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ORIGINAL ARTICLE

A demineralized calf vertebra model as an alternative to classic osteoporotic vertebra models for pedicle screw pullout studies

Atilla Akbay · Gokhan Bozkurt · Ozgur Ilgaz · Selcuk Palaoglu · Nejat Akalan · Edward C. Benzel

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Abstract Screws, clamps and other spinal instrumentation materials are tested using healthy animal and healthy human vertebrae, but the application of similar tests to an osteoporotic vertebra is generally neglected because of high costs and limited availability of high quality and consistent osteoporotic vertebrae. The objective of this study is to develop an in-vitro method to decrease the mineral content of an animal vertebra utilizing decalcifying chemical agents that alters the bone mineral density and some biomechanical properties to such an extent that they biomechanically mimic the osteoporotic spine. This study was performed on 24 fresh calf lumbar vertebrae. Twelve out of these 24 vertebrae were demineralized and the others served as control. A hole was opened in the pedicles of each vertebrae and the bone mineral density was measured. Each vertebra was then placed into a beher-glass filled with hydrochloric acid decalcifier solution. The decalcifier solution was introduced through the holes in the pedicles with an infusion pump. The vertebrae were then subjected to DEXA to measure post process BMD. Pedicle screws were introduced into both pedicles of each vertebrae and pullout testing was performed at a rate of 5 mm/min. The difference of BMD measurements between pre- and post-demineralizing process were also statistically significant ($p < 0.001$). The difference of pullout loads between pre- and post-demineralizing process were also statistically significant ($p < 0.001$). The acid demineralizing process may be useful for producing a vertebra that has some biomechanical properties that are consistent with osteopenia or osteoporosis in humans.

Keywords Osteoporosis · Invitro demineralized vertebra model · Bone mineral density · Pedicle screw · Pullout test

A. Akbay (✉) · G. Bozkurt · S. Palaoglu · N. Akalan
Department of Neurosurgery,
Hacettepe University School of Medicine,
06100 Sihhiye-Ankara, Turkey
e-mail: atik@hacettepe.edu.tr

E. C. Benzel
The Cleveland Clinic Foundation, Spine Institute,
Cleveland, OH, USA

O. Ilgaz
Engineering Faculty, Mechanical Engineering,
Istanbul University, Istanbul, Turkey

Introduction

Studying the osteoporosis related bone loss and instrumentation of osteoporotic vertebra is important in understanding bone mechanics and development of new and improved implant designs [8, 11]. Access to consistent and high quality human cadaveric tissue is limited. Furthermore, human cadaveric specimens are very heterogeneous from a biomechanical perspective. Since Swartz et al. [20] have shown that the mean tissue density, equivalent mineral density, compressive modulus and strength of calf spine are similar to a young healthy human spine; the calf spine has been a common substitute for human cadaveric spines for the biomechanical studies. Nevertheless, the development of an animal model for severe osteoporosis is not possible unless dietary restrictions or hormonal interventions are performed. This significantly increases experiment time and cost. Living animal models simulating the osteoporotic condition induced by overectomy and dietary restrictions are used to test drugs developed to treat osteoporosis [6, 10, 17, 21]. A

living animal model, however, is not necessary for the majority of biomechanical studies. Hence, there exists a need to develop a reproducible in-vitro osteoporotic vertebra model for pedicle screw pullout investigations.

Therefore, an in-vitro method of decreasing the mineral content of an animal cadaveric vertebra, utilizing decalcifying chemical agents, was developed. Such a technique should alter bone mineral density (BMD) and some biomechanical properties as dramatically as it occurs in osteoporotic human bone. The testing of this model is presented herein.

Material and methods

Four fresh lumbar spines from 12–14 weeks old calves were obtained for analysis. All specimens were cleaned of surrounding musculature, ligaments and periosteum, and separated into individual vertebrae, yielding 24 individual vertebrae. The specimens were then wrapped with saline-soaked gauze, sealed in plastic bags, and stored frozen at −20°C until the day of testing. Prior to testing, the specimens were thawed at room temperature for 24 h. Care was taken to keep the specimens moist throughout the experiment. Specimens were randomly divided into two groups of equal size: demineralized and control. The details of the demineralization procedure are described below.

Demineralization procedure

a) Specimen preparation

A hole into the pedicles of each vertebra was prepared with an awl at the junction of midlines of transverse process and facet. This hole was extended into the vertebra corpus with a blunt-tip pedicle probe to a depth of 40 mm. The diameter of holes, which is equal to the diameter of blunt type pedicle probe, is about 4 mm. The specimen was scanned using a Dual Energy X-ray Absortiometry (DEXA) scanner to determine BMD and bone mineral content (BMC) data. Pre-process BMD and BMC data were collected in the antero-posterior direction.

b) Demineralization

We observed that when a colored solution (methylen blue) was perfused with a syringe through one of the holes in the pedicles into the vertebra; the solution permeated all the trabecular bone network of the vertebral body. It left the vertebra from basivertebral vein foramina and the hole on the other pedicle. This implied that the infused decalcifying solution would permeate throughout as well. To examine this hypothesis and to determine the optimum time parameters, pilot experiments were performed with two or three vertebra (Two vertebrae for 1 h, two vertebrae for 6 h, two vertebra for 12 h, and three vertebrae for 24 h.). In pilot experiments, each vertebra, prepared as described above, was placed into a beher-glass filled with 500 cc hydrochloric acid decalcifier solution. The decalcifier solution in 50 cc volumes was then introduced through the holes into the pedicles with a syringe at 1 h intervals. After keeping and irrigating, the vertebrae in the decalcifier solution for 1, 6, 12 and 24 h vertebrae were washed under running tap water. Saline was given through pedicular holes until the decalcifier solution was completely removed. It was observed that the extent of demineralization (BMD and BMC) is correlated to exposure time to the acid bath, provided all other related variables (i.e., temperature, volume and concentration of demineralization solution) remained constant. The percent change of before and after demineralization procedure to time graph is presented in Fig. 1.

The favorable results from the pilot study described above led to the main research where each vertebra was individually placed into beher-glasses filled with 500 cc hydrochloric acid decalcification solution (Shandon TBD-1 Decalcifying Reagent, Thermo Electron Co. Pittsburgh, PA.). The temperature of the solution was 20–22°C. As the first step of the demineralization procedure, 50 cc of the decalcifier solution was flushed through a hole in the pedicle. Then, 480–500 cc of the decalcifier solution was introduced through the same hole into the vertebra with a controlled syringe type infusion pump (IVAC P 6000, IVAC Medical systems Hampshire, RG24 8WA) which has an infusion rate of 40 cc/h. The same procedure was

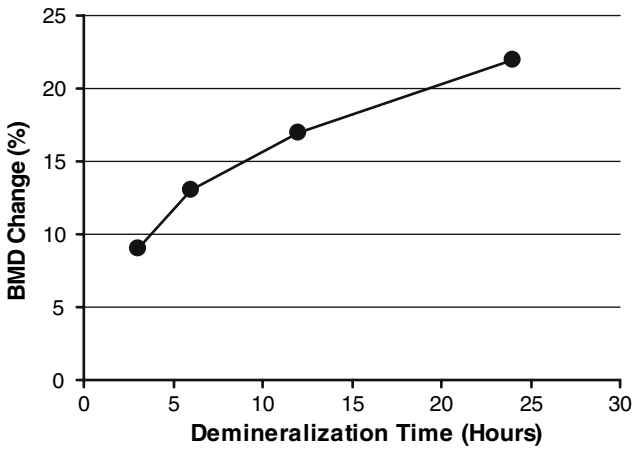

Fig. 1 The percent change of the BMD to demineralization time graphic. Data were achieved from pilot studies. As clearly seen, the percent change of the BMD increases with demineralization time

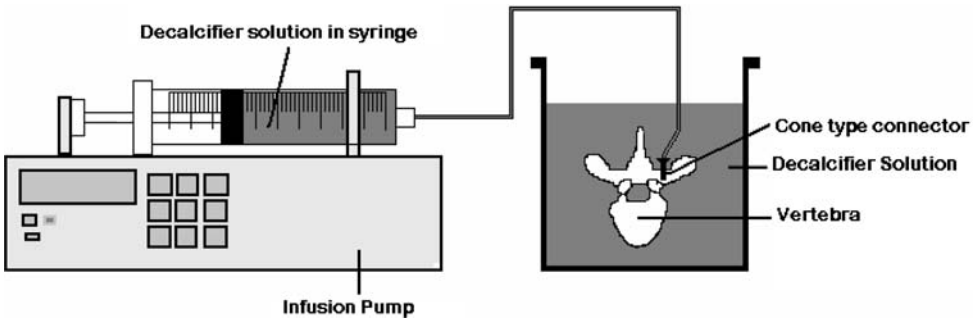

Fig. 2 Schematic diagram showing the experiment setup used for the demineralization process

repeated for the other pedicle for another 12 h. The overall duration of the demineralization time was 24 h.

The infusion pump and vertebra were connected by a 60 cm length of plastic tubing and a plastic cone type connector that snuggly fits the hole on the pedicle. The experimental setup is shown as a schematic diagram in Fig. 2. The vertebrae were then washed under running tap water and flushed with 500 cc of saline solution through the pedicle holes to completely washout the decalcifier solution from the specimen.

Control group

The control group consisting of 12 vertebrae was placed into a beher-glass filled with saline solution for 24 h. For measuring the post-process BMD, the specimens were DEXA-scanned as described above.

Pullout testing

After the BMD measurement process, both pedicles of each vertebra were tapped through the previously prepared infusion holes and instrumented with pedicle screws with a size of 6.5 × 40 mm (Tasarimmed, Istanbul, Turkey). Following this procedure, each vertebra was embedded in a metal alloy with low melting temperature (Cerrobend, Cerro Metal Products Co., Bellefonte, PA) and secured into MTS Alliance RT/10 materials testing machine (MTS Co. Eden Praire, MN). Pullout test was done at the rate of 5 mm/min and the data were collected at 50 Hz. At the end of the pullout test, Cerrobend was melted and vertebrae were removed.

Statistical analysis

Since the paired *t* test is used when measurements are taken from the same subject before and after some manipulation; pre-process and post-process BMD values of vertebrae were statistically analyzed using a paired *t* test to determine significant differences at the 95% confidence level. Descriptive statistics and non-paired *t* test were used to detect differences of the pullout load between decalcified and control groups

Results

The pre-process and post-process BMD and BMC values are shown in (Table 1). Mean and standard deviations of BMD measurements for pre- and post-process were found as 1.43 ± 0.08 and 1.12 ± 0.07 g/cm^2, respectively. A paired *t* test showed that the difference between pre- and post- process BMD measurements were statistically significant ($p < 0.001$). Mean and standard deviations of pullout loads were found as 668.13 ± 170.78 N in the decalcified group and $1{,}602.50 \pm 528.72$ N in the control group, as shown in Fig. 3. An unpaired *t* test demonstrated that the difference was statistically significant ($p < 0.001$).

Table 1 The BMD values of vertebra before and after demineralizing procedure

Specimen No.	BMD before demineralization (gr/cm^2)	BMD after demineralization (gr/cm^2)	Change (%)
1	1,572	1,072	32
2	1,517	1,012	33
3	1,460	1,174	20
4	1,515	1,241	19
5	1,417	1,148	19
6	1,395	1,076	23
7	1,332	1,072	20
8	1,27	1,065	17
9	1,432	1,148	20
10	1,472	1,175	21
11	1,404	1,084	23
12	1,372	1,174	15
Mean ± SD	1,430 ± 0,084	1,120 ± 0,065	21,833 ± 5,458

The percent change is presented in the last column. (Specimens were demineralized for 24 h)

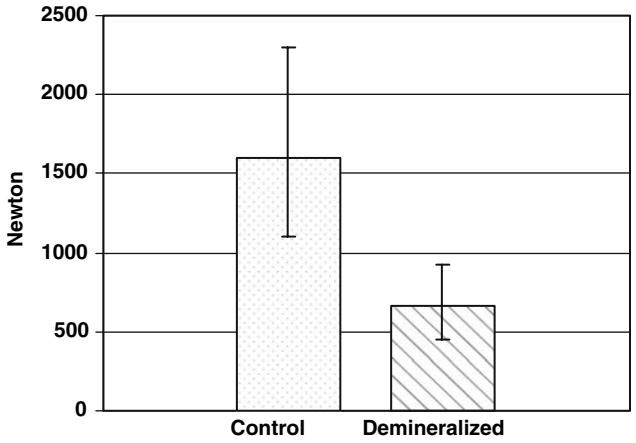

Fig. 3 Bar graph with error bands showing comparison of mean pullout loads of the control and demineralized vertebra groups

Discussion

Gonadectomized laboratory animals such as monkeys, minipigs, sheep, goats, rats and dogs are well known as models of experimental osteoporosis. They, however, present several problems such as unpredictable reproducibility of the extent of osteopenia, high costs, the consumption of time. Living animal models have been considered the ideal model to evaluate therapeutic chemical agents and to investigate the pathophysiological mechanisms of the osteoporosis. The cadaveric calf spine, however, is commonly used to evaluate biomaterials and prosthetic systems for spine fixation. Nevertheless, a calf model for osteoporosis has not been reported.

In the present study, we were able to create a reproducible demineralized vertebra model, in which a graded result was achieved—depending on the time of exposure to the decalsifier solution. The acid demineralization technique altered bone density and the screw pullout characterstics of the vertebrae as dramatically as they occured in osteoporotic vertebrae. It is well known that the bony part of the vertebrae is composed of an organic matrix, mineral and water components. Bone cells and collagen fibers are the main aspects of the organic matrix. The collagen fibers extend in all directions in the bone. The collagen fibers are surrounded by flat, plate-like mineral crystals that are known as hydroxyapatites. Hydroxyapatite crystals constitute the mineral components of bone [2, 3, 7, 21]. The mechanical properties of the vertebra (stiffness, strain, ultimate load, etc.,) are related to the extent of mineralization of the bone matrix [7, 22]. Currey [5] demonstrated that observed torsional strength is proportional to and most depended on mineral content. Calcium hydroxyapatite crystals of the bone could be dissociated by some acids or chelating agents. This process is known as demineralization. Demineralization of hard tissue has been utilized as a step in the preparation for histological and pathological microscopic examination. The reaction between acid and hydroxyapatite crystals is considered to be instantenous. The rate of demineralization is influenced by the concentration of the acid utilized [12, 13]. In the static acid bath, the rate of penetration of the demineralization is related to the molecular size of acid employed [1].

In our study, the acid solution was perfused through the pedicle holes by an infusion pump. In this way, the acid solution could be delivered to nearly all the trabecular bone in the vertebra.

Osteoporosis is defined in epidemiologic studies when bone mineral density is 2.5 standard deviations (SDs) or more below the mean for healthy young adult women at the spine, hip, or wrist [14, 15]. The Dual Energy X-ray Absortiometry scanning (DEXA) is considered the gold standard for BMD measurements, because it is the most extensively validated test against fracture outcomes [15]. The in vivo osteoporosis model, created with gonadectomy, has been, however, shown to provide only 11 and 13% decreases in BMD in primates and sheep, respectively [21]. In the present study, to determine the efficiency of demineralization and decrease the inter-specimen variability, the pre-process and post-process DEXA scan examinations were done for each vertebra individually. The *t* test for paired data demonstrated that the difference between the mean values of BMD in pre-process and post-process specimens to be statistically significant ($p < 0.001$). The mean BMD value for pre-process specimens was 1.43 ± 0.08 g/cm^2 and for post-process specimens was 1.12 ± 0.07 g/cm^2. In our model, the difference between pre-process and post-process BMD values was 21.68%. Therefore, it is reasonable to state that our demineralization protocol was able to successfully decrease the bone mineral density of a calf vertebra. It is also shown in Fig. 4 that the demineralization procedure caused thinning of cortex and demineralizing of the trabecular component of the vertebra.

The efficacy of a pedicle screw fixation system defines the ability of the bone structure to transmit skeletal loads to the fixation system and to withstand the forces applied to bone, such as screw pullout forces. BMD is an important determinant of the holding power of the pedicle screws in the vertebra [4, 18, 19]. Okuyama and coworkers [16] have shown that DEXA scan accurately assesses bone mineral density, which in turn influences biomechanical properties by determining, among many other factors, axial screw pullout strength. In addition, the trabecular architecture of the pedicle is different from that of vertebral body. Trabeculae of the pedicle are greater in thickness and ensemble having less spacing network, which is different from that of vertebra. Age related changes are observed as thinning but not loss of trabecula (the inner structure of the

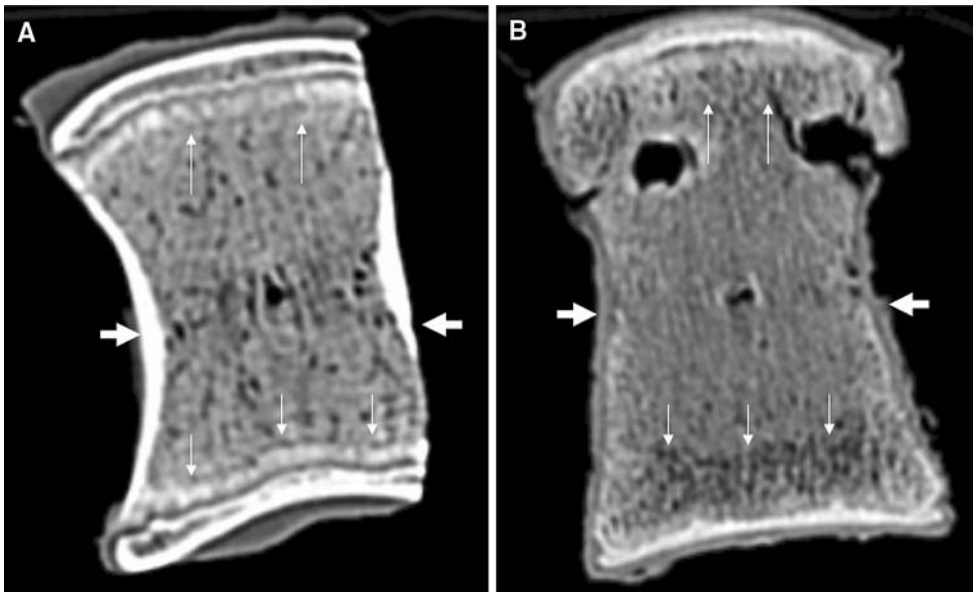

Fig. 4 Representative CT scans of vertebra. Before (**a**) and after (**b**) demineralizing process. Cortex thinning (*bold arrows*) and the dense subcondral bone which seen under the endplates loosening are observed (*fine arrows*)

pedicle is not substantially altered). Thinning of trabecula is the result of mineral loss. The presented model is just mimicking this pattern; hence, we examined the pedicle screw pullout forces in both groups [9]. The statistical analysis showed that the difference between the mean pedicle screw pullout forces of demineralized and control groups to be statistically significant ($p < 0.001$). Our results showed that the decalcification protocol presented here was also capable of mimicking the degradation in mechanical properties of the bone-pedicle screw interface as seen in the osteoporotic human spine.

Consequently, both DEXA measurements and pedicle screw pullout forces were consistent with the demineralization that occurred via the acid demineralization process. Hence, this process is a readily available method for creating a vertebra, which harbors the biomechanical properties of a vertebra with advanced osteoporosis. The calf vertebrae processed with this method could be used for pedicle screw pullout studies.

It is noteworthy that the histology and morphology associated with the model presented herein is more consistent with osteomalacia than osteoporosis. In order to truly mimic osteoporosis, the collagen content and in fact the structure of the trabeculae itself, would require removal (which does not occur in the model presented herein). Unlike osteoporosis, the structure of the trabeculae remains after decalcification. This histological and morphological differentiation should not detract from the experimental significance of this model. From a biomechanical perspective, the structural characterics are of the greatest importance. That is what was studied here. Therefore, the biomechanical observations and correlations made herein are of experimental and ultilmately clincal significance.

Conclusion

The acid demineralizing process may be useful for the creation of an invitro animal model that harbors the some biomechanical properties that exist in osteopenic or osteoporotic vertebra. BMC and pedicle screw pullout loads were analyzed in calf lumbar vertebrae by the acid demineralizing method presented herein. More studies are required to correlate stiffness, compressive strength, and the other biomechanical properties with the extent of demineralization.

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Important

Decalcifier solution used in this study is a hydrochloric acid derivative. Safety glasses and gloves should be utilized for personel protection and a good ventilation must be ensured in the laboratory when studying with the decalcifier solution. Material safety data sheets could be seen for warnings and precautions.

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