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Bone microstructure and atomic periodic disharmonization in osteoporosis

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ABSTRACT

BACKGROUND
Both cortical and cancellous bone display a complex, porous microstructure whose properties depend on the macrostructure of bone as well as age and health of the individual. The aim of this study was to compare the microstructure and characteristics of mineral atoms in osteoporotic and normal bone.

METHODS
A prospective laboratory experimental study was conducted from August to December 2010 at several hospitals in Banjarmasin. Twenty patients with osteoporosis and twenty six normal patients were involved in this study. Bone obtained from surgery was analyzed for microstructure by scanning electron microscopy (SEM), while mapping of mineral atoms was performed by means of SEM-Energy dispersive X-ray spectroscopy (SEM-EDAX) at the Brawijaya University, Malang.

RESULTS
The osteoporotic subjects with mean age of 64.65 ± 16.41 years were older than the normal bone subjects with mean age of 39.38 ± 17.16 years. The body mass index was similar in both groups of subjects. From SEM-EDAX results, three patterns of mineral atoms were apparent in osteoporotic and normal bone. In osteoporotic bone, these patterns were indicative of a disordered substitution or incorporation process. SEM results showed degeneration of microarchitecture (resorption cavities, perforations, and prominent granules) in osteoporotic but not in normal bone. There was a significant difference in microstructure between osteoporotic and normal bone, which was caused by differences in atomic properties.

CONCLUSIONS
Microstructural abnormalities of bone and disharmonization of mineral atoms in the periodic system were found in osteoporosis.

Keywords: Mineral atoms, composite, microstructure, osteoporosis

Mikrostruktur dan disharmonisasi periodik atom pada osteoporosis

ABSTRAK

LATAR BELAKANG

Pada osteoporosis terjadi degenerasi mikrostruktur yang meningkatkan fragilitas dan risiko terjadinya fraktur. Insidens osteoporosis meningkat sesuai bertambahnya umur dan terjadinya fraktur biasanya relatif disebabkan oleh trauma minor. Penelitian ini bertujuan untuk membedakan mikrostruktur dan karakteristik atom mineral antara tulang osteoporosis dan tulang normal.

METODE

Sebuah penelitian prospektif dengan uji eksperimental laboratorium telah dilakukan antara bulan Agustus-Desember 2010 di beberapa rumah sakit di Banjarmasin. Dua puluh pasien osteoporosis dan dua puluh enam pasien normal terlibat dalam penelitian ini. Tulang yang diambil ketika dilakukan operasi fraktur, kemudian dianalisis mikrostruktur dengan menggunakan scanning electron microscope *(SEM) dan mapping atom dengan* SEM-Energy Dispersive X-ray spectroscopy *(SEM-EDAX) di Laboratorium Fisika Universitas Negeri Malang.*

HASIL

Subjek osteoporosis berusia rata-rata 64,65 ± 16,41 tahun jauh lebih tinggi dibandingkan subjek dengan tulang normal yang berusia rata-rata 39,38 ± 17,16 tahun. Indeks massa tubuh antara kedua kelompok tidak berbeda. Gambaran SEM menunjukkan degenerasi mikroarsitektur (kavitas resorpsi, lubang, dan granula yang tinggi) pada osteopotosis dibandingkan tulang normal. Hasil SEM-EDAX ditemukan tiga pola mapping atom pada osteoporosis dan tulang normal. Pada osteoporosis pola mapping atom mengindikasikan ketidakteraturan dalam sistem periodik akibat substitusi atau inkorporasi. Terdapat perbedaan mikrostruktur yang bermakna antara osteoporosis dan tulang normal, yang mengakibatkan adanya perbedaan pada pola mineral atom.

KESIMPULAN

Mikrostruktur tulang yang disertai adanya disharmonisasi atom mineral dalam sistem periodik ditemukan pada osteoporosis.

Kata kunci: *Atom mineral, komposit, mikrostruktur, osteoporosis*

INTRODUCTION

Bone is multifunctional in character, comprising support for body structures, protection and storage of regenerative cells, and homeostasis of mineral ions.[1] This material is non-uniform and heterogenous. Increasing age results in increased mineralization of bone tissue and the development of localized hypermineralization, such that it is prone to develop cracks that affect its overall mechanical characteristics. The effects of its mineralization pattern are more complex than that observable from an overall increase in tissue mineralization. With overall increase in its mineral content, bone becomes harder and stronger. When the optimum point or limit of mineralization for a given mineral is exceeded, the material loses its strength.[2]

Osteoporosis is characterterized by low bone mineral density (BMD) and microarchitectural degradation, both of which increase bone fragility and the risk of fractures.[3-5] The incidence of osteoporosis increases with age and fractures commonly occur from relatively minor injury. Several studies have stressed the role of environmental and social factors as predisposing factors of osteoporosis.[6]

Bone mineralization changes are strongly influenced by the properties of atoms that are capable of substitution to form composites. Previous studies have determined the atomic composition of bone,[7-9] which determines the dimensions of the hydoxyapatite crystals, which are larger[10] or smaller[11] in osteoporotic bone than in normal bone. To date the various atomic compositions of bone have only been assessed quantitatively, and there have been no studies for detection of atomic mapping in bone structures. The purpose of the present study was to compare atomic mapping and microstructural aspects of normal and osteoporotic bone. Assessment of atomic mapping of bone may supply further information on the determiner of bone quality, which should not be based solely on the quantitative aspect of mineral atomic content.

METHODS

Design of the study

A prospective laboratory experimental study was conducted from August to December 2010 at several hospitals in Banjarmasin.

Study subjects

The subjects of this study were women undergoing surgery in the Department of Orthopedics of Ulin General Hospital in Banjarmasin and other centers, with as inclusion criteria menopausal and nonmenopausal women; with trabecular fracture of the proximal femur; and osteoporotic and normal BMD scores. Exclusion criteria: concomitant disease (diabetes mellitus, stroke, and chronic renal failure) and use of steroids.

Measurements of bone microstructure

At the time of surgery, a sample of the fermoral head was taken for determination of bone microstructure by means of scanning electron microscopy (SEM) and mineral atomic mapping of the bone using SEM-Energy-dispersive X-ray spectroscopy (SEM-EDAX). SEM has been routinely used for the non-destructive study of bone surface structure and morphology. In conjunction with EDAX, SEM is capable of analyzing the atomic composition of materials by detecting atomic structures from levels of about 0.1 wt.

The subjects were divided according to BMD scores into a group with normal bone and a group with osteoporotic bone. For the diagnosis of osteoporosis we used BMD data (T scores) for young adults. According to these criteria, a T score of ≤ -2.5 indicates osteoporotic bone, while a T score of > -1 indicates normal bone.[5] SEM and EDAX-SEM examinations were performed at the Physics Laboratory of Brawijaya University in Malang. Analysis of EDAX-SEM data was based on atomic distribution patterns on SEM micrographs, classified according to the position of the respective elements in the periodic table of Mendeleev.

Statistical analysis

The characteristics of the subjects, comprising age, weight, height, BMI and proximal femoral BMD, were compared between the osteoporosis and normal groups, using independent t-test. The SEM and SEM-EDAX results were analyzed by visually comparing their respective images. SEM-EDAX results were compared by evaluating the distribution of the colored points on the bone surface, indicating the mineral atomic distribution.

Ethical clearance

This study obtained ethical clearance from the Research Ethics Commission of the Faculty of Medicine, Brawijaya University, Malang.

RESULTS

This study involved 20 patients with osteoporotic BMD and 26 patients with normal BMD, whose characteristics are presented in Table 1. The osteoporotic subjects with mean

Table 1. Characteristics of osteoporotic and normal subjects

	Osteoporosis (n=20)	Normal (n= 26)	p value
Age (years)	64.65 ± 16.41	39.38 ± 17.16	0.000
Weight (kg)	56.94 ± 15.87	60.20 ± 8.91	0.096
Height (m)	1.55 ± 0.04	1.62 ± 0.06	0.001
BMI (kg/m^2)	23.95 ± 6.81	22.75 ± 3.21	0.913
BMD of proximal femur (g/cm^2)	-2.93 ± 2.03	0.52 ± 1.48	0.000

BMI=body mass index; BMD=bone mineral density

Table 2. Atomic mapping patterns in osteoporotic and normal bone according to period

Pattern	Osteoporosis	Normal	Period
1	Ca, P	Ca, K, Ti	IV
2	Zn, Cu	Zn, Cu, Fe	IV
3	Al, K, Mg, S, Ti, Fe	Al, P, Mg, S	III

age of 64.65 ± 16.41 years were older than the normal subjects with mean age of 39.38 ± 17.16 years. The BMI of both groups of subjects were comparable.

As presented in Table 2, this study found deviations in atomic patterns of osteoporotic bone in relation to the periodic system of Mendeleev, while the atoms in normal bone still followed the regularity of the Mendeleev periodic system. Calcium (Ca), potassium (K), and titanium (Ti) atoms, which showed a homogenous pattern in normal bone (period IV), became non-homogenous as a result of the presence of phosphorus (P) (period III). The atoms of Al, P, Mg, and S, with a homogenous pattern in normal bone (period III), showed a non-homogenous pattern due to the presence of K, Ti and Fe from period IV.

On the basis of SEM analysis, this study found microstructural differences between osteoporotic and normal bone. At 50 x magnification, SEM images of osteoporotic bone showed trabecular structural degradation, with cavities due to excessive resorption of the trabecular arches. The degraded bone was marked by trabeculae with thin and flat walls and numerous cracks, and the beginings of a granular structure. In normal bone the trabeculae were still massive, with interconnected thick-walled arches and rare instances of trabecular cracks, without apparent granular structure (Figures 1a and 1b).

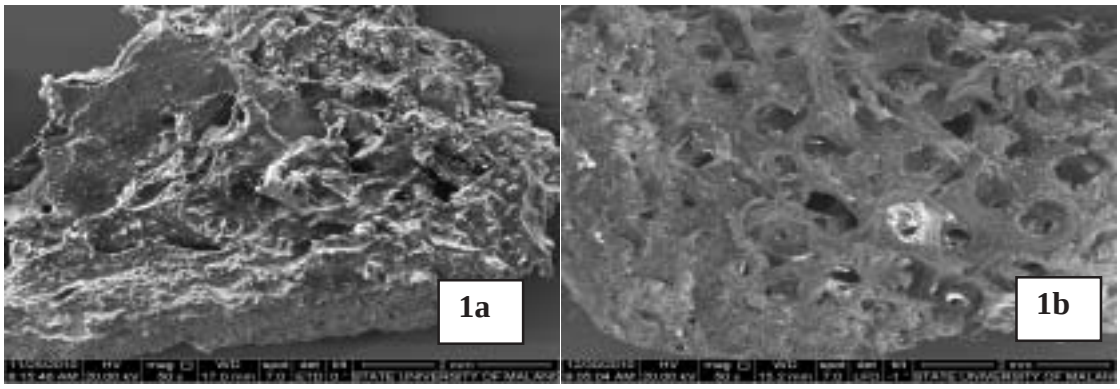

Figure 1. Appearance of trabecular structures in osteoporotic (1a) and normal (1b) bone at 50 x magnification

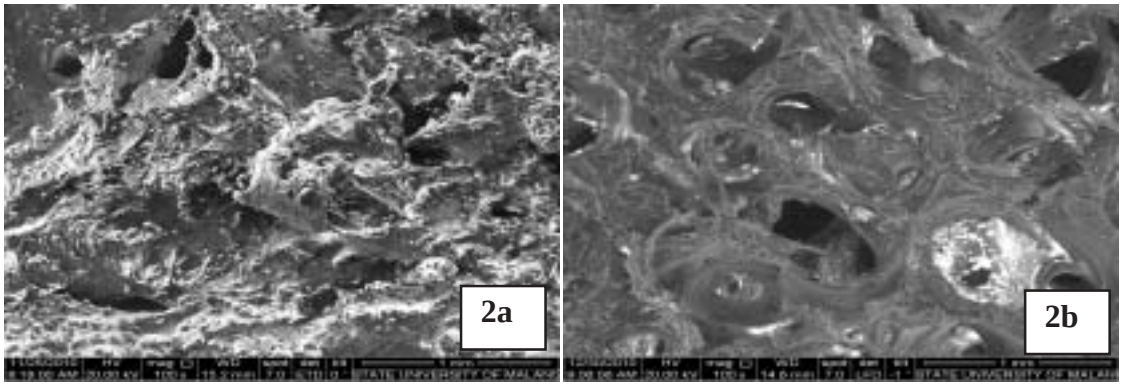

Figure 2. Appearance of trabecular structures in osteoporotic (2a) and normal (2b) bone at 100 x magnification

At 100 x magnification, the SEM images of osteoporotic bone showed the presence of perforations at several locations, surrounded by resorption cavities and trabecular stumps. The surface of the remaining trabecular structures appeared flattened and thin. In contrast, normal bone showed resorption cavities that had not yet become large perforations; the trabecular walls were still thick with a knobby surface, without any cracks (Figures 2a and 2b).

At 3000 x magnification osteoporotic bone showed granular structures of variable dimensions surrounding resorption cavities and trabecular stumps. The formation of granules resulted in increased porosity of the osteoporotic bone. In normal bone no granular structures were yet apparent and its dominant feature was the presence of a network of interconnected fibrillar strands (Figures 3a and 3b).

On EDAX-SEM analysis there were differences in patterns of mineral atoms between osteoporotic and normal bone. Ca and P had similar atomic mapping patterns in osteoporotic bone. However, in normal bone the Ca mapping pattern differed from the P mapping pattern, and resembled the K and Ti mapping patterns (Figure 4).

The second mapping pattern in osteoporotic bone was that shown by Zn and Cu. In normal bone the mapping patterns of Cu and Zn resembled that of Fe (Figure 5).

The third mapping pattern in osteoporotic bone was the pattern shown by Al, K, Mg, S, Ti, and Fe, while in normal bone the mapping patterns of Al, P, Mg, and S were similar (Figure 6).

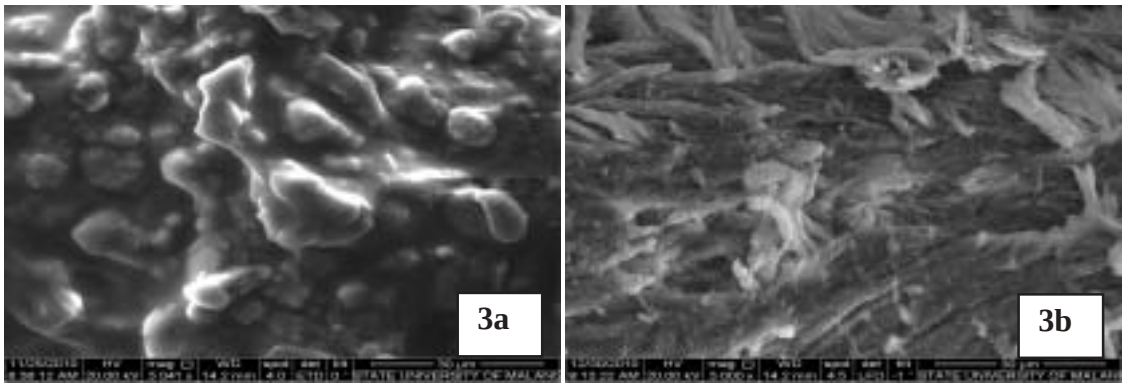

Figure 3. Appearance of trabecular structures in osteoporotic (3a) and normal (3b) bone at 3000 x magnification

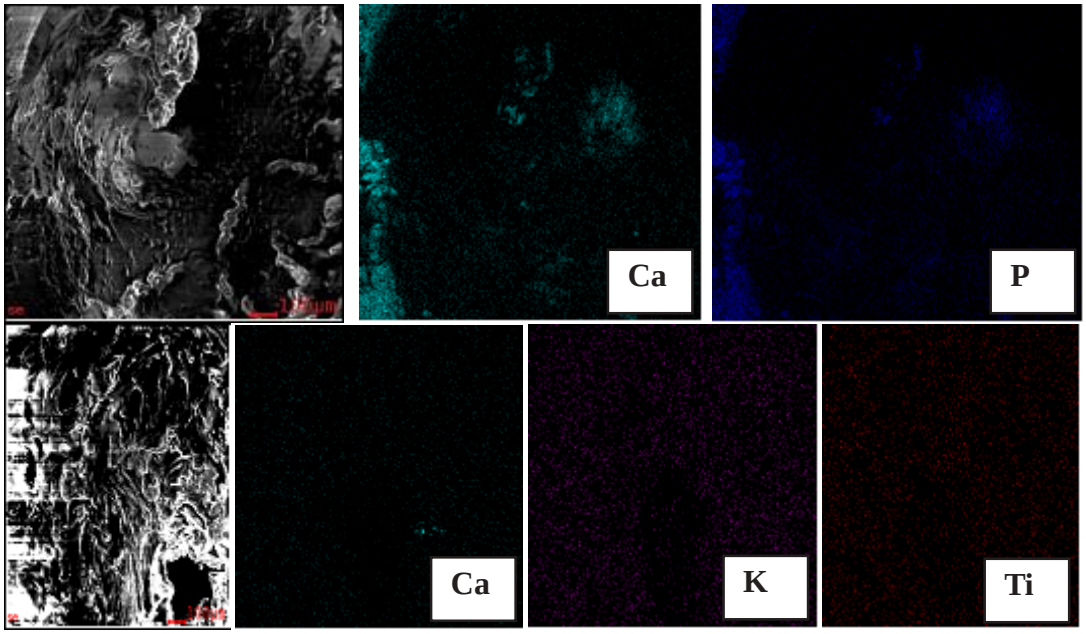

Figure 4. Mapping patterns of Ca and P in osteoporotic bone (top) and mapping patterns of Ca, K, and Ti in normal bone (bottom).

DISCUSSION

Bone undergoes continuous changes throughout life under the influence of hormonal and physical factors. Trabecular bone is sensitive to hormonal or biological factors involved in its metabolism. The mechanical properties of bone are determined by the microarchitecture of the trabeculae, comprising their numbers and thickness, and the presence of cavities.[12]

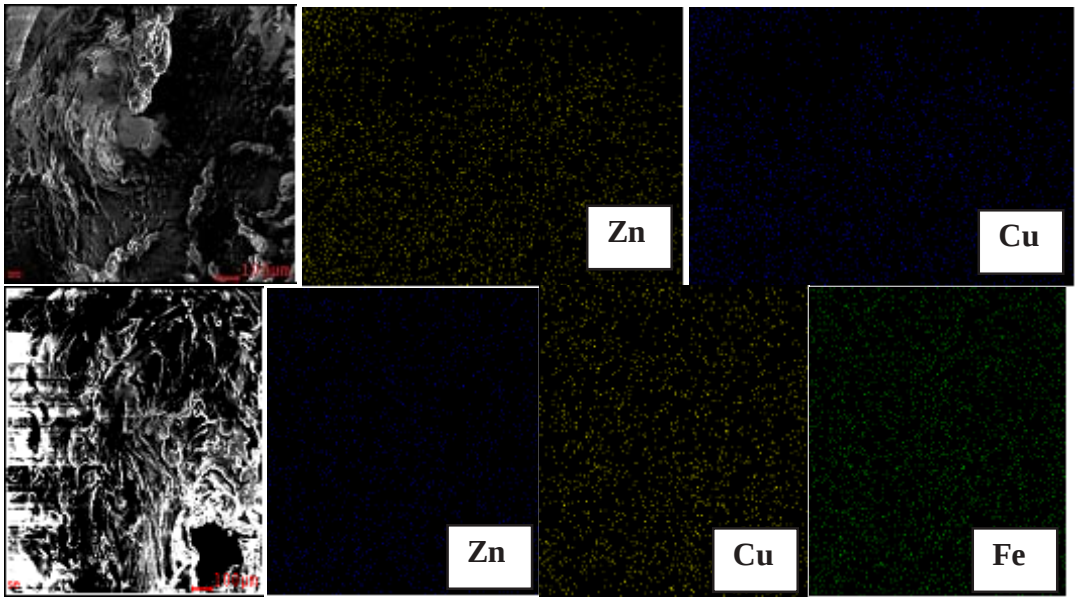

Figure 5. Mapping patterns of Zn and Cu in osteoporotic bone (top) and mapping patterns of Zn, Cu and Fe in normal bone (bottom)

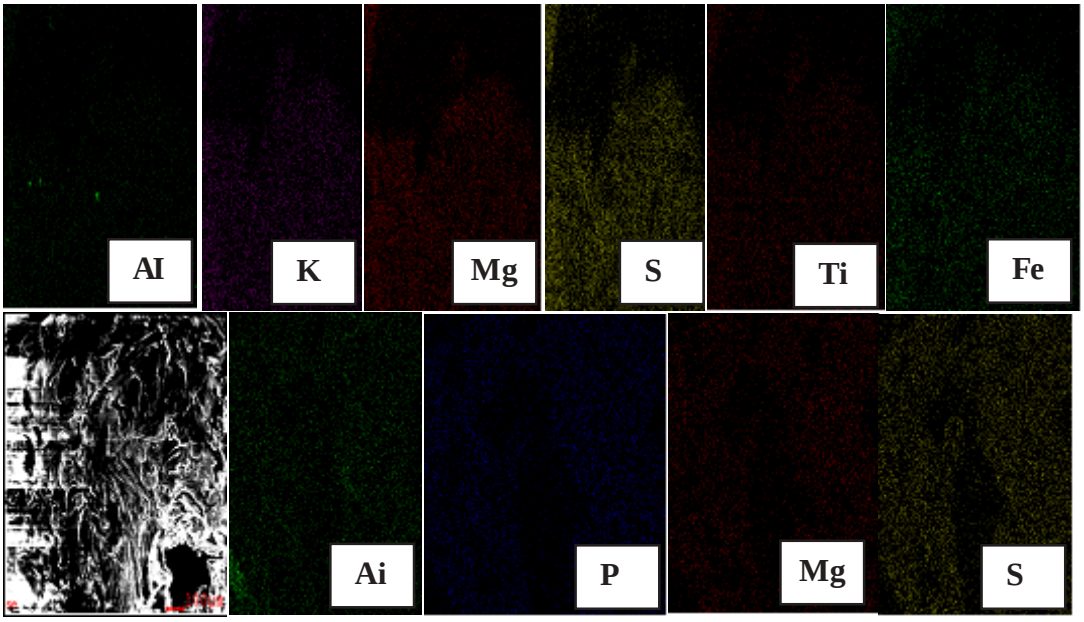

Figure 6. Mapping patterns of Al, K, Mg, S, Ti, and Fe in osteoporotic bone (top) and mapping patterns of Al, P, Mg, and S in normal bone (bottom)

The microstructure of osteoporotic bone shows a diminished amount of bone and a degraded microarchitecture. At 50x magnification osteoporotic bone shows a thinning and reduction of bone structures, and the presence of cracks and perforations, resulting in loss of integrity of the trabecular arches. At 100 x magnification osteoporotic bone displays flattened trabeculae with perforations, while at 3000 x magnification granules are found. Qualitatively, these results are similar to those obtained by Shen et al[12] and Jasiuk.[13]

Differences were also found between osteoporotic and normal bone with regard to atomic mapping patterns, as a result of differences in subsitutions or incorporations in the atomic structure of the bone. Substitution is the replacement of one type of atom with another type, as a result of similarities in the length of the radii and electric charges of these atoms. Incorporation is the joining of one type of atom into the structure/system of other types of atoms, thus affecting the overall integrity of the molecule. In addition, the presence of molecular cavities surrounded by functional groups with affinities to certain mineral atoms also determines the type of atom involved in substitution or incorporation. The affinity of a functional group may attract or repel one or more types of mineral atoms. Overall, the role of substitution and incorporation determines the growth of the bone matrix.[14]

This study found that in osteoporotic bone there were deviations in atomic patterns in relation to the periodic system of Mendeleev, whereas in normal bone the regulatity of the periodic system was still present. These abnormalities were presumably caused by substitution or incorporation of mineral atoms. In addition, these patterns also determine the potential for substitution or function of each atom within the patterns. In normal bone, the first pattern showed a similar mapping between Ca, K, and Ti. These three elements are in the same period IV in the periodic system of Mendeleev. The potassium (K) atom substitutes for calcium (Ca) in the hydroxyapatite crystal, given the presence of a molecular cavity, because the K atom has a longer ionic radius than Ca.[15] The titanium atom imparts hardness and strength to the bone. In the periodic system, the position of Ti is adjacent to that of Ca, in other words, Ti

is homogenous with Ca. The smaller ionic radius (2.23 Å) of the Ti atom enables it to substitute for Ca, or to reach homeostasis to support the strenght of bone. The periodic disharmonization in osteoporosis is marked by similar mapping patterns of Ca atoms (period IV) and P atoms (period III). This disharmonization is due to atomic dishomeostasis, porosity, or decreased size of the hydroxyapatite crystal in osteoporosis. Apparently the first pattern is related to the strength of bone.

The second pattern of normal bone indicates the potentials for substitution between Zn, Cu, and Fe atoms as a result of their adjacent positions in the periodic system. The ionic radius of Fe (1.73 Å) is longer than that of Cu (1.57 Å) and Zn (1.53 Å), such that Fe may be substituted by both Cu and Zn. Zn has been proved to be important in organic structures as well as in crystals. Cu plays a role in the cross-linking of bone with collagen. Both Zn and Cu are mutually antagonistic in bone, where a decrease in Zn levels increases the Cu content of bone.[16-18] Regarding the role of these three mineral atoms as connectors to the organic structures of bone, all three (Fe, Cu, Zn) are involved in this function in normal bone, whereas in osteoporotic bone only Cu and Zn are involved. Apparently the second pattern is related to binding anorganic to organic structures.

The third pattern in normal bone involves Al, P, Mg, and S in period III of the periodic system of Mendeleev, while in osteoporotic bone there are periodic irregularities comprising Al, K, Mg, S, Ti, and Fe. Presumably Fe forms a complex with S, which hampers the function of Fe as a connector with the organic structure. In addition, the remote position of Ti relative to Ca potentially decreases substitution of Ca by Ti.

Previous studies have revealed an association between bone microstructure and the structure and dimensions of its hydroxyapatite crystals. The present study explores another aspect of microstructure development, i.e. the mapping of mineral atoms on the microstructural surfaces involved in the development of osteoporosis. This study revealed disharmonization of mineral atoms in the periodic system of Mendeleev in osteoporotic bone based on substitution and incorporation. On the other hand, in normal bone there is still a Mendeleev periodic harmonization (the atoms within one period form a homogenous pattern in the structure of bone). These results extend the findings of Busse [19] on Ca and P hypermineralization in osteoporotic bone.

One limitation of this study was the qualitative nature of the data analysis, The study of the detailed mechanism of substitution and incorporation in the development of osteoporosis as a result of exposure to metals is the subject of further studies.

CONCLUSIONS

This study demonstrates that the microstructure of trabecular bone in osteoporosis differs from that of normal bone, which is associated with disharmonization of mineral atoms in the periodic system. Atomic disharmonization indicates that osteoporosis is a process leading to amorphous state.

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DAFTAR PUSTAKA

1. Mano JF. Viscoelastic properties of bone: mechanical spectroscopy studies on a chicken model. Mater Sci Eng 2005;25:145-52.
2. Tho JY, Zioupos P, Currey JD, Pharr GM. Microstructural elasticity and regional heterogeneity in human femoral bone of various ages examined by nano-indentation. J Biomech 2002;35:189-98.
3. Huang Q, Kung AWC. Genetic of osteoporosis. Mol Genet Metab 2006;88:295-306.
4. Duncan EL, Brown MA. Genetic studies in osteoporosis – the end of the beginning. Arthritis Res Ther 2008;10:214.

5. Brandao CMR, Lima MG, da Silva AL, Silva GD, Guerra AA Jr, Acurcio FA. Treatment of postmenopausal osteoporosis in women: a systematic review. Cad Saude Publica 2008; 24Supl4:592-606.
6. Handa R, Kalla AA, Maalouf G. Osteoporosis in developing countries. Best Pract Res Clin Rheumatol 2008;22:693-708.
7. Wiechula D, Jurkiewicz A, Loska K. An assessment of natural concentrations of selected metals in the bone tissue of the femur head. Sci Total Environ 2008;406:261-7.
8. Nielsen FH, Stoecker BJ. Boron and fish oil have different beneficial effects on strength and trabecular microarchitecture of bone. J Trace Elem Med Biol 2009;23:195-203.
9. Odabasi E, Turan M, Aydin A, Akay C, Kutlu M. Magnesium, zinc, copper, manganese, and selenium levels in postmenopausal women with osteoporosis: can magnesium play key role in osteoporosis. Ann Acad Med Singapore 2008; 37:564-7.
10. Noor Z, Sumitro SB, Hidayat M, Rahim AH, Taufiq A. Assesment of microarchitecture and crystal structure of hydroxyapatite in osteoporosis. Univ Med 2011;30:29-35.
11. Sastry TP, Chandrasekaran A, Sundaraseelan J, Ramasastry M, Sreedhar R. Comparative study of some physico-chemical characteristics of osteoporosis and normal human femur heads. Clin Biochem 2007;40:907-912.
12. Shen Y, Zhang Z, Jiang S, Jiang L, Dai L. Postmenopausal woman with osteoarthritis and osteoporosis show different ultrastructural characteristics of trabecular bone of the femoral head. BMC Musculoskeletal Disord 2009;10:35. doi:10.1186/1471-2474-10-35.
13. Jasiuk IM. Analysis of trabecular bone as a hierarchial material. XXI ICTAM, Warsaw, Poland, 15-21 August, 2004.
14. Noor Z, Sumitro SB, Hidayat M, Rahim AH, Sabarudin A, Umemura T. Atomic mineral characteristics of Indonesian osteoporosis by high-resolution inductively coupled plasma mass spectrometry. The Sci World J 2012. doi: 10.1100/2012/372972.
15. Vallet-Regi M, Arcos D. Biomimetic nanoceramics in clinical use: from materials to applications. Cambridge: The Royal Society of Chemistry, Thomas Graham House, Science Park;2008.
16. Ovesen J, Moller-Madsen B, Nielsen PT, Christensen PH, Simonsen O, Hoeck HC, et al. Differences in zinc status between patients with osteoarthritis and osteoporosis. J Trace Elem Med Biol 2009;23:1-8.
17. Holloway WR, Collier FM, Herbst RE. Hodge JM, Nicholson GC. Osteoblast-mediated effects of zinc on isolated rat osteoclasts: inhibition of bone resorption and enhancement of osteoclast number. Bone 1996;19:137–42.
18. Aina V, Perardi A, Bergandi L. Cytotoxicity of zinc-containing bioactive glasses in contact with human osteoblasts. Chem Biol Interact 2007; 167:207-18.
19. Busse D, Hahn M, Soltau M, Zustin J, Puschel K, Duda GN, et al. Increased calcium content and inhomogeneity of mineralization render bone toughness in osteoporosis: mineralization, morphology and biomechanics of human single trabeculae. Bone 2009;45:10341-3.