whereas, fibrosis in the NP compartment was moderate in the US-guided method. Also, there were marked loss of normal AF lamella organization and structure and several clefts through the depth of AF in the open surgery group. The total histological grading scores indicated significant differences between the two groups (n =8) at week eighteen (Fig.5G, P=0.0039).

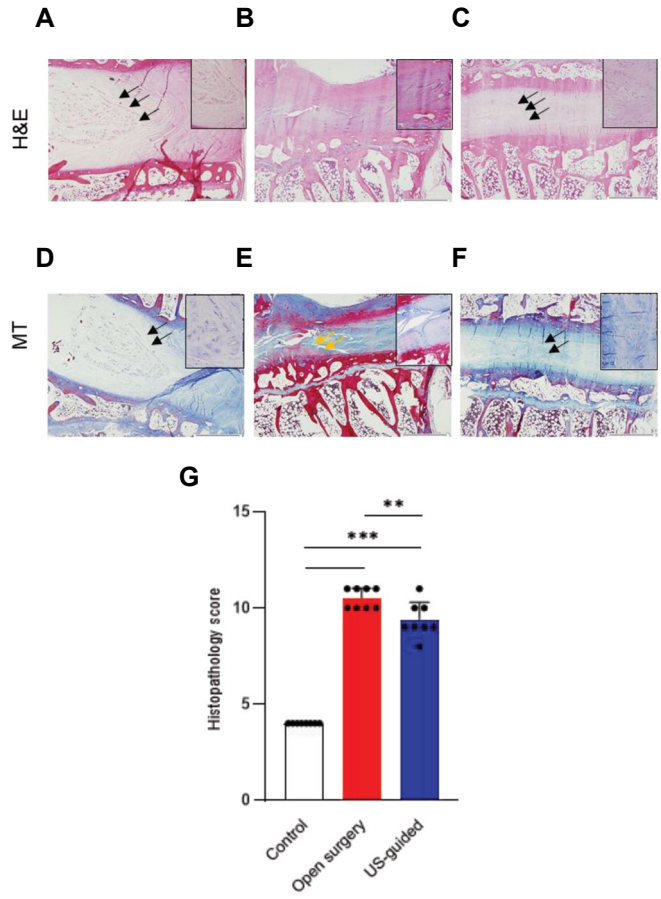

Fig.5: Histological comparison of the adjacent uninjured, injured degenerated disc by open surgery and US-guided in rabbits. **A, B.** The uninjured discs demonstrated numerous large, vacuolated cells within a normal extracellular matrix and a clear border between the AF and NP. **C, D.** The degenerated disc in the open surgery group demonstrated a high grade of degeneration, including severe NP matrix disorganization and dense matrix clumps, and loss of border between the AF and NP with no trace of NP-like cells. **E, F.** In contrast, the degenerated disc in the US-guided group demonstrated loss of normal disc height, milder disorganization of the AF lamellae and loss of the gel-like appearance of the NP but the border between AF and NP was visible. **G.** Changes in histological grading at eighteen weeks in each group. Black arrow shows the border between the AF and NP. MT stained section shows some mineralization in the open surgery group (yellow arrow). **, P<0.01, ***; P<0.001, Biological replicate=4 rabbits; 2 discs of each animal (scale bar: 1000 µm). AF; Annulus fibrosus, NP; Nucleus pulposus, H&E; Hematoxylin-eosin, MT; Masson's Trichrome, and US; Ultrasound.

Discussion

As a common cause of LBP, intervertebral disc degeneration (IDD) is an unavoidable phenomenon in today's lifestyle and occurs as a natural consequence of aging (3). Furthermore, animal models are one of the key tools in developing new therapeutic strategies through *in vivo* evaluations. However, because of the excessive complexity in this field, no suitable model has been presented currently.

Therefore, we investigated the feasibility and efficiency of the TNF-α-induced disc degeneration model with two different approaches in order to shed light on this subject and suggest and better demonstrate the optimal and newly developed method for disc degeneration. In this study, rabbits were used as the animal model, the most widely used model in experimental studies of LBP (25). It has more homology to human intervertebral discs, and the thickness of its cartilaginous endplate can better demonstrate the changes during degradation (3, 7). Also, rabbits have larger bodies which make the technical aspects of surgery more plausible (3).

The most common practice for such studies is to perform open surgeries on subjects for inducing disc degeneration. In the open surgery group in this study, animals underwent surgery for disc degeneration induction and also received TNF-α in order to better mimic the disease condition. Our results indicated that the animals after surgery had to be kept in a clean environment and have postoperative care in order to avoid infection, because wound contamination is the single most important risk factor for abdominal wound disruption (26). As mentioned, in this approach, after six weeks of disease induction by using 18-G needle with depth of 5 mm, the T2-Weighted MRI image showed reduced fluid signal within the discs, disc herniation and decreased DHI, demonstrating disc degeneration. These results are aligned with the study by Masuda et al. (8) as they showed the 18G needle provided the most predictable, slow and progressive disc degeneration during 4-8 weeks. They indicated that this model basically simulates disc herniation and subsequent degenerative changes after an initial injury.

Also, after comparing four-disc injury models, Kim et al. (27) demonstrated that one annular puncture with a 18-G needle in the disc can cause significantly decreased DHI and water content in rabbits which was similar to our findings in both open surgery and US-guided methods.

Although, in Masuda's study (8), a slower rate of progress in the process of disc destruction was observed during 4-8 weeks, the changes in the sixth week in our study was more severe in the open surgery group. This may be due to the use of the inflammatory factor TNF-α in our study. This suggests that the simultaneous use of TNF-α and annular puncture may lead to more severe destruction in discs in a shorter time period. Our findings were similar to others in this field. In 2011, Ye et al. (15) showed that IDD could be induced by the over-expression of TNF-α, through inhibiting the synthesis of the extracellular matrix of the intervertebral disc. Also, Lai et al. (14) used TNF-α in their study, which they managed to indicate that the severity of disc degeneration was significantly associated with annular injury and TNF-α. Their proposed model was comparable in clinical conditions to human cases. Although it was possible that injected TNF-α diffuses to neighboring regions (28), we did not observe any changes in the adjacent uninjured discs in MRI and pathology evaluations in both studied groups. The structures of all the neighboring discs were carefully evaluated and no changes were seen.

On the other hand, in the US guided method, we used ultrasonic waves to guide our needles into designated discs. Decreased DHI and increased Pfirrmann grade compared to the adjacent uninjured discs indicated the accuracy and feasibility of this approach. These findings were similar to Song and colleagues (21), as they managed to induce the model as early as four weeks by performing a 18-G needle annular puncture using the US-guided approach. Moreover, although fluoroscopy is a more commonly used non-invasive technique for such operations, the US-guided method offers several benefits over fluoroscopy including shorter procedure time, being portable and ease of use, no ionizing radiation and better visualization of soft tissue structure such as blood vessels and therefore less complications (25).

In our study, based on Pfirrmann's grades and DHI changes, the severity of degeneration in the US-guided group was significantly lower than in the open surgery group after six and eighteen weeks, which may be due to less inflammation resulting from the non-invasive procedure and reduced destruction of adjacent muscles and ligaments around the discs. Also, Masuda et al. (8) have indicated that osteophyte formation in the vertebral body was caused by extreme dissection or irritation of the perivertebral ligamentous structure. Similarly, in our study, osteophyte formation was observed only in the open surgery group after the sixth week which may have been caused due to the operation. In their study, it was stated that this problem can be reduced with the care and skill of the surgeon to avoid damage to the periosteum of the vertebral body.

In our study, in the open surgery group, each surgery lasted about one hour, which is considered a long procedure and can be a great contributing factor to the surgeon's error. On the other hand, in the US-guided method, the procedure on each case lasted less than five minutes, which can significantly reduce fatigue-induced errors. It is worth noting that in studies related to LBP, usually there should be two surgeries, one for model induction and another for therapeutics administration. The first surgery often leads to further complications including excessive adhesions and angiogenesis making subsequent surgeries even more difficult.

Evaluation of degeneration progress after 18 weeks revealed further disc degeneration in the open surgery group based on DHI, Pfirrmann grade and histology analysis; which was more severe than the findings by Kong et al. (29). Their results showed a higher grade of degeneration and decreased DHI after 15 weeks compared to uninjured discs after annular puncture by a 16-G needle with a depth of 5 mm. Although, they used a thicker needle than us, their reported mean of Pfirrmann grade after 15 weeks was similar to our results after 6 weeks which could possibly be due to the concurrent effect of the TNF-a injection in our study.

Although the assessment of DHI and histology was inherently subjective, we minimized bias by blinding the observer during the evaluation. In spite of that, the data should be confirmed with more comprehensive histopathological evaluations. Although we tried to perform immunohistochemistry for specific genes such as collagen I and II, unfortunately, we could not achieve reportable results. Due to the large disc size in rabbits, there are many crucial steps, especially during tissue sample processing, that need to be taken in order to achieve reportable histological result.

Conclusion

Our results indicated the feasibility of the US-guided method in developing the animal model of IDD and its advantages compared to open surgery. A good model that properly resembles the LBP, should demonstrate slow progression similar to the natural process of the disease in humans, which according to MRI and histopathology evaluations this newly developed method created a milder progression pattern. Therefore, the US-guided procedure could be a promising alternative to conventional methods. Nonetheless, further studies are required to better evaluate the changes in the IDD animal model.

Acknowledgments

The authors deeply appreciate the efforts by their collaborators at Royan ATMP Technology Development Center (ATMP-TDC) and the Department of Stem Cells and Developmental Biology at Royan Institute. Funding was received from Royesh Venture Capital to Dr. Hossein Baharvand and Royan Stem Cell Technology Company (1396/1013) to Dr. Ensiyeh Hajizadeh. There is no conflict of interest in this study.

Authors' Contributions

B.T., M.M., M.B., H.B., S.-N.H., E.H.-S., M.R.B.E.; Conceptualization. B.T., M.M., M.B., S.-N.H., E.H.-S., M.R.B.E., E.E.; Methodology. B.T., M.M., M.B., N.M.Gh.; Performed data collection and analysis, and original draft preparation. B.T., M.B.; Writing-review and editing. B.T., M.M., M.R.B.E., E.H.-S., N.H.; Surgery and clinical evaluation. M.H., F.M.; US-guided method. M.M., F.M.; MRI assessments. M.M., E.E.; The final manuscript was prepared with input from all authors by B.T., M.M., S.N.H., E.H.S. All authors read and approved the final manuscript.

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