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ABOUT COVER

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Clinical and Translational Research

Application of molybdenum target X-ray photography in imaging analysis of caudal intervertebral disc degeneration in rats

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Abstract

BACKGROUND

Conventional plain X-ray images of rats, the most common animals used as degeneration models, exhibit unclear vertebral structure and blurry intervertebral disc spaces due to their small size, slender vertebral bodies.

AIM

To apply molybdenum target X-ray photography in the evaluation of caudal intervertebral disc (IVD) degeneration in rat models.

METHODS

Two types of rat caudal IVD degeneration models (needle-punctured model and endplate-destructed model) were established, and their effectiveness was verified using nuclear magnetic resonance imaging. Molybdenum target inspection and routine plain X-ray were then performed on these models. Additionally, four observers were assigned to measure the intervertebral height of degenerated segments on molybdenum target plain X-ray images and routine plain X-ray images, respectively. The degeneration was evaluated and statistical analysis was subsequently conducted.

RESULTS

Nine rats in the needle-punctured model and 10 rats in the endplate-destructed model were effective. Compared with routine plain X-ray images, molybdenum target plain X-ray images showed higher clarity, stronger contrast, as well as clearer and more accurate structural development. The McNemar test confirmed that the difference was statistically significant ($P = 0.031$). In the two models, the reliability of the intervertebral height measured by the four observers on routine plain X-ray images was poor ($ICC < 0.4$), while the data obtained from the molybdenum target plain X-ray images were more reliable.

CONCLUSION

accepted principles for the use of laboratory animals.

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Molybdenum target inspection can obtain clearer images and display fine calcification in the imaging evaluation of caudal IVD degeneration in rats, thus ensuring a more accurate evaluation of degeneration.

Key words: Molybdenum target inspection; Routine plain X-ray; Intervertebral disc degeneration model; Animal experiment; Imaging analysis; McNemar test

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Core tip: Imaging evaluation is a crucial, easy to use, low-cost and effective technique, especially for evaluating intervertebral disc space height, and is the most commonly used quantitative indicator of intervertebral disc degeneration in animal models. However, in rats with small vertebral bodies and narrow intervertebral disc spaces, conventional plain X-ray images have poor definition and low contrast, micro-calcifications are unclear, and the imaging effect on the boundary of the narrow intervertebral disc space is poor. This is the first study to apply molybdenum target X-ray photography to the imaging evaluation of caudal intervertebral disc degeneration in rat models.

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INTRODUCTION

Intervertebral (IV) disc degeneration is a pathological basis for a series of spinal degenerative diseases^[1]. However, its pathogenesis remains elusive, so it is vital to establish a feasible, highly repeatable, and effective animal model of intervertebral disc degeneration (IVD) to study the cause and pathogenesis of this disease^[2]. At present, the most frequently used models include the rat model of needle-puncture injury-induced caudal IVD and IVD caused by destroying the cartilaginous endplates in rats^[3-5].

The established degeneration models can be evaluated by various methods including imaging, histomorphological and molecular biological analyses. The easiest and most intuitive is imaging evaluation, such as conventional plain X-rays, micro-computed tomography and magnetic resonance imaging (MRI)^[6]. Because it is expensive and takes a long time, the application of micro-computed tomography for evaluation of disc degeneration is not very common. Conventional plain X-ray examination and MRI tend to be the preferred options to evaluate animal degeneration models.

Plain X-ray images of IVD show narrowing of the IV disc space, vertebral wedge-shaped deformities, endplate calcification and marginal osteophyte growth, of which the most characteristic change is the narrowing of IV space height. However, conventional plain X-ray images of rats or mice, the most common animals used as degeneration models, exhibit unclear vertebral structure and blurry IV disc spaces due to their small size, slender vertebral bodies and narrow IV disc spaces. As a result, the measurement of IV disc space height is not a precise indicator of disc degeneration^[7].

The present study is the first to apply molybdenum target X-ray photography to the imaging evaluation of the rat model of caudal IVD. Molybdenum targets can produce soft rays with a long wavelength of 0.063-0.071 nm, low penetrability, high intensity, high contrast, and high sensitivity to micro-calcification, so they are often applied to clinical breast examinations^[8-10]. In this study, rat models of caudal IVD induced by needle-puncture injury and endplate destruction were used to compare and analyze conventional X-ray examination and molybdenum target X-ray photography. It was hypothesized that molybdenum target X-ray photography would produce clearer images for a more precise evaluation of caudal IVD in rats.

MATERIALS AND METHODS

Animals

A total of 21 10-12-week-old clean-grade male Sprague-Dawley rats (Specific Pathogen Free, Shanghai SLAC Laboratory Animal Co., Ltd., China) were selected to prepare the models of single-segment caudal IVD. The laboratory animals were examined to ensure they were healthy and free of diseases. With one rat as the blank control, the discs of 10 rats were punctured using needles while the remaining 10 rats were used as a model for IVD following destruction of the endplate. Shanghai East Hospital (East Hospital Affiliated to Tongji University) Medical Ethics Committee approved the study protocol, which met the relevant guidelines and regulations of the Shanghai Medical Ethics Committee in accordance with internationally accepted principles for the use of laboratory animals.

Model preparation^[1,11,12]

First, 4% chloral hydrate solution was prepared using deionized water, and then injected intraperitoneally into the rats at 10 mL/kg/rat for anesthesia. Subsequently, deep anesthesia (loss of consciousness, slow corneal response, muscle relaxation, and loss of pain reflex) was confirmed in the animals prior to the operations.

To establish the needle puncture model, the ilium and sacrum of rats were palpated to identify the caudal vertebral segments near the hairless site at the trunk-tail junction. Generally, the caudal hair of rats ends at the 7th vertebra, and the 8th/9th or 9th/10th caudal IV discs are punctured. Rats have four microscopically visible large subcutaneous vessels in the tail, located in the dorsal midline, the middle of both sides and in the ventral midline. The midpoint of the connection between each set of two vessels with the IV disc plane was punctured, with 4 puncture points for each segment, during which the vessels were avoided. An 18-G syringe needle was inserted slowly into the IV disc, and upon completion of the insertion, the needle was rotated 360°, stopped for 30 s and then slowly withdrawn, followed by disinfection. After completely regaining consciousness, the rats were fed normally in an animal room for 2-3 wk (Figure 1A).

Similarly, the superior endplate of the 9th or 10th caudal vertebra was selected to establish the model of endplate injury-induced IVD. First, a 0.5 cm-long longitudinal incision was made in the skin adjacent to the IV disc on the superior part of the vertebra, avoiding the vessels. The subcutaneous fascia and ligaments were separated to expose the superior portion of the vertebra. Subsequently, a drill with a bit diameter of 1.5 mm was used to drill into the vertebra near the superior endplate at a 45° angle to destroy the endplate and its blood supply. The skin incision was then sutured and disinfected. Upon completely reviving, the rats were fed normally in the animal room for 2-3 wk (Figure 1B).

Imaging evaluation

After 2-3 wk, all rats received tail MRI using a magnetic resonance imager (UMR770, United-Imaging, Shanghai, China) and Pfirrmann classification^[13]. Rats with grade III-IV degeneration, indicating a significant decrease in the height of the IV disc space, underwent conventional X-ray examination using an X-ray machine (uDR588i, United-Imaging, Shanghai, China) and molybdenum target X-ray photography using the Mammomat Inspiration (Siemens, Germany) (Figure 1C).

The height of the space between degenerative vertebral segments in the conventional plain X-ray images and molybdenum target plain X-ray images was measured by four clinicians. Specifically, the height of both sides and midpoint of the IV disc space was measured and averaged together (Figure 2)^[14].

Statistical analysis

All statistical analyses were generated using SPSS 20 (Chicago, United States). The height of the IV disc space and data dispersion are presented as means and standard deviations. The inter-rater reliability and test-retest reliability of measurements by the four clinicians were determined and evaluated using intraclass correlation coefficients (ICC). The difference in the clarity of degeneration between molybdenum target X-ray photography and conventional X-ray examination was analyzed using McNemar's test at a significance level of 0.05.

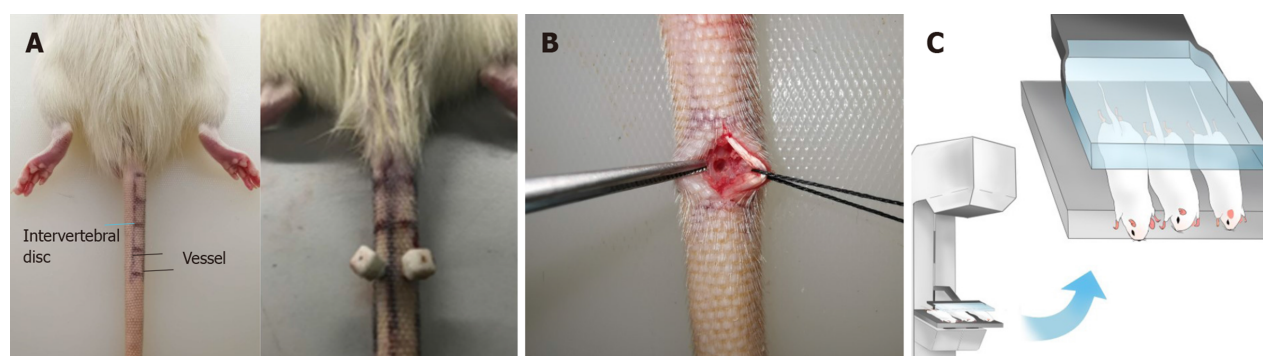


Figure 1 Schematic diagram of the approach to establish rat caudal intervertebral disc degeneration. A: Needle puncture of the intervertebral disc; B: Endplate destruction; and C: Molybdenum target X-ray photography in rats.



Figure 2 Measurement of mean intervertebral disc space height.

RESULTS

The present study included 21 rats, of which 20 were used in the final analysis; one rat died without an obvious cause. Of these 20 rats, nine were used to establish the needle puncture model, and 10 were used to establish the endplate destruction model. One rat was used as the blank control.

MRI, conventional plain X-ray image and molybdenum target plain X-ray image of the normal tail of the blank control rat are shown in [Figure 3](#). It can be seen from these images that compared with the conventional plain X-ray image, the molybdenum target plain X-ray image had higher definition, greater contrast and more clearly and precisely displayed anatomical structures, especially the IV disc space.

[Figure 4](#) displays MRI, conventional plain X-ray images and molybdenum target plain X-ray images of the two IVD models. Based on MRI, the two degeneration models demonstrated very high stability, feasibility and repeatability. Pfirrmann grade^[13] III-V degeneration was achieved after approximately two weeks. In addition, the molybdenum target plain X-ray images clearly showed narrowing of the IV spaces between degenerative vertebral segments, fine endplate calcification and even the drill holes in the endplate destruction-induced degeneration model. The conventional plain X-ray images were blurred, with less clear micro-calcification. In particular, it was difficult to pinpoint the boundary of the IV disc space, thereby affecting the measurement of disc space height. As shown in [Figures 4C and D](#), the conventional plain X-ray examination produced images that less clearly showed degeneration, so that the measurement of intervertebral disc space height was more difficult and uncertain. Based on the results of McNemar's test for such uncertainty, there was a statistically significant difference in the definition of IVD between molybdenum target plain X-ray images and conventional plain X-ray images ($P = 0.031$) ([Table 1](#)).

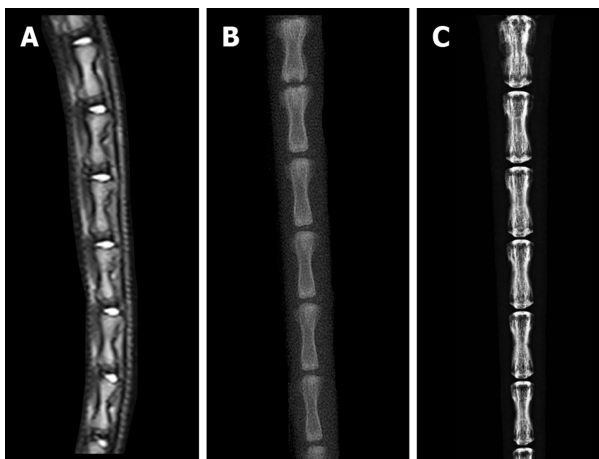
The height of the IV disc space in different plain X-ray images of degenerative vertebral segments in the 19 rats was measured by four observers ([Table 2](#)). According to the ICC value^[15], the measured height of the IV disc space in the conventional plain

Table 1 McNemar's test results of degeneration visualized using molybdenum target X-ray photography and conventional X-ray examination ($n = 19$)

		Molybdenum target inspection (No.)		^a <i>P</i>
		Obvious	Unobvious	
Plain X-ray (No.)	Obvious	12	0	0.031
	Unobvious	6	1	

^a*P*: McNemar's test, $P < 0.05$ **Table 2** Measured height of space between degenerative vertebral segments in different plain X-ray images

	mean $\pm$ SD ¹ (mm)	Observer 1	Observer 2	Observer 3	Observer 4	ICC
Needle puncture model ($n = 9$)	X-ray	2.0 $\pm$ 0.7	1.8 $\pm$ 0.5	2.3 $\pm$ 0.2	1.6 $\pm$ 0.4	0.41
	Molybdenum	1.7 $\pm$ 0.2	1.9 $\pm$ 0.2	1.9 $\pm$ 0.3	1.8 $\pm$ 0.1	0.81
Endplate-injured model ($n = 10$)	X-ray	1.7 $\pm$ 0.3	2.2 $\pm$ 0.5	2.6 $\pm$ 0.3	1.8 $\pm$ 0.1	0.35
	Molybdenum	2.1 $\pm$ 0.1	1.9 $\pm$ 0.3	2.1 $\pm$ 0.1	2.0 $\pm$ 0.2	0.92

¹mean $\pm$ SD: Mean height (Figure 2) $\pm$ standard deviation. ICC: Intraclass correlation coefficient.**Figure 3** Imaging of the normal rat tail. A: Magnetic resonance imaging; B: Conventional plain X-ray image; and C: Molybdenum target plain X-ray image.

X-ray images of the two models was less reliable ($ICC < 0.4$) than the measured height of the IV disc space in the molybdenum target plain X-ray images and was preferable and higher ($ICC > 0.75$). The measurements of the four observers on the conventional plain X-ray images had slightly larger standard deviations and a higher degree of dispersion, so it can be inferred that the boundary of the space between the degenerative vertebral segments in the conventional plain X-ray images was ill-defined for the four observers, greatly affecting the reliability of results.

DISCUSSION

A reliable evaluation method should be developed to establish a feasible, highly repeatable, effective animal model of IVD. Imaging evaluation is a crucial, easy to use, low-cost and effective technique, especially for the evaluation of IV disc space height, and is the most commonly used quantitative indicator of IVD in animal models^[16]. Therefore, the precision of plain X-ray images for the definition of degenerative vertebral segments is particularly important. However, for rats with small vertebral bodies and narrow intervertebral disc spaces, conventional plain X-ray images have

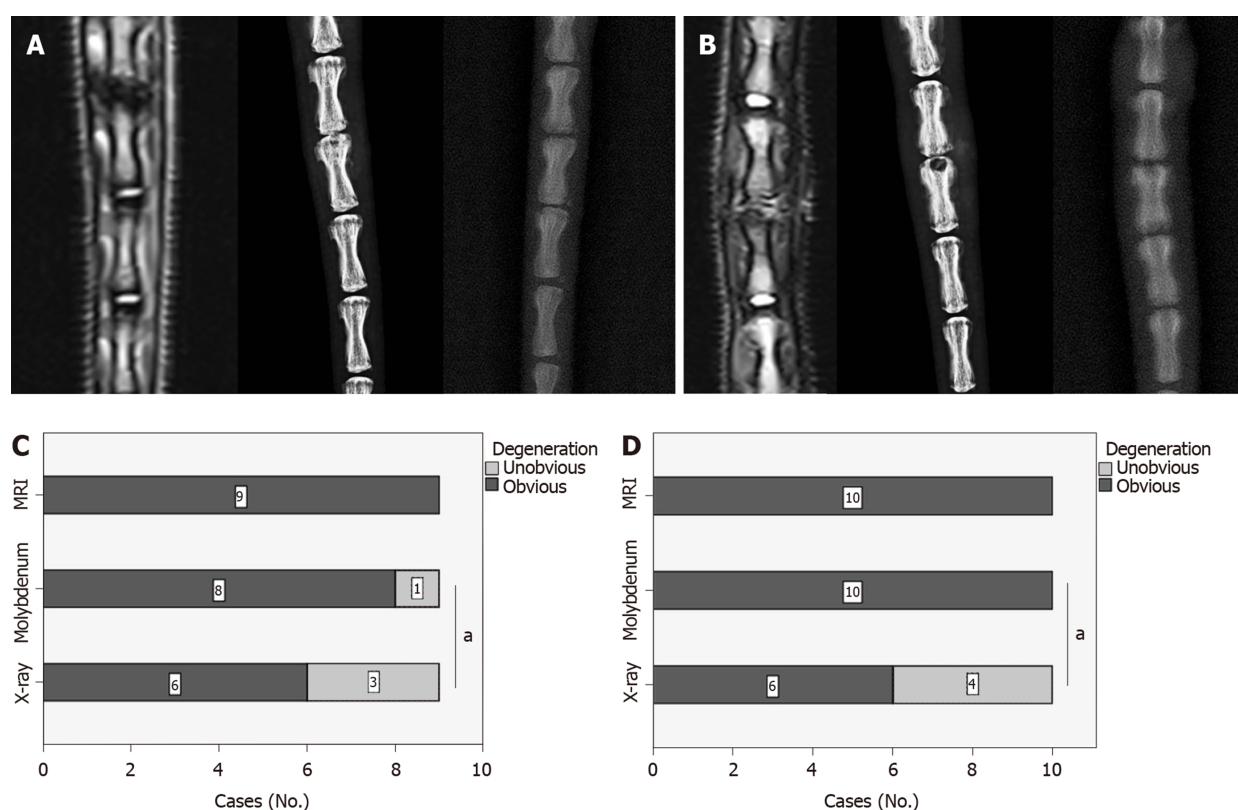


Figure 4 Imaging of the two models. Magnetic resonance imaging, molybdenum target plain X-ray images and conventional plain X-ray images. A: The needle-puncture injury-induced degeneration model; B: The endplate destruction-induced degeneration model; C: Statistics defining images of the needle-puncture injury-induced degeneration model ($n = 9$, ^aTable 1: McNemar's test); and D: Statistics defining images of the endplate destruction-induced degeneration model ($n = 10$, ^aTable 1: McNemar's test).

poor definition and low contrast, micro-calcifications are unclear, and the imaging effect on the boundary of the narrow IV disc space is poor, making it difficult to define the boundaries. This is the first study to apply molybdenum target X-ray photography to the imaging evaluation of caudal IVD in the rat models.

As molybdenum target X-ray photography has high contrast, high resolution and favorable repeatability and produces clear images of micro-calcifications, it is often used for breast examination, especially the screening of breast cancer^[17]. A conventional X-ray machine, with the tube anode surface made of tungsten, produces short waves, hard X-rays with strong penetrability. A molybdenum target X-ray photography device has a molybdenum anode surface and it produces soft X-rays characterized by constant wavelength, strong intensity, strong monochromaticity and high contrast and high resolution to distinguish subtle density differences, such as micro-calcifications^[18].

The needle-puncture injury-induced degeneration model and endplate destruction-induced degeneration model, the two most common rat models of caudal IVD, were used to verify the reliability, precision and universality of the molybdenum target X-ray photography in the present study. It was confirmed by MRI that Pfirrmann grade^[13] III-IV degeneration was produced after approximately two weeks. The models were highly repeatable and reliable, laying the foundation for subsequent research. In the needle puncture injury-induced degeneration model, molybdenum target X-ray photography provided very clear images for the measurement of IV disc space height and more accurately displayed the decrease in the height of the disc space compared with the conventional X-ray examination. Molybdenum target X-ray photography also provided more advantages in the endplate destruction-induced degeneration model, producing clear images of minor fractures and micro-calcifications, with higher contrast, making it possible to precisely and comprehensively evaluate the degeneration (Figure 4).

The measurement of IV disc space height is performed manually, so the measurement data vary due to differences among observers, reducing the reliability of the results. The standard deviations of measurements with conventional X-ray imaging were larger with higher dispersion. The ICC results also showed that the

measurement data of the four observers were greatly different and less reliable than the molybdenum target X-ray images (Table 2). Therefore, it can be concluded that the data from the molybdenum target X-ray photography are more reliable and precise, and reduce the uncertainty of observers in defining and measuring the IV disc space. However, molybdenum target X-ray photography is not suitable for examining mass samples simultaneously due to the structure of the machine.

As rats are small and have long, thin vertebrae and narrow IV disc spaces, precise and clear imaging data are particularly important to achieve accuracy and precision of measurement. Molybdenum target X-ray photography provides better definition, contrast and precision than plain X-ray images. As shown in Figure 4, molybdenum target X-ray photography excelled in the evaluation of micro-calcifications and minor fractures. Molybdenum target X-ray photography can be applied in the evaluation of small bones and joints (Figure 5), such as phalanges and interphalangeal joints, metacarpal bones, and metacarpophalangeal joints. Molybdenum target X-ray photography may be superior to conventional X-ray examination in the evaluation of arthritis or minor fractures in small joints.

This study had some shortcomings. First, the sample size was small, and more samples are needed for further verification. Only two models of IVD were used; thus, additional models are needed to further investigate the utility and limits of molybdenum target X-ray photography. The samples, model types and number of observers will be increased in a subsequent multi-center randomized controlled study.

Molybdenum target X-ray photography has more advantages and can provide clearer X-ray images and more comprehensive and precise data than conventional X-ray examination. We suggest that it may be very useful when used for clinical imaging evaluation of small bones and joints.

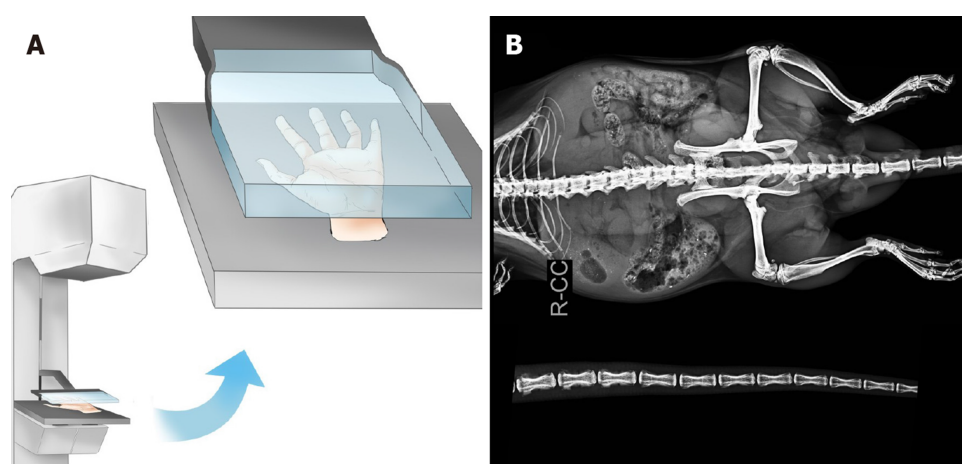


Figure 5 Schematic diagram of molybdenum target X-ray photograph of small joints and bones in the palms and the bones and joints in the whole body of the rats.

ARTICLE HIGHLIGHTS

Research background

Plain X-ray images of intervertebral disc degeneration show narrowing of the IV disc space, vertebral wedge-shaped deformities, endplate calcification and marginal osteophyte growth, of which the most characteristic of these changes is the narrowing of intervertebral space height.

Research motivation

However, in rats with small vertebral bodies and narrow intervertebral disc spaces, conventional plain X-ray images have poor definition and low contrast, microcalcifications are unclear, and the imaging effect on the boundary of the narrow intervertebral disc space is poor.

Research objectives

The present study is the first to apply molybdenum target inspection in the imaging evaluation of caudal intervertebral disc degeneration in rat models, and compare its effectiveness with routine X-ray examination.

Research methods

Two types of rat caudal intervertebral disc degeneration models (needle-punctured model and endplate-destructed model) were established, and their effectiveness was verified using nuclear magnetic resonance imaging. Molybdenum target inspection and routine plain X-ray were then performed on the effective models. Additionally, four observers were assigned to measure the intervertebral height of degenerated segments on molybdenum target plain X-ray images and routine plain X-ray images, respectively. The degeneration was evaluated and statistical analysis was subsequently conducted.

Research results

Nine rats were included in the needle-punctured model and 10 rats in the endplate-destructed model. Compared with routine plain X-ray images, molybdenum target plain X-ray images showed higher clarity, stronger contrast, as well as clearer and more accurate structural development. The McNemar test confirmed that the difference was statistically significant ($P = 0.031$). In the two models, the reliability of the intervertebral height measured by the four observers on routine plain X-ray images was poor ($ICC < 0.4$), while the data obtained from the molybdenum target plain X-ray images were more reliable.

Research conclusions

Molybdenum target X-ray photography has more advantages and can provide clearer X-ray images and more comprehensive and precise data than conventional X-ray examination.

Research perspectives

We suggest that molybdenum target X-ray photography may be very useful when used for basic medicine and clinical imaging evaluation of small bones and joints.

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