

## STUDY OF CALCITROPIC HORMONES IN POSTMENOPAUSAL WOMEN AND THERAPEUTIC RESPONSES TO ESTROGEN CALCIUM AND VITAMIN D SUPPLEMENTATION

Dr. Narendra Vaghela

Ex. Assistant Professor Dept. of OBGY Govt. Medical College, Surat, Gujarat

**Article Info:** Received 17 October 2021; Accepted 27 December 2021

**DOI:** <https://doi.org/10.32553/ijmbs.v5i12.2380>

**Corresponding author:** Dr. Narendra Vaghela

**Conflict of interest:** No conflict of interest.

### Abstract

**Background:** A range of techniques, such as histomorphometry, densitometry, calcium flux measurement, and estimate of biochemical bone markers, can be used to assess the status of the skeleton. Histomorphometry is an invasive, costly, time-consuming procedure that can only be performed on a single bone site. Densitometry is accurate and noninvasive, but it takes a long time to detect changes. Technically, measuring calcium fluxes is tough. Biochemical indicators of bone remodelling, on the other hand, are noninvasive and respond to intervention more quickly than densitometry. Biochemical bone markers can be found in the blood or urine and represent the process of bone remodelling. There are three types of byproducts produced during the creation of new bone: (a) enzymes or proteins released by bone cells participating in the remodelling process, (b) breakdown products formed during the resorption of old bone, and (c) byproducts generated during the resorption of old bone.

**Aim:** Study of biochemical markers of calcitropic hormones in postmenopausal women and therapeutic responses to estrogen, calcium and vitamin D supplementation.

**Material and Method:** Over a period of six months 150 women were recruited, to study. Among 150 subjects, 100 women had normal hepatic and renal function and were euthyroid. These 100 subjects were divided into two groups based on their menstruation. The women who have cessation of menstruation were included into study or post menopause group and the women who have regular menstrual cycles were considered as control or pre menopause group.

**Results:** The biochemical markers of bone resorption such as serum TRAP, urine free hydroxyproline and urine free deoxypyridinoline levels were significantly elevated in postmenopausal women. Also, the levels of urinary calcium and the indices of calcium/creatinine ratio were elevated in postmenopausal women over control group. Highly elevated levels of follicle stimulating hormone and luteinizing hormone and highly declined levels of estradiol were observed in postmenopausal women. The serum 25-hydroxy vitamin D levels were found to be insufficient in both control and study groups. However, the parathyroid status was found to be in the normal range and were identical in both groups in the background of insufficient vitamin D status and deficient calcium intake.

**Conclusion:** The further extensive studies are required to arrive at final definite conclusions, regarding the therapeutic approach in postmenopausal women of Central India. Speculations were made at many instances where the situation demanded, and of course, speculations are inevitable in any scientific study to pave the way for further investigation. For instance, the estimation of interleukins, interferon and other cytokines, which influence bone turn over would be more useful in understanding overall spectrum of bone remodeling.

**Keywords:** vitamin D, TRAP, calcitropic, estrogen and calcium

### Introduction

Osteoporosis is a disorder marked by a loss of bone mass and a disturbance of bone microarchitecture, leading to increased bone fragility and fracture risk. The World Health Organization (WHO) recently recommended a more therapeutically meaningful definition based on bone mineral density measurements (BMD).<sup>(1,2)</sup> As per WHO definition, bone mass in a given individual is compared to the mean peak bone mass of young normal individuals. Osteoporosis is characterised by fractures. Hip fractures are the most dangerous of the hip, spine, and wrist fractures because of their high morbidity and mortality rates. Fractures are predicted to climb dramatically as the population ages and

life expectancy continues to improve. The greatest increase is likely to occur in Asia, Africa, and Latin America, where osteoporosis awareness is significantly lower than in the West.<sup>(3)</sup>

To reduce accelerated bone loss in early postmenopausal women<sup>(4)</sup> and to minimize the fracture risk, a variety of prophylactic treatments like calcium, vitamin D and antiresorptive therapies (estrogen alone [Estrogen Replacement Therapy], estrogen combined with progestin [Hormone replacement therapy], bisphosphonate, calcitonin, and selective estrogen receptor modulators [SERMs] and

synthetic parathyroid hormone) have been used. There were several studies on the effects of alendronate, risedronate, salmon calcitonin and raloxifene on fracture incidence but the studies on estrogen therapy are sparse.<sup>(5)</sup> Hormone replacement therapy is the treatment of choice for postmenopausal osteoporosis prevention and treatment. In postmenopausal women, it can prevent bone loss, enhance bone density in the spine and hips, and lower the risk of hip and spinal fractures. Even if started after the age of 70, it is effective.<sup>(6,7)</sup> Estrogen replacement therapy (ERT) alone has been used for prevention and treatment of postmenopausal osteoporosis for over 50 years.<sup>(5)</sup> Chen and Chow (2001) reported that combination of vitamin D3 analogues with estrogens were more effective than estrogen alone for the prevention of bone loss in postmenopausal women under calcium supplementation.<sup>(8)</sup>

There were only few studies documented the impact of estrogen replacement therapy (ERT), calcium and vitamin D supplementation on bone turnover in postmenopausal women in Western countries. Whereas in India, there was no study of this nature was carried out so far. Further, there is a lack of normative literature about biochemical markers of bone turnover, vitamin D status and therapeutic suitability of postmenopausal women pertaining to the Indian scenario, particularly in Central India.<sup>(9)</sup>

In the present study, according to the suggestion of Morgan (2001), the supplementation of calcium and vitamin D was given as an adjunctive therapy together with estrogen replacement therapy to treat both medical and surgical menopause women.<sup>(12)</sup>

### Material and Methods

Over a period of six months 150 women were recruited to study. Among 150 subjects, 100 women had normal hepatic and renal function and were euthyroid. These 100 subjects were divided into two groups based on their menstruation. The women who have cessation of menstruation were included into study or post menopause group and the women who have regular menstrual cycles were considered as control or pre menopause group. Of 100 subjects, 50 belonged to study group whereas the rest of the subjects belonged to control group. Anthropometric characteristics, the levels of calcium and phosphorus in diet, serum and urine, calcitropic hormone status and the levels of biochemical markers of bone turnover were studied in postmenopausal women along with control group.

**Inclusion criteria:** All postmenopausal women (natural and surgical menopause) who have cessation of menstruation were considered as post menopause group. Fertile women with regular menstrual cycles were considered as pre menopause (control) group.

**Exclusion criteria:** The subjects who have thyroid disorders, parathyroid disorders, liver or renal disorders (information obtained by laboratory assays) were excluded from the study and control groups. Women who had used calcium supplements, vitamin D preparations, antiresorptive therapy, thiazides, steroids, or other drugs that could influence bone or vitamin D metabolism (information gleaned from their medical records) were also excluded.

### Clinical assessment

In every patient detailed history of menstrual cycles, postmenopausal symptoms, diet history, exposure to Sunlight and other general examinations were elicited. Examination of pulse, blood pressure, anemia, jaundice, lymph nodes, cataract, bone deformity, bone tenderness, fractures, skin lesions, thyroid palpable, secondary sex characters, tetany, height, weight etc., was recorded in each patient (data not presented). Body mass indices were calculated in each subject from their body weight and height.

### Study design

The postmenopausal women who have no evidence of malignancy in mammogram were advised to take calcium carbonate (800 mg/day), 1,25dihydroxy vitamin D3 (0.25 µg/day) and conjugated equine estrogen (0.625 mg/day) in tablet form. All the treated subjects were advised to attend every month to examine therapeutic response clinically. In this fashion, sixty-one postmenopausal women completed one-year therapy

### Methods:

- Serum calcium, phosphorus, total alkaline phosphatase, creatinine, bilirubin, serum glutamate-oxaloacetate transaminase (SGOT), serum glutamate-pyruvate transaminase (SGPT), serum glutamate-pyruvate transaminase (SGPT), and 24-hour urine calcium, phosphorous, creatinine were measured using an automated analyzer.
- The levels of follicle stimulating hormone in human serum samples were measured using an immunoradiometric test kit.
- Luteinizing hormone levels were quantitatively determined by using immunoradiometric assay kit in human serum samples.
- Coated tube radioimmunoassay kit was used for the quantitative determination of estradiol in human serum.
- The intact PTH levels in human serum were estimated by immunoradiometric assay (IRMA).
- 25-hydroxy vitamin D levels (25-OH vitamin D) estimation: Radioimmunoassay kits were used to measure 25-hydroxy vitamin D (25-OH vitamin D) levels.

### Result:

**Table 1: Levels of Luteinizing hormone, biochemical bone markers and calcitropic hormones in serum and urine samples of pretreatment and post-treatment groups of postmenopausal women**

| Parameters (Units)                       | Pre menopause Control group Mean $\pm$ SE | Post-treated postmenopausal group Mean $\pm$ SE |
|--|---|---|
| Serum LH (IUIL)                          | 4.63 $\pm$ 0.14                           | 52.6 $\pm$ 0.35                                 |
| Serum FSH (IU/L)                         | 5.44 $\pm$ 0.28                           | 24.16 $\pm$ 0.62                                |
| Serum Estradiol (Pg/ml)                  | 99.22 $\pm$ 0.066                         | 35.49 $\pm$ 1.44                                |
| Serum TSH ( $\mu$ IU/ml)                 | 1.77 $\pm$ 0.25                           | 0.84 $\pm$ 0.19                                 |
| Serum Creatinine (mg/dl)                 | 0.55 $\pm$ 0.07                           | 0.76 $\pm$ 0.12                                 |
| Serum total ALP (IU/L)                   | 69.31 $\pm$ 2.14                          | 79.66 $\pm$ 1.015                               |
| Serum TRAP (U/L)                         | 3.56 $\pm$ 0.28                           | 5.51 $\pm$ 0.83                                 |
| Urine free OH-Pro (pmol/mmol Creatinine) | 4.17 $\pm$ 0.24                           | 7.03 $\pm$ 0.27                                 |
| Serum intact-PTH (pg/ml)                 | 19.07 $\pm$ 0.56                          | 21.47 $\pm$ 0.87                                |
| Serum 25-hydroxy vitamin D (ng/ml)       | 14.53 $\pm$ 0.69                          | 11.19 $\pm$ 0.55                                |

The luteinizing hormone (LH) levels were found to be significantly elevated in post-treatment group when compared with controls. The bone resorption markers such as serum TRAP and wine free hydroxyproline were increased in post-treatment group over controls and were significant. A significant reduction in the levels of intact-PTH and significant elevation in the levels of 25-OH vitamin D were observed in post-treatment group over controls.

**Table 2: Serum and urine levels of calcium, phosphorus, and calcium/creatinine ratios in control group and post-treatment group of postmenopausal women**

| Parameters (Units)               | Pre menopause Control group Mean $\pm$ SE | Post-treated postmenopausal group Mean $\pm$ SE |
|----------------------------------|---|---|
| Serum Calcium (mg %)             | 7.44 $\pm$ 0.021                          | 7.67 $\pm$ 0.030                                |
| Serum Phosphorus (mg %)          | 2.29 $\pm$ 0.082                          | 2.44 $\pm$ 0.036                                |
| Urine Calcium (mg/day)           | 116.3 $\pm$ 6.215                         | 138.6 $\pm$ 3.294                               |
| Urine Phosphorus (mg/day)        | 378.1 $\pm$ 17.81                         | 356.4 $\pm$ 5.291                               |
| Urine Calcium/ Creatinine ratio" | 0.124 $\pm$ 0.005                         | 0.157 $\pm$ 0.003                               |

The results of the serum and urine levels of calcium and phosphorus, and urine calcium/creatinine ratios in control group and post-treatment group of postmenopausal women were mentioned in table 2. The mean values of serum calcium, urine calcium and urine calcium/creatinine ratios were increased in post-treatment group of postmenopausal women over the control group. The percent increase in the levels of serum calcium was significant level and the percent increase in the levels of wine calcium and urine calcium/creatinine ratios were significant. The serum and urine levels of phosphorus in post-treatment group and controls were found to be similar.

### Discussion

To reduce accelerated bone loss in postmenopausal women and to minimize the risk of fracture, various treatments like calcium, vitamin D and antiresorptive therapies (hormone replacement therapy, bisphosphonate, calcitonin, selective estrogen receptor modulators and synthetic parathyroid hormone) have been used. (Shoemaker and Klemes, 2003)<sup>(4)</sup>

Reginster et al. (1992)<sup>(13)</sup> reported that postmenopausal women receiving estrogen replacement therapy (0.625

mg/day) for at least six months have shown raised estradiol levels to a minimal value 60 pg/ml. The increase in estradiol levels were known to decrease LH levels by acting on pituitary gland.(West et al., 1974)<sup>(14)</sup> In the present study also, a reduction in LH levels after therapy indicates that the circulating estradiol levels were raised with therapy.

Reginster et al. (1992)<sup>(13)</sup> also opined that, a significant reduction in bone resorption is achieved when circulating levels of estradiol reach a value 60 pg/ml. In the present study also a significant reduction in bone resorption was observed after therapy as evidenced by the decreased levels of bone resorption markers such as serum TRAP and urine free hydroxyproline levels. Not only bone resorption markers but also bone formation marker was declined to a lower extent. Significant decline in bone formation markers is due to significant reduction in bone resorption markers, since bone resorption and bone formation are independent and coupled processes. Estrogen replacement therapy is known to diminish bone resorption and bone formation markers. In the present study also, the increased estradiol levels through estrogen replacement therapy might have diminished both the markers of bone formation and resorption to a variable extent so as to reduce or to minimize the bone loss.

Generally, most of the absorbed calcium is deposited in bone. Besides the deposition of calcium in bone, calcium also plays a crucial role in vital functions such as muscular contraction, neurotransmission, membrane transport, enzyme reactions, hormone secretion, and blood coagulation and as a second messenger. If the levels of calcium present in extracellular fluid are more than the physiological concentrations, the body regulatory system removes the excess calcium from extracellular fluid through kidneys to maintain calcium homeostasis. In the present study, consequent to calcium treatment the extracellular calcium levels might have increased beyond the physiological concentrations thereby the regulatory system might reported to excrete the excess calcium as a mechanism to maintain calcium homeostasis. Hence, the increased levels were observed in urine calcium and urine calcium/creatinine ratios.

Generally, dietary deficiency of calcium and malabsorption of calcium enhance the synthesis of PTH, which thereby acts on bone to mobilize calcium reserves into extracellular fluid to maintain calcium homeostasis. During the supplementation of calcium and vitamin D, sufficient physiological concentrations of calcium are maintained. Hence, there is no need for excess synthesis of PTH from parathyroid gland to maintain calcium homeostasis.

The increased intact-PTH levels were suppressed after calcium and vitamin D supplementation. Therefore, a significant decline in increased intact-PTH level was observed with therapy. This was confirmed by several studies, which suggested that PTH secretion is suppressed by 24-hour calcium infusion (**Ledger et al., 1995**)<sup>(15)</sup> or long term calcium supplementation (**McKane et al., 1996**)<sup>(16)</sup> and by short-term treatment with calcitriol. (**Ledger et al., 1994**)<sup>(17)</sup> In a study (**McKane et al., 1996**)<sup>(16)</sup>, increased levels of bone turnover markers in elderly women were found to be reversed by long term calcium and vitamin D supplementation, which means that the prolonged supplementation of calcium and vitamin D inhibits PTH-mediated bone resorption. **Khosla et al. (1997)**<sup>(18)</sup> studied 138 premenopausal women (mean age: 35 years) and 213 postmenopausal women (mean age: 68 years). Among the 213 postmenopausal subjects, 47 (22%) were receiving estrogen replacement therapy. In the present study, bone resorption markers (serum TRAP and urine free hydroxyproline) significantly elevated in post-treatment group of postmenopausal women over control group. From this finding, it is obvious that some more therapeutic period is necessary to normalize the levels of bone resorption markers with controls or to bring down the levels of bone resorption markers to the levels of controls.

### Conclusion:

The further extensive studies are required to arrive at final definite conclusions, regarding the therapeutic approach in postmenopausal women of South India. Speculations were made at many instances where the situation demanded, and of course, speculations are inevitable in any scientific study

to pave the way for further investigation. For instance, the estimation of interleukins, interferon and other cytokines, which influence bone turn over would be more useful in understanding overall spectrum of bone remodeling. Due to constraints of funding, facilities, besides the time, the above estimations could not be carried over. Nevertheless, the author is hopeful to carry out these studies in detail in his post-doctoral pursuit.

### References:

1. Peck WA, Burckhardt P, Christiansen C, Fleisch HA, Genant HK, Gennari C, Martin TJ, Martini L, Morita R, Ogata E, Rapado A, Shulman LE, Stem PH, Young RIT. Consensus development Conference: diagnosis, prophylaxis and treatment of osteoporosis. *Am J Med* 1993;94: 646-650.
2. Kanis JA. Assessment fracture risk and its application to postmenopausal osteoporosis. *Osteoporosis in* 1994;14(6): 368-381.
3. Pande KC. Prevalence of low bone mass in healthy Indian population. *J Indian Med Assoc* 2002;100(10): 598-600,602.
4. Shoemaker JR, Klemes AB. Alternate options to hormone replacement therapy for osteoporosis. *J Osteopath Assoc* 2003;103: S1-5.
5. Marcus R, Wong M, Heath III H, Stock JL. Antiresorptive treatment of postmenopausal osteoporosis: comparison of study designs and outcomes in large clinical trials with fracture as an endpoint. *Endocr Rev* 2002;23: 16-37
6. Greenspan FS, Baxter JD. Basic and clinical Endocrinology. Chapter 5 - The calcitropic hormones and metabolic bone diseases, 4<sup>th</sup> edition: 1994;293-296.
7. Prestwood KM, Pilbeam CC, Burleson JA, Woodiel FN, Delmas PD, Deftos LJ, Raisz LG. The short-term effects of conjugated estrogen on bone turnover in older women. *J Clin Endocrinol Metab* 1994;79: 366-371.
8. Chen M, Chow SN. Additive effect of  $\alpha$ -calcitriol on bone mineral density of the lumbar spine in Taiwanese postmenopausal women treated with hormone replacement therapy and calcium supplementation: a randomized 2-year study. *Clinical Endocrinology* 2001;55: 253-258.
9. Chailurkit L, Ongphiphadhanakul B, Piaseu N, Saetung S, Rajatanavin R. Biochemical markers of bone turnover and response to bone mineral density to intervention in early postmenopausal women: an experience in a clinical laboratory, *Clinical Chemistry* 2001;47: 1083-1088.
10. Nguyen TV, Sambrook PN, Eisman JA. Source of variability in bone mineral density measurement: implications for study design and analysis of bone loss. *JBone Miner Res* 1997;12: 124-135.
11. Ravn P, Ciemmesen B, Christiansen C. Biochemical markers can predict the response in bone mass during alendronate treatment in early postmenopausal women. *Bone* 1999;24: 237-244.

12. Morgan SL . Calcium and vitamin D in osteoporosis. *Rheum Dis Clin North Am* 2001;27: 101-130.
13. Reginster J, Minne HW, Sorensen OH, Hooper M, Roux C, Brandi ML, Lund B, Ethgen D, Pack S, Roumagnac I, Eastell R. Randomized trial of the effects of risedronate on vertebral fractures in women with established postmenopausal osteoporosis. *Osteoporosis Int* 2000;11: 83-91
14. West ESES, Todd WR! Mason HS, Bruggen JTV . *Textbook of biochemistry*. 4' edition, Chapter 34, The Hormones. 1974;1471-1472.
15. Ledger GA, Burritt MF, Kao PC, O'Fallon WM, Riggs BL, Khosla S. Role of parathyroid hormone in mediating nocturnal and age-related increases in bone resorption. *J Clin Endocrinol Metab* 1995;80: 3304-3310.
16. McKane WR, Khosla S, Egan KS, Robins SP, Burritt MF, Riggs BL. Role of calcium intake in modulating age-related increases in parathyroid function and bone resorption. *J Clin Endocrinol Metab* 1996;81: 1699-1703.
17. Ledger GA, Burritt MF, Kao PC, O'Fallon WM, Riggs BL, Khosla S. Abnormalities of parathyroid hormone secretion in elderly women that are reversible by short term therapy with 1,25-dihydroxy vitamin D3. *J Clin Endocrinol Metab* 1994;79: 211-216.
18. Khosla S, Atkinson EJ, Melton LJ 111, Riggs BL. Effects of age and estrogen status on serum parathyroid hormone levels and biochemical markers of bone turnover in women: A population-based study. *J Clin Endocrinol Metab* 1997;82: 1522-1527.