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Age-Related Changes in Bone Turnover Markers and Ovarian Hormones in Premenopausal and Postmenopausal Indian Women

Meena P. Desai,* K.V. Bhanuprakash, M. Ikram Khatkhatay, and Uday M. Donde
Division of Molecular Immunodiagnostics, National Institute for Research in Reproductive Health, Mumbai, India

This study characterizes age-related changes in bone turnover markers in relation to ovarian hormones. The data (N = 236) were divided into 5-year age bands and three groups: premenopausal (Group I, N = 139), perimenopausal (Group II, N = 30), and postmenopausal (Group III, N = 67). Age-related increases in mean parathyroid hormone (PTH), osteocalcin (OC), and collagen telopeptide (CTx) levels were observed. Women in Group II (N = 37) with osteopenia had lower levels of E_1G ($P<0.001$) with normal FSH levels as compared to 50 women in the same group with normal bone mineral density (BMD). Their mean OC levels were reduced ($P<0.05$) and CTx levels were significantly elevated ($P<0.01$). The mean E_1G levels were significantly lower ($P<0.001$) and mean CTx levels were significantly higher ($P<0.001$) in 30 perimenopausal women (Group II) compared to premenopausal women. In 28 postmenopausal women (group III) the mean BMD levels and E1G were significantly lower ($P<0.001$) with elevated FSH levels ($P<0.001$). Increased CTx levels ($P<0.0001$) reflected a higher rate of bone resorption. These observations suggest that perimenopausal women with low E_1G, elevated FSH should be screened for osteoporosis, and it may be valid to combine simultaneous measurements of bone turnover markers with ovarian hormones when screening women at risk for osteoporosis. J. Clin. Lab. Anal. 21:55–60, 2007.

Key words: bone turnover markers; ovarian hormones; osteoporosis

INTRODUCTION

Age-related bone loss has been attributed to a decline in bone formation due to decreased function of osteoblasts and the poor calcium absorption efficiency of the intestines. However, studies on the relationship among age, menopausal status, and indices of bone turnover have shown conflicting results (1,2). It is well known that the rate of bone loss from all skeletal sites accelerates during the first postmenopausal years and then declines, and is generally associated with a high turnover (3–5). The accelerated bone loss is a result of declining ovarian function. A gradual decline in estrogen concentration with a postmenopausal hormonal pattern is also followed by a regular ovulatory cycle (6). However, little is known about how these hormonal changes affect bone turnover, since they fluctuate in individual women from time to time and in different populations. Changes in the biochemical markers of bone turnover in relation to estrogen withdrawal have been studied in most countries all over the world and a few countries in Asia, but yet not in India.

Osteoporotic syndromes in postmenopausal women appear to represent a major public health problem in India as well. It is essential to understand the normal rates of bone change during the pre- and postmenopausal periods in Indian women; however, such information is lacking. The purpose of this study was therefore to characterize age-related changes in hormonal profiles and bone metabolism as reflected by changing levels of

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*Correspondence to: Dr. Meena Desai, Division of Molecular Immunodiagnostics, National Institute for Research in Reproductive Health, J.M. Street, Parel, Mumbai 400 012, India.
E-mail: mends@rediffmail.com

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bone turnover markers, and to define the levels of bone turnover markers in Indian adult women. Based on the current results, we recommend that women with low hormonal profiles should be screened for osteoporosis.

MATERIALS AND METHODS

The Ethics Committee of the National Institute for Research in Reproductive Health approved the research protocols. Upon entry into the study, all participants were interviewed and their clinical and obstetrical histories were recorded in the prescribed format. An average dietary recall of their food was noted. The exclusion criteria for this study were women with: 1) a history of fractures; 2) taking drugs known to affect bone metabolism, or evidence of a medical or surgical condition known to affect bone loss; 3) pregnant or breast-feeding; and 5) smoking. We calculated the body mass index (BMI) in kg/m^2 by measuring the subjects' height in meters without footwear, and weight in kilograms with the subjects in light clothing. Women who had regular menstrual cycles for the last 6 months were defined as regularly cycling, and those with a slight deviation in their menstrual pattern were defined as perimenopausal. Those who had amenorrhea for >12 months were considered postmenopausal.

A total of 236 women (age = 21–70 years) participated in the study willingly because of prior awareness about the consequences of menopause and osteoporosis. Written consent was obtained from each subject. A total of 139 women were in the premenopausal phase (Group I) since they were regularly cycling with their study cycle ovulatory as confirmed by urinary estrone glucuronide (E_1G), pregnanediol glucuronide (PdG), and follicle stimulating hormone (FSH) levels. Group II consisted of 30 perimenopausal women (38–47 years) with cycle lengths ranging between 33 and 45 days. Group III consisted of 67 postmenopausal women who had a menopause duration of more than 1 year and elevated FSH levels (>50 mIU/mgC). Pregnanediol glucuronide was estimated only in pre- and perimenopausal women to assess their ovulatory status and confirm cyclicity.

Women who participated in the study had undergone bone mineral density (BMD) measurements during last 3 months, and made their reports available for the study. They were classified as having normal and abnormal bone mass based on BMD measurements and/or markers of bone turnover.

Blood samples were collected from all of the women between 10:00 a.m. and 11:00 a.m. For women in the pre- and perimenopausal phases, blood was drawn between days 5 and 9 of the cycle, with day 1 considered as the first day of menstrual bleeding. First morning urine specimens were collected thrice in a cycle (between days 5 and 9, days 11 and 14, and days 21 and 24). For women in the postmenopausal group (group III) a single blood and first morning urine sample were collected. Both serum and urine samples were stored in duplicates at $-20^{\circ}C$ and $-70^{\circ}C$ until they were analyzed. The aliquot stored at $-70^{\circ}C$ was used to estimate bone markers and PTH. Care was taken to estimate PTH within a fortnight.

Serum osteocalcin (OC), intact parathyroid hormone (PTH), and urinary collagen telopeptide (CTx) were assayed using kits from Diagnostics Systems Laboratories, Inc. (Texas), and in-house-developed enzyme-linked immunosorbent assays (ELISAs) were used to estimate E_1G, FSH, and PdG (7–9). Other biochemical indices, such as calcium, phosphorous, and creatinine, were measured by standard methods (10–12).

Analysis of the Data

We divided the subject population into 5-year age bands (21–25 years, 26–30 years, 31–35 years, 36–40 years, 41–45 years, 46–50 years, 51–55 years, and 56–60 years) to assess age-related changes. We also divided the population into three groups to assess changes in estrogen levels and bone turnover markers. All statistical analyses of the data were performed with Graph Pad Prism version 4.0 software. For statistical comparisons, the arithmetic mean was taken into consideration and the data were expressed as the means $\pm$ SD, with statistical significance as defined by $P<0.05$, $P<0.01$, and $P<0.001$ using one-way analysis of variance (ANOVA) with Tukey's multiple-comparison test (13).

RESULTS

The inter- and intra-assay coefficients of variation in the quality assurance pools (QAP) ranged between 10–12% and 15–16% for OC, CTx, and PTH, as provided by the manufacturer. All of the QAP estimates were within the accepted limits assigned by the manufacturer, which ensured the reliability of the results. The internal QAP results of E_1G, FSH, and PdG ranged between 8–10% and 10–14%, which were also within the acceptable limits. The characteristics of the women enrolled in the study are given in Table 1. The mean BMI was elevated in peri- and postmenopausal women as compared to premenopausal women.

Levels of PTH, OC, and CTx in Subjects Across the Age Groups

Age-related increases in the mean PTH, OC, and CTx levels were observed across the 5-year age bands (Figs. 1,2,3). Intact PTH levels in the study cohort

TABLE 1. Characteristics of women enrolled

	Group I (premenopausal) (N = 139)	Group II (perimenopausal) (N = 30)	Group III (postmenopausal) (N = 67)
Age (years)			
Range	21–45	38–47	46–60
Mean ± SD	33.6 ± 5.20	40.5 ± 2.3	53.2 ± 3.8
BMI (kg/m^2)			
Range	18–25	23–28	24–29
Mean ± SD	22.03 ± 1.24	25.06 ± 1.28	26.98 ± 1.25
Cycle length (days)			
Range	28–32	33–45	>1 year menopause
Mean ± SD	30.2 ± 1.5	38.6 ± 2.5	–

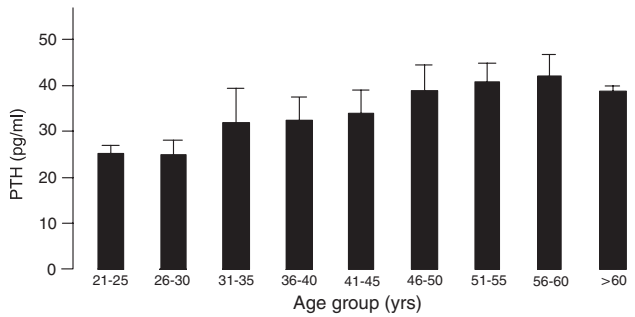

Fig. 1. Mean levels of PTH across the age bands.

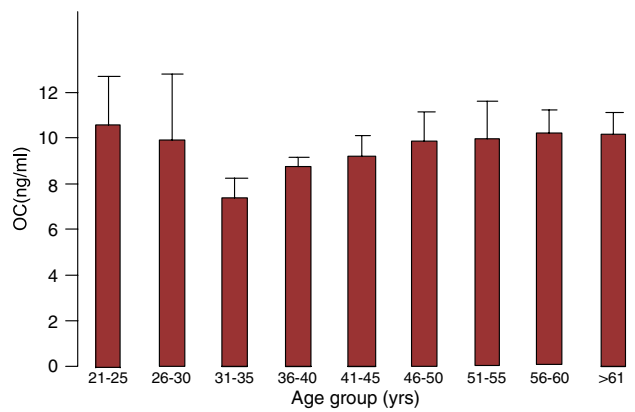

Fig. 2. Mean levels of OC across the age bands.

ranged between 16 and 72 pg/mL across the 5-year age bands, and a significant increase (17.38%, $P<0.001$) in these levels was observed in the age band of 21–45 years. Peak bone mass was achieved at around 30 years, and the mean OC levels dropped significantly in the subsequent age band (25.85%, $P<0.001$). The subsequent increase in bone resorption was reflected by a sharp rise in CTx levels (52%, $P<0.001$) over the mean levels seen in the age span of 21–35 years. OC levels also increased thereafter from a mean level of 7.35 ng/mL to 9.32 ng/mL seen in the age band of 41–45 years (an increase of 26.82%, $P<0.001$) due to increased bone

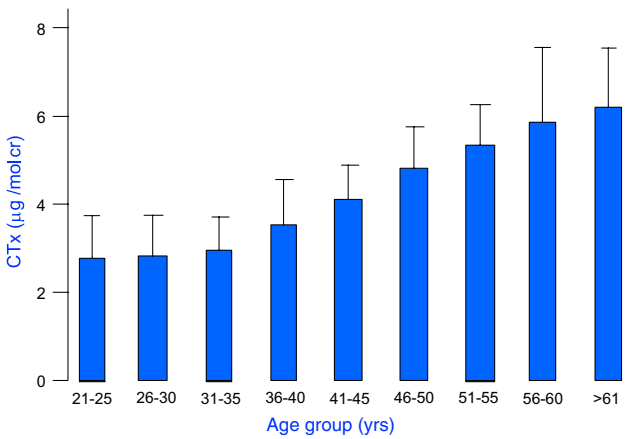

Fig. 3. Mean levels of CTx across the age bands.

remodeling observed during that phase. The levels increased to 9.96 ng/mL (an increase of 6.86%, $P<0.05$) in the age range of 46–50 years and subsequently normalized at 10.5 ng/mL in the age range of 51–60 years. During the menopausal phase, CTx levels showed a profound and significant rise reflecting increased bone resorption (295.2–547.3 µg/mol Cr, $P<0.001$). However, serum calcium and phosphorous levels were in the normal range (8.5–11.5 mg/dL and 3.0–5.6 mg/dL, respectively) across the age groups (Table 2).

Hormonal Levels and Bone Turnover in Subjects

The levels of hormones and the bone turnover markers in the three groups are depicted in Fig. 4. Fifty premenopausal women had a normal BMD, and 37 women were osteopenic (BMD = 0.8897 ± 0.0403 g/cm^2). These osteopenic women (Group I) had significantly lower levels of E_1G (39.34 ± 6.5 ng/mgCr; $P<0.001$) as compared to the premenopausal women with normal bone mass (47.75 ± 7.64 ng/mgCr). Their mean OC levels were moderately reduced (8.93 ± 1.76 ng/mL; $P<0.5$), while the CTx levels were significantly elevated (295.2 ± 101.7 µg/mol Cr; $P<0.001$,

TABLE 2. Mean levels of calcium, phosphorous, PTH, OC and CTx across the age bands

Age group	N	Calcium (mg/mL)	Phosphorus (mg/mL)	PTH (pg/mL)	OC (ng/mL)	CTx (μg/mol Cr)
21–25	24	9.6±0.4	3.9±0.1	25.1±2.1	10.6±2.2	260.1±98.1
26–30	38	9.9±0.7	4.1±0.3	25.1±3.1	9.9±1.2	256.5±75.8
31–35	30	9.2±0.8	4.1±0.4	32.3±7.7	7.4±1.0	295.2±86.5
36–40	30	9.3±0.3	5.4±0.3	32.9±5.2	9.0±1.9	342.0±96.6
41–45	25	9.3±0.9	4.1±0.3	34.5±5.2	9.3±0.8	383.0±87.1
46–50	20	9.5±1.0	4.2±0.2	39.9±5.7	10.0±1.2	464.4±89.0
51–55	25	9.6±1.0	4.2±0.2	40.7±4.2	10.0±1.7	486.9±89.4
56–60	20	9.6±1.3	4.1±0.5	42.6±4.9	10.5±0.9	547.3±151.2
>60	24	9.1±1.2	4.1±1.1	39.6±1.6	10.8±1.5	627.3±171.1

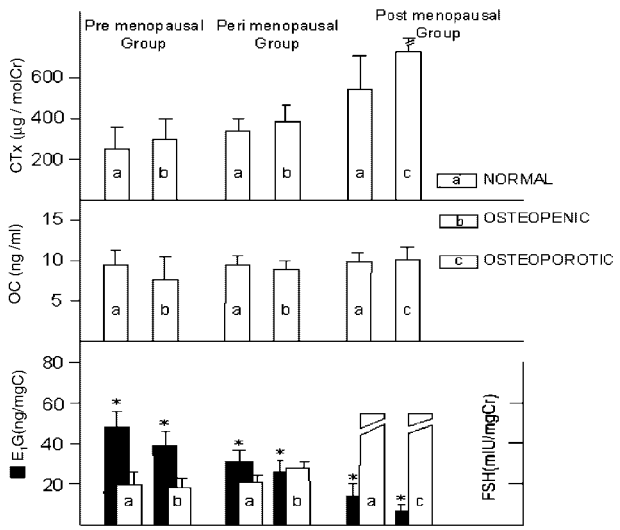

Fig. 4. Mean levels of E_1G, FSH, CTx, and OC measurements in pre-, peri-, and postmenopausal women.

respectively) as compared to the premenopausal women with normal bone mass (9.50±1.66 ng/mL; 246.6± 117.45 μg/mol Cr). The mean E_1G, FSH, CTx, and OC levels in the remaining 52 women were essentially the same as those observed in 50 women with normal BMD measurements, suggesting normal bone homeostasis.

The mean E_1G levels were significantly lower (28.82±5.8 ng/mgCr; $P<0.001$) in 30 perimenopausal women as compared to the premenopausal women. All of these women also had slightly elevated FSH (mean = 30.4± 4.0 mIU/mgCr; $P<0.001$) and CTx (mean = 390.6±83.88 μg/mol Cr; $P<0.001$) levels as compared to the premenopausal women, reflecting perimenopausal changes. The mean BMD levels were significantly lower in all of the postmenopausal women in Group III (0.786±0.083 g/cm^2 $P<0.001$) as compared to the premenopausal women, indicating osteoporosis. In normal postmenopausal women E_1G levels were lower (14.82±4.8 ng/mgCr; $P<0.001$) with elevated FSH levels (>50 mIU/mgCr; $P<0.001$). A high rate of bone resorption was observed in these women, as reflected by increased CTx levels (mean = 547.2± 106.2 μg/mol Cr, $P<0.001$). However, the OC level (10.02±1.68 ng/mL) was only marginally increased. In the remaining 39 postmenopausal osteoporotic women the CTx levels were further elevated (mean = 686.1 ±103.2 μg/mol Cr, $P<0.0001$), with a drop in E_1G levels (mean = 6.84±3.05 ng/mgCr; $P<0.001$) and a marginal increase in OC (mean = 10.29±1.02 ng/mL). Based on the mean±2 SD, we formulated a cutoff range for E_1G, FSH OC, and CTx as shown in Table 3.

DISCUSSION

Osteoporosis is a silent thief that leaves no evidence of progressive bone loss. Measurement of BMD by dual energy X-ray absorptiometry (DEXA) and estimation of bone turnover markers are widely used to assess bone health. However, these parameters are expensive, which prevents their widespread use to screen women who are at higher risk of developing osteoporosis. It is therefore necessary to adopt alternative parameters that could be used to warn women who are at likely at risk of developing osteoporosis. Estrogen deficiency is a dominant factor in bone loss, and we observed significant and simultaneous changes in the E_1G levels and the bone turnover markers in the pre- and postmenopausal women. Moreover, estimation of E_1G is relatively inexpensive.

Several cross-sectional studies have shown an increase in the concentration of PTH with age that probably progresses after menopause in response to the decrease in calcium absorption. It is believed that increased secretion of PTH with aging increases bone turnover by increasing the number of bone remodeling units when resorption and formation are uncoupled, thereby increased bone loss (14,15). Our observations are similar to previous findings suggesting that PTH has a permissive role in

TABLE 3. Mean levels and cut off ranges (mean ± 2 SD) of E1G, FSH, OC and CTx in premenopausal, perimenopausal and postmenopausal women

	Premenopausal		Perimenopausal	Menopausal	
Analyte	Normal (N = 102)	Osteopenic (N = 37)	(N = 30)	Normal (N = 28)	Osteoporotic (N = 39)
E_1 G (ng/mgCr)	47.75 ± 7.64 (32.47–63.03)	39.34 ± 6.5 (26.34–52.34)	28.82 ± 5.82 (17.18–40.46)	14.82 ± 4.8 (5.22–24.42)	6.84 ± 3.05 (0.74–12.94)
FSH (mIU/mgCr)	20.22 ± 4.1 (12.22–28.62)	22.64 ± 2.9 (16.84–28.44)	30.45 ± 4.05 (22.35–38.55)	> 50 (50–80)	> 50 (50–80)
OC (ng/mL)	9.50 ± 1.66 (6.18–12.82)	8.93 ± 1.78 (5.37–12.49)	9.49 ± 1.16 (7.17–11.81)	10.02 ± 1.68 (6.66–13.38)	10.29 ± 1.04 (8.21–12.37)
CTx (μg/molCr)	246.6 ± 117.6 (11.4–481.8)	295.2 ± 101.7 (91.8–498.6)	390.6 ± 83.88 (222.81–558.35	547.2 ± 106.2 (334.8–759.6)	686.2 ± 103.2 (479.8–892.2)

age-related bone loss. The levels of bone turnover markers also increased progressively with age.

The subjects enrolled in the present study were heterogeneous and drawn from middle and higher socioeconomic groups with adequate nutritional intake (average dietary Ca intake = 400–600 mg), as observed by random 24-hour recall, and had normal reproductive endocrine function. The Ca levels were within the normal range; however, Indian women have a far lower Ca intake compared to that reported for Western populations (600–800 mg/day).

In this study the ovulatory status of the women was monitored by urinary hormone estimations (E_1G, PdG, and FSH), which provide an integrated picture of hormonal levels. The perimenopausal women showed low E_1G levels with decreased bone mass and elevated levels of CTx, indicating increased bone resorption. In the postmenopausal group extremely low levels of E_1G were observed, and this group had a comparatively high bone turnover as reflected by elevated OC and CTx levels. In the postmenopausal group the E_1G levels further decreased with a high bone resorption with elevated CTx levels. The disturbances in ovulation may not have been reflected in a single estimation of ovarian hormones in serum. An inverse relation between changes in bone turnover markers and estrogen concentration was observed, in agreement with earlier observations (16). Moreover, the urinary hormone levels of E_1G and FSH also reflect a menopausal transition with a concomitant increase in FSH concentrations, which indicates a decline in follicle numbers, and early changes in ovarian function (17).

In conclusion, the present study documents age-related changes in PTH, OC, CTx, and hormone levels in Indian women. Clear cutoff limits of OC, CTx, E_1G, and FSH with no overlap in values were observed among the pre-, peri-, and postmenopausal groups. The results of this study suggest that it may be valid to combine simultaneous measurements of bone turnover markers and ovarian hormones when screening peri- and postmenopausal women at risk for osteoporosis.

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