The Beneficial Role of Some Bone Markers in Evaluating Women with Osteoporosis under Different Therapeutic Regimens

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Abstract

Osteoporosis is a systemic disease of the skeleton, characterized by low bone mass and alteration in the micro-architecture of the bone tissue that lead to an increase in brittleness with the ensuing predisposition to bone fracture. Global statistics shows that women are more exposed to this disease than men and in particular at menopause. This study was designed to evaluate the use of some bone markers: serum osteocalcin (Ost), alkaline phosphatase (ALP), as bone formation markers, also parathyroid hormone (PTH), calcium and inorganic phosphate level, for the assessment of patients with osteoporosis and to evaluate their role in monitoring of several types of therapeutic interventions (such as bisphosphonates, hormonal replacement therapy, and Ca and vit.D) in postmenopausal women.This study comprised of 36 women (age 51.67±5.14 years) those diagnosed to have osteoporosis, to be allocated randomly into three groups according to the type of therapy to be given as;

group A: received bisphosphonates (sodium alendronate 10mg/day) for twelve weeks (N=12).

group B: treated with hormonal replacement therapy (conjugated estrogen 0.625mg/day) for twelve weeks (N=12).

group C: received Ca and vit. D (Ca1500mg/day and cholecalciferol 1000IU/day) for twelve weeks(N=12).

In addition to 15 perimenopausal healthy women to serve as a control group (age 51.13±7.62 years).The studied parameters were measured in serum obtained before starting treatment and after 12 weeks of therapy. Result indicated that the baseline values of both serum Ost and ALP were significantly higher in postmenopausal patients as compared to controls and serum Ost showed a significant reduction after treatment with alendronate compared to those treated with either HRT or Ca and vit. D.From this study we recommend estimating the baseline bone markers (Ost and ALP) status for newly diagnosed osteoporotic patients to be used as a guide for deciding the initial therapeutic intervention, and detection of non responder instead of waiting until patients develop further fracture while they are on therapy.

Key words: Osteoporosis, Postmenopause, Osteocalcin, Alendronate

الخلاصة

يعتبر مرض هشاشة العظام "ترقق العظام" من الأمراض التي تصيب الهيكل العظمي وتسبب خللا في النسيج العظمي مما يؤدي لنقص في كثافة العظام وتغيرات دقيقة في النسيج العظمي والتي تؤدي إلى زيادة هشاشة العظام وزيادة احتمالية التعرض لكسر العظام. وتدل الإحصائيات العالمية إن معدل إصابة النساء بهذا المرض أكثر من الرجال خاصة بعد سن اليأس. يهدف البحث إلى تقيم فعالية استخدام بعض مؤشرات العظام في تشخيص المرض منها الاستيوكالسين ومحلل الفوسفات القاعدي كمؤشرات لبناء العظام, إضافة إلى قياس مستوى هرمون الباراثيروید إلى جانب الكالسيوم والفوسفات اللاعضوية وفي متابعة المرض باستخدام علاجات مختلفة مثل الفوسفونات الثنائية وعلاج الهرمونات التعويضي والكالسيوم وفيتامين دي. تضمنت الدراسة ٣٦من النساء اللاتي بعانين من مرض هشاشة العظام وتم تقسيمهن إلى ثلاثة مجاميع وفقا للعلاج الذي أعطي لهن لمدة اثنا عشر أسبوعا .(بمعدل أعمار ٥١,٦٧± ٥,١٤ سنة).

المجموعة ا- أعطيت عقار الاندرونيت ١٠ملغم/اليوم لمدة اثنا عشر أسبوعا وتضمنت ١٢ مريضة.

المجموعة ب- أعطيت علاج الهرمونات التعويضي من الاستروجين(٠,٦٢٥ملغم/اليوم)لمدة اثنا عشر أسبوعا وتضمنت ١٢ مريضة.

المجموعة ج- أعطيت الكالسيوم مع فيتامين دي(١٥٠٠ملغم و١٠٠٠ وحدة دولية يوميا) وتضمنت ١٢ مريضة.

إضافة الى خمسة عشر من النساء الاصحاء كمجموعة ضابطة بمعدل عمر(٥١,١٣±٧,٦٢ سنة), تم قياس المؤشرات قيد الدراسة في مصل المريضات قبل وبعد بدء العلاج بأثنا عشر أسبوعا .اظهرت النتائج بان المستويات البدائية للاوستياكالسين ومحلل الفوسفات القاعدي في المصل كانت مرتفعة في النساء في سن انقطاع الطمث والمصابات بترقق العظام مقارنة بالمجموعة الضابطة وانخفض مستوى الاوستياكالسين في مصل المريضات بعد علاجهن بالاندرونيت . من نتائج الدراسة يمكننا التوصية بتقييم مستوى مؤشرات العظام للمريضات حديثات التشخيص بترقق العظام لتكون دليلا على اختيار العلاج المناسب وكذلك للكشف عن عدم الاستجابة بدلا من الانتظار لحدوث الكسور خلال استمرارهم على العلاج.

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Introduction

Bone consists of an extracellular matrix, the organic phase of which is composed of type I collagen, proteoglycans, and noncollagenous proteins including: osteocalcin, bone sialoprotein, osteonectin, thrombopondin, and osteopontin. Bone matrix also contains growth factors and cytokines that have an important regulatory role in bone remodeling [(1)]. Bone modeling prevents the occurrence of damage by adapting bone structure-and hence strength-to its loading circumstances [(2)].Bone in the adult skeleton is renewed continuously in response to a variety of stimuli by the process of bone remodeling. This involves removel of trenches or tunnels of bone from the surfaces of trabecular and cortical bone, respectively, by osteoclasts (bone resorbing cell) and osteoblasts that subsequently fill in these trenches by laying down new bone matrix in them [(3)]. In normal adult bone, the processes of resorption and formation are coupled both in space and time, thus bone resorption always precedes formation [(4)]. Bone loss is the result of an imbalance in bone turnover with bone resorption occurring at a faster rate than new bone formation [(5)].Osteoporosis most commonly affects postmenopausal women, placing them at a significant risk of fracture. Hip fractures are important causes of mortality and morbidity among postmenpausal women. Approximately 20% of patients with hip fractures die within a year, most of the deaths occurring within the first 6 months after a fracture. Among the survivors, 30%-50% never regain their prefracture functional status [(6)]. Within geriatric population in most countries, disorders of bone and mineral metabolism are becoming increasingly relevant to every day clinical practice. Consequently, the interest in, and the need for effective measures to be used in the screening, diagnosis and follow-up of such pathologies has markedly grown. Together with the clinical and imaging techniques, biochemical tests could play an important role in both the assessment and differential diagnosis of osteoporosis [(7)], but yet have not been practically used in osteoporosis [(8)]. In recent years, a panel of new simple to use kits to determine bone markers had became available for the diagnosis, monitoring, of osteoporosis, and other metabolic diseases of bone tissue[(9)].

This study was designed to evaluate:

1. The role of some biochemical bone formation markers, such as, Osteocalcin, Parathyroid hormone, Alkaline phosphate, serum Calcium and serum Inorganic phosphatase, in the assessment of osteoporosis status in postmenopausal women.
2. The effect of three therapeutic interventions applied in osteoporosis (i.e. Bisphosphonates, supplement of vit. D and Ca and hormonal replacement therapy) on the studied bone formation parameters after 12 weeks of starting their therapies.

Subjects and Methods

This study comprised 36 postmenopausal women with newly diagnosed osteoporosis by a senior physician based on (x-ray and sign and symptoms), according to WHO diagnostic criteria for osteoporosis [(10)] with a mean age of 51.67±5.14 years. They were classified into three main groups to be treated for twelve weeks as follows:

GROUP A; include 12 patients to be treated with alendronate 10 mg/day.

GROUP B: include12 patients to be treated with hormone replacement therapy (HRT) (conjugated estrogen 0.625mg /day).

GROUP C: include 12 patients to be treated with Ca and vit .D (Ca1500 mgand cholicalciferol 1000IU /day).

In addition to a control group of 15 perimenopausal of apparently healthy women with a mean age of 51.13±7.62years.

A fasting 5 ml venous blood sample was withdrawn from each subject before and twelve weeks after treatment. Serum was separated and divided into aliquots to be used for immediate determination of total ALP [(11)]. Other aliquots were stored frozen (-20°C) for determination of osteocalcin[(12)], PTH[(13)], serum total Ca[(14)], and serum inorganic phosphate levels[(15)]. Statistical analysis was performed using Microsoft excel 2007 in order to estimate mean± SEM, as well as, the estimation of paired t-test values, considering $p<0.05$ to be significant.

Results

1. Effect of treatment with either alendronate, HRT, or calcium and vitamin D on serum level of osteocalcin in women with postmenopausal osteoporosis.

Figure (1) showed that the Serum levels of osteocalcin in osteoporotic women were significantly elevated ($p<0.05$) as compared to controls by 29.18% ,19.33% ,and 40.33%, respectively in the three groups A,B and C .There was a significant reduction ($P<0.05$) in serum osteocalcin levels after treatment with

alendronate by -33.03%, in comparison with corresponding values before treatment. Meanwhile, alendronate administration induced a significant decrease in serum osteocalcin in comparison with HRT, and (Ca and vit. D) treated groups.

2. *Effect of treatment with either alendronate, HRT, or calcium and vitamin D on serum level of ALP in women with postmenopausal osteoporosis.*

Table(1) indicates a significant increase ($p<0.05$) in serum total ALP activity before starting treatment with alendronate. Whereas, non significant alteration in serum level of ALP($P>0.05$) were observed following treatment with the three tested therapies in comparison with their pre-treatment values.

3. *Effect of treatment with either alendronate, HRT, or calcium and vitamin D on serum level of PTH in women with postmenopausal osteoporosis.*

Table (1) showed that the serum PTH levels were non-significantly altered baseline values in comparison to the control group. Also non significant changes in serum levels of PTH were observed following the treatment with either one of the three tested therapies in comparison with their pre-treatment values.

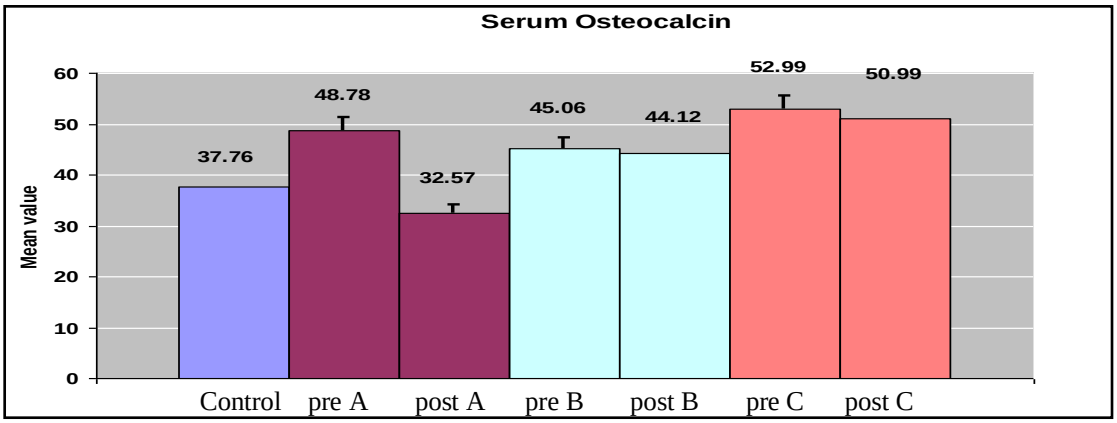

Figure 1: Effect of alendronate(10mg/day), HRT(0.625 mg /day conjugated estrogen), and Ca and vit. D (Ca and vit. D 1500 mg /day and 1000 IU /day respectively), for twelve weeks on serum osteocalcin level in post menopausal osteoporosis women .

Group A= Treated with alendronate.
Group B= Treated with HRT
Group C= Treated with Ca and Vit.D

Table 1 : Effect of alendronate (10 mg/day), HRT(0.625 mg/day conjugated estrogen),and Ca and vit. D (Ca and vit. D 1500 mg/day and 1000 IU /day respectively) for twelve weeks on serum total ALP and PTH levels in all patients.

Marker	Drug	Control (N=15)	Before Treatment (N=12)	After Treatment (N=12)
ALP (U/L)	ALN	88.47 ±13.82	133.92±37.91*	125.58±44.21
	HRT		100.83±26.88	93.42± 20.89
	Ca and vit D		100.25±27.61	91.08±16.41
PTH (ng/ml)	ALN	27.77±14.55	27.93±17.19	28.92± 16.15
	HRT		32.77± 16.88	32.29±16.69
	Ca and vit D		33.37±10.8	31.17±10.0

values are presented as mean ± SEM.
* significant difference from control at $p < 0.05$
# significant difference from before treatment at $p<0.05$
ALN= Alendronate
HRT= Hormone replacement therapy

4. Effect of treatment with either alendronate, HRT, or calcium and vitamin D on serum level of calcium and inorganic phosphate in women with postmenopausal osteoporosis.

Table (2) indicate that there was non significant flacutuation in serum total Ca and inorganic phosphate levels in all patients groups in comparison with control group , nor after treatment with either alendronate ,HRT, or Ca and vit D or in comparison with their pre treatment values .

Table 2:Effect of alendronate (10 mg/day) , HRT(0.625 mg /day conjugated estrogen) ,and Ca and vit. D(Ca and vit. D 1500 mg /day and 1000 IU /day respectively) for twelve weeks on serum total Calcium and inorganic phosphate levels in postmenopausal osteoporosis women.

Marker	Drug	Control (N=15)	Before Treatment (N=12)	After Treatment (N=12)
Ca (mg/dl)	ALN	8.78±0.69	8.45±1.16	8.50±1.13
	HRT		8.32±1.12	8.21±1.07
	Ca and vit D		8.98±1.12	9.39±0.54
PO_4^{-3} (mg/dl)	ALN	3.85±0.38	4.13±1.61	4.47±1.70
	HRT		4.11±0.95	4.25±1.01
	Ca and vit D		3.67±0.51	3.73±0.47

values are presented as mean ± SEM.
ALN= Alendronate
HRT= Hormone replacement therapy

Discussion

In our study the levels of serum Ost, was significantly higher in untreated patients with postmenopausal osteoporosis as compared to controls (Figure 1). These results are in agreement with (Aysan T et al, 2007 and Peipchl P et al, 2000) [16,17]. This could be attributed to the accelerated bone loss occurring within the first years following menopause [18].Since during both menopause and aging, the coordinated balance to disturbance of bone formation and resorption can be disturbed, resulting in excessive bone loss and osteoporosis [19]. Thus serial measurement of Ost is indicated to be an excellent marker to assess the long term effects of antiresorptive therapy [20].The significant reduction in serum Ost level of postmenopausal osteoporosis following alendronate administration in comparison with the baseline values. This result is confirmed by other studies. [21, 22, 23] And this is because aminobisphosphonates can decrease bone remodeling by decreasing osteoclast activity and by inducing osteoclast apoptosis. [24,25] The hypothesis that alendronate, like other nitrogen-containing bisphosphonates, inhibits a rate-limiting step in the mevalonic acid pathway that is essential for osteoclast function[26,27], this could be explained that the first measurable effect of bisphosphonate is the decrease of the rate of bone resorption that is followed by a slower decrease of the rate of bone formation, due to the coupling of the two processes and the attainment of a new steady state at a lower rate of bone turnover[28].In the present study the level of Ost was non significantly lowered following twelve weeks of treatment with HRT therapy compared to their level before treatment (table1) this is in agreement with previous study [29,30], indicated that serum Ost was able to decreases only after 6 months of treatment with HRT[17]. The Possible mechanism could include a decrease in osteoclastogenesis, osteoclast apoptosis and additional effect on calcium homeostasis [31]. Estrogens may affect osteoclasts directly, in addition estrogens certainly exerts effect on osteoclasts indirectly by suppressing the production of bone resorbing cytokines from osteoblast and bone marrow stromal cells [32].The possible explanation to the lack of response of bone markers to treatment with calcium and vitamin D (Figure 1 and Table 1) may be simply because this treatment has a bone stimulant effect but no or very minimal anti-resorptive effect [33]. However, previous studies have shown some minimal improvement of bone mineral density (BMD) in patients treated with calcium and vitamin D but this did not correlate with any reduction of fracture risk [34]. (Shinchuk et al, 2005) had reported high prevalence of vitamin D deficiency in men and women with osteoporosis [35]. Therefore calcium and vitamin D is now considered as a complementary treatment to osteoporosis medications such as bisphosphonates rather

than a sole line of treatment by itself[36,37], although (Palacios et al, 2005) had reported a significant reduction in serum bone markers after treatment with calcium and vitamin D[38]. This supports the notion found in (Figure1 and Table 1) that alendronate is a stronger antiresorptive agent than both HRT[39,40] and calcium and vitamin D [41]. And that alendronate may have a very beneficial effect when added to HRT regimen in patients with postmenopausal osteoporosis [39]. Additionally total serum alkaline phosphatase in untreated postmenopausal women showed a significant increase in level as compared to control (Table 1). These results are in agreement with other studies [16, 42]. As this enzyme function in the metabolism of bone tissue, thus increased activity could be a way to compensate for lost calcium/phosphorus by recruiting more of these elements to build new bone tissue [16, 42]. However, no differences were noted in serum ALP levels following treatment with all tested medications in postmenopaul osteoporotic patients. This may be due to the fact that total ALP measurements is less specific to bone than measurements of the bone isoenzyme [7] and also may be due to short time for follow up period (twelve weeks) in this study. Meanwhile, PTH levels, in the current work, showed non significant changes in untreated postmenopausal osteoporosis groups in comparison with controls. Such finding could be explained through the changes that take place in calcium metabolism related to estrogen deficiency, which induces a decrease in the intestinal absorption of calcium and reduces the biological activity of vitamin D. It also enhances the renal calcium excretion and diminishes the ability of parathyroid hormone to reduce calciuria. Serum calcium remains unchanged or slightly increased due to compensatory increased bone resorption and PTH remains unchanged resulting in increased bone resorption and this is in agreement with our result[43]. Results in table 2 elucidated non significant fluctuations in serum calcium and inorganic phosphate before and after the treatment of postmenopausal women. Our results are in agreement with other studies [42,44,45,46]. As serum calcium is homeostatically controlled and the integrity of bone may be sacrified to maintain serum calcium within the normal range [47]. Consequently, serum calcium is a poor predictor of histological features and is not indicative of bone resorption in osteoporotic patient [48]. Meanwhile non significant alteration in serum inorganic phosphate before and 12 weeks after treatment in postmenopausal women and this was in agreement with previous study [42,44,45,46], indicating that the determination of serum inorganic phosphate was of no significant values for the diagnosis and follow up of osteoporosis.Thus effective treatment with antiresorptive agents can show up significant changes in abnormal bone markers after treatment supporting the value of measuring them in the patients with osteoporosis. Hence bone markers (Ost) can be used for monitoring the response to therapy and also as aguide for patients compliance with treatment.Calcium and vitamin D supplementation is recommended as a complementary treatment even if dietary supply is sufficient. Thus we can recommend checking the baseline bone marker status (Ost) for all osteoporotic patients.

References

1. Dogan E. and Posasci C.: Monitoring hormone replacement therapy by biochemical markers of bone metabolism in menopausal women Postgrad. Med. J. 2002;78:727-731
2. Martin T. and Seeman E.: Bone remdelling; its local regulation and the emergence of bone fragility. Pub. Med. 2008;22(5):701-22
3. Brendan F. and Lianpingxing: Functions of RANKL/RANK/OPG in bone modeling and remodeling. Arch. Biochem. Biophys. 2008;15:473(2):139-146.
4. Kenny A.M. and Prestwood K.M.: Osteoporosis: pathogenesis, diagnosis and treatment in older adults. Rheum. Dis. Clinc. North. Am. 2000;26(3):569-591.
5. Jean, Yves R. and Veronique R.: Patients preference in the management of postmenopausal osteoporosis with bisphosphonates. Clinc. Interv. Aging. 2006;1(4):415-423.
6. Jun I, Yoshihiro, Tsuyoshi T, et al : Hip fracture protection by alendronate treatment in postmenopausal women with osteoporosis. Clini. Intervene. In Aging ; 2008 ;3(3):483-489.
7. Markus J. Seibel: Biochemical markers of bone turnover part 1; biochemistry and variability. Clin. Biochem. Rev. 2005;26:97-122.
8. Chailurkit L, Ongphiphadhanakul B, Piaseu N, et al :Biochemical markers of bone turnover and response of bone mineral density to intervention in early postmenopausal women : an experience in a clinical laboratory. Clin. Chem.. ;2001: 47:1083-1088.
9. Filip RS : Age and BMD related differences in biochemical markers of bone metabolism in rural and urban women

from Lublin region, Poland. Ann Agric Environ Med ;2004:11:255-259.

10. WHO: Assessment of fracture risk and its application to screening for postmenopausal osteoporosis: report of a WHO study group. Geneva Tech. Rep. Series. 1994; 843:1-129.

11. Belfield A and Goldberg DM: Revised assay for serum phenyl phosphatase activity using 4-amino- antipyrine enzyme;1971;12:561-573.

12. B. Demiaux, M.E, Arlot and et al: Serum osteocalcin is increased in patients with biochemical and histomorphometric finding, Journal of Clinical Endocrine. and Metab.;1992:74(5):1146-1151.

13. Kruger L., Rosenblum S, Zaazra J, et al : Intact PTH is stable in unfrozen EDTA plasma for 48 hours prior to laboratory analysis. Clin. Chem. ;1995:41(6):s47.

14. Stern J and Lewis WHP :The colorimetric estimation of calcium in serum with o-cresophthalein complexone Clinc. Chim. Acta.;1957:2:576.

15. Eugene S, Slawa S, and Bennie z : Direct serum inorganic phosphate. Clinc. Chim. Acta;1967:15:155

16. Aysan T, Aksoyz B, Asuman, et al : Correlation of serum IL-6 levels and prolidase activity between bone turnover markers and BMD in postmenopausal women with and without osteoporosis. Turkish Journal of Medical Sciences ;2007:37(3):129-134.

17. Peichl P, Griesmacher A, Muller MM, et al : Serum osteocalcin and urinary crosslaps are suitable markers to short-term hormone replacement therapy. Gynaecological Endocrinology;2000:14(5) : 374-381.

18. Garnero P, Borel O, and Delmas PD : Evaluation of a fully automated serum assay for c-terminal cross- linking telopeptide of type I collagen in osteoporosis.Clin. Chem. ; 2001:47(4):694-702.

19. Apurva KS, Elizabeth LV, Michael LE, et al ; Clinical use of serum and urine bone markers in the management of Osteoporosis. Current Medical Res Opin ;2005:21(7):1015-1026.

20. Indumati V and Patil VS : Biochemical markers of bone remodeling. JCDR. net.article;2010.

21. Raisz L, Smith JA, Trahiotis M, et al : Short term risedronate treatment in postmenopausal women:effects on biochemical markers of bone turnover. Osteopor. Int.;2000:11(7):615-620.

22. Kadir Y, Garhan G, Saliha K, et al : Comparison of the effect of alendronate, risedronate and calcitonin treatment in postmenopausal osteoporosis. Journal of Back and Musculos Rehabilitation ; 2005:18(3-4):1878-6324.

23. Gur A, Colpan L, Cevik R, et al : Comparison of zink excretion and biochemical markers of bone remodeling in the assessment of the effects of alendronate and calcitonin on bone in postmenopausal osteoporosis. Clin. Biochem.;2005:38(1):66-72.

24. Manolagas S C :Birth and death of bone cells: basic regulatory mechanisms and Implications for the pathogensis and treatment of osteoporosis.Endocr. Rev. ;2000:21:115:137

25. Stepan JJ, Alenfeld F, Boivin G, et al :Mechanism of action of antiresorptive therapies of postmenopausal osteoporosis.Endocr. Regul. ;2003: 37(4) : 225-238

26. Dunford JE, Thompson K, Coxon FP, et al ; Structure activity relationships for inhibition of farnesyl diphosphate synthase in vitro and inhibition of bone resorption in vivo by nitrogen-containing bisphosphonates. J Pharm. Exp. Ther.; 2001: 296(2):235-242.

27. Kavanagh Kl, Guo K, Dunford JE, et al :The molecular mechanism of nitrogen-containing bisphosphonates as Antiosteoporosis drugs. Proc. Natl. Acad. Sci. USA;2006:103(20):7829-7834.

28. Papapoulos S : Clinical aspect of bisphosphonates. ASBMR,2007.

29. Okabe R, Nakatsuka K, Inaba M, et al :Clinical evaluation of the elecsys β-crosslaps serum assay, a new assay for degradation products of type I collagen C-telopeptides.Clin. Chem. ; 2001: 47:1410-1414.

30. Okabe R, Nakatsuka K, Inaba M, et al : Significance of serum crosslaps as a predictor of changes in BMD during estrogen replacement therapy; comparison with serum carboxyterminal telopeptide of type I collagen and urinary deoxypyridinoline. J. Bone Miner. Metab.; 2004:22(2):127-131.

31. Riggs BL, Khosla S, and Melton I J :Sex steroids and the construction and conservation of the adult skeleton. Endocr. Rev.;2002:23:279-302.

32. Nuti R : Osteoporosis: bone balance, disturbance leading to bone loss. Medieographia;2007:29(2):120-125.

33. Buckley KM, Leib ES, Cartularo KS, et al :Calcium and vitamin D_3 supplementation

prevents bone loss in the spine secondary to low-dose corticosteroids in patients with rheumatoid arthritis. a randomized, double-blind, placebo-controlled trial. Ann. Intern. Med. ;1996: 125(12):961-968.

34. Cranny A, Guyatt G, Griffith L, et al :Meta-analyses of therapies for postmenopausal osteoporosis. IX:summary of meta-analyses of therapies for postmenopausal osteoporosis. Endocr. Rev. ;2002:23(4):570-578.

35. Shinchuk L, Haanacahuari N, and Ingersill D :Vitamin D deficiency and osteoporosis: highly prevalent in men and women admitted to subacute rehabilitation facility bosten massuchsetts during summer. Am. J. Physical Med. Rehab.; 2005: 84 (3):203.

36. Adachi JD, and Ioannidis G :Calcium and vitamin D therapy in corticosteroid induced bone loss:what is the evidence?. Calcif. Tissue. Int. ;1999:65(4):332-336.

37. Laszlo A :Postmenopausal osteoporosis. Orv. Hetil.;2004:145(1):3-13.

38. Palacios S, Castelo-Branco C, Cifuentes I, et al :Changes in bone turnover merkers after calcium-enriched milk supplementation in healthy postmenopausal women: a randomized, double blinded, prospective clinical trial.Menopayse ;2005: 12(1):63-68.

39. Tiras MB, Noyan V, Yildiz A, et al: Effect of alendronate and hormone replacement therapy, alone or in combination, on bone mass in postmenopausal women with osteoporosis: a prospective, randomized study.Hum. Reprod.; 2000:15(10):2087-2092.

40. Evio S, Tiitinen A, Laitinen K, et al Effect of alendronate and hormone replacement therapy, alone and in combination, on bone mass and markers of bone turnover in elderly women with osteoporosis. J. Clin. Enndocrinol. Metab.;2004:89(2):626-631.

41. Grados F, Brazier M, Kamel S, et al : Prediction of bone mass density variation by bone remodeling markers in postmenopausal women with vitamin D insufficiency treated with calcium and vitamin D supplementation. J. Clin. Endocrinol.Metab.;2003:88(11):5175-5179.

42. Ashuma S, Shashi S, Anju , et al ; Study of some common biochemical bone turnover markers in postmenopausal women.Indian Journal of Clinical Biochemistry ;2005: 20(1):131-134.

43. Rizzoli R :Control of bone turnover and bone balance: a target for antiosteoporosis treatment. Medicographia.;2007;29(2):133-136.

44. Jasminka Z, Llich RD, Jean EK, et al :Nutrition in bone health revisited: a story beyond calcium.American College of Nutrion.;2000:19(6):715-737.

45. Omarani GR, Masoompou SM, et al:Effect of menopause and renal function on vitamin D status in Iranian Women. Health Journal.;2006.

46. Sireen S, Hisham M, Hilow, et al :The efficacy and safety of calidon tablets for management of osteoporosis in Jordanan women: a randomized clinical trial Saudi Pharmaceutical journal. ;2004: vol(12): no.(2-3).

47. Sasaki N, Kusano E, Ando Y, et al :Glucocorticoid decreases circulating osteoprotegerin (OPG):possible mechanism for glucocorticoid induced osteoporosis Nephrol. Dial. Transplant.; 2001: 16(3): 479-482.

48. Malluche HH, and Faugere MC :Bone biopsies: history and histomorphometry. In: metabolic bone Disease and clinically related disorders, 2nd edition, avioli L.V. and Krane S.M.(editor). Philadelphia, W.B. Saaunders Co., P.;1990:283-328.