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AGE- AND BMD-RELATED DIFFERENCES IN BIOCHEMICAL MARKERS OF BONE METABOLISM IN RURAL AND URBAN WOMEN FROM LUBLIN REGION, POLAND

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Abstract: The utility of biochemical markers of bone metabolism has not been proven in the diagnosis of metabolic diseases of the bone tissue; however they are widely used as a tools for treatment monitoring. Their serum concentrations are influenced by a number of factors, like gender, health status, anthropometric and environmental factors. All the factors listed above should be taken into consideration during clinical use. The aim of the study was to determine the reference values and evaluate the influence of environmental and anthropometric variables on biochemical markers of bone turnover for women from Lublin Region (Poland). Subjects of the study were 188 normal women aged 30-79, all residents of Lublin Region. Analysed markers of bone turnover were: osteocalcin (OC) and C-terminal cross-linking telopeptide of type I collagen (CTX-I), both assessed using ELISA method. All blood samples were taken and analyzed at the Clinical Chem. Laboratory and Patho-morphology Department at the Institute of Agricultural Medicine in Lublin. The lumbar spine (L_2-L_4) of all subjects was examined in a-p position using the dual X-ray absorptiometry-DXA (DPX-A LUNAR Corp.) at the Department of Metabolic and Degenerative Diseases of Bone Tissue of Institute of Agricultural Medicine in Lublin. Data pertaining to factors affecting bone tissue were collected using a specially prepared questionnaire. Serum levels of OC and CTX-I in women in every age range were different, generally increasing with age. Serum levels of OC and CTX-I in the analysed population strongly depended of both menopausal status and bone mineral density. In conclusion, this study demonstrates that age and menopausal status variations need to be considered when interpreting laboratory measurements of biochemical markers of bone metabolism.

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INTRODUCTION

Biochemical markers of bone metabolism are tools of great importance in understanding the pathophysiologic basis for bone metabolic diseases. In recent years, a panel of new, simple to use kits for determining bone markers became available for the diagnosis and treatment monitoring of osteoporosis and other metabolic diseases of bone tissue. Determination of protein fragments produced by osteoblasts like osteocalcin (OC) or enzymes excreted during osteogenesis such as bone alkaline phosphatase (bone ALP), are commonly used to asses osteoblastic activity. On the other hand, one of the most valuable assessments for osteoclastic activity is the measurement of degradation products of collagen type I, such as C- or N-cross-linked telopeptides of collagen type I [4, 36].

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In women, serum concentrations of bone markers during the period of adult life are at low levels when compared with their levels during childhood and puberty, when higher values of bone formation markers are noticeable [5, 30, 33]. Both pregnancy and lactation accelerate bone turnover, mainly by a rise of bone resorption [3, 40]. Most biochemical markers of bone metabolism reveal a linear increase with age, with high acceleration after menopause. Markers have been studied most intensively in postmenopausal women, based on their usefulness as a tool for monitoring response to different osteoporosis therapies including HRT, bisphosphonates, SERMS, and Calcitonin. Most recently, bone turnover markers have been studied for their ability to predict bone loss rates [2, 7, 11, 14, 21, 24, 25, 34, 39, 42]. There are also data indicating their usefulness in detecting and monitoring several diseases affecting bone tissue, eg. rheumatological diseases, Paget disease, renal osteodystrophy, bone metastases [6, 8, 16, 29]. Interpretation of the markers concentrations must take into account methodological and seasonal differences as well as biological differences such as gender, age, race and ethnicity. Wide clinical use of osteocalcin (OC) and especially - C-terminal cross-linked telopeptide of collagen type 1 (CTX-I) needs validation with special emphasis on establishing normal values, based on measurements in representative groups of healthy subjects. There are few studies that clarify the characteristics of bone turnover in healthy women. However, racial, ethnic, anthropometrical, seasonal differences in bone turnover have been clearly defined for some populations, although there is a lack of information in the literature on serum values of OC, and CTX-I in Polish subjects [15, 17, 21, 23].

Our primary objective was to investigate biochemical markers of bone turnover in relation to age and menopausal status, and to attempt to determine the reference values for women from the Lublin Region (Poland).

MATERIALS AND METHODS

Lublin Region is located in central-eastern Poland. The economy of this region is based mostly on agriculture and a large proportion of the inhabitants work as farmers. 1,200 women were drawn from the computer census at Lublin (urban population) and Urzędów (rural population) Town Halls and invited by post to participate in the study. Urzędów district was chosen because of its typically regional rural features. Approximately 860 volunteers were examined from which 202 fulfilled all study protocol criteria. Finally, the study population comprised 188 healthy women from 30–79 years of age. A medical history was taken on each subject using a specially designed questionnaire to evaluate previous diseases, fractures, reproductive history, age at menopause, drugs and other factors affecting bone metabolism. Exclusion criteria were: history of chronic diseases affecting bone tissue and medication which could interfere with bone mass (such as bisphosphonates, calcitonin, anabolic steroids, fluoride, vitamin D, corticosteroids).

Biochemical markers of bone turnover. The blood samples were collected between 11.00 a.m. and 11.30 a.m after an overnight fast. All participants had routine laboratory evaluations, including the measurement of serum total calcium (Ca) and creatinine (Cr), both performed immediately after phlebotomy. All serum samples were stored at -75ºC. Serum levels of Osteocalcin (OC) and C-terminal cross-linking telopeptide of type I collagen (CTX-I) were measured using an enzyme-linked immunosorbent assays - N-Mid Osteocalcin One Step ELISA (Osteometer BioTech A/S, Denmark) for OC and Serum CrossLaps One Step ELISA (Osteometer BioTech A/S, Denmark) for CTX-I in Pathomorphology Department at the Institute of Agricultural Medicine. For OC intra- and interassay variations were 5.4–6.8%, 2.8–6.8% respectively, sensitivity was 0.5 ng/ml. For CTX-I intra- and interassay variations were 4.7–4.9% and 5.4–8.1% respectively, and sensitivity was 92 pM. Both assays were run according to the manufacturer's directions and tested in duplicate on all serum samples. Serum total calcium (Ca) and creatinine (Cr) concentrations were measured by Chem. Laboratory at the Institute of Agricultural Medicine using standard colorimetric methods.

Bone densitometry. The lumbar spine (L_2-L_4) of all subjects was examined in a-p position using the dual X-ray absorptiometry-DXA (DPX-A, LUNAR Corp.) at the Densitometric Laboratory of the Institute of Agricultural Medicine in Lublin. Scan printouts were assessed independently by 2 research scientists. Spine scans with massive osteophytes were excluded from final data analysis.

All data including medical history, serum levels of Osteocalcin (OC), C-terminal cross-linking telopeptide of type I collagen (CTX-I) bone mineral density, age, weight, and height were entered into a computer database and 188 subjects were selected according to study criteria.

Statistical analysis. Statistical analysis was performed using regression analysis and analysis of variance with software Statistica 5.0. The Students test and F-test were used to examine the significance of differences between groups for non-paired data. For non-normal distribution data the χ^2 test was used. Probabilities $p \leq 0.05$ were considered significant.

RESULTS

We examined mean serum levels of OC and CTX-I by using 6 age groups, according to 5 year intervals (≤ 44, 45-49, 50-54, 55-59, 60-64, ≥ 65). To examine the influence of menopausal status and bone mineral density (BMD) on OC and CTX-I, the analyzed population was divided in 5 groups as follows: before menopause with normal BMD, before menopause with osteopenia (L_2-L_4 <

-1 SD > -2,5 SD), after menopause with normal BMD, after menopause with osteopenia (L_2-L_4 < -1 SD > -2,5 SD), and after menopause with osteoporosis (BMD L_2-L_4 ≤ -2,5 SD).

Osteocalcin (OC). Medium serum OC concentration for all of the analyzed population was 17.19 ng/L. Serum OC levels generally increased with age, being about 2-fold higher in women aged over 65 years of age compared to the women under 44 years of age (Tab. 1). Mean serum OC levels were highest in the 60-64 and ≥ 65 years of age groups (mean values 24.12 and 25.82 ng/L respectively). The lowest mean serum OC levels were observed in women under 45 years of age (mean value 12.16 ng/L). Mean serum levels of OC in women with different menopausal status and BMD values are summarized in Table 2. The lowest mean levels of serum OC were observed in women before menopause with normal BMD, insignificantly higher in the group with osteopenia (mean values 13.07 ng/L and 14.78 respectively). Irrespective of BMD values, in women after menopause, mean serum levels OC were 2 fold higher compared to the women before menopause. In the group with normal BMD, the mean OC value was 20.7 ng/L, and in the osteoporotic group 25.8 ng/L. The highest individual OC values (exceeding 80 ng/L) were observed in women after menopause. In women before menopause, the highest observed individual OC values were about 30 ng/L. There were no statistically significant differences in serum levels of OC between rural and urban women in this study (mean values 8.9 and 8.4 ng/L respectively).

C-terminal cross-linking telopeptide of type I collagen (CTX-I). Medium serum CTX-I concentration for all of the analyzed population was 3,574.9 pM. The mean serum CTX-I values presented in Table 1 generally increased with age. This trend was altered in women in the age range ≥ 65 years of age, in which a significant decrease of mean CTX-I levels was observed. The highest mean level of CTX-I was observed in the group 60-64 years of age, and the lowest in women under 45 years of age (mean values 5,113.9 pM and 2,540.9 pM respectively). Mean serum levels of CTX-I in women with different menopausal status and BMD values are summarized in Table 2. The lowest mean levels of serum CTX-I were observed in women before menopause with normal BMD, and insignificantly higher in group with osteopenia (mean values 2,820.0 pM and 3,018.5 pM respectively). Irrespective of BMD values in women after menopause, mean serum levels of CTX-I were 2-fold higher compared to the women before menopause. Bone mineral density (BMD) values strongly influenced mean serum CTX-I levels in women after menopause. The following mean serum CTX-I values were observed: in the group with normal BMD: 3,870.6 pM, in the osteopenic group: 4,455 pM, and in the osteoporotic group: 5,502.4. The highest individual CTX-I values (exceeding 11,000 pM) were observed in women after menopause with osteopenia. In women before menopause, the highest observed individual OC values were about 7,000 pM. There were no statistically significant differences in serum levels of CTX between rural and urban women in this study (mean values 1,797.9 and 1,750.4 pM respectively).

Table 1. Serum levels of osteocalcin (OC) and C-terminal crosslinking telopeptide of type I collagen (CTX-I) by age in a population-based random sample of 188 women from Lublin Region (Poland).

Age		OC (ng/mL)		CTX-I (pM)	
	n	Mean	SD	Mean	SD
≤ 44	35	12.16	5.91	2,540.9	1,255.6
45-49	48	15.13	6.73	2,895.0	1,378.5
50-54	46	15.78	7.10	3,936.1	2,211.7
55-59	38	22.01	17.07	4,178.8	2,382.0
60-64	14	24.12	12.63	5,113.9	2,569.2
≥ 65	7	25.82	26.35	4,676.8	3,857.2
Total	188	17.19	11.76	3,574.9	2,155.9

Table 2. Serum levels of osteocalcin (OC) and C-terminal crosslinking telopeptide of type I collagen (CTX-I) by menopausal status and bone mineral density (BMD) in a population-based random sample of 188 women from Lublin Region (Poland).

Menopausal status and BMD result		OC (ng/mL)		CTX-I (pM)	
	n	Mean	SD	Mean	SD
Before menopause andnormal BMD	77	13.07	5.99	2,820.0	1,408.9
Before menopause and BMD L_2-L_4 < -1 SD > -2.5 SD*	14	14.78	6.35	3,018.5	1,327.4
After menopause and normal BMD	53	20.70	14.07	3,870.6	1,889.3
After menopause and BMD L_2-L_4 < -1 SD > -2.5 SD*	33	19.34	12.72	4,455.0	2,856.3
After menopause and BMD L_2-L_4 ≤ -2.5 SD*	11	25.80	20.34	5,502.4	3,598.1

* according to the criteria of osteopenia and osteoporosis from WHO Technical Study Series Geneva 1994, p. 843.

Calcium (total Ca). Medium serum total Ca concentration for the analyzed population was 8.88 mg/dL. The influence of age and menopausal status on serum total Ca was not observed. In 4 subjects, serum total Ca levels were insignificantly below the laboratory norm (8.2-10.2 mg/dL).

DISCUSSION

We have attempted to define the relationships between OC, CTX-I, age and menopausal status to clarify the influence of these factors on serum levels of bone formation and resorption markers. Not surprisingly, in our

Table 3. Correlation coefficients for the relationships between CTX-I and OC in women from Lublin Region (Poland) with respect to menopausal status. Underlined values are statistically significant at $p < 0.05$.

Before menopause	BGP	CTX-I
BGP	—	0.30
CTX-I	0.30	—
After menopause		
BGP	—	0.59
CTX-I	0.59	—

study of women from Lublin Region in Poland, older women had higher bone turnover compared with their younger counterparts. The progressive increase in OC and CTX-I mean serum concentrations with advancing age was noticeable in all age ranges. It should be noticed that positive and statistically significant correlations between analyzed markers were found (Tab. 3), e.g. elevated serum concentration of CTX-I was accompanied by elevated OC serum concentrations and vice versa, elevated serum OC concentrations were accompanied by elevated CTX-I. In the regression analyses, age consistently correlated with both of the markers of bone turnover. This conclusion, this not consistent with some previous studies where it has been suggested that bone loss in aging women is a result of increased bone resorption, rather than decreased bone formation [9, 12, 18, 31]. Marked menopausal status differences in bone turnover markers were also found in the analysed population. Serum OC and CTX-I were elevated in the postmenopausal women compared with women before menopause. According to previous studies, menopause is associated with significant increase in bone turnover rate, as measured by the variety of formation and resorption markers, including OC and CTX-I measured using the ELISA method. [10, 32] These trends were more significant when analysed together with bone mineral status (BMD). We found negative associations between OC, CTX-I and bone mineral density measured by the DXA method at the lumbar spine. Other anthropometric factors, such as height, weight, BMI, did not show any relation in regression analysis. BMD generally decreased with age, accompanied by increase in bone turnover determined by serum levels of OC and CTX-I. With this being a cross-sectional study, the age-related difference in bone mineral status may reflect losses over time, but it may also be due to differences in lifestyle, nutritional habits, level of physical activity and other environmental factors. Although bone turnover does not directly depend on the BMD, and reports about the possibility of prediction of bone loss using markers are incoherent, some authors emphasize their usefulness in the determination of fast and slow bone losers [13, 43].

The increased bone turnover in older subjects from our study is consistent with previous studies in White, Black and Chinese women [1, 26, 41, 44]. It is also consistent with studies conducted in both male and female subjects, in which women after menopause present much higher levels of bone markers compared with older men [27, 35, 37]. It can also be concluded from the present study that bone turnover, which is significantly elevated after menopause, remains at a high level over the years. [19, 20, 22, 28, 38].

Although our analyses were performed on a limited sample size in the various subgroups to establish commonly used reference values, the data gives solid feedback on OC and CTX-I mean serum values in women from Lublin Region in Poland. It should be also concluded, that in any case, age and menopausal status variations need to be considered when interpreting laboratory measurements of biochemical markers of bone metabolism.

In conclusion, our study demonstrates changes in bone turnover markers that are affected by age and menopausal status. Age consistently and independently correlates with all markers of bone turnover measured in this study. The menopausal status difference in bone markers concentrations may well reflect the physiologic pathway responsible for the bone mineral density (BMD) decrease in women after menopause.

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REFERENCES

1. Adachi JD: The correlation of bone mineral density and biochemical markers to fracture risk. *Calcif Tissue Int* 1996, **59**, 16-19.

2. Adami S, Passeri M, Ortolani S, Broggini M, Carratelli L, Caruso I, Gandolini G, Gnessi L, Laurenzi M, Lombardi A, *et al.*: Effects of oral alendronate and intranasal salmon calcitonin on bone mass and biochemical markers of bone turnover in postmenopausal women with osteoporosis. *Bone* 1995, **17**, 383-390.

3. Anim-Nyame N, Sooranna SR, Jones J, Alaghband-Zadeh J, Steer PJ, Johnson MR: A longitudinal study of biochemical markers of bone turnover during normal pregnancy and pregnancies complicated by pre-eclampsia. *BJOG* 2002, **109**, 708-713.

4. Bikle DD: Biochemical markers in the assessment of bone disease. *Am J Med* 1997, **103**, 427-436.

5. Bouillon R, Bex M, Van Herck E, Laureys J, Dooms L, Lesaffre E, Ravussin E: Influence of age, sex, and insulin on osteoblast function: osteoblast dysfunction in diabetes mellitus. *J Clin Endocrinol Metab* 1995, **80**, 1194-1202.

6. Calvo MS, Eyre DR, Gundberg CM: Molecular basis and clinical application of biological markers of bone turnover. *Endocr Rev* 1996, **17(4)**, 333-368.

7. Crandall C: Parathyroid hormone for treatment of osteoporosis. *Arch Intern Med* 2002, **162**, 2297-2309.

8. Deftos LJ, Olfert RL, Hill CS: Bone alkaline phosphatase in Pagets disease. *Horm Metab Res* 1991, **23**, 559-561.

9. Dennison E, Eastell R, Fall CHD, Kellingray S, Wood PJ, Cooper C: Determinants of bone loss in elderly men and women: A prospective population based study. *Osteopor Int* 1999, **10**, 384-391.

10. De Leo V, Ditto A, la Marca A, Lanzetta D, Massafra C, Morgante G: Bone mineral density and biochemical markers of bone turnover in per- and postmenopausal women. *Calcif Tissue Int* 2000, **66**, 263-267.

11. Devogelaer JP, Broll H, Correa-Rotter R, Cumming DC, De Deuxchaisnes CN, Geusens P, Hosking D, Jaeger P, Kaufman JM, Leite M, Leon J, Liberman U, Menkes CJ, Meunier PJ, Reid I, Rodriguez J, Romanowicz A, Seeman E, Vermeulen A, Hirsch LJ, Lombardi A, Plezia K, Santora AC, Yates AJ, Yuan W: Oral alendronate induces progressive increases in bone mass of the spine, hip and total body over 3 years in postmenopausal women with osteoporosis. *Bone* 1996, **18**, 141-150.

12. Eastell R, Delmas PD, Hodgson SF, Mann KG, Riggs BL: Bone formation rate in older normal women: Concurrent assessment with bone histomorphometry, calcium kinetics, and biochemical markers. *J Clin Endocrinol Metab* 1988, **67**, 741-748.

13. Ebeling PR, Atley LM, Guthrie JR, Burger HG, Dennerstein L, Hopper JL, Wark JD: Bone turnover markers and bone density across the menopausal transition. *J Clin Endocrinol Metab* 1996, **81**, 3366-3371.

14. Eriksen EF, Melsen F, Sod E, Barton I, Chines A: Effect of long-term risendronate on bone turnover in women with postmenopausal osteoporosis. *Bone* 2002, **31**, 620-625.

15. Finkelstein JS, Sowers M, Greendale GA, Lee MT, Neer RM, Cauley JA, Ettinger B: Ethnic variation in bone turnover in pre- and early perimenopausal women: effects of anthropometric and lifestyle factors. *J Clin Endocrinol Metab* 2002, **87**, 3051-3056.

16. Fohr B, Dunstan CR, Seibel M: Clinical review 165: Markers of bone remodeling in metastatic bone disease. *J Clin Endocrinol Metab* 2003, **88**, 5059-5075.

17. Garnero P, Mulleman D, Munoz F, Sornay-Rendu E, Delmas PD: Long term variability of markers of bone turnover in postmenopausal women and implications for their clinical use: the OFELY study. *J Bone Mineral Res* 2003, **18**, 1789-1094.

18. Garnero P, Sornay-Rendu E, Chaupuy MC, Delmas PD: Increased bone turnover in late postmenopausal women is a major determinant of osteoporosis. *J Bone Miner Res* 1996, **11**, 337-349.

19. Garnero P, Delmas PD: New developments in biochemical markers for osteoporosis. *Calcif Tissue Int* 1996, **59**, 2-9.

20. Guerrero R, Diaz Martin MA, Diaz Diego EM, Rapado A, de la Pietra C: New biochemical markers of bone resorption derived from collagen breakdown in the study of postmenopausal osteoporosis. *Osteoporosis Int* 1996, **6**, 297-302.

21. Gundberg CM, Looker AC, Nieman SD, Calvo MS: Paterns of osteocalcin and bone specific alkaline phosphatase by age, gender, and race or ethnicity. *Bone* 2002, **31**, 703-708.

22. Han ZH, Palnitkar S, Sudhaker Rao D, Nelson D, Parfitt AM: Effect of ethnicity and age of menopause on the remodelling and turnover of iliac bone: Implications for mechanisms of bone loss. *J Bone Miner Res* 1997, **12**, 498-508.

23. Henry YM, Eastell R: Ethnic and gender differences in bone mineral density and bone turnover in young adults: effect of bone size. *Osteopor Int* 2000, **11**, 512-517.

24. Hochberg MC, Greenspan S, Wasnich RD, Miller P, Thompson DE, Ross PD: Changes in bone density and turnover explain the reductions in incidence of nonvertebral fractures that occur during treatment with antiresorptive agents. *J Clin Endocrinol Metab* 2002, **87**, 1586-1592.

25. Johnel O, Scheele WH, Lu Y, Reginster JY, Need AG, Seeman E: Additive effects of raloxifene and alendronate on bone density and biochemical markers of bone remodeling in postmenopausal women with osteoporosis. *J Clin Endocrinol Metab* 2002, **87**, 965-992.

26. Kleerekoper M, Nelson DA, Peterson EL, Wilson PS, Jacobsen G, Longcope C: Reference data for bone mass, calciotropic hormones, and biochemical markers of bone remodeling in older (55-75) postmenopausal white and black women. *J Bone Miner Res* 1994, **9**, 1267-1276.

27. Krall EA, Dawson-Huges B, Hirst K, Gallager JC, Sherman SS, Dalsky G: Bone mineral density and biochemical markers of bone turnover in healthy elderly men and women. *J Gerontol Med Sci* 1997, **52A**, 61-67.

28. Kushida K, Takahashi M, Kawana K, Inoue T: Comparison of markers for bone formation and resorption in premenopausal and postmenopausal subjects, and osteoporosis patients. *J Clin Endocrinol Metab* 1995, **80**, 2447-2450.

29. Mansson B, Carey D, Alini M, Ionescu M, Rosenberg LC, Poole AR, Heinegard D, Saxne T: Cartilage and bone metabolism in rheumatoid arthritis. Differences between rapid and slow progression of disease identified by serum markers of cartilage metabolism. *J Clin Invest* 1995, **95(3)**, 1071-1077.

30. Matsukura T, Kagamimori S, Nishino H, Yamagami T, Iki M, Kajita E, Kagawa Y, Yoneshima H, Matsuzaki T, Marumo F: The characteristics of bone turnover in the second decade in relation to age and puberty development in healthy Japanese male and female subjects. Japanese Population-based Osteoporosis Study. *Ann Human Biol* 2003, **30**, 13-25.

31. Melton LJ, Khosla S, Atkinson EJ, O'Connor MK, O'Fallon WM, Riggs BL: Cross-sectional versus longitudinal evaluation of bone loss in men and women. *Osteopor Int* 2000, **11**, 592-599.

32. Melton LJ, Khosla S, Atkinson EJ, O'Fallon WM, Riggs BL: Relationship of bone turnover to bone density and fractures. *J Bone Miner Res* 1997, **12**, 1083-1091.

33. Rauch F, Schonau E, Woitge H, Remer T, Seibel M: Urinary excretion of hydroxy-pyridinium cross-links of collagen reflects skeletal growth velocity in normal children. *Exp Clin Endocrinol* 1994, **102**, 94-97.

34. Reid IR, Brown JP, Burckhardt P, Horowitz Z, Richardson P, Trechsel U, Widmer A, Devogelaer JP, Kaufman JM, Jaeger P, Body JJ, Brandi ML, Broell J, Di Micco R, Genazzani AR, Felsenberg D, Happ J, Hooper MJ, Ittner J, Leb G, Mallmin H, Murray T, Ortolani S, Rubinacci A, Saaf M, Samsioe G, Verbruggen L, Meunier PJ: Intravenous zoledronic acid in postmenopausal women with low bone mineral density. *N Engl J Med* 2002, **346**, 653-661.

35. Rudnicki M, Thode J, Jorgensen T, Heitman BL: Effects of age, sex, season, and diet on serum ionized calcium, parathyroid hormone and vit. D in random population. *J Intern Med* 1993, **234**, 195-200.

36. de la Piedra C, Traba ML, Cabera DC, Henriques MS: New biochemical markers of bone resorption in the study of pomenopausal osteoporosis. *Clin Chim Act* 1997, **265**, 225-234.

37. Sherman SS, Hollis BW, Tobin JD: Vitamin D status and related parameters in a healthy population: The effect of age, sex, and season. *J Clin Endocrinol Metab* 1990, **71**, 405-413.

38. Sone T, Miyake M, Takeda N, Fukunaga M: Urinary excretion of type I collagen crosslinked N-telopeptides in healthy Japanese adults: age- and sex-related changes and reference limits. *Bone* 1995, **17**, 335-339.

39. Sorensen OH, Crawford GM, Mulder H, Hosking DJ, Gennari C, Mellstrom D, Pack S, Wenderoth D, Cooper C, Reginster JY: Long-term efficacy of risedronate: a 5-year placebo-controlled clinical experience. *Bone* 2003, **32**, 120-126.

40. Sowers M, Eyre D, Hollis BW, Randolph JF, Shapiro B, Jannausch ML, Crutchfield M: Biochemical markers of bone turnover in lactating and nonlactating postpartum women. *J Clin Endocrinol Metab* 1995, **80**, 2210-2029.

41. Tsai KS, Pan WH, Hsu SJH: Sexual difference in bone markers and b one mineral density in normal Chinese. *Calcif Tissue Int* 1996, **59**, 454-460.

42. Woitge HW, Scheigt-Nave C, Kissling C, Leidig-Bruckner G, Meyer K, Grauer A, Scharla SH, Ziegler R, Seibel MJ: Seasonal variation of biochemical indexes of bone turnover: results of a population based study. *J Clin Endocrinol Metab* 1998, **83**, 68-75.

43. Vergnaud P, Garnero P, Meunier PJ, Breart G, Kamihagi K, Delmas PD: Undercarboxylated osteocalcin measured with a specific immunoassay predicts hip fracture in elderly women: The EPIDOS study. *J Clin Endocrinol Metab* 1997, **82**, 719-724.

44. Yan L, Prentice A, Zhou B, Zhang H, Wang X, Stirling DM, Laidlaw A, Han Y, Laskey A: Age- and gender-related differences in bone mineral status and biochemical markers of bone metabolism in Northern Chinese men and women. *Bone* 2002, **30**, 412-415.