Original article **UDC:612.751.08:616.714.1-007.234-021.6**
doi:10.5633/amm.2016.0206

OPTICAL DENSITY OF CORTICAL BONE MATRIX IS DIMINISHED IN EXPERIMENTALLY INDUCED OSTEOPOROSIS

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Osteoporosis is characterized by low bone mineral density (BMD) and abnormalities in bone structural and material properties, with unexplained low trauma fractures. The aim of the present study was to quantify the optical density of cortical bone matrix in rats with experimentally induced osteoporosis by ovariectomy. The experimental group was divided in two equal subgroups, the first sacrificed in the third month after ovariectomy and second sacrificed in the fifth month after ovariectomy. After decalcification, on routinely stained histopathologic sections optical density (OD), standard deviation of OD, mode OD, minimal and maximal OD of cortical bone matrix were estimated. Mean optical density and mode optical density of cortical bone were statistically higher in the control than in the experimental group ($p<0.05$). Maximal optical density of cortical bone was significantly lower in rats three months after ovariectomy than in other groups.

Obtained results indicate that in experimentally induced osteoporosis the optical density of cortical bone matrix is diminished, similarly to low bone mineral density. *Acta Medica Medianae 2016;55(2):35-39.*

Key words: osteoporosis, bone matrix, density

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Introduction

Osteoporosis is characterized by low bone mineral density (BMD) and abnormalities in bone structural and material properties, with unexplained low trauma fractures (1). Bone is a porous viscoelastic anisotropic composite material with three types of constituents; water, a variety of organic constituents (protein and cellular), and an inorganic mineral phase. Accoeding to weight percen-tage, the cellular constituents, matrix, and mineral phase are approx. 8%, 25% and 67%, respe-ctively. Bone is a complex calcified, living, biolo-gical composite with many different cell types: osteoblasts, osteocytes, quiescent bone lining cells, osteoclasts, and mononuclear resorptive cells (osteomacs or reversal cells) found in bones that reside in different locations (2). The ovariectomized animal has been widely used as a model of postmenopausal osteoporosis, as osteopenia that develops after ovariectomy has many qualitative similarities to the human disease (3).

New technologies for the diagnosis of osteoporosis are reference point indentation (RPI), Fourier transform infrared spectroscopy (FTIR), Raman spectroscopy, and magnetic resonance imaging (MRI) (4). All of these methods are based on the bone mineral content. On the other hand, for routine histopathologic sections decalcification is necessary, and comparison of histopathologic features with other diagnostic tehniques is in question. Reports regarding decalcified bone matrix density, on histological sections, are rare (5).

Aim

The aim of the present study was to quantify the optical density of cortical bone matrix in rats with experimentally induced osteoporosis.

Material and methods

Female Wistar rats weighting about 100 g were divided in three groups: control group (n=5) and experimental groups (n=10), with osteoporosis-induced ovariectomy. The experimental group was divided in two subgroups, one sacrificed in the third month after ovariectomy and the second sacrificed in the fifth month after ovariectomy. The left femur was removed and fixed in 10% neutral buffered formalin. Decalcification was

done with 10% formic acid. After routine hematoxillineosin (HE) staining, digital pictures of cortical bone were made at objective x20 on microscope BX50 (Olympus, Tokyo, Japan), in jpg format, resolution 1280x1020 pixels. Bone histomorphometry was done by ImageJ software (W. Rasband, National Institutes of Health, USA, http://imagej.nih.gov/ij), after calibration. Optical density (OD), standard devia-tion of OD, mode OD, minimal and maximal OD of cortical bone matrix were estimated (Figure 1). The obtained values were divided by the value of thickness of tissue section, which was obtained as width of the section fold (5).

Statistical significance of obtained differences was estimated by MANOVA test and post hoc analysis using the Tuckey test. P-values <0.05 were considered statistically significant.

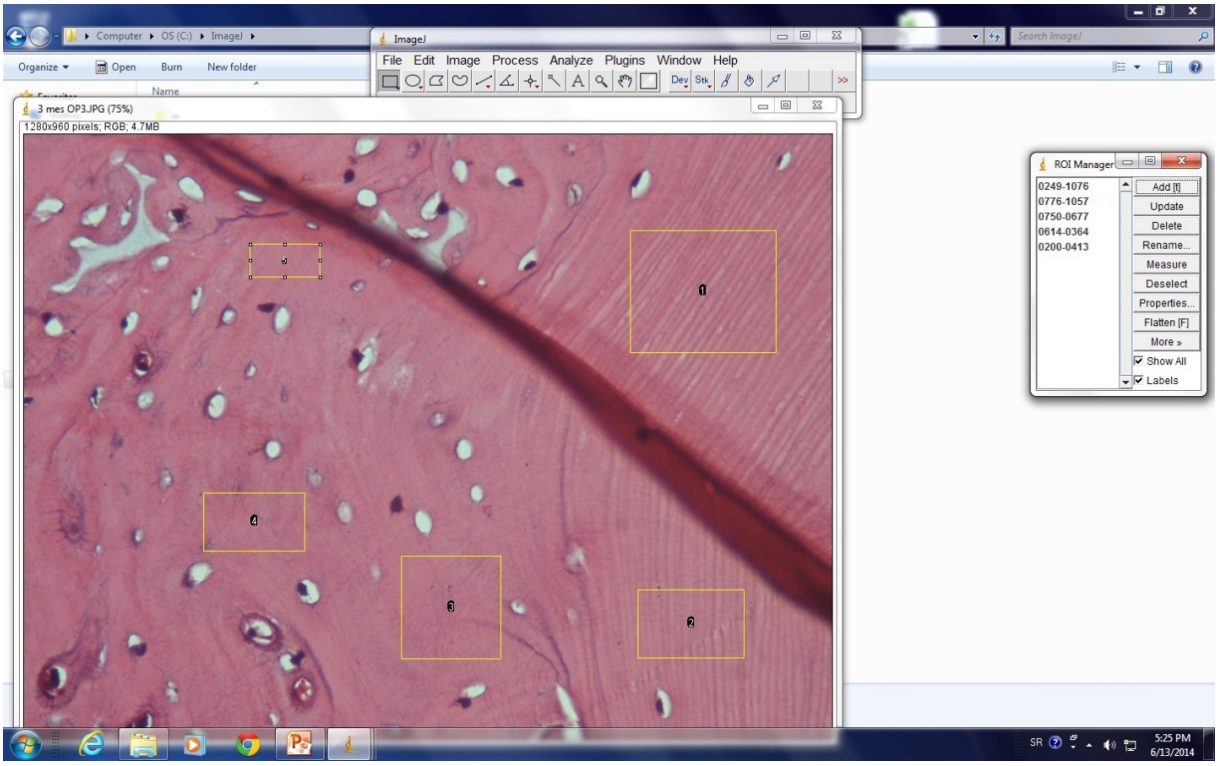

Figure 1. Using ImageJ software, rectangular boxes of various sizes were made on digital picture

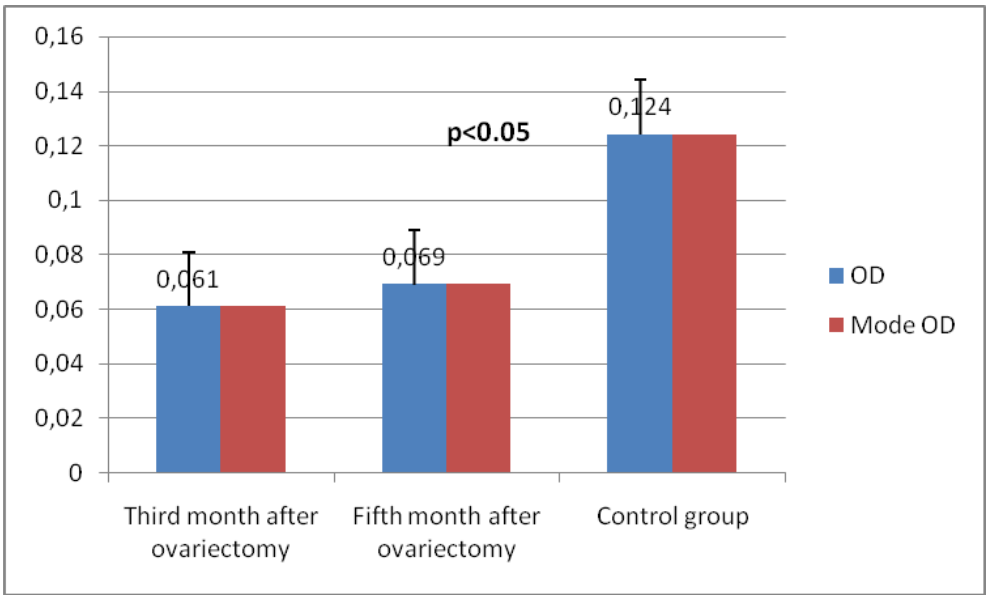

Figure 2. Mean optical density (±SD) and mode optical density divided by section thickness (a.u.)

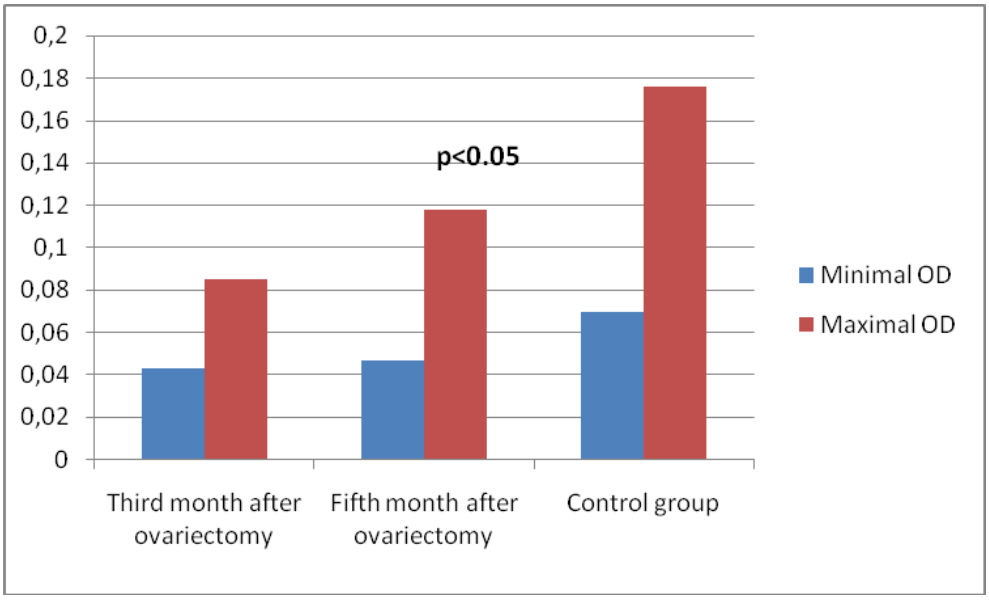

Figure 3. Minimal and maximal optical densities divided by section thickness (a.u.)

Results

Histologically, cortical bone tissues from different groups of rats were very similar.

Mean optical density and mode optical density of cortical bone were statistically higher in the control than in the experimental groups ($p<0.05$). Maximal optical density of cortical bone was sig-nificantly lower in rats three months after ovarie-ctomy than in the other groups ($p<0.01$) (Figure 2 and 3).

Discussion

Despite decades of increased public awareness and medical intervention, osteoporosis remains a major health issue in the elderly. Dietary factors and hormonal status are widely recognized for their importance to bone health. Clinical studies have suggested that the greater risk of bone loss in females may be correlated with osteoporosis after menopause. On the other hand, in women, idiopathic osteoporosis (IOP) is characterized by intact gonadal functions and unknown secondary cause of bone loss. In men, IOP is most commonly associated with lower bone formation (7, 8), thought due to osteoblast dysfunction (9), impaired proliferation (10), or decreased recruitment to remodeling or decreased recruitment to remodeling sites (11). Alterations in bone material properties have also been reported in younger subjects with bone fragility (12), and collagen maturity (as measured by collagen cross-link ratio) was higher in cases with fragility fractures (13).

The results of our study, obtained on decalcified bone tissue, indicates significantly lower mean optical density of cortical bone matrix in female rats with experimentally induced osteoporosis, especially at month three after ovariectomy. On the other hand, maximal optical density was nearly normal at month five after ovariectomy, probably because as a consequence of compensatory mechanism. Our results are similar to the previous study (5) in which rats were sacrificed at week five after ovariectomy, and diminished optical density of cortical bone was found in ovariectomized group. Further inves-tigations are needed to clarify the possible mechanisms of this phenomenon: osteoblast dysfunction, altered proliferation, decreased remodeling, higher collagen maturity, or something else.

Conclusion

According to our results, in experimentally induced osteoporosis, the optical density of decalcified cortical bone matrix is diminished, similarly to low bone mineral density.

Acknowledgments

This study was supported by the Ministry of Science, Republic of Serbia, grant III41017.

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Originalni članak **UDK:612.751.08:616.714.1-007.234-021.6**
doi:10.5633/amm.2016.0206

OPTIČKA GUSTINA KORTIKALNE KOSTI JE SMANJENA U EKSPERIMENTALNO IZAZVANOJ OSTEOPOROZI

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Glavne karakteristike osteoporoze su niska mineralna gustina kosti i promenjena građa kosti, sa pojavom preloma na neznatnu traumu. Cilj ovog rada bio je kvantifikacija optičke gustine matriksa kosti kod pacova sa osteoporozom koja je eksperimentalno izazvana ovariektomijom. Eksperimentalna grupa (n=10) podeljena je na dve podrupe sa po pet pacova, prva je žrtvovana posle tri meseca od ovariektomije, a druga posle pet meseci od ovariektomije. Posle dekalcifikacije, na rutinski bojenim patohistološkim isečcima, određivani su optička gustina (OD), standardna devijacija OD, modna OD, minimalna i maksimalna OD matrika kortikalne kosti. Nađeno je da su srednja i modna optička gustina matriksa kortikalne kosti statistički zanačajno veće u kontrolnoj grupi u odnosu na eksperimentalnu grupu. Dobijeni rezultati pokazuju da u eksperimentalno indukovanoj osteoporozi dolazi do smanjenja gustine matriksa kortikalne kosti, a ne samo do smanjena mineralne gustine kosti. *Acta Medica Medianae 2016;55(2):35-39.*

Ključne reči: *osteoporoza, matriks kosti, gustina*