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Research Article

Demineralized Calf Vertebra Model: Can Be Used in Osteoporosis Research?

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Summary

In osteoporosis researchs, animal models have been used widely, but every model have associated with some advantages and disadvantages. In vitro studies with fresh cadaver look like good solution, however paucity of cadaver restricts its use. Demineralized calf vertebra model have been introduced as a new option. The aim of present study to check this new model on histopathological view and bone mineral density changes. Lumbar spines of seven fresh calves were cleaned from soft tissues and were submitted to DXA exams which provide the areal bone mineral density (BMD) and bone mineral contents (BMC). Than, demineralization procedure was done for each L1 and L5 vertebras of calves whereas other vertebras were used as control group. Following procedure, new DEXA exams were made. Light microscobic evaluation with Hematoxylen-Eosine staining was achieved in control and study groups. As a result, bone mineral density changes was statistically significant between two group, but histological appearance was far from ordinary bone with empty bone lacunes in demineralized vertebras.

Key words: Demineralisation, osteoporosis, calf vertebrae

Demineralize Dana Omurgası Modeli Osteoporoz Araştırmalarında Kullanılabilirmi?

Özet

Osteoporoz araştırmalarında hayvan modelleri geniş olarak kullanılmaktadır fakat her modelin avantajları ve dezavantajları bulunmaktadır. Taze kadavrayla yapılan in vitro çalışmalar iyi sonuçlar vermektedir, bununla beraber kadavra teminideki problemler kullanımını zorlaştırmaktadır. Demineralize dana vertebra modeli yeni bir seçenek olabilir. Bu çalışmanın amacı bu yeni modelde histopatoloji ve mineral dansitesindeki değişiklikleri incelemektir. Yedi adet taze dana omurgasının yumuşak dokuları temizlendi ve DEXA ile kemik mineral miktarları ve mineral dansiteleri ölçüldü. Demineralizasyon her omurgada L1 ve L5 vertebralarına uygulandı. Diğer vertebralar kontrol grubu olarak kullanıldı. İşlemden sonra yeni DEXA ölçümleri yapıldı. Işık mikroskobunda hematoksilen eozin boyaması kullanılarak histolojik inceleme yapıldı. Sonuç olarak kemik mineral dansiteleri iki grup arasında istatistiki olarak farklı bulundu. Demineralize vertebralardaki boş kemik lakünleriyle normal kemik yapıları histolojik olarak birbirinden farklı bulundu.

Anahtar Kelimeler: Demineralizasyon, osteoporoz, dana omurgası

INTRODUCTION

Osteoporosis is a age – related and multifactorial metabolic disease which restricts daily living activities of human. It is one of the major healthy probleme worldwide affecting especially elderly populations.

Osteoporosis is specific for human, because no mammalian specie other than human develops spontaneus bone fractures the its life span. However, the need of material other than human is indispensable for the studies on pathogenesis and surgical strategies of osteoporotic fractures and antiosteoporotic drugs. In vivo animal models are good at pathogenesis and medical treatment studies, so animals such as dog, cow,sheep, cat, dodent, rabbit, pig and minipig have been widely used. Osteoporosis can be induced through immobilisation, by feeding a low calcium or high phosphorus diet, following ovariectomy or with some drugs like corticosteroids and heparin in animal studies[13,3,7,11,5]. But, animal studies have limited benefits in studies with biomechanical measurements and implant use. In vitro studies with fresh cadaver have been used widely, but it has been limited by finding new cadavers. Recently, demineralized calf vertebra model has been reported as an alternative due to similarity to human spine[11,1,12,2].

The objet of present study is testing fresh calf model with corresponding histopathological changes in specimens and bone mineral density measurements following acid demineralisation.

MATERIAL AND METHODS

L1 – L6 spine of seven fresh calves aging 22-24 months were used in the study. They stored frozen at – 20 degree celsius until the day of testing. All lumbar segments were cleaned of surrounding soft tissues excluding intervertebral discs. L6 segments were excluded from the test. L1 and L5 vertebras were separated for osteoporosis model whereas L2, L3 and L4 vertebras were divided for control group.

Before the demineralisation procedure bone mineral dencity of fresh calve bones were measured (Norland X R.Series)

42 lt. 10 % of hydrochloricacid solution were prepared for acid demineralisation and stored until testing days. Demineralisation procedure was done using similar method described by Akbay et al.[1]. Each vertebrae was taped bilaterally from pedicule to the anterior half of the corpus with 11 gauge biopsy needle. It was any damage after insertion of the needle. Physiologic saline was given though right pedicule and its drainage from left pedicule plus foramina nutricium was detected.

14 lt. 10 % hydrochloricacid solution was separeted for demineralisation of vertebraes as 1 lt. in serum pocket for infusion and 1 lt. for filling in beher glass per vertebrae. Connection was achieved between biopsy needle in right pedicule and serum pocked including decalcifier solution. Pocked was fixed onto two metre high from the level of vertebrae. All vertebrae was placed into beher glass filled with decalcifier solution.

HCl. Acid solution was perfused by each pedicule during 12 hours, 24 hours totally with a 40 cc / hour rate. Decalcification procedure was finished following waching under running tap water till obtaining banish of calcium from the vertebrae.

In control group same procedure was performed with physiologic saline.

After completing procedure each vertebrae was scanned again using DEXA scanner for bone mineral density

In histological evaluation, all specimens of both group were processed by fixation in 10 % neutral buffered formalin, decalcification by strong acid (48 hours in 1 liter of water with 200 nitric acid), dehydration in graded alcohol, infiltration with xylene and embedding in parafine

blocks. Serial 5 mm. sections were prepared by microtome, than they were stained with Hematoxylen & Eosine.

For statistical analysis Wilcoxon Signed Rank Tests were used.

RESULTS

When bone mineral density measurements were compared before and after procedure it was seen that acid demineralisation decreased density as high as 37.79 % which is statistically significant ($p < 0.018$). (Table 1-2)

On light microscobic examination, in cross-section of bone tissues it was identified that calcium was succesfully banished from tissues in study group. General appearance of trabecular bone and bone marrow were preserved, but it was noted that bone lacunes were empty and no osteocytes were found inside (Figure 1,2,3).

Calcium was also seen as succesfully banished in control group(but as a result of pathological process), and trabecular bone and bone marrow areas were seen as ordinary morphology. Differently from study group, osteocytes and bone marrow cells were demonstrated noticeably in all cases of control group (Figure 4,5,6).

Table1: Bone Mineral Density (BMD) and Bone Mineral Content (BMC) changes in vertebras (a) before and (b) after the procedure.

	BMD (gr/cm^2)	BMC (gr)	BMD Changes %
Test 1a	0.8277	10.760	
Test 1b	0.5714	6.799	30.96
Test 2a	0.7045	9.307	
Test 2b	0.4028	4.491	42.82
Test 3a	0.7420	12.730	
Test 3b	0.4720	7.410	36.38
Test 4a	0.7587	9.579	
Test 4b	0.5233	7.228	31.02
Test 5a	0.6854	8.563	
Test 5b	0.3370	4.346	50.83
Test 6a	0.7754	10.100	
Test 6b	0.5383	7.663	30.57
Test 7a	0.7166	9.402	
Test 7b	0.4155	4.250	42.01
a(mean)	0.7443		
b(mean)	0.4657		37.79 %

Table 2: Test Statistics (b)

	BMDb-BMDa
A symp. Sig. (2-tailed)	.018

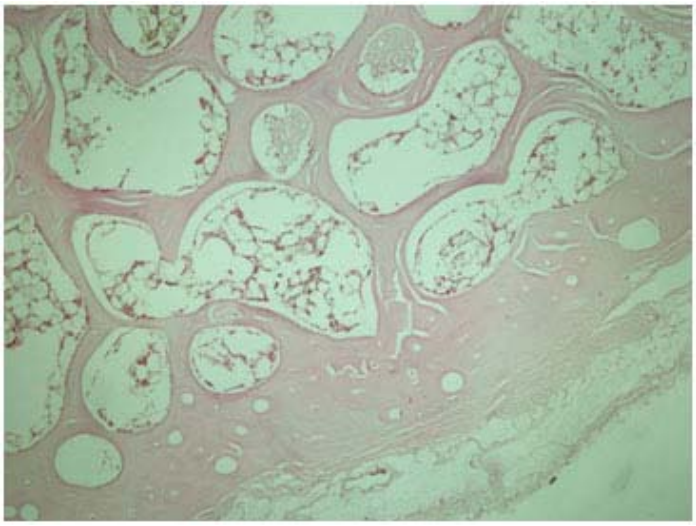

Fig 1: *General view of trabecular bone and bone marrow areas in study group (H&E, 4X).*

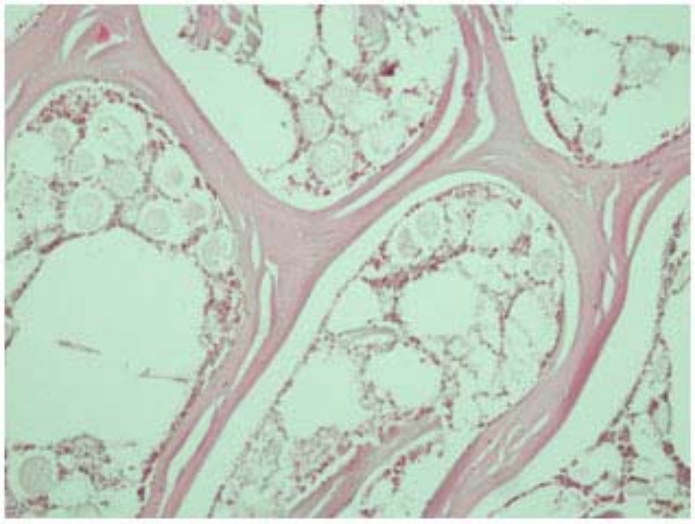

Fig 2: *There is no osteocyte in trabecular bone and cytolitic changes is observed in bone marrow areas (H&E, 10X).*

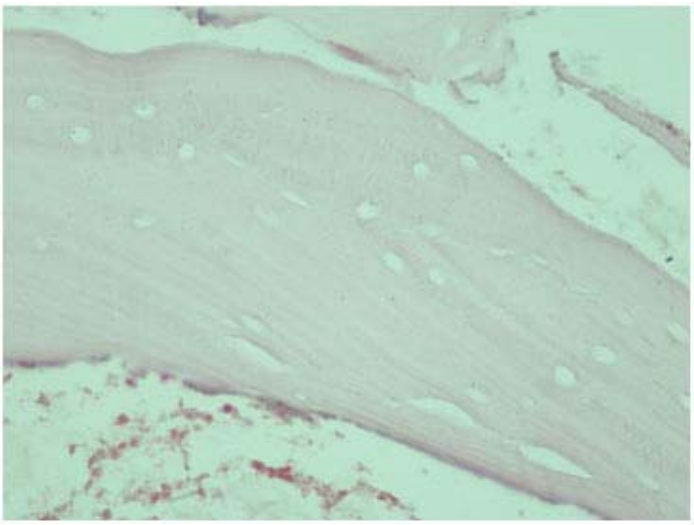

Fig 3: *Empty osteocyte lacunes are observed in trabecular bone (H&E, 40X).*

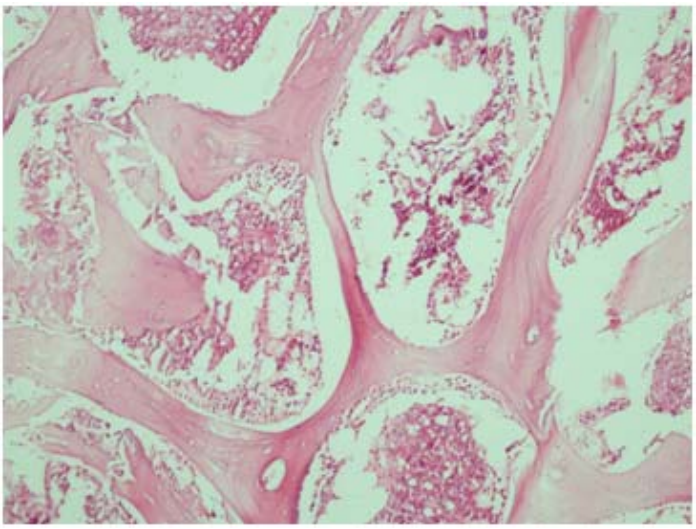

Fig 4: *Normal view of bone and bone marrow areas in control group (H&E, 4X).*

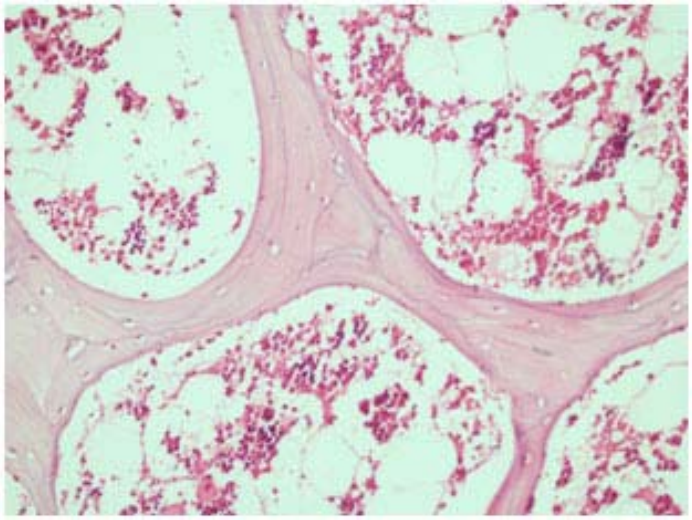

Fig 5: *Normal osteocytes in trabecular bone and normal cell population in bone marrow area (H&E, 10X).*

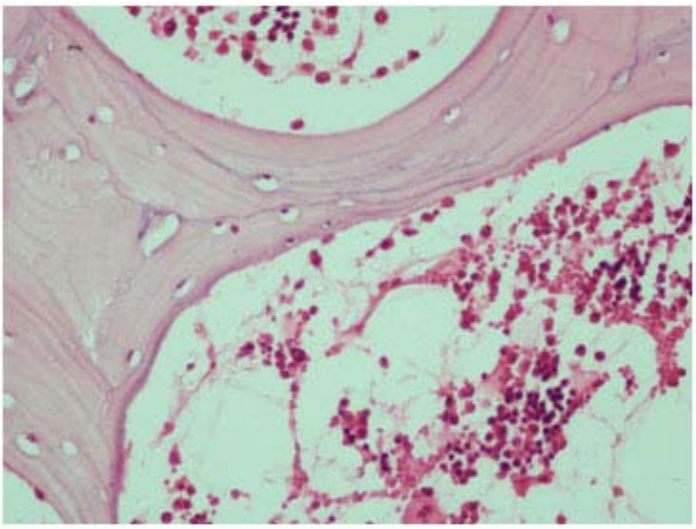

Fig 6: *Normal osteocytes in trabecular bone and normal cell population in bone marrow area (H&E, 40X).*

DISCUSSION

Osteoporosis is a disease of bone charecterised by reduction in bone mass and increased risk of fracture. In osteoporotic conditions, bone microarchitecture is disrupted, fragility is increased, so fracture may develop with a minimal trauma within daily living activities. Experimental osteoporosis models are generally performed in animal with some advantages like easy to hand and house, inexpensive, available in large numbers, spontaneously ovulate and some of them has large enough to evaluate spinal implants[13]. However every animal model has some disavantages; unsufficient control of agressive primate and high risk of zoonotic transmission disease, animal ethic problems of cats and dogs, lack of Haversian system in rats, high costs and rarity of pigs and minipigs, etc[13]. In addition, experiments with animals are time consuming and costly procedures.

Second option is fresh human cadaver and especially biomechanical analysis of spinal instruments, human fresh cadaver are satisfactory, but the problem is the difficulty in obtaining fresh cadaver in some centre. Under these circumstances large animal spines have looked as an alternative source. Although large animals such as baboon, sheep, porcine, calf and deer have some similarity with human spine, they have also some limitations in spine research[11]. Within these animals bovine thoracolumbar spine has been frequently selected as a model for in vitro mechanical studies[2]. The mechanical and physical properties of calf spine research detected that it can be selected as a good model of the young nonosteoporotic human spine and it is useful fort he testing of spinal instrumentation[12].

The prosess of bone demineralisation is used extensively in the preparation of bone specimens for histological study, modifying cortical bone allografts[6] and recently osteoporotic vertebra models[1]. Indeed, in vitro bone demineralisation can provide satisfactory decrease in bone mineral density and similarity in mechanical property of osteoporotic bone, however histological finding are not stated precisely. In our study we focused on if in vitro demineralized calf vertebra model can be used just an osteoporotic vertebra model. Bone mineral density measurements showed that density results were similar to osteoporosis, but microscobic examination revealed that cellularity of bone was also lost in addition of demineralisation of bone which has not seen in animal model of osteoporosis.

In histopathologic examination of osteoporosis induction in animal model; elongated, thinned, perforated and broken appearance of trabecullae, larger lacunar size[4] and reducing osteocyte density[15] have noted. Some changes in bone cells are also seen in human osteoporosis. Immobilisation alone causes an early increase in the trabecular osteoclastic resorption surface and later in the size of periosteocytic lacunae and an early depression of osteoblastic bone formation[9]. All other causes of osteoporosis such as age, menopause, chronic glucocorticoid excess, alcoholism, cigarette smoking result a changes in the number of bone cells, their birth rates, their life span and their death by apoptosis[8]. So decreasing number of osteocytes in osteoporosis is anticipated. Some animal studies demonstrated that osteocyt density was reduced in animal model[8]. Increased apoptosis of osteocytes and osteoblasts has been showed with high- dose glucocorticoid treatment in mice as similar as human osteoporosis[14]. Bone marrow cells did not changed statistically significant meaning in overiectomized animal model[10]. But, all these changes in cellular formation is not identified as much as acellular state of our in vitro model. In our control group osteocytes was seen as ordinary state. It was mean that

demineralisation caused death of all bone cells, so final bone tissues were weak as osteoporosis, but were not similar to osteoporotic bone on histologic aspect.

Contrary to histological discrepancy between in vivo and in vitro studies, ın our study, bone mineral density measurement showed saticfactory decrease to simulating osteoporosis and showed similar response to compressive strength. Therefore we used this model in a research concerning with kyphoplasty.

In conclusion, acid demineralisation technics in calf vertebrae model can be used for mechanical strength evaluation of spine and evaluation of bone fragility, but it is not a similar model of osteoporosis in animal or in human by reason of histological discrepancy.

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