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Effects of Menopause and Osteoporosis on Some Hormone and Biochemical Markers of Bone Turnover

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This study was performed to analyze the effect of menopause and osteoporosis on some hormone and biochemical markers of bone turnover. Eleven young adult premenopausal women (mean age 27.3 ± 0.9 years); 26 premenopausal women (mean age 41.5 ± 0.7 years); 17 perimenopausal women (mean age 48.8 ± 0.7 years); 26 postmenopausal women (mean age, 56.2 ± 2.4 years, menopause duration, 9.7 ± 1.9 years); and 11 postmenopausal osteoporotic women (mean age 73.7 ± 2.3 years, menopause duration, more than 15 years) were recruited for this study. Serum was assayed for estradiol (E_2) follicle stimulating and luteinizing hormones (FSH and LH), insulin-like growth hormone factor-1 (IGF-1), total alkaline phosphatase (TALP) and osteocalcin or bone galacto-protein (BGP). The obtained data indicated that the measurements of E_2, FSH and LH are closely correlated with the bone formation markers IGF-1 and TALP. IGF-1, TALP and BGP are proved to have a good performance for bone formation in the increased bone turnover status.

Key words: BGP, IGF-1, TALP, menopause, osteoporotic women, ovarian function

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Introduction

Menopause represents a critical life step characterized by complex endocrine changes, that affect the musculoskeletal system and its neurological control. Pathogenic of the cessation of ovarian function is a marked decline in the 17β-estradiol level, the major estrogen in women of reproductive age (Greendale and Judd, 1993). The discovery of estrogen receptors in osteoblast (Eriksen *et al.*, 1988) suggests a direct effect of estrogen on bone. Estrogen deficiency leads to increased bone turnover and to a greater increase in bone resorption than bone formation (Heaney *et al.*, 1978). Menopause is also the cause of an increased production of the pituitary hormones FSH and LH. Although the role of these substances in maintaining bone health is unknown, evidence is beginning to emerge indicating that pituitary hormones may modulate the effect of estrogen on bone (Cole *et al.*, 1994). Bone contains a large numbers of growth factors; among the most abundant is insulin-like growth factor-1. IGF-1 peaks during puberty at or about the same time as acquisition of peak bone mass and declines with aging in slop similar to age-related bone loss (Rosen and Conover, 1997). In the last decade, IGF-1 has received the most attention because of the interaction with osteoporosis (Rosen, 1999). Osteoblasts are rich in alkaline phosphatase (ALP). Bone loss, reportedly, could be predicted by the assessment of bone formation markers such as ALP (Christiansen *et al.*, 1990). Osteocalcin is synthesized by osteoblasts and only located in bone and reflected osteoblastic activity (Lian and Gundberg, 1988).

The present study aimed to evaluate a number of biochemical variables related to ovarian function and bone formation in Egyptian women, in order to assess their role on bone metabolism.

Materials and Methods

Subjects: Young adult group (YOUNG) consisted of 11 young adult healthy women with range of age 24 – 32 years (mean ± SE, 27.3 ± 0.9 years) and with regular menstrual cycle.

Premenopausal group (PRE) was 26 premenopausal healthy women with range of age 36 – 49 years (mean ± SE, 41.5 ± 0.7 years) and with regular menstrual cycle. The PRE group was then further classified into three subgroups, each covering a pentad, 49 – 45, 44 – 40 and 39 – 35 years.

Perimenopausal group (PERI) consisted of seventeen women aged 42 – 52 years (mean ± SE, 48.8 ± 0.7), all subjects characterized by irregular menses. The PERI group was further classified into two subgroups each covering a pentad, 54 – 50 and 49 – 45 years.

Postmenopausal group (POST) consisted of 26 healthy women, aged 43 – 80 years (mean age ± SE, 56.2 ± 2.4 years) and with no menstrual bleeding for at least one year (mean menopausal duration ± SE, 9.7 ± 1.9 years). POST group was further classified into four subgroups, each covering a pentad, above 65, 59 – 55, 54 – 50, and 49 – 45 years.

Subjects of these four groups had no previous history of metabolic bone disease.

Osteoporotic group was eleven untreated postmenopausal osteoporotic females aged 65–85 years (mean ± SE, 73.7 ± 2.3 years) with menopausal duration over 15 years. All had symptoms of lumbago. Atrophies, a traumatic wedge or compression fracture of the spine (but no recent fracture) were diagnosed by X-ray films of the thoracic and lumbar spine.

All subjects were receiving no medications that might affects the hormonal balance, the calcium absorption and metabolism.

Samples: Blood samples were obtained from each subject 6 to 9 days after ovulation (mid luteal phase) between 9 and 1 O′ clock after an overnight fast. Serum was separated and stored at -20°C until analysis.

Measurement of biochemical markers: Serum estradiol was measured by a solid phase ^{125}I - RIA (DPC, Los Angeles, CA). The intra-assay and inter-assay CV were < 7.0 and < 8.1%, respectively. Serum FSH was measured by double antibody ^{125}I - RIA (DPC, Los Angeles, CA). The intra-assay and inter-assay CV were < 6.5 and < 7.7%, respectively. Serum LH was measured by a solid phase ^{125}I – IRMA (ICN Biomedicals, Inc, Costa Mesa, CA). The intra-assay and inter-assay CV were < 11.7 and < 8.6%, respectively. Serum IGF-1 was measured by ^{125}I – RIA (Biosource, Belgium). The intra-assay and inter-assay CV were < 6.1 and < 9.9%, respectively. Serum intact osteocalcin was measured by solid phase ^{125}I – IRMA (Biosource, Belgium). The intra-assay and inter-assay CV were < 4.7 and < 6.3%, respectively. Total ALP was measured spectrophotometrically with sodium thymolphthalein monophosphate as substrate (Teco Diagnostics, Anaheim, CA). The intra-assay and inter-assay CV were < 3.1 and < 4.2%, respectively.

Statistical analysis: The statistical significance of the mean values between groups or subgroups were performed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range "t" test, whenever necessary. Unpaired tow tailed "t" test between mean values of the two PERI subgroups were carried out. Simple regression analyses were performed between the markers. The statistical significance of the correlation was confirmed with "t" test. P values less than 0.05 were considered significant. The statistical analysis was performed according to Bahn (1972)

Results

Table 1 shows the means ± SE of the biochemical markers in the groups. E_2 and IGF-1 in the groups of osteoporosis and POST were significantly lower than those in PRE and YOUNG but the level of IGF-1 in the PERI group was non-significantly decreased, compared to the PRE group. On the contrast, FSH, LH and TALP in the osteoporotic, POST, and PERI groups were significantly higher above the PRE and YOUNG with an exception that the increase of TALP level in the PERI group above the PRE and YOUNG groups was non-significant. The levels of BGP were fluctuated throughout the groups, where the highest level revealed from the osteoporotic group but the lowest level from

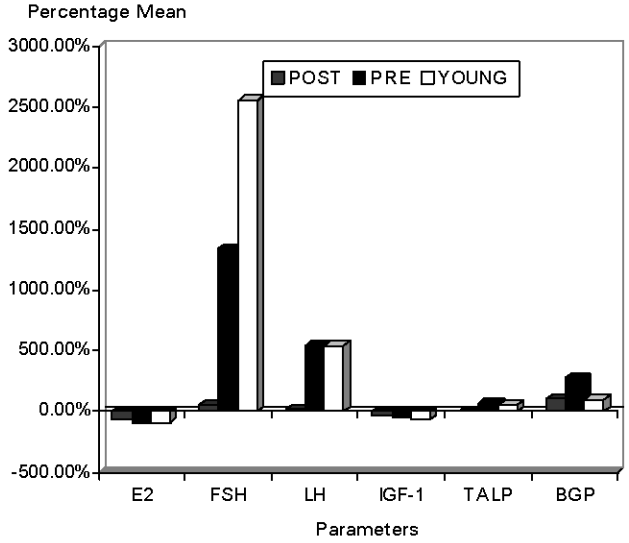

Fig. 1: Percentage mean (%) increase of the hormone and biochemical markers in the osteoporotic group over POST, PRE and Young groups.
E2 = Estradiol FSH = Follicle stimulating hormone
LH = Luteinizing hormone IGF-1 = Insulin like growth hormone factor 1 TALP = Total alkaline phosphatase
BGP = Bone galacto-protein

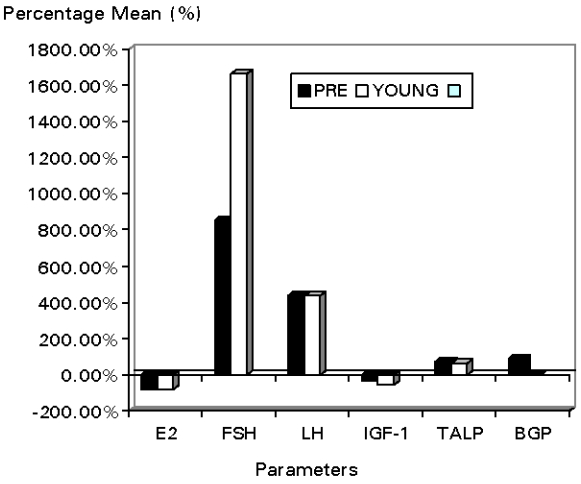

Fig. 2: Percentage mean (%) increase of the hormone and biochemical markers in POST group over PRE and YOUNG group

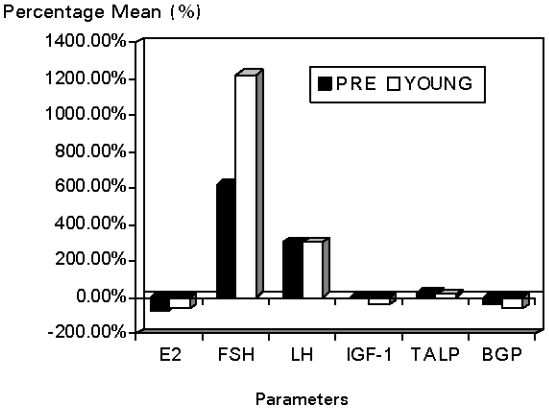

Fig. 3: Percentage mean (%) increase of the hormone and biochemical markers in PERI group over PRE and YOUNG groups.

the PERI group (Table 1). Table 2 shows the levels of all tested markers in the subgroups of POST, PERI and PRE. The subgroup of the oldest postmenopausal women (over 65 years old) showed significant changes in levels of IGF-1 and BGP, compared to the subsequent younger subgroups (59 – 55, 54 – 50, and 49 – 45 years old). Comparisons were also carried out among the age-matched subgroups POST, PERI, and PRE at 49 – 45 years old and revealed that E_2, FSH, and LH levels in the postmenopausal subgroup were significantly changed compared to the PRE subgroup. IGF-1 and TALP showed gradual decrease with loss of the ovarian adequacy (Table 3).

In Fig. 1, BGP in the osteoporotic group shows that the greatest percentage of mean was increase 104%, relative to the POST group whereas, FSH and LH in the osteoporotic group precedes BGP, when compared with PRE and YOUNG groups. Levels of FSH and LH in POST and PERI groups (Fig. 2 and 3, respectively) showed the highest percent mean increases over both of PRE and YOUNG groups followed by BGP in POST group over PRE group (Fig. 2) and in PERI group over YOUNG group (Fig. 3). Table 4 shows the correlation coefficient values among the six-biochemical markers and revealed no significant correlation between BGP and any of the rest markers whereas, significant correlations among E_2, FSH, LH, IGF-1 and TALP markers were revealed ($P < 0.01$ and 0.05).

Discussion

The results of this study demonstrated severe deficiency of serum estradiol in the osteoporotic group, which may reflect the role of estradiol in regulating bone remodeling. This suggestion is depending on several facts that estrogen is one of the hormones among several factors regulates the bone remodeling. Estrogen deficiency may be an etiological agent for postmenopausal osteoporosis and can also alter the balance of multiple growth factors and cytokinase that regulate bone turnover leading to increase bone resorption than bone formation (Watts, 1999; Pacifici *et al.*, 1993; Jilka *et al.*, 1992; Oursler *et al.*, 1991; and Heaney *et al.*, 1978). However, the group of postmenopausal women with lack of estradiol content, showed no manifestations of osteoporosis. The possible explanations are that, the osteoporotic disease has not affect all postmenopausal women with estrogen deficiency, because of the presence of additional factors that operates the bone loss only in the presence of estrogen deficiency (Kassem *et al.*, 1996). In addition, the levels of estradiol in the postmenopausal women were relatively higher than in the osteoporotic women. The levels of estradiol in the subgroups of 59-55, 54-50, and 49-45 years old were higher by 1.5, 3.1 and 3.0 times, respectively, above the osteoporotic group (Tables 1 and 2). The postmenopausal subgroup at age above 65 years showed a dramatic decrease of estradiol levels (Table 2), which may reflect a risk of osteoporosis. These may be confirmed by other studies. In postmenopausal women, higher estradiol concentration appears to have a greater bone density (Cummings *et al.*, 1998) and in elderly subjects, serum estradiol levels are associated with BMD (Gurlek and Gediko, 2001). Moreover, the measurements of serum estradiol among other factors (body mass index, urinary hydroxyproline and calcium) have identified 79% of fast and slow bone losers in the study of Christiansen *et al* (1987). However, no correlation between base line bone mineral density (BMD) and each of estradiol, estrone, estrone sulfate and estrone glucuronide have been detected in the study of Keen *et al.* (1996). The strong negative correlations between E_2 and each of FSH and LH (in this study) confirms the modulator effect of FSH and LH on estradiol, which in turn, modulate the bone (Cole *et al.*, 1994).

Serum IGF-1 positively correlates with lumber BMD in subjects with idiopathic osteoporosis (Kurland *et al.*, 1996) and with BMD at all sites of the hip, radius, and lumbar spine in women (Langlois *et al.*, 1998). Data showed that women of the osteoporotic group had the lowest level of IGF-1 (65.9 ng ml^{-1}), whereas the PRE and YOUNG healthy women had the highest levels (139.2 and 218.8 ng ml^{-1}, respectively). These data are somewhat powerful and suggesting that IGF-1 level may be associated with BMD in the women. Bauer *et al.* (1998) in their study reported on the relationship between IGF-1 and hip fractures that women with low level of IGF-1 less than 80ng ml^{-1} have a 60% greater risk of hip fracture and incident vertebral fracture than the others. Therefore, the two subgroups of the elderly post menopausal women at age above, 65 and 59-55 years with IGF-1 concentrations of 49.4 and 59.5ng ml^{-1}, respectively may reflect a greater risk of osteoporosis. Reed *et al.* (1993) proved the relationship between endogenous estrogen and serum IGF-1.

The effect of ovarian function on serum levels of IGF-1 may be reflected where the levels of IGF-1 decreased with the increase of the ovarian exhaustion (Tables 1 and 3). The osteoporotic and postmenopausal women, who characterized by exhausted ovary, as reflected by the levels of E_2, FSH and LH, showed the lowest levels of IGF-1 in contrast to the premenopausal and young adult women, who characterized by adequate ovarian function, showed the highest levels of IGF-1. The PERI group, with less adequate ovary, revealed lower concentration of IGF-1 than the PRE and YOUNG groups.

Age matched subgroups (49-45 years old) with different ovarian adequacy reflected further confirmation (Table 3). The significant correlations between serum IGF-1 and each of E_2, FSH, and LH further confirmed the relationship between IGF-1 and gonadal

Table 1: The values (mean ± SE) of the studied hormone and biochemical markers in the tested groups.

	Groups				
Parameters	Osteoporosis	POST	PERI	PRE	YOUNG
Age(years)					
Mean ± SE	73.7 ± 2.3	56.2 ± 2.4	48.8 ± 0.7	41.5 ± 0.7	27.3 ± 0.9
Range	65-85	43-80	42-52	36-49	24-32
Hormones					
E_2 (Pg ml^{-1})	8.4 ± 1.3[1,2]	20.3 ± 5.2[3,4]	38.1 ± 7.8[5,6]	119.8 ± 11.7	102.1 ± 13.2
FSH(mlu ml^{-1})	69.0 ± 6.2[1,2,7,8]	45.8 ± 4.8[3,4]	34.4 ± 10.2[5,6]	4.8 ± 0.7	2.6 ± 0.4
LH, (mlu ml^{-1})	67.5 ± 9.2[1,2,9]	56.7 ± 4.8[3,4]	42.0 ± 9.9[5,6]	10.5 ± 1.1	10.5 ± 1.3
Biochemical markers					
IGF-1(ng ml^{-1})	65.9 ± 10.6[1,2,8]	97.3 ± 9.5[4,10]	129.9 ± 14.3[6]	139.2 ± 9.6[11]	218.8 ± 33.1
TALP (lu L^{-1})	39.6 ± 2.4[1,9,12]	41.7 ± 4.0[3,4,13]	29.7 ± 2.1	24.3 ± 1.9	25.3 ± 2.4
BGP (ng ml^{-1})	9.4 ± 1.2[1,8,12,14]	4.6 ± 1.3[15]	1.6 ± 0.5	2.5 ± 0.2	4.7 ± 1.5

[1] P < 0.01 vs PRE, [2] P < 0.01 vs YOUNG, [3] P < 0.01 vs PRE, [4] P < 0.01 vs YOUNG,
[5] P < 0.01 vs PRE, [6] P < 0.01 vs YOUNG, [7] P < 0.05 vs POST, [8] P < 0.01 vs PERI,
[9] P < 0.05 vs PERI, [10] P < 0.05 vs PRE, [11] P < 0.01 vs YOUNG, [12] P < 0.05 vs YOUNG,
[13] P < 0.01 vs PERI, [14] P < 0.01 vs POST, [15] P < 0.05 vs PERI

POST = Postmenopausal group
PERI = Perimenopausal group
PRE = Premenopausal group
YOUNG = Young adult group

E_2 = Estradiol FSH = Follicle stimulating hormone LH = Luteinizing hormone BGP = Bone galacto-protein
IGF-1 = Insulin like growth hormone factor 1 TALP = Total alkaline phosphatase

Table 2: The values (mean ±SE) of the studied hormone and biochemical markers in the tested Subgroups.

	Sub-groups								
	POST				PERI		PRE		
Parameters	> 65	59-55	54-50	49-45	54-50	49-45	49-45	44-40	39-35
Hormone									
E_2 (Pg ml^{-1})	6.4 ± 0.6	12.6 ± 1.5	25.9 ± 9.8	25.3 ± 14.6	36.3 ± 12.3	39.3 ± 10.4	119.3 ± 31.7	128.6 ± 14.7	85.0 ± 17.8
FSH (mlu ml^{-1})	37.6 ± 5.7	41.5 ± 3.6	52.0 ± 8.9	61.3 ± 8.6	30.9 ± 14.5	36.8 ± 14.7	2.7 ± 0.4	4.8 ± 0.9	7.0 ± 2.1
LH (mlu ml^{-1})	55.5 ± 12.1	41.3 ± 0.7	54.5 ± 7.9	67.5 ± 7.8	46.3 ± 18.3	38.9 ± 11.7	10.8 ± 3.2	10.4 ± 1.2	10.7 ± 3.6
Biochemical markers									
IGF-1 (ng ml^{-1})	49.4 ± 18.2[1,2]	59.5 ± 1.8[3,4]	108.0 ± 13.0	129.3 ± 5.9	152.3 ± 32.0	114.3 ± 8.8	109.9 ± 8.8	137.1 ± 7.9	175.0 ± 38.8
TALP(lu l^{-1})	33.3 ± 4.2	42.1 ± 5.7	45.9 ± 7.8	43.5 ± 8.9	33.1 ± 3.4	27.3 ± 2.6	25.3 ± 2.9	22.5 ± 2.8	28.6 ± 4.3
BGP (ng ml^{-1})	12.7 ± 3.0[1,2,5]	0.17 ± 0.04	1.4 ± 0.41	1.8 ± 0.73	0.75 ± 0.41	1.4 ± 0.51	2.0 ± 0.83	2.2 ± 0.67	4.0 ± 3.0

[1] P < 0.01 VS 54 – 50 years, [2] P < 0.01 VS 49–45 years, [3] P < 0.05 VS 54–50 years, [4] P < 0.05 VS 49–45 years, [5] P < 0.01 VS 59-55 years

Table 3: The values (mean ± SE) of the studied hormone and biochemical markers in age-matched subgroups, 49-45 years old.

	Subgroups of 49-45 years.		
Parameters	POST	PERI	PRE
Hormone			
E_2 (Pg ml^{-1})	25.3 ± 14.6 [1]	39.3 ± 10.3[3]	119.3 ± 31.7
FSH (mlu ml^{-1})	61.3 ± 8.6 [2]	36.8 ± 14.7	2.7 ± 0.4
LH (mlu ml^{-1})	67.5 ± 7.8 [1]	38.9 ± 11.7	10.8 ± 3.2
Biochemical markers			
IGF-1 (ng ml^{-1})	129.3 ± 5.9	114.3 ± 8.8	109.9 ± 8.8
TALP (lu l^{-1})	43.5 ± 8.9	27.3 ± 2.6	25.3 ± 2.9
BGP (ng ml^{-1})	1.8 ± 0.73	1.4 ± 0.51	2.0 ± 0.83[1]

[1] P < 0.01 VS PRE, [2] P < 0.05 VS PRE, [3] P < 0.01 VS PRE.

Table 4: The correlation coefficient between hormone and biochemical markers in all subjects

Parameters	FSH	LH	IGF-1	TALP	BGP
Hormone					
E_2	- 0.625 [1]	- 0.614 [1]	+ 0.261 [2]	- 0.499 [1]	- 0.099
FSH		+ 0.881 [1]	- 0.334 [1]	+ 0.325 [1]	+ 0.198
LH			- 0.306 [1]	+ 0.369 [1]	+ 0.203
Biochemical markers					
IGF-1				- 0.270 [2]	- 0.214
TALP					+ 0.184

[1] P < 0.01 ; [2] P < 0.05

function. These findings are in coinciding with others. Serum IGF-1 level is positively related to serum estradiol concentration (Greendale *et al.*, 1997) and the declining sex steroid production negatively impacts the GH/IGF-1 axis (Ho *et al.*, 1987). IGF-1 amplifying the response of ovarian cells to gonadotropins exerting a stimulatory action on granulosa cell replication (Adashi *et al.*, 1985) and also exerts stimulatory action on granulosa aromatase particular FSH (Hsu and Hammond, 1987). Bone alkaline phosphatase (ALP) and BGP are produced by osteoblasts. Bone formation can be assessed either by measuring a prominent enzymatic activity of the bone forming cells, ALP, or by measuring bone matrix components, BGP, released into the circulation during formation. In this study, TALP activities were significantly 1.6 and 1.7 times higher in the osteoporotic and POST groups, respectively, over the PRE group. The PERI group, with early signs of menopause, showed no significant increase of 1.2 times higher than the PRE group. Age-matched subgroups (49-45 years old) reflected increased TALP activities with decreased of the ovarian function (Table 3). These findings may show that TALP can be considered a good bone marker, which coincide with the ovarian function and may reflect the bone turnover, unless there is no increase of hepatic isozyme. The correlation between TALP and each of E_2, FSH, LH, and IGF-1 were significant. Since loss of ovarian hormones cause an increase in bone turnover and results

in a negative bone balance (Manolagas and Jilka, 1995). Also IGF-1 is positively associated with BMD at all sites of the hip, radius, and lumber spine (Langlois *et al.*, 1998). Therefore, the significant correlations between TALP and each of E_2, FSH, LH, and IGF-1, in this study, may confirm that TALP is a good marker in assessing the bone turnover. However, some other authors have reported lack sensitivity of TALP in state of osteoporosis (Ohishi *et al.*, 1998). Wand *et al.* (1992) reported that the rate of bone formation by the radioisotopic method and the concentration of osteocalcin activity as well as E_2 secretion (Erickson *et al.*, 1989). Moreover, synthesis of IGF-1 can be stimulated by the gonadotropins, in have declined progressively after menopause in healthy control, but not decreased in women with vertebral fractures. Because BGP, is produced only by osteoblasts and seems to be specific to the function of the osteoblasts therefore, the dramatic increase of serum BGP in the osteoporotic group, in this study, is indicative to the high bone turnover rate, where bone formation and resorption are coupled but bone resorption exceeds bone formation. It is noticeable, that the high level of BGP in the POST group is entirely attributed to the dramatic increase of this protein in the subgroup of the oldest women at age above 65 years. This dramatic increase may reflect increased bone turnover. This explanation is confirmed by the study of Delmas (1992), where serum BGP is the most sensitive marker of bone turnover. The lack of correlation between BGP and the other tested biochemical markers (Table 4) revealed that the concentration of circulating BGP has not been affected by the ovarian function, the osteoblastic anabolic factor IGF-1 or the osteoblastic enzymatic activity TALP.

In conclusion, differences of the levels of E_2, FSH and LH among the premenopausal, postmenopausal, and osteoporotic groups in addition to their close correlations with the bone formation markers IGF-1 and TALP may reflect the more important determinants than the qualities of these variables in terms of specificity to bone. IGF-1 and TALP have good performance in assessing the bone turnover in the postmenopausal and the osteoporotic status. BGP is a good marker reflecting high bone turnover in the elderly osteoporotic and postmenopausal women.

References

Adashi, E.Y., C. E. Resnick, A.J. D'Ercole, M.E. Savoboda and J.J. Van Wyk, 1985. Insulin-like growth factors as intraovarian regulators of granulosa cell growth and Function. Endocr. Rev., 6: 400-420.

Bahn, A.K., 1972. Basic Medical Statistics. Grune & Stratton, a subsidiary of Harcourt Brace Jovanovich, publishers, New York, San Francisco, London.

Bauer, D.C., C. Rosen, J. Cauley and S.R. Cummings, 1998. For the SOF Research Group. Low serum IGF-1 but not the IGFBP-3 predicts hip and spine fracture: the study of osteoporotic fracture. Bone, 23: 561.

Christiansen, C., B.J. Riis and P. Rodbro, 1990. Screening procedure for women at risk of developing postmenopausal osteoporosis. Osteop. Int., 1: 35-40.

Christiansen, C., B.J. Riis and P. Rødbro, 1987. Prediction of rapid bone loss in postmenopausal women. The Lancet, 16: 1105-1108.

Cole, H.W., S. Chandrasekhar , H. M. Hsuing, M. L. Heiman and M. Sato, 1994. Beneficial effects of ethynyl estradiol (EE_2) and raloxifene in the ovariectomized (OVX) rat are dependent on an intact pituitary. J. Bone Miner. Res., 9: 57.

Cummings, S.R., W.S. Browner, D. Bauer, K. Stone, K. Ensrud, S. James and B. Ettinger, 1998. Endogenous hormones and the risk of hip and vertebral fractures among older women. Study of Osteoporotic Fractures Research Group. N. Engl. J. Med., 339: 733-738.

Delmas, P.D., 1992. Biochemical markers of bone turnover: methodology and clinical use in osteoporosis. Am. J. Med., S5B: 59S-63S.

Eriksen E. F, D. S. Colvard, N. J. Berg, M. L. Graham, K. G. Mann, T. C. Spelsberg, and L. Riggs, 1988. Evidence of estrogen receptors in normal human osteoblast-like cells. Science, 241: 84-86.

Eriksen, G.F., V.G. Garzo and D.A. Magoffin, 1989. Insulin-like growth factor-1 regulates aromatase activity in human granulosa and granulosa-luteal cells. J. Clin. Endocrinol. Metabol., 69: 716-724.

Greendale, G.A., S. Delstein and E. Barrett Connor, 1997. Endogenous sex steroids and bone mineral density in older men and women. J. Bone Miner. Res., 12: 1833-1843.

Greendale, G.A. and H.L. Judd, 1993. The menopause: health implications and clinical management. J. Am. Ger. Soc., 41: 426-436.

Gurlek, A. and O. Gediko, 2001. Endogenous sex steroid GH and IGF-1 levels in normal elderly men: relationships with bone mineral density and markers of turnover. J. Endocrinol. Invest., 24: 408-414.

Heaney, R.P., R.R. Recker and P.D. Saville, 1978. Menopausal changes in bone remodeling. J. Lab. Clin. Med., 92: 964-970.

Ho, K.Y., W. S. Evans, R.M. Blizzard, J. D. Veldhuis, G.R. Merrian, K. E. Samojilk, R. F. Furlanetto, A.D. Rogol, D.L. Kaiser and M.O. Thomer, 1987. Effects of sex and age on the 24-h profiles of growth hormone secretion in man: importance of endogenous estradiol concentrations. J. Clin. Endocrinol. Metabol., 64: 51-58.

Hsu, C.J. and J.M. Hammond, 1987. Gonadotropins and estradiol stimulate immunoreactive insulin-like growth factor-1 by porcine granulosa cells in vitro. Endocrinology, 120: 198-207.

Jilka, R.L., G. Hangoc, G. Girasole, G. Passeri, D.C. Williams, J.S. Abrams, B. Boyce, H. Broxmeyer and S.C. Manolagas, 1992. Increased osteoclast development after estrogen loss: mediation by interleukin-6. Science, 257: 88-91.

Kassem, M., L.J. M. Melton III and B. L. Riggs, 1996. The type I / type II model for involutional osteoporosis. In: Marcus R, Feldman D, Kelsey J, Eds. Osteoporosis. San Diego: Academic Press, 691-702.

Keen, R.W., T. Nguyen, R. Sobnack, L.A. Perry, P.W. Thompson and T.D. Spector, 1996. Can biochemical markers predict bone loss at the hip and spine?: A 4-year prospective study of 141 early postmenopausal women. Osteopor. Int., 6: 399-406.

Kurland, E.S., F. Chan, D. Vereault, C. J. Rosen and J.P. Bilezikian, 1996. Growth hormone IGF-1 axis in men with idiopathic osteoporosis and reduced circulating levels of IGF-1. J Bone Miner. Res., 11: S1323.

Langlois, J.A., C. J. Rosen, M. Visser, M. T. Hannan, T. Harris, P. W. F. Wilson and D. P. Kiel, 1998. Association between insulin-like growth factor 1 and bone mineral density in older women and men: the Framingham Heart Study. J. Clin. Endocrinol. Metabol., 83: 4257-4262.

Lian, J.B. and C.M. Gundberg, 1988. Osteocalcin biochemical consideration and clinical applications. Clin. Orthop., 226: 267.

Manolagas, S.G. and R.L. Jilka, 1995. Mechanisms of disease: bone marrow, cytokines and bone remodeling-emerging insights into the pathophysiology of osteoporosis. N. Eng. J. Med., 332: 305-311.

Ohishi, T., M. Takahashi, K. Kushida, K. Yamazaki, H. Hoshino, A. Kitazawa and T. Inoue, 1998. Urinary collagen crosslinks reflect further bone loss of femoral neck in osteoporotic patients undergoing vitamin D therapy. Endocrinol. Res., 24: 259-267.

Oursler, M.J., C. Cortese, P. Keeting, M. Anderson, S. K. Bonde, B. L. Riggs and B.L. Spelsberg, 1991. Modulation of transforming growth factor-β production in normal human osteoblast-like cells by 17β - estradiol and parathyroid hormone. Endocrinology, 129: 3313-3320.

Pacifici, R., J.L. Vannice, L. Rifas and R.B. Kimble, 1993. Monocytic secretion of interleukin-1 receptor antagonist in normal and osteoporotic women: effect of menopause and estrogen / progesterone therapy. J. Clin. Endocrinol. Metabol., 77: 1135-1141.

Reed, M.J., L. Topping, N. G. Coldham, A. Purolit, M. W. Ghilchik and V.H.T. James, 1993. Control of aromatase activity in breast cancer cells: the role of cytokines and growth factors. J. Steroid Biochem. Mol. Biol., 44: 589-596.

Rosen, C.J., 1999. Serum insulin- like growth factors and insulin-like growth factor-binding proteins: clinical implications. Clin. Chem., 45: 1384-1390.

Rosen, C.J. and C. Conover, 1997. Growth hormone / insulin- like growth factor-1 axis in aging: a summary of a National Institute of Aging-Sponsored Symposium. J. Clin. Endocrinol. Metabol., 82: 3919-3922.

Wand, J.S., J.R. Green, R. Hesp, J. N. Bradbeer, P. N. Sambrook, T. Smith, L. Hampton, J. M. Zanelli and J. Reeve, 1992. Bone remodeling does not decline after menopause in vertebral fracture osteoporosis. Bone Miner, 17: 361-375.

Watts, N.B., 1999. Clinical utility of biochemical markers of bone remodeling. Clin. Chem., 45: 1359-1368.

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