Spontaneous Age-related Cervical Disc Degeneration in the Sand Rat

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Abstract

Background Disc space narrowing, osteophytes, and disc degeneration are common and increase with aging. Few animal models are appropriate for the study of spontaneous age-related cervical disc degeneration.

Questions/purposes We used the sand rat, a member of the gerbil family with well-recognized age-related lumbar disc degeneration, to determine whether spontaneous cervical disc degeneration differed from lumbar degeneration when evaluated by (1) radiologic and (2) histologic measures. Animals 2 to 25 months of age were used in these analyses.

Methods Cervical and lumbar discs of 99 sand rats were analyzed with radiology, and cervical discs of 67 sand rats were studied with histology. Lateral digital radiographs of cervical and lumbar spines were scored for presence or

absence of wedging, disc space narrowing, osteophytes, end plate calcification, and irregular disc margins at C2–C3 through C6–C7 and T12–L1 through L7–S1. Percentages for presence were calculated and statistically analyzed for younger (range, 2–11.9 months old) versus older (range, 12.0–25 months old) animals.

Results Cervical discs in younger animals exhibited a greater proportion of irregular margins compared with lumbar sites (94% versus 83%; $p = 0.02$; 95% CI for difference, 2.7, 19.0%). In older animals, cervical discs showed a greater proportion of osteophytes than did lumbar discs (7% versus 0%; $p < 0.0001$). The incidence of disc space narrowing was greater in cervical versus lumbar sites (99% versus 90%; $p = 0.0008$). Cervical spine sites which contained osteophytes morphologically showed irregular disc margins and revealed an extrusion of herniated disc material in the osteophytes.

Conclusions Radiologic and morphologic studies confirmed age-related disc degeneration in the cervical spine of the sand rat.

Clinical Relevance Clinical cervical aging studies have shown that 14% of asymptomatic subjects younger than 40 years have abnormal MRI scans with an increase to 50% by 50 years old. We studied an economic rodent model for cervical age-related spontaneous disc.

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Introduction

Animal models are important tools in many types of orthopaedic spine research. The proceedings summary of the 1995 National Institutes of Health workshop on back pain emphasized the importance of this costly healthcare problem and recommended areas of future research, including the importance of animal models for the study of disc

degeneration. In this work, Krag commented that "... the usefulness of a particular model derives not from the extent to which it mimics reality (because that cannot be known in advance), but rather from the extent to which it facilitates the formulation and subsequent testing of hypotheses that lead to an improved understanding of that reality" [13].

Basic science studies of disc degeneration are complicated because in most species, disc degeneration must be induced with either surgical injury such as needle puncture (rabbit and other animal models [4, 11]) or chemonucleolysis [13, 16]. Needle puncture models have produced important data, but this method is now known to result in cell death, decreased dynamic modulus, and increased creep of the disc during short-term loading [12]. Needle injury also has been clinically associated with accelerated adjacent segment degeneration [18]. Sobajima et al. [27] commented that the classic stab model causes immediate herniation of the nucleus pulposus and thus cannot mimic slower changes in the nucleus or annulus associated with onset and progression of disc degeneration. It also is not appropriate as a model to test therapies that focus on early degeneration. Therefore, non-injury models, such as the spontaneous age-related lumbar disc degeneration in the sand rat, have great research value.

The sand rat (*Psammomys obesus obesus*), a member of the gerbil family, has age-related spontaneous lumbar disc degeneration develop. This model of lumbar disc degeneration first came to attention in the scientific literature in the 1980s [1, 17, 22–26, 32]. These early studies documented histologic and radiologic changes in animals 2, 3, 6, and 18 to 30 months of age and illustrated the histologic presence of granular debris in the nucleus, small annular tears, herniations into and through cartilage end plates, and ligament calcification. In the sand rat and in the human, the most advanced disc degenerative changes involve the lumbar and thoracolumbar spinal regions.

Human and sand rat lumbar degenerative features share many common features, including disc space narrowing, wedging, end plate calcification, irregular disc margins, and subchondral sclerosis [5, 9]. This model was used successfully in analyses of radiologic changes in the lumbar spine in cross-sectional and prospective animal populations [9, 28, 31], analyses of vascularization of the end plate [8], and experimental autologous disc cell implantation [10].

Clinical studies of the aging cervical spine have shown that 14% of asymptomatic subjects younger than 40 years have abnormal MRI scans with an increase to 50% by 50 years old [2, 6, 7]. Disc space narrowing, osteophytes, and disc degeneration are common and increase with age. Relatively few animal models have been used in analyses of cervical disc degeneration. Panjabi [20] reviewed animal models used in cervical spine biomechanical research, noting previous work in the canine [3] and goat [21, 30], and Yingling et al. [29] used the porcine cervical spine to model human lumbar spine.

In the current study, we used the sand rat, a member of the gerbil family with well-recognized age-related lumbar disc degeneration, and asked whether spontaneous cervical disc degeneration differed from lumbar degeneration as determined by (1) radiologic and (2) histologic measures. Animals 2 to 25 months old were analyzed.

Methods

Animal Model

Animal studies were performed after approval by the Institutional Animal Care and Use Committee. *Psammomys obesus obesus* specimens were obtained from our colony of animals of known ages. One to two animals were housed per cage in a dedicated housing room. Animals were provided tap water and Purina sand rat diet #5L09 (Ralston-Purina, Richmond, IN, USA) ad libitum; this special low-calorie diet is a formulation with 2.42 kcal/g metabolizable energy, which prevents the development of diabetes seen with high calorie ad libitum feeding. Animals were identified by IMI-10000 Implantable Micro Identification chips (BioMedic Data Systems, Inc, Maywood, NJ, USA).

Radiologic Analyses

Study Population

Cervical and lumbar discs of 99 sand rats (48 males and 51 females) 2.1 to 24.7 months old were radiologically evaluated. One author (RP), blinded to the ages of the animals, scored digital radiographic images for the following outcome measures: presence (scored as 1) or absence (scored as 0) of wedging, disc space narrowing, osteophytes, end plate calcification, and irregular disc margins at C2-C3 through C6-C7 and T12-L1 through L7-S1, and percentages were calculated for the presence or absence of these features. When there was poor imaging or bone overlap at a cervical or lumbar site, the respective feature for that site was not scored.

Histologic and Morphologic Studies

At euthanization, cervical spines were removed, trimmed of adherent tissue, fixed in 10% neutral buffered formalin, and decalcified and embedded in paraffin. Four-micron thick midsagittal sections were cut and sections stained with Masson-trichrome, dehydrated, cleared in xylene, and mounted using resinous mounting media. Morphologic studies were performed on 35 specimens from sand rats

2 to 11.9 months old and 32 specimens from sand rats older than 12 months. Histologic studies included qualitative study of sections prepared with routine stains (such as hematoxylin and eosin) and Masson-trichrome staining for assessment of disc and bone features.

Statistical Methods

Standard statistical analyses were performed using calculation of means $\pm$ SD. Radiologic data on presence or absence of wedging, disc space narrowing, osteophytes, end plate calcification, and irregular disc margins were statistically analyzed in younger (2–11.9 months old; $n = 28$) versus older animals (older than 12 months, $n = 63$) using the chi-square test or Fisher's exact test with SAS Version 9.2 (SAS Institute Inc, Cary, NC, USA). A p value of 0.05 or less was considered statistically significant. Radiologic data were collected on all scorable cervical and lumbar discs in the same animal and a final

data set formulated, which contained all cervical and lumbar data for animals of all ages.

Results

Radiologic Findings

Radiologic assessment showed that wedging was common in cervical and lumbar sites in both age groups (Table 1). Disc space narrowing differed in older animals for cervical versus lumbar sites. The incidence of narrowing was significantly greater in cervical sites compared with lumbar sites (98.7% versus 90.2%; $p = 0.0008$) (Table 2). In older animals, cervical discs showed a significantly greater proportion of osteophytes than did lumbar discs (6.5% versus 0%, respectively; $p < 0.0001$) (Table 3). In older animals cervical discs showed a significantly greater proportion of osteophytes than did lumbar discs (6.5% versus 0%, respectively; $p < 0.0001$). End plate calcification was present in both age groups in all

Table 1. Analysis of the radiologic feature of wedging.

Spinal site	Percentage present	Fraction present	Percentage present	Fraction present	p value, younger versus older
	Age 2–11.9 months		Age ≥ 12 months		
Cervical					
C2-C3	82.4%	14/17	100%	32/32	0.078
C3-C4	100%	17/17	100%	32/32	
C4-C5	88.2%	15/17	90.6%	29/32	
C5-C6	87.5%	14/16	93.6%	29/31	
C6-C7	73.3%	11/15	80.8%	21/26	
Overall cervical	86.6%	71/82	93.5%	143/153	
Lumbar					
T12-L1	82.4%	14/17	100%	32/32	0.842
L1-L2	82.4%	14/17	75.0%	24/32	
L2-L3	58.8%	10/17	68.8%	22/32	
L3-L4	82.4%	14/17	75.0%	24/32	
L4-L5	94.1%	16/17	90.6%	29/32	
L5-L6	100%	17/17	100%	32/32	
L6-L7	100%	17/17	100%	31/31	
L7-S1	100%	17/17	96.8%	30/31	
Overall lumbar	87.5%	119/136	88.2%	224/254	
p value, analyses of overall data, cervical versus lumbar	0.845		0.830		

Table 2. Analysis of the radiologic feature of disc space narrowing.

Spinal site	Percentage present	Fraction present	Percentage present	Fraction present	p value, younger versus older
	Age 2–11.9 months		Age $\geq$ 12 months		
Cervical					
C2-C3	88.2%	15/17	100%	32/32	0.098
C3-C4	100%	17/17	100%	32/32	
C4-C5	100%	17/17	96.9%	31/32	
C5-C6	100%	16/16	100%	31/31	
C6-C7	86.7%	13/15	96.2%	25/26	
Overall cervical	95.1%	78/82	98.7%	151/153	
Lumbar					
T12-L1	88.2%	15/17	90.6%	29/32	0.887
L1-L2	88.2%	15/17	81.3%	26/32	
L2-L3	64.7%	11/17	81.3%	26/32	
L3-L4	82.4%	14/17	81.3%	26/32	
L4-L5	94/1%	16/17	90.6&	29/32	
L5-L6	100%	17/17	100%	32/32	
L6-L7	100%	17/17	100%	31/31	
L7-S1	100%	17/17	96.8%	30/31	
Overall lumbar	89.7%	122/136	90.2%	229/254	
p value, analyses of overall data, cervical versus lumbar	0.159		0.0008		

Table 3. Analysis of the radiologic feature of osteophytes.

Spinal site	Percentage present	Fraction present	Percentage present	Fraction present	p value, younger versus older
	Age 2–11.9 months		Age ≥ 12 months		
Cervical					
C2-C3	0	0/17	0%	0/32	0.103
C3-C4	5.9%	1/17	9.4%	3/32	
C4-C5	0%	0/17	12.5%	4/32	
C5-C6	0%	0/16	6.5%	2/31	
C6-C7	0%	0/15	3.9%	1/26	
Overall cervical	1.2%	1/82	6.5%	10/154	
Lumbar					
T12-L1	0%	0/17	0%	0/32	1.0
L1-L2	0%	0/17	0%	0/32	
L2-L3	0%	0/17	0%	0/32	
L3-L4	0%	0/17	0%	0/32	
L4-L5	0%	0/17	0%	0/32	
L5-L6	0%	0/17	0%	0/32	
L6-L7	0%	0/17	0%	0/31	
L7-S1	0%	0/17	0%	0/31	
Overall lumbar	0%	0/136	0%	0/254	
p value, analyses of overall data, cervical versus lumbar	0.379		< 0.0001		

cervical and lumbar discs. Cervical discs in younger animals exhibited a significantly greater proportion of irregular margins compared with lumbar sites (93.9% versus 83.1%, respectively; $p = 0.02$) (Table 4). Disc space narrowing and irregular disc margins were much less evident in younger compared to older animals (Fig. 1).

Morphologic Findings

In cervical discs levels in younger animals, the nucleus pulposus often was observed to still contain notochordal cells (Fig. 2), a finding consistent with previous observations in lumbar disc sites [1]. Histologic evidence of cervical disc degeneration was present in the older animals as reflected in prominent wedging, narrowing (Fig. 2), and osteophyte formation (Fig. 1). With progressive aging, the nucleus in cervical discs became narrowed, and was increasingly difficult to identify in the oldest animals (Fig. 2). Cervical discs with osteophytes often revealed an extrusion of herniated disc material in osteophyte regions

Table 4. Analysis of the radiologic feature of irregular disc margins.

Spinal site	Percentage present	Fraction present	Percentage present	Fraction present	p value, young versus old
	Age 2–11.9 months		Age ≥ 12 months		
Cervical					
C2-C3	82.4%	14/17	87.5%	28/32	0.413
C3-C4	100%	17/17	93.8%	30/32	
C4-C5	94.1%	16/17	96.7%	31/32	
C5-C6	100%	16/16	93.6%	29/31	
C6-C7	93.3%	14/15	80.8%	21/26	
Overall cervical	93.9%	77/82	90.8%	139/153	
Lumbar					
T12-L1	76.5%	13/17	93.8%	30/32	0.200
L1-L2	64.7%	11/17	75.0%	24/32	
L2-L3	71.9%	9/17	71.9%	23/32	
L3-L4	76.5%	13/17	75.0%	24/32	
L4-L5	94.1%	16/17	93.8%	30/32	
L5-L6	100%	17/17	96.9%	31/32	
L6-L7	100%	17/17	96.8%	30/31	
L7-S1	100%	17/17	100%	31/31	
Overall lumbar	83.1%	113/136	87.8%	223/254	
p value, analyses of overall data, cervical versus lumbar	0.021		0.341		

(Fig. 1). Representative morphologic images of all discs in a complete cervical spine section of an 18-month-old animal are shown (Fig. 3).

Discussion

There are few animal models of cervical spine degeneration. The objective of our study was to determine whether cervical disc degeneration differed from lumbar degeneration using radiologic and histologic analyses of the spine in the sand rat, a small animal model of age-related, spontaneous lumbar disc degeneration. Animals 2 to greater than 12 months old were studied. Radiologic and morphologic studies confirmed age-related disc degeneration in the cervical spine of the sand rat.

Challenges and potential experimental limitations of cervical spine studies in the sand rat were the small size of this spine segment in very young animals and the expert care that was needed when dissecting the highest cervical levels from the skull. Attention to detail also was needed

Fig. 1A–C Representative radiologic sagittal views of cervical spines are shown for (A) a 2.1-month-old sand rat and (B) a 22.7-month-old animal. Prominent irregular disc margins (IM) and disc space narrowing (N) can be seen in the older animal. *an osteophyte that is shown in

histologic section in Illustration C. The white bar to the left of the spine is an animal ID chip. (C) A histologic section through the osteophyte region of the older animal shows the presence of extruded herniated disc material (Stain, Masson-trichrome; bar = 500 μ m).

Fig. 2A–D Representative midsagittal histologic images of cervical discs are shown from (A) a 3.9-month-old animal with normal morphologic features, (B) an 11.6-month-old animal with prominent wedging, (C) an 11.9-month-old animal with disc space narrowing,

and (D) advanced disc narrowing and degeneration with osteophyte formation and possible disc herniation in the osteophyte body in an 18-month-old animal (Stain, Masson-trichrome; bars = 500 μ m).

Fig. 3A–C Histologic images are shown for (A) the C7-T1 level, (B) C6-C7, C5-C6, and C4-C5 levels, (left to right, respectively), and

(C) C3-C4, C2-C3, and C1-C2 levels (left to right, respectively) from an 18-month-old animal (Stain, Masson-trichrome; bar = 500 μ m).

when sectioning the paraffin blocks to obtain good midsagittal dissection for histologic examination. Careful positioning was needed to obtain good radiologic images without the presence of other overlapping bones. When there was poor imaging or bone overlap at a cervical or lumbar site, the respective feature for that site was not

scored. Our study has numerous strengths, including analysis of cervical and lumbar sites in the same animal, and histologic analyses of morphologic features of the cervical spine, complementary to our digital radiologic imaging, with histologic views of the midsagittal cervical spine. The sand rat is a quadruped, thus it does not reproduce

biomechanical forces as seen in the human biped. In addition, this cervical model would be difficult to use for therapeutic investigations such as stem cell injections or other therapeutic testing.

Radiologic documentation of the sand rat cervical spine showed disc space narrowing, wedging, irregular disc margins, and osteophyte formation—features which mimic documented clinical degenerative features present in the aging human cervical spine [5, 9]. Our data identified a significantly higher incidence of irregular disc margins in the cervical spines of younger animals compared with lumbar sites; this suggests that disc degeneration may be proceeding at different rates in cervical and lumbar sites and points to the need for future study of cervical disc changes in this interesting and important animal model.

Histologic studies complemented the radiologic features and showed cellular and tissue changes in the nucleus related to cervical disc space narrowing, prominent wedging, and osteophyte formation. Morphologic examination of cervical discs with osteophytes revealed the novel finding of the presence of extruded herniated disc material in osteophyte bodies.

Literature on the clinical disc contains relatively few studies that assessed the association between cervical and lumbar disc changes and progression in healthy subjects [14, 15, 19]. Cervical spines of asymptomatic healthy subjects examined by Boden et al. [2] exhibited abnormal MRI scans in 14% of subjects younger than 40 years; the percentage increased to 50% in subjects 50 years old. In general, clinical studies show that stenosis in cervical or lumbar sites would positively predict stenosis in other spine regions 15% to 32% of the time. Okada et al. [19] suggested that individual factors (possibly genetic) may stimulate degeneration in cervical and lumbar discs simultaneously. Okada et al. [19] also found that cervical spine disc degeneration was more prevalent in patients with lumbar disc herniation than in healthy subjects, with the suggestion of the importance of attention to cervical discs in patients with lumbar herniations.

The data presented here provide validation of an economic, small rodent model with spontaneous, age-related degeneration of the cervical spine. Cervical changes were assessed versus features of lumbar changes present in the same animal. A significantly higher incidence of irregular disc margins in the cervical spines of young animals was found versus lumbar sites; this suggests that disc degeneration may be proceeding at different rates in cervical and lumbar sites. Future sand rat studies designed to provide detailed quantitative analyses of bone features in micro-CT cervical spine models and immunohistochemical analyses of matrix features in cervical discs would be a useful contribution to the literature. Since the sand rat is a member of the gerbil family, lack of specific antibodies for

extracellular matrix analyses may be continue to be challenging in future work.

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